Synthetic Carbohydrate

An Aid to Nutrition in the Future

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FINAL REPORT

SYNTHETIC CARBOHYDRATE

AN AID TO NUTRITION IN THE FUTURE

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ABSTRACT

This report discusses the synthetic production of carbohydrate on
a large scale. First of all, the world food situation is studied in
some detail. Next, three possible nonagricultural methods of making
starch are presented in detail and discussed. The simplest of these,
the hydrolysis of cellulose wastes to glucose followed by polymeriza-
tion to starch, appears a reasonable and economic supplement to agri-
culture at the present time.

The conversion of fossil fuels to starch was found to be not com-
petitive with agriculture at the present time, but tractable enough to
allow a reasonable plant design to be made. This design may serve as
a basis for future analysis, should needs, costs, or technology change.
It may also serve as a model for the design of similar but more compli-
cated multienzyme, large scale processes, such as mock photosynthesis.

A reconstruction of the photosynthetic process using isolated en-
zyme systems proved technically much more difficult than either of the
other two processes. Particular difficulties relate to the replacement
of expensive energy carrying compounds, separation of similar materials,
and processing of large reactant volumes. Problem areas have been pin-
pointed, and technological progress necessary to permit such a system to
become practical has been described. This process is of special interest
because of its potential use to recycle metabolic waste (CO_2) on prolonged
space flights.

Next, the possible use of synthetically produced carbohydrate materi-
al is discussed in some detail. Finally, an analysis of the implementation
of the cellulose process in a representative area and its impact on the
social, economic, and political factors of the area is made.

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ACKNOWLEDGMENTS

The study participants and advisors are indebted to these speakers for the time and effort they spent in preparing and presenting a uniformly outstanding series of lectures. The talks contributed immensely to the knowledge of the class and the success of the study.

As director of the Stanford portion of the program, I would like to thank all of the NASA, university, and industry people who participated in the various informal discussions and criticisms which helped form this report. I would like to thank the NASA/Ames Research Center for its hospitality and for the enthusiasm it has shown for the program. I would like to thank the incomparable Ms. Caroline Duff for her patience, humor, organizational competence, and conspicuously overadequate secretarial skills. I would like to thank Jerry Berman and Kate Murashige for an excellent job of editing this report and Inga Lof for a beautiful job of organizing and typing. I would like to thank Channing Robertson and Jake Shapira for the time and energy which they put into the study as advisors to the chemical and process groups and Dr. John Billingham, my NASA/Ames Co-director, for the many hours he put into this effort above and beyond his busy schedule, for his enthusiasm and technical contributions, and for initially convincing me that this project was not really insane. Lastly, I would like to thank the Faculty Fellows who participated in this project and their most beautiful families. I have never worked with a more enjoyable summer group and will long remember their virtuosity with matters as sophisticated as enzyme chemistry and as humble as cornstarch. I hope that they enjoyed the program as much as I did and benefited sufficiently from the program to repay them for the many hours of work which they contributed.

J. L. Adams

Chapter 1

INTRODUCTION AND SUMMARY

1.1 General

This report is the result of an eleven week systems design project
sponsored jointly by the National Aeronautics and Space Administration
and the American Society of Engineering Education. The group consisted
of eighteen faculty members from across the nation, representing a number
of disciplines. Stanford University and Ames Research Center were the
host institutions for the workshop. The purpose was both to give the
participants experience in systems engineering and to produce a useful
study.

The project involved applying a systems approach to study nonagricul-
tural carbohydrate production on a large scale. The project grew out of
work done at the Ames Research Laboratory in the general area of synthe-
sizing food for long-duration manned space missions. Since approximately
80% by weight of a person's diet is carbohydrate, and since carbon dioxide
and water are available from the metabolic cycle, the Ames work naturally
focused toward the production of carbohydrate from carbon dioxide and water.
Once into this work, it was impossible to avoid considering the possibili-
ties of using such a synthetic process to provide food on a large scale
here on earth.

The decision was made to use this problem as a focus for the summer
workshop for a number of reasons. The problem was extremely cross disci-
plinary, since social, economic, and political considerations were involved
as well as technical ones. Since preliminary work at Ames indicated that
the production of carbohydrates would almost certainly require processes
mediated by enzymes, it was obvious that the technology involved would be
exciting. Finally, it was both real and timely and of great interest to
many agencies, industries, and individuals.

Even the most optimistic forecasts of world population growth indicate
a severe challenge to the ability of agriculture to meet future food needs.
Malnutrition and under-nutrition are serious problems in large parts of
the world, even today. World projections which estimate a population of
the order of 7 billion by the year 2000, fail to depict the plight of the
developing countries, where the food needs in 1985 will be 2 1/2 times
that of 1962.

The possible means of increasing the world food supply are improvements
in conventional agriculture and the use of unconventional food sources.
Although there is great potential in the so-called "Green Revolution",
it must be recognized that there are limits to the earth's land and water
resources, and to the capacity of plants to produce, even in the face of
extensive enrichment. The area of potentially arable land exceeds that
cultivated by more than a factor of 3, but more than half of this land
is in the tropics. Furthermore, in Asia, where the population problem is
currently most severe, essentially all of the arable land is already in
use. Better control of water, implying drainage as well as irrigation,
involves high cost. Irrigation alone is conservatively estimated to cost

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$400 per acre to develop at present, and in parts of the world, the use of normal river flow is about at the limit. The Green Revolution offers, at best, a breathing spell in the world's food crisis, while alternatives, such as artificial food processes, are developed.

After the decision was made to investigate this problem, a preliminary study contract was let by Ames Research Center to a group at the University of California at Berkeley to do some process investigations which would be needed by the workshop. The group was headed by Dr. James A. Bassham and produced valuable guidance on various pathways from carbon dioxide and water to starch. This preliminary work, the previous Ames work, and some preliminary thinking about the problem done by the Stanford and Ames advisors was available to the workshop participants when they arrived.

The summer study proceeded through three phases. The Fellows were divided into three groups, each group with a Stanford-Ames advisor. One group was responsible for the chemistry of the process, another for the system hardware aspects, and the third for social, economic, political, and legal aspects of the problem. From time to time ad-hoc groups were formed to work on specific problems which did not fall cleanly within the areas of the three main groups. Each of the three groups selected a leader for each of the three phases of the project. These leaders, a project manager who was selected for each phase, and the Stanford Ames advisory group formed the project management team. The result was that most of the participants played a direct management role in the study.

The first four weeks of the study period were spent in gathering information, attempting to place data in parametric form, examining alternative system concepts, and further defining the problem. The first two weeks made intensive use of lectures by outside experts in areas of interest to the study (specific titles and names are included in the acknowledgments at the end of this document). During this first three week period no attempt was made to consolidate the results of the information gathering into a cohesive whole. In fact, if anything, an effort was made to cause the study to diverge.

The next four weeks were spent in converging upon a system definition. The outline of the final report was developed early in this phase and responsibility for developing various sections of the report assigned to groups and to individuals. Constraints were firmed up and a large amount of time spent in system interface problems. The final three weeks were spent in working out details of the system, providing the necessary cohesiveness, producing a summary report for the final presentation, and in writing the final report.

At the outset of the study, it appeared possible to imitate the reactions used by plants to manufacture carbohydrates, using sources of energy other than sunlight. Although the sequence of reactions leading from carbon dioxide to sugar in plants has been known for some time, the study necessarily concerned itself with the nature of the support systems needed to keep these reactions going. It is these systems that are difficult to reproduce.

Consideration of population trends, political, economic, and legal aspects associated with food production and consumption, and potentials of enzyme chemistry led the group to consider three chemical processes,

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or pathways, which would lead to the production of glucose and starch. The first is a reasonably competitive process which digests cellulose into its edible components. The second is a comparatively simple enzyme mediated process which converts a petrochemical into carbohydrates. The third is a process which mimics photosynthesis, using hydrogen to reduce carbon dioxide to sugar in the same approximately 17 steps used in nature.

This report outlines each of these processes in some detail and points out advantages, disadvantages, and critical problems involved with each. The "cellulose" and "fossil fuel" processes were carried into fairly detailed designs of plants with 100 ton/day capacity. The "CO_2 fixation" process was not carried to detailed plant design, but was given a thorough feasibility study. Political, economic, and social implications of the "cellulose" plant were thoroughly considered.

The world food situation is discussed in considerable detail in Chapter 2 of this report. Chapter 3 is an explanation of the essential concepts of the three pathways to synthetic carbohydrate. Chapter 4 is a detailed chemical treatment of the pathways in the processes. Chapter 5 contains details on process and plant design including flow diagrams, economic balances, and equipment details. Chapter 6 is concerned with the use of the synthetic carbohydrate after it is manufactured. Chapter 7 discusses a sample application of the cellulose system. The appendices include information which was generated during the study and is of possible interest to people interested in this area, but which is of too detailed a nature to be included in the main body of the report.

Chapter 2, 6, and 7 are somewhat self-contained and simple to read. However, Chapters 3, 4, and 5 contain a vast amount of technical detail and may prove somewhat difficult for those new to this problem area. We will therefore conclude this introductory chapter with some summary descriptive material on each of the three processes considered and summary cost data. This material should expedite reading of the more detailed material which follows in later chapters.

1.2 Cellulose Process

The production of food from cellulose has a reasonably long history. Acid hydrolysis of wood chips was carried out on an industrial scale both in the U.S. and in Germany during the first half of this century. However, the use of enzymatic degradation has been a comparatively recent development. Two other groups, one at LSU and one at Berkeley have studied similar processes with similar results in terms of cost data, but no plant has yet been built. A flow diagram and an outline of the chemical reactions used in the process we are proposing appear in Figures 1.1 and 1.2.

The cellulose process utilizes the extracellular enzymes of Trichoderma viride to digest inedible cellulose polymers into glucose or fruit sugar. In the plant designed by this group, bagasse (sugar cane wastes) is used as raw material. After mechanical disruption by chopping and milling, alkali treatment serves to loosen up the structure sufficiently to allow attack by the cellulases from the fungus. (It also dissolves off the hemicellulose impurities contained in the raw material.) The pretreated material is then sent to a reactor along with the exudate of

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Hemicellulose

```
Structured                                    Loosened
Cellulose        ╔═══════════╗                Cellulose
           ══▶   ║   Alkali  ║   ══▶
    +            ╚═══════════╝                    +
  Lignin                                        Lignin
    +
Hemicellulose
```

```
                                        ╔═══════════╗
                                        ║ Cellulase ║  ──▶ Lignin
                                        ╚═══════════╝
```

Glucose Syrup

```
        ADP
         ▲
Glucose-6-   ╔═══════════╗
Phosphate ◀══║ Hexokinase║ ◀══ Glucose Syrup
         ║   ╚═══════════╝
         ║        ▲
         ▼       ATP
  ╔═══════════════╗
  ║     PGM       ║  ══▶   Glucose-1-P
  ║ Phosphorylase ║
  ╚═══════════════╝              +
         │                 Glucose-6-P
         ▼
        P_i                      +

                               Starch
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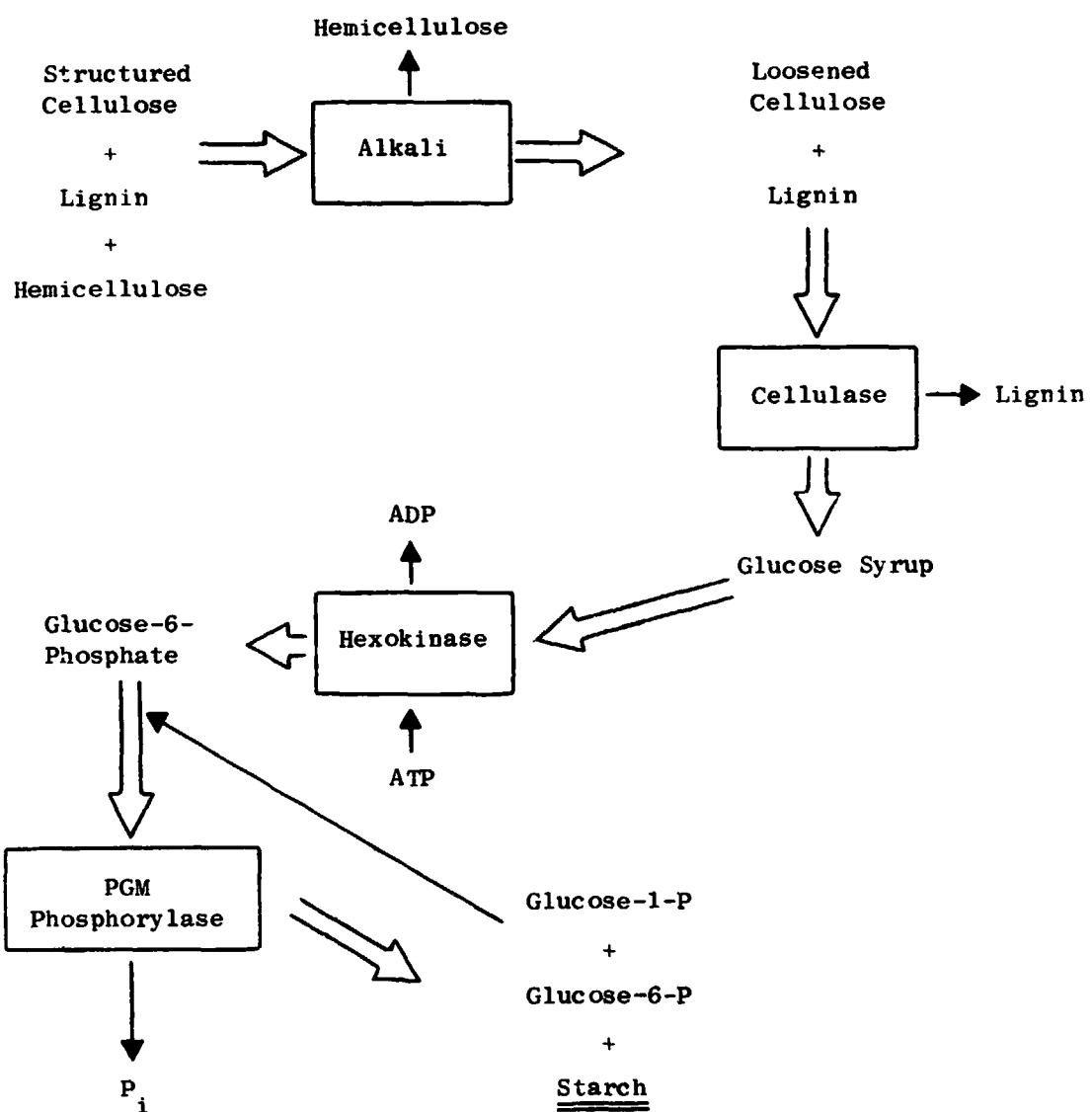
Figure 1.1.   SIMPLIFIED FLOW DIAGRAM--CELLULOSE TO STARCH.
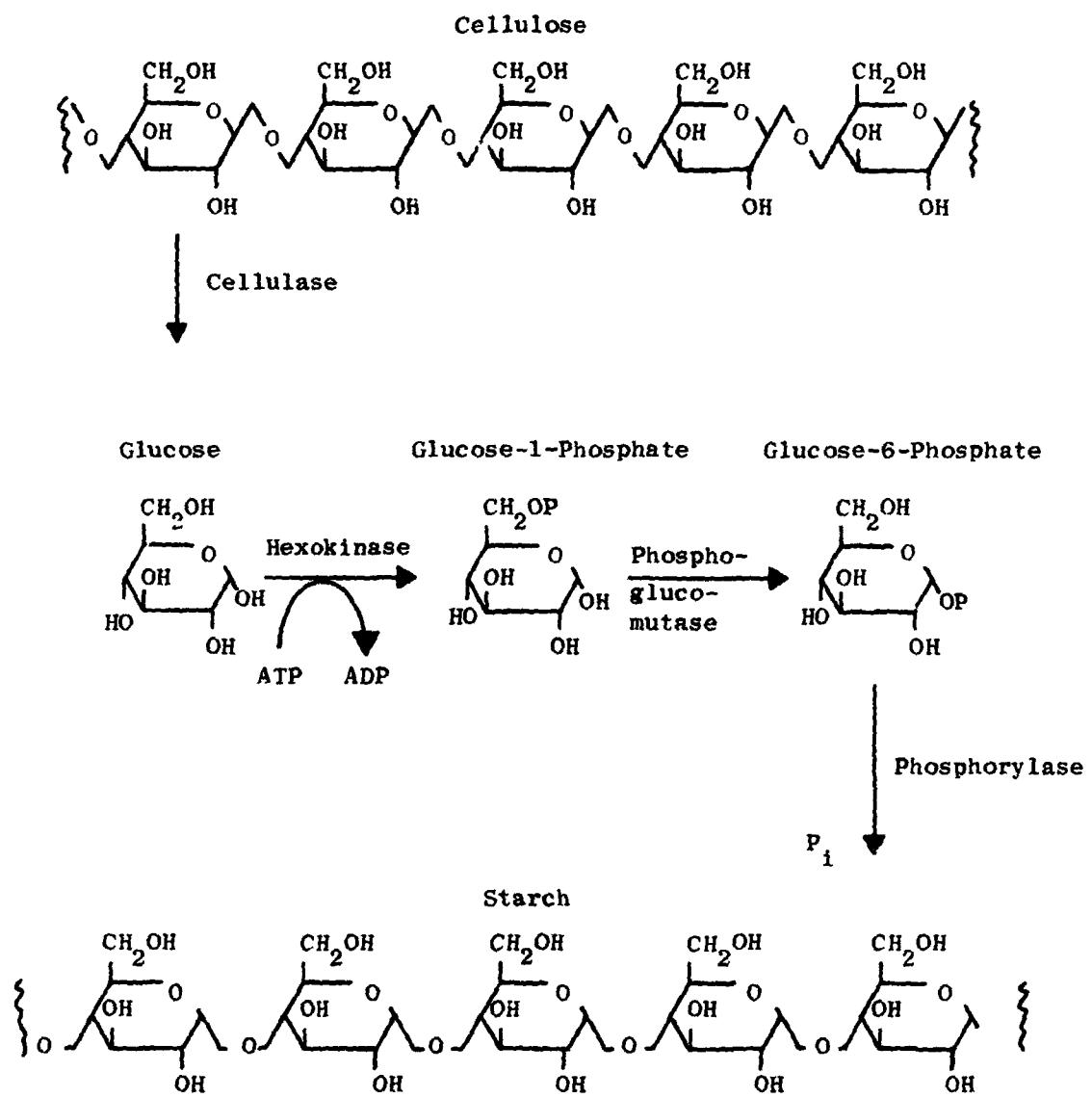
Figure 1.2.   CHEMICAL PATHWAY--CELLULOSE TO STARCH.

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the Trichoderma. (The organism is grown in a fermenter in parallel with the reactor and uses the raw bagasse as an energy source in the culture medium.) After hydrolysis in the reactor, which produces a 10 to 15% glucose solution, the contents are filtered allowing the product glucose to pass, but retaining the enzyme and unused cellulose for recycling and reuse.

The glucose produced is converted to starch in an additional three step process using microbial enzymes. The first two steps add a phosphate to glucose; due to the peculiarities of available enzymes this must be done by adding it first to one carbon atom in the glucose molecule and then transferring it. The source of the phosphate is ATP, a well known but expensive biochemical, and two enzymes are used, hexokinase from yeast and phosphoglucomutase from Escherichia coli. The third step causes the phosphorylated glucose to polymerize, giving starch.

The design of this segment of the process presented several challenges. There are few design precedents on which to base estimates and to delineate problems, as enzymatic conversions on an industrial scale have been restricted to very small quantities of material (as in drug production) or have involved the use of whole organisms in fermentations (as in wine production).

First, the large scale initial production of three relatively purified enzymes is required, and although yeast and E. coli (the sources) are easily grown, considerable quantities (on the order of 50 tons for a 100 ton/day plant) are needed. The production facility must also necessarily provide for replenishment of these enzymes as they deteriorate.

Another concern was the requirement that adenosine triphosphate (ATP) be constantly regenerated. Because of the cost of ATP, the breakdown products of ATP which are sloughed off at different points must be recombined. In order to accomplish this, a satellite regenerator is proposed. In this regenerator, the breakdown products [adenosine diphosphate (ADP) and inorganic phosphate (P_i)] are mixed in the presence of potassium cyanate and an enzyme from E. coli which condenses the intermediately formed carbamyl phosphate with ADP to form ATP. To complete the cycle, the potassium cyanate is also regenerated from the products of this reaction. Aside from replacement of losses then, no additional raw materials are required.

A third hurdle was the design of separation systems which would discriminate on a large scale between such similarly charged chemicals as ADP and glucose-6-phosphate. Ion exchange chromatography is used because the alternative method, selective membranes, requires excessive areas and pressure. This involves considerable scaling up of what is ordinarily a laboratory procedure. It is also necessary to glean the final starch product away from its smaller precursors and the enzymes that make it. The problem of precipitating this out and allowing the recovery of the soluble enzyme, as well as recycling the phosphate and unreacted sugar-phosphate, involves a complex regime of centrifugation, ion exchange adsorption and enzyme stabilization.

The cost of construction of a plant to produce 100 tons of glucose from cellulose per day is estimated to be about $3.5 million. At operating and material costs based on present day prices, the glucose could be produced at about 4.5¢ per pound. Cost estimates for the conversion of

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glucose to starch run 5.8¢/lb. This gives a total cost per pound of starch produced from cellulose as 10.3¢/lb. (This is in the range of current carbohydrate costs which range from 2¢ to 18¢ per pound of metabolizable starch. To the extent that glucose could be tapped off and used as a food supplement, the price would be even more competitive.)

A sugar cane mill processing 4000 tons of cane per day for a four month crushing season would provide enough bagasse to operate a 100 ton per day plant* the year round. Bagasse can be easily handled and stored to retain its value as a raw material. A starch factory in conjunction with a sugar mill would provide both jobs and food where they are most needed, as well as contributing to general economic development through maximizing resource utilization.

Starch offers at least three attractive possibilities for improving food supplies: (1) a basic component in a prepared food, (2) an extender for conventional food components, and (3) a basic component in animal feeds. The first is the most appealing from the standpoint of increasing the quantity of foods meeting basic nutritional needs. A high starch product widely used in Japan is "Instant Ramen", a noodle packed in individual servings which requires only the addition of boiling water for preparation. Such a product would be generally acceptable over the world and could be easily fortified as well as flavored to suit regional tastes. Starch is already used with flour in baked goods in many parts of the world and commercial recipe formulations could dramatically increase that use. The third use, as animal feed, offers promise of improving diets through meat protein, most likely chicken. Chicken feeds are high in energy, and the energy component represents close to one-half of feed costs. Consumer acceptance of starch as a food would no longer present a problem, and availability of food grains for human use would be increased.

1.3 Fossil Fuel Process

The fossil fuel process has considerably higher projected costs than the cellulose process and was, therefore, looked upon more as a design challenge than as an immediately applicable solution to the world's food problems. A flow diagram for the process is shown in Figure 1.3 and the chemical reactions in Figure 1.4.

The sequence uses glycidaldehyde, an intermediate in the industrial conversion of petroleum to glycerol, as starting material. The glycidaldehyde is distilled into aqueous solution in contact with an acid resin to convert it to a mixture of glyceraldehyde and dihydroxyacetone, both 3-carbon sugars. An enzyme, triokinase, then phosphorylates these small sugars (trioses) so that they can be condensed using aldolase to fructose diphosphate (FDP) (a phosphorylated 6-carbon sugar). The FDP is then hydrolyzed to fructose (a component of invert sugar) which could, if desired, be tapped off as a product. Since fructose is 1.7 times as sweet as sugar, it is a valuable food chemical.

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*
A plant producing 100 tons of starch per day would provide 1/2 of the carbohydrate requirement for 500,000 people.

Glycid-
aldehyde → water, acid → D-glyceraldehyde
L-glyceraldehyde
dihydroxyacetone → Triokinase

ATP

→ ADP

↓

L-glyceraldehyde
+
D-glyceraldehyde
phosphate
+
dihydroxyacetone
phosphate

Fructose ← Triose
1,6-diphosphate   Phosphate
Isomerase

Aldolase

↓

P$_i$ ← Alkaline
Phosphatase

↓

Fructose → Glucose
Isomerase → Fructose
+
Glucose → Hexokinase → ADP

ATP

↓

Glucose-6-Phosphate

Glucose-1-P
+
Glucose-6-P
+
Starch ← Phosphogluco-
mutase

Phosphorylase

↓

P$_i$

Figure 1.3. SIMPLIFIED FLOW DIAGRAM--GLYCIDALDEHYDE TO STARCH.

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glycidaldehyde

CHO
|
CH
| O
CH₂

H^+

H_2O

D,L-glyceraldehyde   +   dihydroxyacetone

CHO
|
CHOH
|
CH₂OH

CH₂OH
|
C=O
|
CH₂OH

triokinase

ATP

ADP

D-glyceraldehyde-
phosphate

dihydroxyacetone-
phosphate

CHO
|
H-C-OH
|
CH₂OP

CH₂OH
|
C=O
|
CH₂OP

triose phosphate
isomerase

aldolase

fructose

CH₂OH
|
C=O
|
HO-C-H
|
H-C-OH
|
H-CO-H
|
CH₂OH

alkaline

phosphatase

P_i

fructose-1,6-diphosphate

CH₂OP
|
C=O
|
HO-C-H
|
H-C-OH
|
H-CO-H
|
CH₂OP

glucose
isomerase

glucose   glucose-6-phosphate   glucose-1-phosphate

CH₂OH
O
OH
HO
OH

hexokinase

ATP   ADP

CH₂OP
O
OH
HO
OH

phosphogluco-
mutase

CH₂OH
O
HO
OP
OH

phosphorylase

<u><u>starch</u></u>

Figure 1.4.   CHEMICAL PATHWAY.

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The fructose is then converted partially to glucose using glucose isomerase (an enzyme which has been used on an industrial scale by the Japanese to carry out the reverse reaction in order to sweeten syrup). The glucose is then converted to starch in a three step process identical with that used in the cellulose route.

In the total process, the large scale initial production of eight enzymes along with provision for their replenishment is required. In general, microbial sources are to be used--three of the seven will be prepared from yeast, three from E. coli, one from Streptomyces albus, and one from a Bacillus subtilis mutant. It may be possible eventually to use one source for all these enzymes, but only known sources and established purification procedures have been considered. The quantities of starting materials, in terms of dry cell weight, range from 48 lbs (to isolate triose phosphate isomerase) to 70 tons (to isolate the triokinase).

It is planned to run the process as far as glucose-6-phosphate with stirred tank reactors using the enzymes on solid supports so that they can be filtered out. Four of the six enzymes in this portion of the sequence have been immobilized on various particle backings by others. It is assumed that attempts to apply these techniques to the remaining two would be successful.

The production cost of glucose from this process would be 10.1¢ per pound exclusive of the raw material, and carrying this to starch in the same facility would add another 5¢. The total cost of 15.1¢ per lb would then be in the competitive range if it were not for the cost of the glycidaldehyde, which is estimated at 17¢/lb. Since the precursor of glycidaldehyde (propylene) is quite cheap (corresponding to 1.5¢ per lb of carbohydrate) a breakthrough in this industrial process could make the process practical.

As they now stand, however, these figures are much higher than those for either agriculturally produced starch, or cellulose produced starch, so the product could hardly be expected to be economically competitive at the present state of technology. Its use would be restricted to "emergency" applications.

1.4 CO_2 Fixation Process

The CO_2 fixation process is by its very nature an order of magnitude more complex than the fossil fuel process. There are 16 steps in the route from CO_2 to starch (as drawn in Figure 1.5), catalyzed by 13 different enzymes, but the additional number of steps is the least problem.

The pathway is cyclic. In plants, a single CO_2 is attached to a 5-carbon recipient to give a 6-carbon compound. However, this does not work unless the 5-carbon recipient can be regenerated. The plant does this by restructuring 5/6 of these 6-carbon products into 5-carbon recipients. This means the process cannot be carried out in a straightforward way, putting in reactants at the beginning, and getting out products at the end. It implies rather, a controlled separation of 1/6 of the product formed at some point.
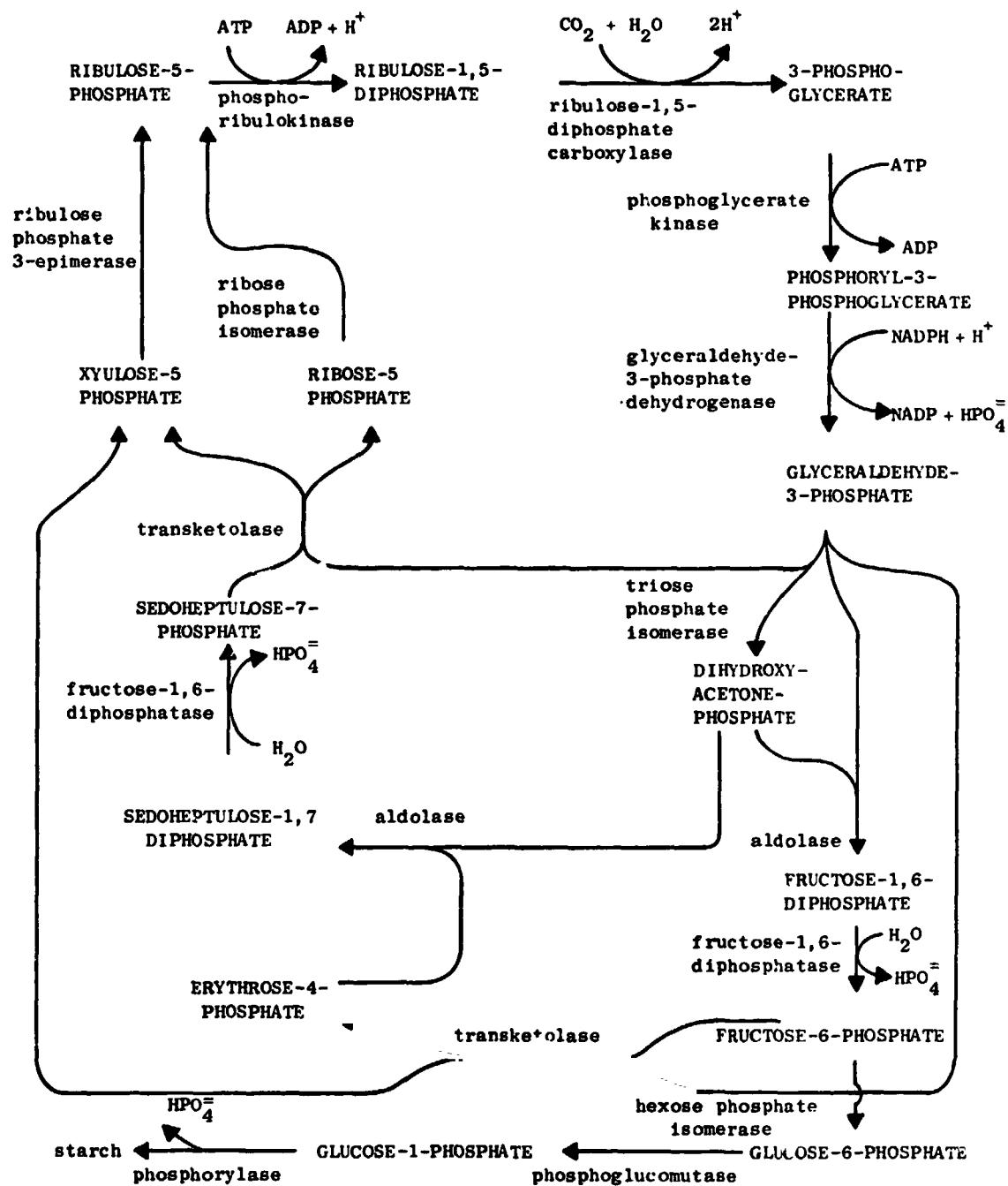
Figure 1.5. CHEMICAL REACTIONS--CO_2 TO STARCH.

Regeneration processes are also escalated; in the formation of one glucose, 18 ATP are required, rather than 2 as in the fossil fuel sequence or 0 as in the cellulose process. ATP is still needed in the glucose-to-starch sequence, and a whole new problem arises due to the oxidation state of the starting material. Reducing power needs to be supplied in the form of NADPH, an unstable biochemical which is oxidized in the process to nicotine adenine diphosphate (NADP$^+$), and must, of course, be re-reduced, since NADPH costs $250/gram at present. This re-reduction implies the use of an external material rather than recyclable intermediates. The possibility of using alcohol dehydrogenase to catalyze its reduction by ethyl alcohol was explored but the enzyme mediated use of H_2 to reduce ferrodoxin (a chemical serving this function in the natural photosynthetic process) was preferred. There is no experimental precedent on which to base this, however.

Enzyme production is also a source of considerable concern. Three of the necessary enzymes have so far been prepared only in crude form. Only four of the 12 enzymes on the sequence from CO_2 to "glucose" have been immobilized on solid supports--two of them with less than spectacular success (the activity falls by a factor of 1000). Quantities required also appear to be high. There would be obvious advantage in obtaining all the enzymes from one source, such as spinach, but even operating at the maximum efficiency of the green plant itself, 500 tons of spinach would be required. If purified enzymes are needed, the amounts increase to 40,000 tons or so of source material.

When these difficulties are quantified, the increments added to the cost of product starch are extremely high. The make-up quantities of NADPH and ATP alone are excessive even at very low attribution rates. In view of this, no attempt was made to design a 100 ton/day factory in detail, but a preliminary cost estimate of 77¢ per pound has been made on the basis of the flow diagram in Figure 1.6.
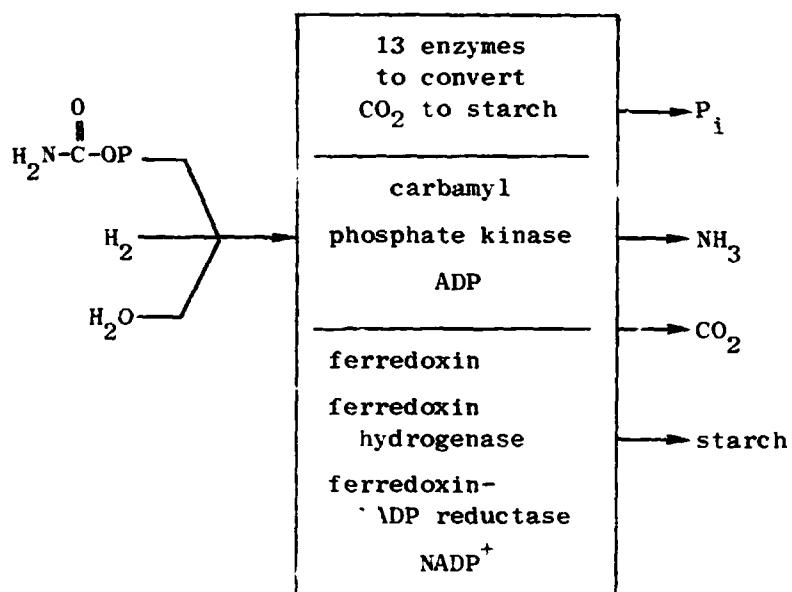
Figure 1.6. SIMPLIFIED FLOW DIAGRAM-CO_2 TO STARCH.

1.5 Cost Data Comparison

A summary of the cost data compiled for each of the three processes is shown in Table 1.1. It is clear that in the cellulose process, ATP regeneration in the production of starch is a heavy factor. Although it becomes larger in the fossil fuel process, it is outweighed by the raw material cost, which then becomes the major cost contributor. In the CO_2 fixation process, again ATP regeneration is costly, and due to the number of enzymes involved, their production is a large item also.

Table 1.2 shows the price of the equivalent of a pound of starch that could be obtained by consuming various agricultural products. (The values assume the product is eaten as is, but are corrected for non-nutritional content.) They are U.S. prices for the year 1970, and are subjected to considerable variation at other times and places. The table is included for the purpose of allowing a rough comparison with synthetic starch costs.

Table 1.1

MANUFACTURING COST FACTORS IN ϕ PER POUND OF PRODUCT

	Cellulose Process		Fossil Fuel Process		CO_2 Fixation
	Cellulose Glucose	Glucose Starch	Glycidaldehyde Glucose	Glucose Starch	CO_2 Starch
Processing	3.0	1.0	2.3	1.0	3[*]
Enzyme Production	0.4	1.4	1.8	1.4	10[*]
ATP Regeneration	0	3.4	6.0	2.6	60[*]
Raw Material	1.1	___	17.0	___	4
Total	4.5ϕ	5.8ϕ	27.1ϕ	5.0ϕ	77ϕ
Total for Process	10.3ϕ		32.1ϕ		77ϕ

[*]
Extrapolated from the corresponding costs in the other two processes.

Table 1.2

COST PER EQUIVALENT POUND OF
STARCH FROM AGRICULTURAL SOURCES
1970 U.S. Prices

Corn	2.6¢
Wheat	2.6¢
Rice	6.7¢
Sorghum	2.3¢
Potatoes	13.6¢

Chapter 2

THE WORLD FOOD SITUATION

2.1 Introduction

This chapter will present a discussion of the world food situation leading to a justification for nonagricultural food production. First of all, population trends will be examined. Then present and projected food supplies will be studied aiong with the nutritional requirements of individuals. Finally, economic and social impacts of the world food situation will be discussed.

2.2 World Population Growth Trends: Past, Present, and Future

2.2.1 Magnitude of World Population Growth During Last Two Centuries

In the past two centuries, the world has experienced a rate of population growth unique in the history of mankind. Three-fourths of the total increase of the human species occurred in the last 200 years. The magnitude of this growth is ill··;trated vividly in Figure 2.1 below.
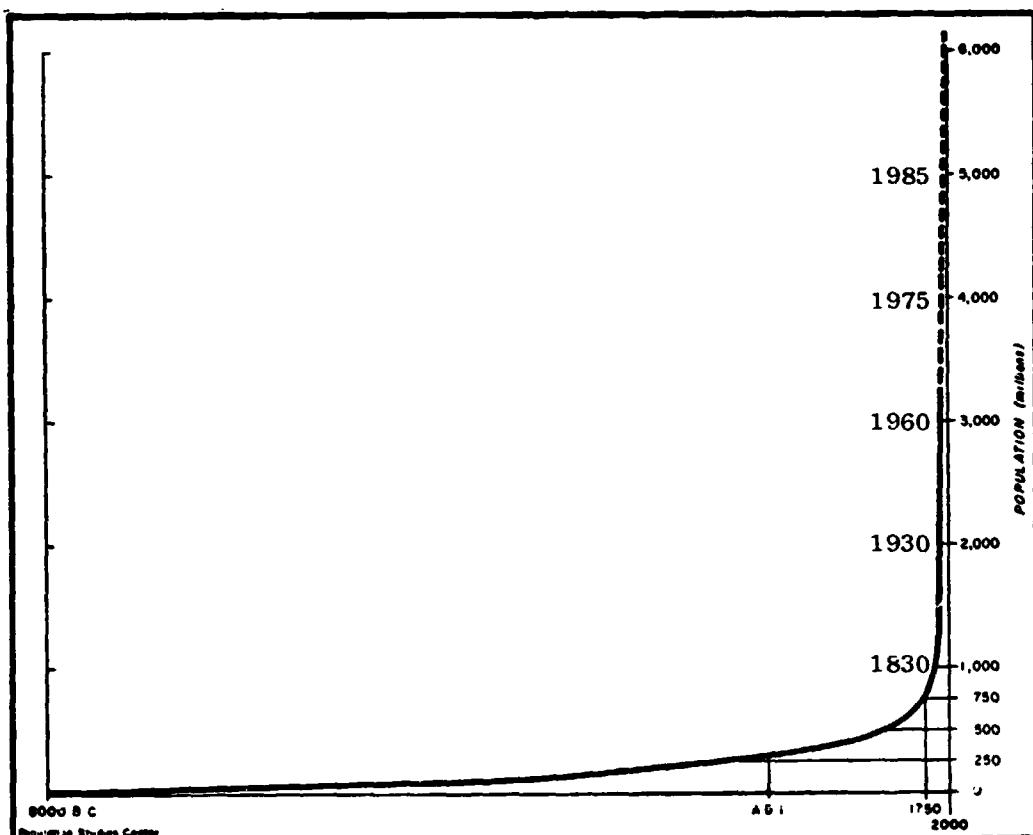
Figure 2.1. LONG-RANGE TREND OF WORLD POPULATION GROWTH. Source: John D. Durand, "The Modern Expansion of World Population" in Thomas R. Detwyler (ed.) Man's Impact on Environment, McGraw-Hill, N.Y., 1971, p. 41.

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In 1830 there were one billion people on the earth. By 1930 there were two billion, and by 1960 there were three billion. Presently, world population is estimated to be close to 3.7 billion. If present trends continue, the fourth billion is likely to be attained by 1975, the fifth by 1985, and the seventh by 2000.

These statistics represent an exponential or geometric rate of increase. Until two hundred years ago, the rate was two percent every thousand years. The present rate is over two percent each year. It took many thousands of years to produce the first billion people; the next billion took a century; a third came after thirty years; the fourth will be produced in just fifteen years. At present growth rates, the fifth billion would be added in a decade and each new addition of one billion would come in ever shorter intervals.

2.2.2 Prospect for Continuing Population Expansion: 1970-2000

What are the prospects for continued population growth to the end of the century? Demographers generally agree that the present expansion of world population will continue for some time to come, probably for more than a hundred years. This is true even with optimistic assumptions about controlling fertility rates. However, the magnitude of this growth is still a matter of debate. The answer depends on what happens to the birth and death rates in the developing countries in the next thirty to forty years.

Figure 2.2 summarizes the most recent set of United Nations population projections for the years 1965-2000 which were published in
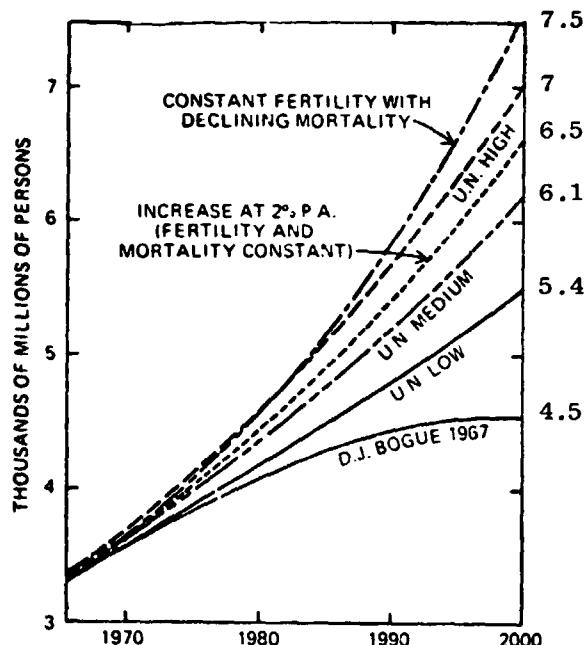
Figure 2.2. SIX ESTIMATES OF WORLD POPULATION, 1965-2000. Sources: World Population Prospects as Assessed in 1963. Population Studies No. 41, Department of Social and Economic Affairs, United Nations, New York, 1966. Unpublished estimate by D. J. Bogue, 1967.

the year 1966. It also includes a separate projection made by demographer D. J. Bogue about the same time. According to these projections, population will grow from its present size of 3.6 billion to a range within a low of 4.5 billion and a high of 7.5 billion by the year 2000. The spread between the lowest and highest estimate is very wide indeed-- some 3 billion people, almost the present size of the world population.

Because of considerable concensus among demographers that the assumption of fertility decline in the "medium" variant is unduly optimistic, this study will use the projections of the United Nations "high" variant for estimating the dimensions of the food problem during the next thirty years.

Table 2.1 below summarizes this projection. Under this variant, world population would almost double from today's 3.6 billion to 7 billion by the turn of the century. At that time, the now developing countries would number about 5.4 billion persons; the economically advanced nations, about 1.6 billion people. Moreover, the developing countries would increase by some 3 billion people in the thirty year period as opposed to one-half billion by the developed nations. Because the growth of the population would be six times greater in the developing countries, by the end of the century, about 77 percent of the world's population would live in the now developing countries. In brief, during the next three decades, most of the huge population growth would occur in those parts of the world that already are overcrowded, poorest, and least able to feed more people.

Table 2.1

WORLD POPULATION BY MAJOR AREAS 1960 TO 2000 ACCORDING TO THE "HIGH"
VARIANT PROJECTION OF THE UNITED NATIONS (Population in Millions)

Area	1960	1970	1980	1990	2000
World Total	2,998	3,659	4,551	5,690	6,994
More Developed Areas[*]	976	1,102	1,245	1,402	1,574
Less Developed Areas[**]	2,022	2,557	3,306	4,288	5,420

[*] Includes Europe, the U.S.S.R., Northern America, Japan, Temperate South America, Australia and New Zealand.

[**] Includes East Asia less Japan, South Asia, Africa, Latin America less Temperate South America and Oceania less Australia and New Zealand.

Source: United Nations, World Population Prospects as Assessed in 1963 (United Nations, New York, 1966), p. 135.

1970 figures are from 1971 World Population Data Sheet of the Population Reference Bureau.

2.2.3 Projections After 2000 A.D.

What are the prospects for population growth beyond the year
2000? There a·e three principal models of population trends, all of
which project an end to growth. The first might be labeled the "Modified
Irish Curve," the second, the "Population Crash Curve," and the third,
the "Gradual Transition to Zero Population Growth." While all predict
that the present expansion will eventually run its course sometime during
the twenty-first century, they differ fundamentally as to how the final
equilibrium between births and deaths will be achieved. Both the "Irish
Curve" and the "Population Crash Curves" are Malthusian in outlook with
a balance between fertility and mortality brought about by the traditional
brutal forms of population control--disease, famine, war and death. Only
the third model, the "Gradual Transition Curve," assumes that an equilib-
rium level of population can be reached in a less inhuman way--namely, the
voluntary reduction of family size by rational means. A summary of their
predictions appears below.

2.2.3.1 Modified Irish Curve

Figure 2.3 shows a curve based on an analogy to the results
of the Irish potato famine of the 1840's. It predicts that population
will grow at an expanding rate unt.1 about 1990 when a number of serious
disasters cause temporary declines in population. This is followed by a
substantial downward adjustment in population which will eventually sta-
bilize around 2150 at levels below those achieved in 1970.
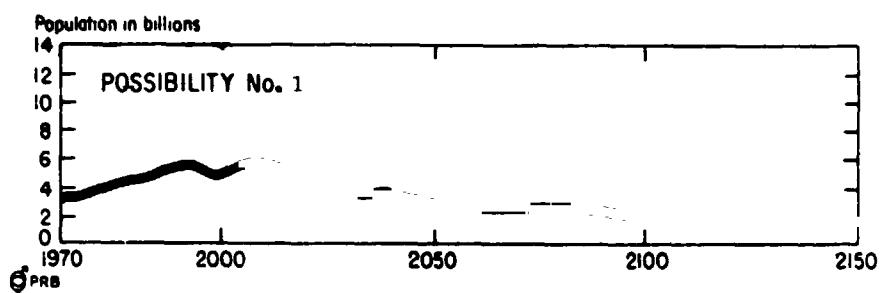
Figure 2.3. THE "MODIFIED IRISH CURVE". "A number of
serious disasters could cause temporary declines in
population and be followed by a consensus to keep
population down." Source: "Man's Population Predic-
ament," Population Bulletin, a publication of the
Population Reference Bureau, Vol. 27, No. 2, April
1971, p. 37.

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2.2.3.2 Population Crash Model

Figure 2.4 illustrates the "Population Crash Curve." This
type of a population curve is similar to the one which biologists use to
describe populations of living organisms. When a population of organisms
grows in a finite environment, sooner or later it will encounter a re-
source limit--a phenomenon described by ecologists as reaching the "car-
rying capacity" of the environment. As soon as a population overshoots
its carrying capacity, growth is brought to an abrupt end through a dra-
matic increase in the death rate. In this model such a "crash" is pre-
dicted to occur during the 21st century.
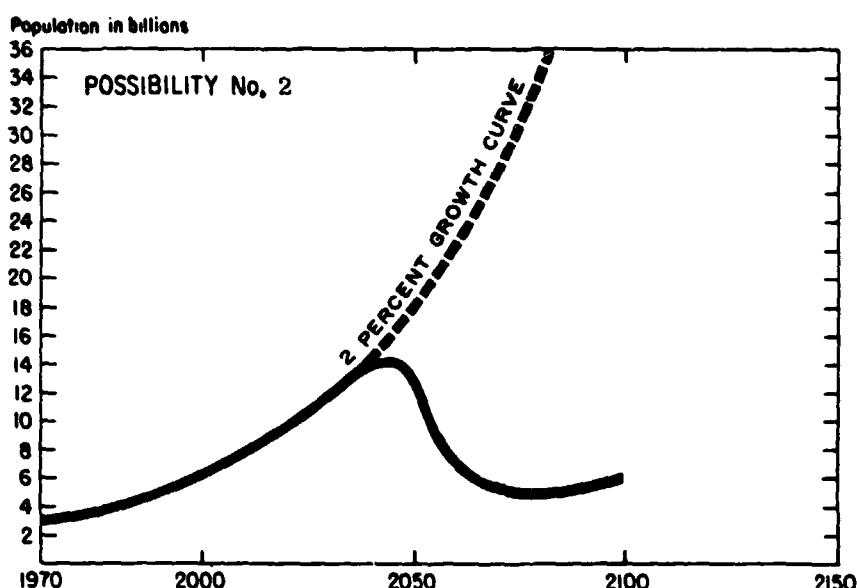
Figure 2.4. THE "POPULATION CRASH CURVE". "Continued
   population growth at the current 2 percent rate could
   lead to a calamitous population crash sometime in the
   twenty-first century." Source: "Man's Population Pre-
   dicament," Population Bulletin, a publication of the
   Population Reference Bureau, Vol. 27, No. 2, April
   1971, p. 34.

2.2.3.3 Zero Population Growth Model

Figure 2.5 illustrates the less catastrophic future pre-
dicted by two variants of this model. Each variant shows a prediction
for the world as a whole, and for the developing countries. (The dif-
ference between the variants lies in the predicted date when family
planning becomes effective.)

According to this model, if reduction to the replacement
rate of two children per family is reached by the developed countries

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Alternatives of Population Growth for the
Developing Countries and the World
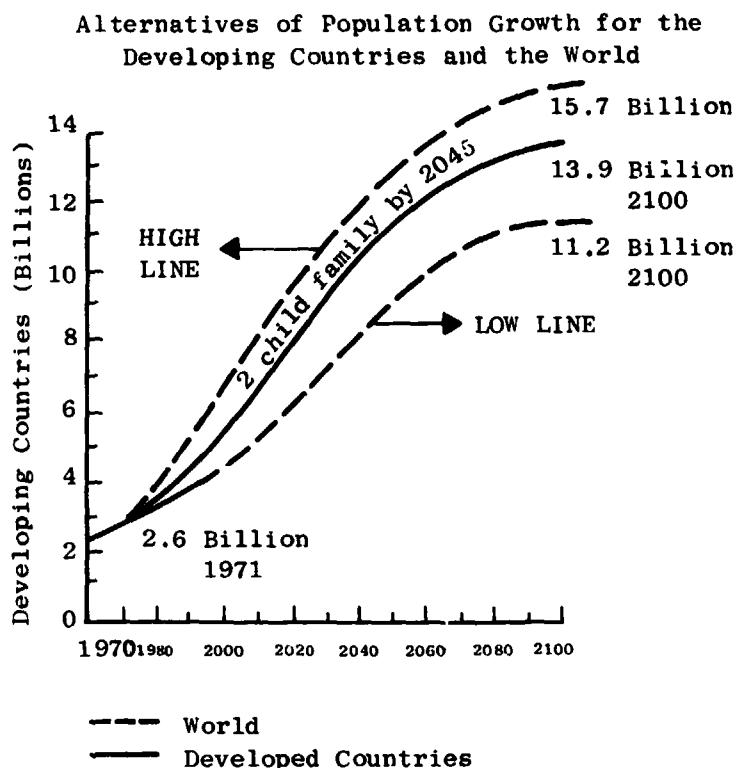
"Population will not stop growing when the two-
child family is reached." It will continue to
grow for about 60 to 70 years before it stabil-
izes either at the high of 15.7 billion or the
low of 11.2 billion. Source: Tomas Frejka,
Population Council ("Alternatives of World Popu-
lation Growth," monograph in process of publica-
tion). Cited by Robert S. McNamara, "A Burden
on Development," The New York Times Supplement,
Section 12, April 30, 1972, p. 16.

Figure 2.5. POSSIBILITY NO. 3: "GRADUAL
APPROACH TO ZERO POPULATION GROWTH."

in 2020 and the developing countries in 2040, world population will
stabilize at 15.7 billion people, of whom 13.9 (or 89%) billion will
live in the developing countries. If this goal is achieved 2 decades
earlier, the world population would stabilize at 11.2 billion, of whom
9.6 billion (or 85%) will live in the developing countries. Because
living standards of the masses have not shown the ability to improve
sufficiently to create the climate for successful family planning pro-
grams, higher estimates appear more likely.

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2.2.4 Summary

Based on the data of past population curves, trends in economic development and family planning, we have used, for the purposes of this study, the "high" variant of the UN projection to the year 2000, and the higher estimate of the ZPG model. According to these predictions, the population of the world will grow from its present total of 3.7 billion people, with 70% in the developing countries, to 7.0 billion in 2000 with 78% in the developing countries, and reach a leveling off total, late in the 21st century, of 15.7 billion with 89% in the developing parts of the world. This then, is the number of people whose nutritional needs must be met during the next 100 plus years.

2.3 Present and Projected World Food Needs and Supplies

2.3.1 Introduction

Given the demographic projections previously discussed, can the world match population growth with proportionately increased food supplies? In the section to follow, we will attempt to appraise future world needs and supplies of food in order to determine whether in fact we face growing food shortages in the next three decades. To provide background for our analyses, we will first review world food trends since the 1930's. Then we will look at projections of food requirements and supplies though the year 2000 based on the following variables: (1) the U.N. demographic projections to the year 2000, (2) the availability of new lands which can be brought into cultivation, (3) the availability of new technology to improve yields from the land already under cultivation, (4) possible new sources of food, and (5) the impact of various alternative sources of food supplies on private costs and social costs (i.e., damage to the environment).

2.3.2 World Food Trends: Prior to 1970

2.3.2.1 History of Production

Since 1960 the per capita food in developing countries has been diminishing. Between 1960 and 1965 population in the developing countries was growing by 11.5 percent while the food supply had increased by only 6.9 percent [1]. Although the world wide food output expanded by 18% between 1930 and 1968 [2], the expansion occurred principally in the developed countries of North America, Western Europe, Japan, and the Soviet Union. In 1962, for example, the developing countries had an average daily calorie intake of 2200, compared with 3000 for the developed countries [3]. While food intake in the advanced countries significantly exceeded requirements, in the bulk of the poor countries calories consumed fell short of minimum needs by at least 10 percent and probably by much more [4]. The only reason widespread famine did not occur was the sale or gift of surplus foods by the developed nations in North America and Western Europe.

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The average person in the developing countries not only was not getting enough to eat in terms of calories, but his nutritional needs were also insufficient to provide minimum safeguards to health. The 1962 figures of daily per capita protein intake were 85 grams for developed countries, and 57 grams for developing countries as a group [5]. As a result, today three hundred million children in developing countries suffer "grossly retarded physical growth" [6]. A larger number in these countries are mentally retarded because of protein deficiency. This means perhaps as many as half to two-thirds of all children in these countries are already blocked from ever achieving full intellectual potential.
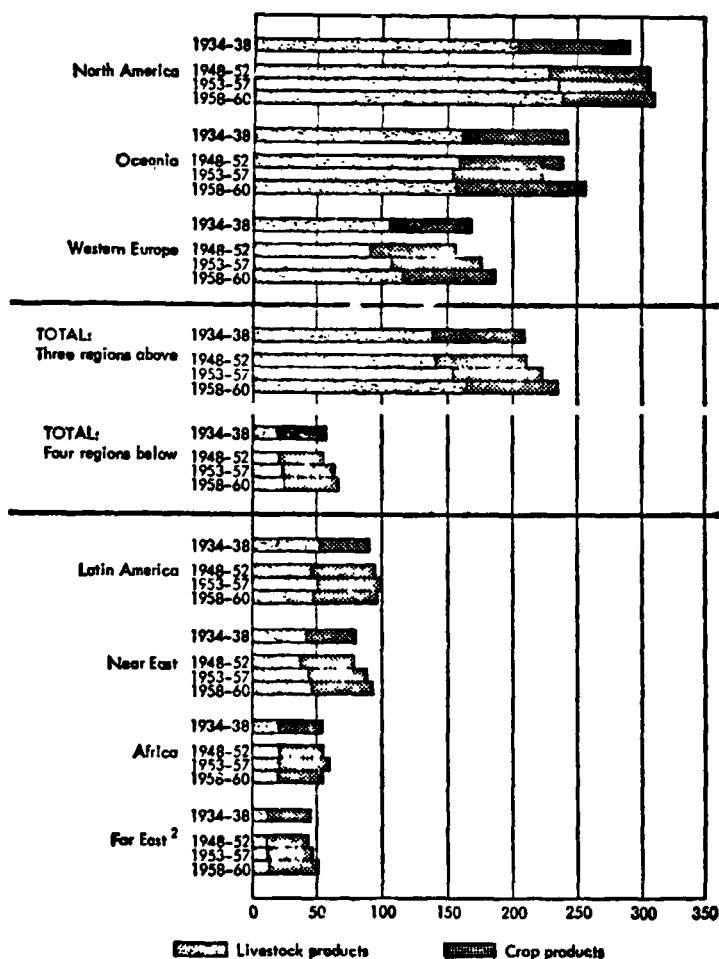
Figure 2.6. ESTIMATED VALUES OF FOOD SUPPLIES PER CAPITA BY WORLD REGIONS, PREWAR TO 1958-60 (PRICE-WEIGHTED INDICES WORLD[1] AVERAGE FOR ALL FOOD, 1948-52 = 100). Cited in the American Assembly, The Population Dilemma, Philip M. Hauser, ed., 2nd ed., Prentice-Hall, Inc., 1969, p. 118.

2.3.2.2 Attempts to Increase Production: Prior to 1970

Prior to 1965 most of the growth in agricultural production came from bringing more land into cultivation--land which was often of marginal quality. For a variety of reasons, these areas of the world had yet to participate in the agricultural revolution that had already brought such large increases in output per man and per acre in North America and Western Europe.

After 1965 there came a further breakthrough in agricultural productivity, the so-called Green Revolution. A central feature of the green revolution has been the development of certain new seeds, particularly in wheat and rice, that produce very large increases in crop yield and which, moreover, are especially suited to conditions in tropical nations. Heretofore, most agricultural technology, like most industrial technology, had been developed with respect to the environment of developed nations--in the case of farming, tne climate and soil conditions associated with the temperate zone. The new seeds, however, were developed with particular reference to tropical and subtropical weather and especially to the great availability of solar energy in areas near the equator.

Figure 2.7 shows the kind of dramatic changes in yields per acre made possible by the new seeds.
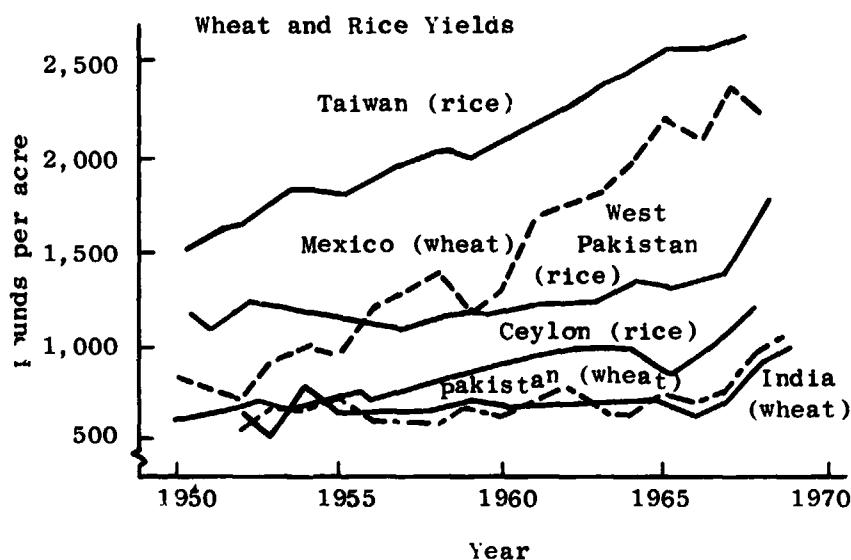
Wheat yields in Mexico, Pakistan, and India. Rice yields in West Pakistan, Ceylon.

Source: Lester R. Brown, Seeds of Change (New York: Praeger Publishers, 1970), pp. 37 and 39.

Figure 2.7. DRAMATIC CHANGES IN YIELDS PER ACRE MADE POSSIBLE BY THE NEW SEEDS.

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In the case of Mexico (wheat) and Taiwan (rice), the intro-
duction of new seed began some time ago and hence the upward movement is
very pronounced; in the case of other countries, we see only the begin-
ning of the process. But even in the early stages of application, the
new agricultural technology can have a substantial effect. World food
production rose 14 percent in the period 1967-1969. But note: in India
and Pakistan, the increase was 27 percent!

2.3.3 World Food Projections: 1970-2000

We now return to the original question: can the world ade-
quately feed a population projected to grow to 5.0 billion people by
1985 and 6.9 billion by 2000? Two authoritative studies which attempt
to answer these questions will be analyzed and compared in the section
to follow:

(1) A study by Resources for the Future, Inc., prepared by its
     staff members, Joseph L. Fisher and Neal Potter, in 1969, and

(2) The FAO's "Indicative World Plan for Agricultural Development"
     also prepared in 1969.

2.3.3.1 Resources for the Future Study

In considering the adequacy of food supplies by the year
2000, Fisher and Potter [7] begin by comparing the world's caloric re-
quirements at three levels: (1) the extension of recent consumption
trends, (2) the world at the U.S. per capita consumption level of 1965,
and (3) the world at the per capital level of Western Europe in 1965.
By combining the projected changes in per capita consumption with the
U.N. "high" projection (Table 2.2) [8], they computed the number of
calories that would be consumed under each of the above three assump-
tions. This is summarized in Table 2.3.

Actual world consumption in 1965 was estimated at 7,800
billion calories a day. Attainment of level (1) at the end of the cen-
tury would require 19,800 billion calories a day; of level (2), 22,100
billion; and of level (3), 21,100 billion.

To achieve any of the levels of calorie consumption in
the year 2000 summarized in Table 2.3, world food output would have to
be from 2 1/2 to 3 times the 1965 figure. This would require an annual
increase of about 3 percent of the 1965 figure.

According to Fisher and Potter, an increase in food output
2 1/2 to 3 times the 1965 figure is not beyond the range of possibility.
The most likely means for attaining this food "target", in their view, is
through increases in crop yield per acre, although the expansion of cul-
tivated area also offers some promise in Africa and Latin America. If
the developed countries were to attain per acre yields already achieved
in the developed countries, they would stand a good chance of meeting
their basic calorie requirements by 2000. However, they would need to
increase agricultural productivity beyond this if they were to have
grains left over for animal feed to increase their protein consumption.

Table 2.2

POPULATION TRENDS: HISTORICAL 1920-65, AND PROJECTIONS
FOR 1980 AND 2000, BY WORLD AREAS (MILLIONS)

	1920	1938	1950	1965	1980	2000
World**	1860	2170	2517	3295	4551	6994
Northern America	116	142	166	214	275	376
Latin America	90	125*	163	246	383	686
Western Europe	233*	260*	286	324	350*	390*
East Europe and USSR	250*	300*	285	352	440*	570*
China, Communist Asia	490*	520*	580*	730	1010*	1400*
Non-Communist Asia	530*	670*	800*	1100	1600*	2700*
Africa	143	186*	222	311	463	864
Oceania	8.5	11.0*	12.7	17.5	23	35

*Rough estimate by the authors, Fischer and Potter.
**Total will not add due to rounding.
Sources: United Nations, Demographic Yearbook, 1960, 1966; U.N. Department
of Economic and Social Affairs, World Population Prospects, as Assessed in
1963 (United Nations, New York, 1966: Population Studies, No. 41), Table
A3.3 and others. The "high" variant is used throughout.

Table 2.3 [9]

PROJECTIONS OF CALORIE CONSUMPTION IN THE YEAR 2000 COMPARED TO
PREWAR AND 1965 ACTUAL CONSUMPTION (BILLIONS OF CALORIES PER DAY)

	Actual		Calorie consumption in year 2000 if:		
	Prewar	1965	Trend of 1952-56 to 1963-65 continues (1)	World is at U.S. 1965 per capita consumption level (2)	World is at West Europe 1965 per capita level (3)
World	5200	7800	19,800	22,100	21,100
Northern America	460	680	1,190	1,190	1,130
Latin America	280	640	2,350	2,170	2,060
Western Europe	750	980	1,440	1,230	1,170
East Europe and USSR	800*	1100*	2,600*	1,800	1,720
Communist Asia	1100*	1400*	2,900*	4,400	4,200
Non-Communist Asia	1400*	2200*	7,000*	8,500	8,100
Africa	400*	710*	2,200*	2,700	2,600
Oceania	36	60	110	110	100

*Very rough estimate by the authors, Fischer and Potter.
Sources: Prewar and 1965 Actual: Population data of Table 2.2 multiplied
by indicated consumption levels from FAO, The State of Food and Agriculture,
1968, pp. 176-177, U.S. Dept. of Agriculture; Economic Research Service, The
World Food Budget 1970, pp. 100-102; and FAO, Production Yearbook 1958, p. 239.
Columns (1), (2), and (3): Population projections of Table 2.2 multiplied
by levels of consumption as indicated.

Fisher and Potter take into account the recent breakthrough
in wheat and rice production in Asian countries, which is introducing the
developing countries to the "agricultural revolution." However, they
point out that improved yields outside Asia depend upon the development
and distribution of advanced technology throughout the developing world.
Unlike medical and industrial technology, agricultural technology does
not travel well. Local conditions, especially in tropical areas, require
modifications of what has proved successful elsewhere and often entail a
long and painful learning process. Moreover, the agricultural technology
that we in the U.S. take for granted is supported by an enormous infra-
structure of research, credit, marketing and repair facilities, as well
as by a congenial institutional structure commonly absent in the develop-
ing countries. Problems of capital investment in agriculture, land terure
and distribution, as well as resistance of traditional societies to change,
still loom as obstacles. For these reasons, Fisher and Potter's prognosis
is only guardedly optimistic. In any case, success in achieving such lev-
els, and indeed the very effort to reach them, requires a more than propor-
tional increase in the use of the agricultural pesticides and fertilizers
whose use is creating some of the environmental problems in more advanced
countries.

2.3.3.2 FAO's Indicative World Plan for Agricultural Development

The basic economic analysis of the FAO study rests on two
interacting parts, the demand for and supply of food. The demand for
food in the developing countries is projected to increase at an annual
rate of 3.9 percent. About 71 percent of this increase will result from
an increase in the number of people to be fed (the "population effect")
and about 29 percent will be due to higher individual purchasing power
(the "income effect").

To meet the projected annual increase in demand of 3.9
percent for food by 1985, food production will have to expand to 2 1/2
times over the base year 1962. This would represent an increase in the
value of agricultural production from $55 billion in 1962 to $122 billion
by 1985. By comparison, food production during the 10-year period 1955-
57 to 1965-67 increased only 2.7 percent per year, and during the first
6 months of the 1960's, 2.4 percent. Thus, an annual increase of 3.9
percent would require a 60 percent increase in the rate of growth of the
food supply over that achieved during the first half of the 1960's (3.9
percent compared to 2.4 percent).

Is such an increase feasible? The FAO's answer is yes, if
farming changes from extensive to intensive methods. Before 1965, much
of the growth of agricultural production in the developing countries came
from bringing new land into cultivation. According to the IWP, new land
is no longer the answer because many countries have already used most of
their arable land. India, for example, is presently cropping 402 millior
acres out of a potential 410 million. However, even where the potential
cultivated area is several times the area now cultivated--as in Latin
America and Africa--the benefit/cost ratio appears to be heavily in favor
of more extensive use of lands already cultivated. The reasons will be
discussed elsewhere.

In addition to more intensive agriculture (increases in yield per acre), the IWP includes three basic policies: (1) further improvements of the high yielding varieties of cereals, (2) development of high-yielding varieties of other crops, and (3) improving the protein content and general dietary balance. Implementation of these policies would take place in two stages. In the first stage, new cereal varieties would yield enough to provide caloric sufficiency for human beings. New varieties would not only produce more food but gradually free land for other crops.

In the second stage, agriculture would shift toward multiple cropping and mixed crops. Lands released from cereal production would now be planted to pulses, oilseeds, fruits and vegetables, as well as fodders, raw materials, and pastures. Finally, land would be used to expand the production of beef, veal, poultry and eggs, in order to improve protein sufficiency. The history of progress in this plan to date casts some doubt on whether the predicted need for a 4% increase in food production can be met.

2.3.4 The World Food Situation

Three factors which control the increase in food production by agricultural means are the amount of land under cultivation, irrigation, and the application of improved technology.

2.3.4.1 Land and Soils

The physical limitation of land area is the most easily visualized constraint on conventional food production. How much of that land area is potentially usable for agriculture depends on topography, soil characteristics, temperature, day length, water supply, and the availability of development capital. A detailed tabulation of land areas by use classification is presented in the annual FAO Production Yearbook. The outlook for increased agricultural production from bringing additional land area into use is placed in its proper perspective by the reference of Robert McNamara to "the scarcest factor--land" in the Agriculture and Rural Development section of his address to the 1971 Annual Meeting, World Bank/IFM/IDA.

Estimates of the world's potentially arable land have recently been attempted in a more quantitative and detailed manner than has hitherto been possible, as summarized in Table 2.4. These estimates, however, must be tempered with an understanding that much of the land which might be brought under cultivation cannot be expected to be as productive as land presently in use. Further, some marginal land now being cropped must revert to less intensive use. Although the potentially arable land is more than three times the area actually harvested in any given year, more than half lies in the tropics.

Table 2.4

ESTIMATES OF THE WORLD'S POTENTIALLY ARABLE LAND

Total land area of earth, excluding Antarctica (in thousands of millions of hectares)	Arable land	Meadows and pas- tureland areas	Forest areas	Desert areas, unsuit- able land, built-up areas	Arable land can be increased to the following totals (in thousands of millions of hectares)		
					With existing methods of cultivation and no capital investment	With capital investment	With new methods of cultivation and sub- stantial capital investment
	(In thousands of millions of hectares, 1961-62)						
13.6	1.43	2.58	4.10	5.42	2.67	5.49	9.33

Source: Malin, K. M., Food Resources of the Earth, Vol. 3, p. 383, Proceedings of the World Population Conference.

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In the tropics, even the basic data on soil properties and climatic conditions are scanty or lacking. Crops must be grown on soils which are severely leached of nutrients and with high infestations of pests whose depredations are never interrupted by winter. Tropical soils range from highly leached ones of the rain forest, through alkali-saturated soils of the desert, rich volcanic soils of Java, alluvial soils of the Nile delta, to impoverished soils of the ancient uplands. On a few of these soils, cultural methods similar to those employed in temperate zones have been successful in giving annual yields far in excess of those of temperate regions. In other areas, these same cultural methods have failed completely and often have resulted essentially in the destruction of the soils.

According to the FAO there are large areas of potentially arable land in temperate parts of North America and Australia but the potential for increasing the net cultivated area is very small in Europe and Asia and relatively small in the Soviet Union.

In Asia, if the potentially arable land area is subtracted in which water is so short that one four-month growing season is impossible, there is essentially no excess of potentially arable land over that actually cultivated.

2.3.4.2 Water

Water is another key to increased agricultural production. Better control of water would possibly be the greatest single factor in increasing food output. Control implies drainage as well as irrigation. The two must be considered together, a cardinal rule often overlooked by planners unfamiliar with intensive agriculture. Irrigated area drainage is a requirement for continued agricultural production in order to prevent a build-up of salinity. Drainage in humid areas is necessary to improve aeration of the root zone.

Attempts to assess the possible benefits of further conventional irrigation in the world are largely speculative. Since World War II hundreds of millions of dollars have been invested in irrigation projects. Some of these have been highly disappointing, if not outright failures, because lands or water were unsuitable. At other times water made available by a big dam or other project was the only factor in agricultural development and there was no attempt to see that the water was made available at grass-roots levels through canals and ditches linked to the main project. Each irrigation project, to have a chance of success, must be based on a fairly intensive study of local land and water and should involve careful planning for providing necessary inputs to production besides water. The cost of irrigation and water management is high, especially on a large scale. The largest project in recent years has been the Indus basin scheme in West Pakistan. It began in 1960 and includes the Mangla Dam, one of the largest earth dams in the world. Including lateral distribution works and other investments, costs in India may well exceed $250 per acre for irrigation development. Cost data from modern projects in other parts of the world indicate that complete development costs of less than $400 per acre are unlikely.

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Global estimates of land potential through water use are difficult, but Table 2.5 gives some idea for parts of Asia, Africa, and South America.

Table 2.5

ESTIMATE OF IRRIGATION POTENTIAL IN PARTS OF
ASIA, AFRICA, AND SOUTH AMERICA

Region	Major river basins and annual runoff (million acre-feet per year)	Arable area (10^6 acres)	Arable area in irrigable climate zones (10^6 acres)	Potential Irrigation (10^6 acres)
India (810 million acres)	Indus, Brahmaputra, Ganges, Godaveri, Kistna: 1,233	403	(266) (116)	187
Pakistan (234 million acres)	Indus, Brahmaputra, Ganges: 1,127	86	(48) (25)	43
Southwest Asia (1,704 million acres)	Tigris, Euphrates: 50			80
Continental Southeast Asia (511 million acres)	Irrawaddy, Mekong, Salween: 690	...	(102)	25
Brazil (2,102 million acres)	Amazon, San Francisco, Paraná: 2,679	966	10	10
Tropical South America less Brazil (945 million acres)	Orinoco, Magdalena: 549	312	14	10
Middle South America (372 million acres)	Paraná: 378	125	24	10
Southern South America (913 million acres)	Paraná, Uruguay, Bueno, Valderia, Bío-Bío, Negro; 574	266	246	125
Tropical Africa (2,350 million acres)	Congo, Niger, Zambesi: 1,520	967	250	150
Total	7,673	3,225	1,101	640

Source: President's Committee, World Food Problem, Vol. 2, p. 447.

2.3.4.3 Technology and Capital

Where adequate water is available, the optimum food production comes through the development of improved farming systems involving more fertilization, better seeds, better machinery, and improved cultural practices, including water management. This is the "package of practices" approach of the so-called Green Revolution. While it would be difficult to overestimate the importance of the "Green Revolution," it must also be noted that by itself it will not bring about an agricultural revolution everywhere. There is also a time factor in the mobilization of resources to meet the approaching avalanche of population; and in the long run, the fundamental fact of the land-man ratio must not be overlooked.

2.3.4.4 Better Seeds and Improved Plant Breeding

The past success of new cereal varieties has sometimes been spectacular. For example, use of the "high-yielding" wheat varieties and acceptance of "miracle" rice in India have this year resulted in an 8,000,000 ton wheat surplus and at least self-sufficiency in rice. Today Mexico can grow all the corn it needs on less land than before, even though its population has almost doubled since 1943, and several countries of the rice-bowl area of Southeast Asia now indicate plans for rice export.

These new varieties are not improved varieties in the traditional sense of 10 percent or 20 percent better than the old varieties. They are twice as good. They are also largely independent of season, that is, they can be planted at any suitable time of the year. This makes multiple cropping feasible in a way that was not possible before. The result is that individual farmers are not replacing a low-yielding variety with a high-yielding one but are replacing one crop of a low-yielding variety with two crops (or more in some cases) of a high-yielding variety.

However, lest one be carried away by the prospect of plenty, a number of constraints must be mentioned.

(1) Not all land is suitable for "high-yielding" varieties. For example, perhaps only 14 to 20 percent of the tropical rice growing area has adequately controlled irrigation and drainage, productive soils, and reasonable availability of inputs (improved varieties, nitrogen, pesticides, power). The new varieties have done little to increase yields on the vast areas of problem soils upon which a sizable proportion of the rice-eating people depend.

(2) Continued improvement of yields through plant breeding and the development of new varieties is a slow process which must be followed by adaptation to local conditions and, finally, seed multiplication and adoption by the farmer.

(3) The widespread introduction of very closely related germ plasm of a given crop over a substantial portion of the total cultivated acreage presents a very real hazard in terms of the potential for catastrophic disease or insect infestations. The

disease problem of the potato crop in Ireland during the past
century amply illustrates this point.

(4) Ther~ is a theoretical limit to yielding ability.

Thus, although results have been promising, better seeds
and improved plant breeding do not offer capacity for infinite increase.

2.3.4.5 Fertilizers, Pesticides, and Machinery

On land now under cultivation, estimates of fertilizer
needed to double agricultural production in the developing countries
require an increase from the six million metric tons used in 1966 to
67 million metric tons. In addition, the 120,000 metric tons of in-
secticides, fungicides, herbicides, etc. currently used would need to
increase to 700 metric tons. The ecological problems related to such
an expansion would clearly be massive. The capital investment required
would also be considerable--an estimated $17 billion for fertilizers
and $1.8 billion for pest controlling chemicals.

Costs for implementing machinery usage at the level cur-
rently employed in the United States and Western Europe in Asia, Africa
and Latin America are estimated at $2.5 billion. At present these areas
employ only a fraction of the 0.5 horsepower per acre considered neces-
sary for an efficient agriculture.

2.4 Nutritional Requirements

2.4.1 Kinds of Food Requirements

The constituents in foods, nutrients, can be divided into
five major categories defined as proteins, fats, carbohydrates, vitamins,
and minerals. The body, in order to operate efficiently from the stand-
point of meeting energy requirements, growth and general health, requires
the essential nutrients in adequate amounts and balance in order to opti-
mize physical and mental health throughout the life cycle. The following
discussion outlines briefly the nature of these nutrients.

2.4.1.1 Protein

Approximately 50 percent of the dry weight of the human
body is protein. The proteins in the body are not fixed unchanging sub-
stances deposited for a lifetime, but instead are in a constant state of
exchange. This state of exchange is common to all living things. Because
of this constant breaking down and building up, proteins are necessary in
the diet for both growth and replacement.

Protein is not the name of a single compound but of a cate-
gory of nitrogeneous polymers. Proteins are produced by living cells and
are constructed of building blocks known as amino acids. There are twenty
of these, of which eight are "essential," i.e., human metabolism is unable

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to synthesize these eight and must obtain them from an external source. The other twelve amino acids can be synthesized within the body from one or the other of the eight essential acids as well as the other nitrogen sources present in normal foods.

Unfortunately, the interrelation of the amino acids and auxiliary nitrogen sources is very complex and involves multiple pathways to protein synthesis. There is a present insufficient information about essential amino acids and their relation to total nitrogen needs [10,11].

To build the proteins necessary for body growth and maintenance, all of the essential amino acids have to be on hand at the same time in sufficient quantities. If one is missing or is present in too small a quantity, the deficiency would limit the use of the remaining amino acids. A high biologic value protein contains enough of the essential amino acids to maintain body tissue and to promote a normal growth rate, such complete proteins are usually from animal sources.

Because of the important role proteins have in promoting growth and maintenance of the body they are an essential part of any diet whether it be natural or artificial. Many diseases occur around the world as a result of protein deficiency.[*] Minimum protein requirements have been established by the National Research Council (1968) [12] and are shown in Table 2.6.

2.4.1.2 Carbohydrates

Carbohydrates are molecules composed of carbon, hydrogen and oxygen. Some carbohydrate molecules are relatively small whereas others are larger and more complex consisting of a few or many of the smaller molecules linked together in chains which may be straight, branched, ringed, or combinations.

The simplest class of carbohydrates consist of a single unit and are called monosaccharides, the commonest of which is glucose. Carbohydrates made up on long chains of monosaccharides are known as polysaccharides, among them are the starches.

The body has no minimum requirement for carbohydrates as such, but does require calories for energy. The number of calories needed varies primarily with the size and age of the individual. The number required by an average size adult is in the vicinity of 2800K calories. (See Table 2.6 for exact figures recommended by the National Research Council.) Traditionally these calories are supplied in the form of carbohydrates because of their general abundance and low price compared to protein and fat calories. The body does not store carbohydrates, as such to any degree, but converts the carbohydrates to fats for energy storage. If a sufficient amount is not taken into the body to supply energy needs, protein is used to make up the deficit.

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[*] Kwashiorhor is a major international public health problem.

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An important function of carbohydrates in the diet is that of producing conditions which are necessary for the complete oxidation of fat. Without carbohydrates an accumulation of the products of incomplete fat oxidation which are known as ketone bodies occurs and ultimately leads to a deleterious condition known as ketosis [13].

Because of the sweetness of carbohydrates in the form of sugars, foods are made more palatable by their addition or natural content. This may be an advantage or a disadvantage! For example, if a generally unacceptable but nourishing food is made acceptable by sweetening, then, the advantage is clear. On the other hand, if a sweet but non-nourishing food is available, it may become part of the diet at the expense of more nourishing items.

Carbohydrate molecules vary in size and solubility hence their osmotic pressure or water-attracting power varies. When glucose is eaten, the osmotic pressure of the solution in the stomach is increased and water passes from the tissues into the stomach to dilute the solution. This can cause distention of the stomach and reduce the desire to eat.

2.4.1.3 Fats

Fats are composed of glycerol (a three carbon alcohol) and fatty acids. The fatty acids are hydrocarbons consisting of chains of carbon atoms of various lengths terminated at one end by a carboxyl group which gives them their acid nature. The nature and consistency of a fat depends on the length of the carbon chain of its constituent fatty acids and whether the chain links are fully or partially filled with hydrogen atoms. In general fats containing long-chain fatty acids tend to be solid at room temperature whereas fats made up of shorter chains are likely to be liquids, called oils, at room temperature. Furthermore, those in which the carbon chain is not completely filled with hydrogen (unsaturated) are likely to be more liquid than those which are saturated [14]. A significance of unsaturated dietary fats is their association with lower levels of blood cholesterol. Cholesterol, a complex fat-like substance occurring in all animal tissue is an important fraction of the blood lipoprotein and is associated with heart disease.

Fats in the diet make meals palatable and satisfying. They promote efficiency in the utilization of protein and carbohydrates and facilitate the utilization of fat-soluble vitamins. They are important sources of vitamins A, D, E, and K, and provide linoleic acid known to be the essential fatty acid in the diet.

The amount of fat in any particular diet is affected partly by the customs of the people being considered and partly by the economic conditions. In general, fat consumption increases in proportion to wealth.

2.4.1.4 Vitamins and Minerals

Vitamins are organic compounds which act as body regulators. They are needed in a diet in small amounts and are necessary for normal growth and the maintenance of health. For the most part, vitamins cannot

be synthesized or manufactured by the human body but must be supplied in the diet.

Vitamins are classified on the basis of their solubility. Vitamin C and the vitamins of the B complex are water soluble. Vitamins A, D, E, and K in their natural forms are soluble in fats. Recommended daily vitamin allowances have been established by the National Research Council and are shown in Table 2.6. The recommended allowances are designed for the maintenance of good nutrition of practically all healthy people in the U.S.A. Individual allowances must take into consideration the age, general health, and height and weight of the person as well as the location and climate of the region in which he resides.

There are a number of diseases associated with vitamin deficiencies [16]. Most are striking and debilitating. A deficiency in vitamin A injures the epithelial tissues throughout the body, impairs vision, lowers resistance to respiratory and other infections and can seriously impair tooth development in growing children.

The B complex is composed of a number of vitamins which have little in common except that they are water soluable. The group is comprised of vitamins B_1, B_2, B_6, B_{12}, niacin, pantothenic acid, biotin, choline and folic acid. There are a number of specific physical disorders and diseases associated with deficiencies of each, including beriberi, dermatitis, pellagra, conjunctivitis, anemia, etc.

Vitamin C or ascorbic acid as it is frequently called is a vitamin which is needed in the largest amount by man. It occurs naturally in a number of fruits and vegetables. Its deficiency leads to loss in weight, appetite, and general weakness. Growth stunting in the young occurs if the deficiency is severe as well as a condition known as scurvy which is manifested by debility, skin degeneration, spongy gums, hemorrhages in body tissues and sometimes death. It is not clear how ascorbic acid functions in metabolism, however, it is recognized to play a vital role in maintaining the welfare of the cells and tissues throughout the body.

Vitamin D is responsible for the prevention of rickets, a deficiency disease, which primarily occurs in infants, children and young adults. It is manifested by flabby toneless muscles, a distended abdomen and severe bone deformations. The D vitamins belong to a class of light-sensitive organic compounds known as sterols which are widely distributed in animal and plant tissues. Human skin contains a sterol called cholecalciferol which is transformed into vitamin D_3 when it is exposed to ultraviolet light. Well nourished adults who lead normal lives and receive sufficient sunshine generally require no additional vitamin D. For infants, children, adolescents, pregnant women and nursing mothers, the National Research Council recommends 400 units per day.

Two other fat soluble vitamins are E and K. The relationship of each of these vitamins to nutrition has not yet been clearly established but a number of significant symptoms and diseases occur when either is absent from the diets of test animals.

Young test animals, after several months on diets deficient in vitamin E failed to grow properly. Other symptoms also occurred, such

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as: weakness and degeneration of skeletal muscles, heart and brain lesions, and liver damage. No minimum requirements of vitamin E have been determined for humans but recommended allowances have been established by the National Research Council and can be seen in Table 2.6.

Vitamin K is essential for normal function of the liver and for the formation of prothrombin, a protein necessary for the clotting or coagulation of blood. Because it is found in sufficient quantities in a number of common foods, no recommended allowance has been set. Under certain conditions where internal hemorrhaging, injuries, or surgical operations have been performed, supplemental vitamin K may be administered.

All living things contain a variety of minerals. Some occur in larger proportions than others. For example, approximately 2 percent by weight of the adult body is composed of calcium, whereas only about 0.00004 percent is iodine. In order to maintain the life processes it is necessary that a number of minerals be supplied in the diet. Deficiency syndromes of minerals include symptoms that range from serious bone and teeth disorders when calcium and phosphorous are deficient to goiters when iodine is deficient. Recommended allowances have been established for some of the minerals and can be found in Table 2.6.

2.4.2 Present Diet Composition

The energy requirement of the body varies with age, level of activity, body weight, sex, and climate. Both FAO and the Food and Nutrition Board of the U.S. National Research Council have adopted an approach based on a reference man and woman [17,18]. The Food and Nutrition Board propose a reference man and woman 22 years old and living at 20°C with weights of 70 and 58 kg, respectively. They assume daily allowances of 2800 and 2000 kcals for the man and woman based on a detailed energy consumption regime. Since the factors influencing caloric requirement are sensitive to location, the recommended values will change from country to country. Table 2.7 indicates the assumed values in several representative countries [19].

On the basis of the above set of recommended allowances, it is possible to set up a required daily carbohydrate requirement. A working average including the male and female requirement, the variation from country to country and from age to age will be taken at 2800 kcalories of energy and 65 grams of protein. Using a caloric density of 4 kcal/gm for protein and carbohydrates and 9 kcal/gm for fats, the energy requirement to be supplied from carbohydrates, protein and fats is 2540 kcalories, as indicated in Figure 2.8.

The present food composition pattern in the United States is such that 40 percent of the caloric intake is in the form of fats. This is not representative of the world fat intake as seen from Table 2.8. Assuming a fat-carbohydrate caloric intake ratio of 25-75, the net caloric intake from carbohydrates would be 1905 k calories or about 475 grams of carbohydrates.

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In order to assess the significance of these figures, a compilation of the nutritional value has been made in Table 2.9 for selected carbohydrate oriented foods. Protein is evaluated on a gross basis only. It will be examined in detail later.

2.4.3 Analysis of Nutritional Status

The results of the previous discussion indicates that about one pound of carbohydrate is needed each day to satisfy our energy requirements. They indicate further that most of the foods used in the world as a carbohydrate source also furnish a substantial percentage of the protein requirement and a smaller, but not negligible, amount of minerals and vitamins. On]y soybeans contain an appreciable amount of fat. Rice, wheat, corn, millet, and soybeans can be classified as polynutrients insofar as they supply substantial percentages of more than one nutrient requirement. By contrast, cassava is a mononutrient insofar as it is almost exclusively a carbohydrate. Potatoes also lean to a mononutrient status. This means that any introduction of artificial glucose into areas consuming a polynutrient carbohydrate source should be done on a supplementation basis. Otherwise a protein deficiency could result. Any introduction of artificial glucose into a mononutrient carbohydrate consuming area can be done on a substitution basis. These latter areas include portions of South America, Central Africa, and Indonesia.

In order to achieve nutritional balance, artificial glucose shou]d be combined with other natural or artificial nutrients unless carb...ydrate deficiency is the primary problem. An example of this approach is Incaparina, a complete polynutrient food designed for Central American consumption.

In order to quantitatively assess the polynutrient status of selected carbohydrate oriented foods, a "standard balanced protein" was defined. This was done by averaging the essential amino acide content of meat, eggs, and milk [20]. The raw data and resultant mean values of the standard balanced protein are shown in Table 2.10. The figures are expressed in milligrams of amino acid per gram of protein. In a similar fashion, the essential amino acid content of several common carbohydrate oriented foods is shown in Table 2.11. Table 2.12 shows a comparison on a percent basis of the foods listed in Table 2.11 to the "standard balanced protein."

The results of this comparison are exceedingly interesting. Corn, wheat, and millet are lacking primarily in lysine, but are relatively "balanced" with regard to the other amino acids. Rice is somewhat weak in lysine. Millet is particularly good source of all essential amino acids, except lysine.

Manioc is a poor source not only of protein, but also of the more valuable protein constituents. Potatoes also are a weak source of the essential amino acids.

Soy flour is particularly nutritious. The mixture of soy flour with other types of flour is an excellent combination.

Table 2.6

FOOD AND NUTRITION BOARD, NATIONAL ACADEMY OF SCIENCES–NATIONAL RESEARCH COUNCIL

RECOMMENDED DAILY DIETARY ALLOWANCES,* Revised 1968

Designed for the maintenance of good nutrition of practically all healthy people in the U.S.A.

	Age[b] (years) From–Up to	Weight (kg)	Weight (lbs)	Height (cm)	Height (in.)	kcal	Protein (gm)	Fat Soluble Vitamins — Vit-min A Activity (IU)	Vita-min D (IU)	Vita-min E Activity (IU)	Ascor-bic Acid (mg)	Fola-cin[c] (mg)	Nia-cin (mg equiv)[d]	Ribo-flavin (mg)	Thi-amin (mg)	Vita-min B6 (mg)	Vita-min B12 (µg)	Cal-cium (g)	Phos-phorus (g)	Iodine (µg)	Iron (mg)	Mag-nesium (mg)
Infants	0–1/6	4	9	55	22	kg × 120	kg × 2.2[e]	1,500	400	5	35	0.05	5	0.4	0.2	0.2	1.0	0.4	0.2	25	6	40
	1/6–1/2	7	15	63	25	kg × 110	kg × 2.0[e]	1,500	400	5	35	0.05	7	0.5	0.3	0.3	1.5	0.5	0.4	40	10	60
	1/2–1	9	20	72	28	kg × 100	kg × 1.8[e]	1,500	400	5	35	0.1	8	0.6	0.4	0.4	2.0	0.6	0.5	45	15	70
Children	1–2	12	26	81	32	1,100	25	2,000	400	10	40	0.1	8	0.6	0.5	0.5	2.0	0.7	0.7	55	15	100
	2–3	14	31	91	36	1,250	25	2,000	400	10	40	0.2	8	0.7	0.6	0.6	2.5	0.8	0.8	60	15	150
	3–4	16	35	100	39	1,400	30	2,500	400	10	40	0.2	9	0.8	0.7	0.7	3	0.8	0.8	70	10	200
	4–6	19	42	110	43	1,600	30	2,500	400	10	40	0.2	11	0.9	0.8	0.9	4	0.8	0.8	80	10	200
	6–8	23	51	121	48	2,000	35	3,500	400	15	40	0.2	13	1.1	1.0	1.0	4	0.9	0.9	100	10	250
	8–10	28	62	131	52	2,200	40	3,500	400	15	40	0.3	15	1.2	1.1	1.2	5	1.0	1.0	110	10	250
Males	10–12	35	77	140	55	2,500	45	4,500	400	20	40	0.4	17	1.3	1.3	1.4	5	1.2	1.2	125	10	300
	12–14	43	95	151	59	2,700	50	5,000	400	20	45	0.4	18	1.4	1.4	1.6	5	1.4	1.4	135	18	350
	14–18	59	130	170	67	3,000	60	5,000	400	25	55	0.4	20	1.5	1.5	1.8	5	1.4	1.4	150	18	400
	18–22	67	147	175	69	2,800	60	5,000	400	30	60	0.4	18	1.6	1.4	2.0	5	0.8	0.8	140	10	400
	22–35	70	154	175	69	2,800	65	5,000	—	30	60	0.4	18	1.7	1.4	2.0	5	0.8	0.8	140	10	350
	35–55	70	154	173	68	2,600	65	5,000	—	30	60	0.4	17	1.7	1.3	2.0	5	0.8	0.8	125	10	350
	55–75+	70	154	171	67	2,400	65	5,000	—	30	60	0.4	14	1.7	1.2	2.0	6	0.8	0.8	110	10	350
Females	10–12	35	77	142	56	2,250	50	4,500	400	20	40	0.4	15	1.3	1.1	1.4	5	1.2	1.2	110	18	300
	12–14	44	97	154	61	2,300	50	5,000	400	20	45	0.4	15	1.4	1.2	1.6	5	1.3	1.3	115	18	350
	14–16	52	114	157	62	2,400	55	5,000	400	25	50	0.4	16	1.4	1.2	1.8	5	1.3	1.3	120	18	350
	16–18	54	119	160	63	2,300	55	5,000	400	25	50	0.4	15	1.5	1.2	2.0	5	1.3	1.3	115	18	350
	18–22	58	128	163	64	2,000	55	5,000	400	25	55	0.4	13	1.5	1.0	2.0	5	0.8	0.8	100	18	350
	22–35	58	128	163	64	2,000	55	5,000	—	25	55	0.4	13	1.5	1.0	2.0	5	0.8	0.8	100	18	300
	35–55	58	128	160	63	1,850	55	5,000	—	25	55	0.4	13	1.5	1.0	2.0	5	0.8	0.8	90	18	300
	55–75+	58	128	157	62	1,700	55	5,000	—	25	60	0.4	13	1.5	1.0	2.0	6	0.8	0.8	80	10	300
Pregnancy						+200	65	6,000	400	30	60	0.8	15	1.8	+0.1	2.5	8	+0.4	+0.4	125	18	450
Lactation						+1000	75	8,000	400	30	60	0.5	20	2.0	+0.5	2.5	6	+0.5	+0.5	150	18	450

* The allowance levels are intended to cover individual variations among most normal persons as they live in the United States under usual environmental stresses. The recommended allowances can be attained with a variety of common foods, providing other nutrients for which human requirements have been less well defined. See text for more detailed discussion of allowances and of nutrients not tabulated.

[b] Entries on lines for age range 22–35 years represent the reference man and woman at age 22. All other entries represent allowances for the midpoint of the specified age range.

[c] The folacin allowances refer to dietary sources as determined by Lactobacillus casei assay. Pure forms of folacin may be effective in doses less than ¼ of the RDA.

[d] Niacin equivalents include dietary sources of the vitamin itself plus 1 mg equivalent for each 60 mg of dietary tryptophan.

[e] Assume 3 protein equivalent to human milk. For proteins not 100 percent utilized factors should be increased proportionately.

CARBOHYDRATE	PROTEIN	FAT	VITAMINS AND MINERALS
SUPPLY ENERGY NEEDS	GROWTH AND REPLACEMENT	TASTE HELP UTILIZE PROTEINS VEHICLE FOR FAT SOLUBLE VITAMINS LINOLEIC ACID FOR GROWTH	BODY REGULATORS ENZYME COMPONENTS GROWTH AND HEALTH
NO REAL MINIMUM REQUIREMENT ~4 KCAL/GM	~65 GM/DAY ~4 KCAL/GM	~FEW GRAMS/DAY ~9 KCAL/GM	MG/DAY

260 KCALORIES

2800 KCAL/DAY NEEDED
-260 KCAL/DAY PROTEIN
2540 KCAL SUPPLIED BY FATS AND CARBOHYDRATES

Figure 2.8.   AVERAGE DIETARY NEEDS.

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Table 2.7

COMPARATIVE DIETARY STANDARDS OF SELECTED COUNTRIES AND UN AGENCIES

COUNTRY	SEX	AGE (YR)	WGT (KG)	ACT.	KCALS	PRO (GM)
USA	M	22	70	NORMAL	2800	65
	F	22	58	NORMAL	2000	55
FAO	M	25	65	NORMAL	3200	46
	F	25	55	NORMAL	2300	39
COLUMBIA	M	20-29	65	NORMAL	2850	68
	F	20-29	55	NORMAL	1900	60
INDIA	M	25.4	55	NORMAL	2800	55
	F	21.5	45	NORMAL	2300	45
JAPAN	M	26-29	56	NORMAL	3000	70
	F	26-29	49	NORMAL	2400	60
E. GERMANY	M	18-35		LIGHT WORK	2700	85
	F	18-35			2300	75

Source: Recommended Dietary Allowances Food and Nutrition Board, National Research Council, National Academy of Science, Pub. #1964, 1968.

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Table 2.8

PERCENTAGE OF TOTAL CALORIES FROM FAT

GREAT BRITAIN	38	BRAZIL	18
HOLLAND	34	EGYPT	12
SWEDEN	38	I!!DIA	13
USA	41	MEXICO	14

Source: Aykrody, W. R. Food For Man, Pergamon Press 1964.

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Table 2.9

CONSTITUENTS OF SELECTED CARBOHYDRATE RICH NATURAL FOODS IN 100 GRAMS OF EDIBLE PORTION

	FOOD ENERGY (KCALS)	PROTEIN (GM)	FAT (GM)	CARBOHYDRATE (GM)
WHEAT (WHOLE GRAIN, DURUM) #2434	332	12.7	2.5	70.1
CORN (WHOLE GRAIN, RAW) #843	348	8.9	3.9	72.2
RICE (BROWN, RAW) #1869	360	7.5	1.9	77.4
POTATOES (RAW) #1785	76	2.1	0.3	17.1
TAPIOCA (DRY) #2268	352	0.6	0.2	86.4
TARO (RAW) #2271	98	1.9	0.2	23.7
MILLET (WHOLE GRAIN) #1338	327	9.9	2.9	72.9
SOYBEANS (RAW MATURE SEEDS) #2139	403	34.1	17.7	33.5

Numbers refer to source reference entry. Source: Composition of Foods, USDA Agricultural Handbook #8, 19.3.

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Table 2.10

BALANCED PROTEIN SOURCE BASED ON RELATIVE AMOUNTS IN WHOLE EGGS, WHOLE MILK, AND
SIRLOIN TIP BEEF   (All values in milligrams of amino acid per gram of protein)

	TRYPTOPHAN	LEUCINE	LYSINE	METHIONINE	PHENYLALANINE	ISOLEUCINE	VALINE	THREONINE
MILK, WH FRESH	13.6	99.1	78.2	23.6	48.2	63.6	69.1	46.4
SIRLOIN, TIP LEAN	11.7	81.9	87.4	24.9	41.1	52.3	55.6	44.2
EGG, WHOLE	6.0	88.0	64.0	31.0	58.0	66.0	74.0	50.0
MEAN (BALANCED PROTEIN)	13.8	89.7	76.6	26.5	49.1	60.6	66.2	46.9

Raw Data Source: Bowes and Church, Food Values of Portions Commonly Used, 11th Ed., 1970.

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Table 2.11

COMPARISON OF AMINO ACID CONTENT IN THE PROTEIN IN SOME SELECTED FOODS WITH THE NORM AMINO ACID BALANCE. (All values in milligrams of amino acid per gram of protein)

	TRYPTOPHAN	LEUCINE	LYSINE	METHIONINE	PHENYLALANINE	ISOLEUCINE	VALINE	THREONINE
CORN FLOUR	6.04	130	29.0	19.0	45.0	46.0	51.0	40.0
WHEAT FLOUR	12.0	77.0	23.0	13.0	55.0	46.0	43.0	29.0
RICE FLOUR, GRANULATED	10.9	86.0	38.9	18.0	50.0	46.9	70.0	38.9
MANIOC FLOUR	13.1	41.2	41.2	6.25	28.1	28.1	30.6	27.6
MILLET	11.0	179	22.0	30.0	72.0	81.0	74.0	33.0
SOY FLOUR, FULL FAT	15.0	85.0	69.0	15.0	54.0	59.0	58.0	43.0
POTATOE, RAW WHITE	10.0	50	52.9	11.9	43.8	43.8	52.9	41.0
NORM	13.8	89.7	76.6	26.5	49.1	60.6	66.2	46.9

Table 2.12

PERCENTAGE COMPARISON OF AMINO ACID CONTENT IN SOME SELECTED FOODS TO THE NORM BALANCED PROTEIN. (All in percent)

	TRYPTOPHAN	LEUCINE	LYSINE	METHIONINE	PHENYLALANINE	ISOLEUCINE	VALINE	THREONINE
CORN FLOUR	43.8	144	37.8	71.7	91.6	75.9	77.0	85.2
WHEAT FLOUR	87.0	85.8	30.0	49.0	112	75.9	65.0	61.8
RICE FLOUR, GRANULATED	79.0	95.8	50.8	67.9	102	77.3	106	88.0
MANIOC FLOUR	94.9	45.9	53.8	23.6	57.2	46.4	46.2	58.8
MILLET	79.7	199	28.7	113	147	134	112	239
SOY FLOUR, FULL FAT	109	94.7	90.0	56.6	110	97.3	87.6	91.7
POTATO, RAW WHITE	72.4	55.7	69.1	44.9	89.2	72.3	79.9	87.4

These results point to several definite conclusions. With the exception of cassava and potatoes, the common cereals are potentially important sources of high quality proteins. Lysine enrichment, either by genetic modification or by external process addition is a very advantageous course of action for corn, wheat, rice, and millet usage in order to improve their "biologic value."

2.5 Alternative Methods of Meeting Food Needs

2.5.1 Intensive Agriculture

Intensified agriculture takes the form of four distinct practices:

Water Availability
Varietal Optimization
Pest Control
Fertilizer Usage

These four are considered a total approach or a package of practices. They are applied simultaneously to achieve a high yield. Each technique is heavily interrelated to the other three.

It is important to realize what factor is not included in this set of practices. There is no mention of land. The Green Revolution is a revolution of yield per acre, not more acres.

Figures 2.9, 2.10, and 2.11 show the impact of the application of these four practices on several crops in the U.S. The yields per acre increase by factors of 2 or 3, and labor inputs become correspondingly less. Figure 2.12, which indicates the consumption of fertilizer, can be viewed as a measure of intensification of agriculture in various areas of the world. Europe is the largest consumer of fertilizer and appears to be saturating in its total tonnage. The proportion of the world consumption taken by the United States has risen substantially since 1945. Asia is in a very steep period of increasing fertilizer usage and is expected to become a much greater consumer of fertilizer in the near future. Oceania, Africa, and South America represent agricultural areas where little intensification has taken place.

It appears that there is still room for increased production of food by intensification of agriculture in some areas of the world. The application of such techniques raises production levels by less than a factor of 10. Whether such methods could be totally effective, however, in meeting demands for food in the most economically viable way is uncertain. Limitations on this approach have been outlined in Section 2.3.

2.5.2 Controlled Environment Approach

Another possibility for food production is the use of controlled environment greenhouses. Intensive agricultural techniques have produced yields as high as 100 tons/acre of tomatoes and cucumbers.
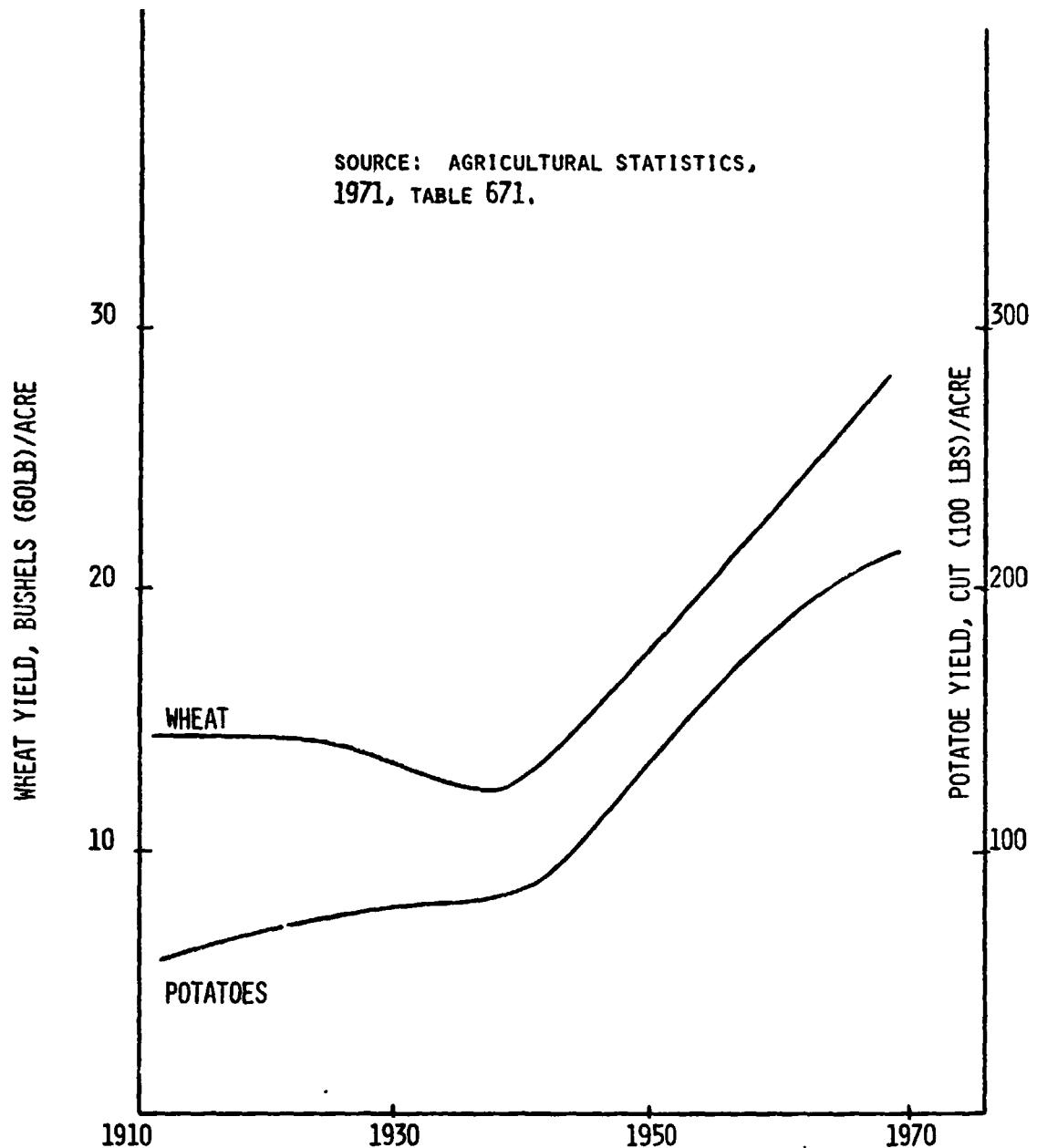
Figure 2.9. HISTORY OF WHEAT AND POTATO YIELD IN THE UNITED STATES.
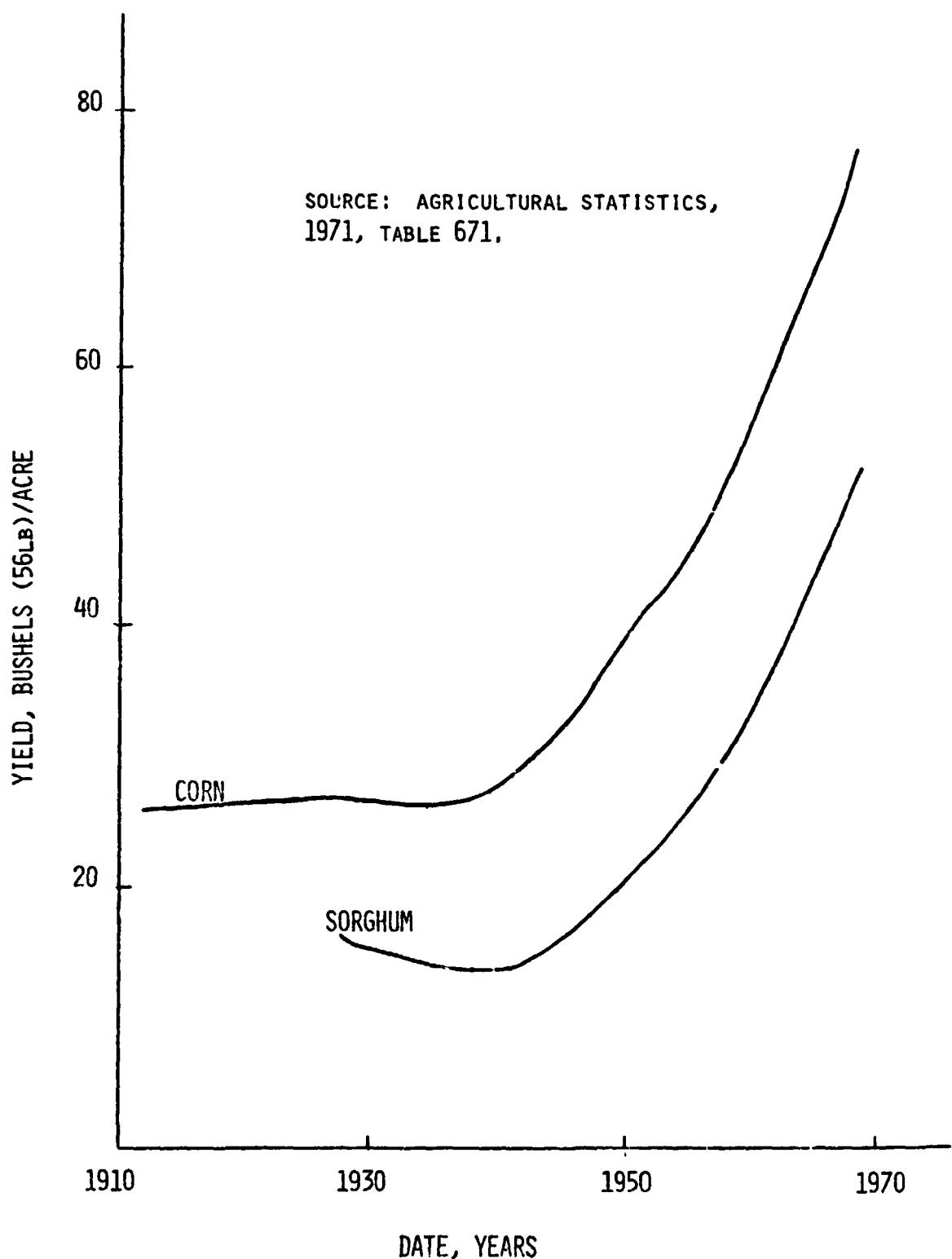
Figure 2.10. HISTORY OF CORN AND SORGHUM YIELD IN THE UNITED STATES.

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SOURCE: AGRICULTURAL STATISTICS, 1971. TABLE G71.
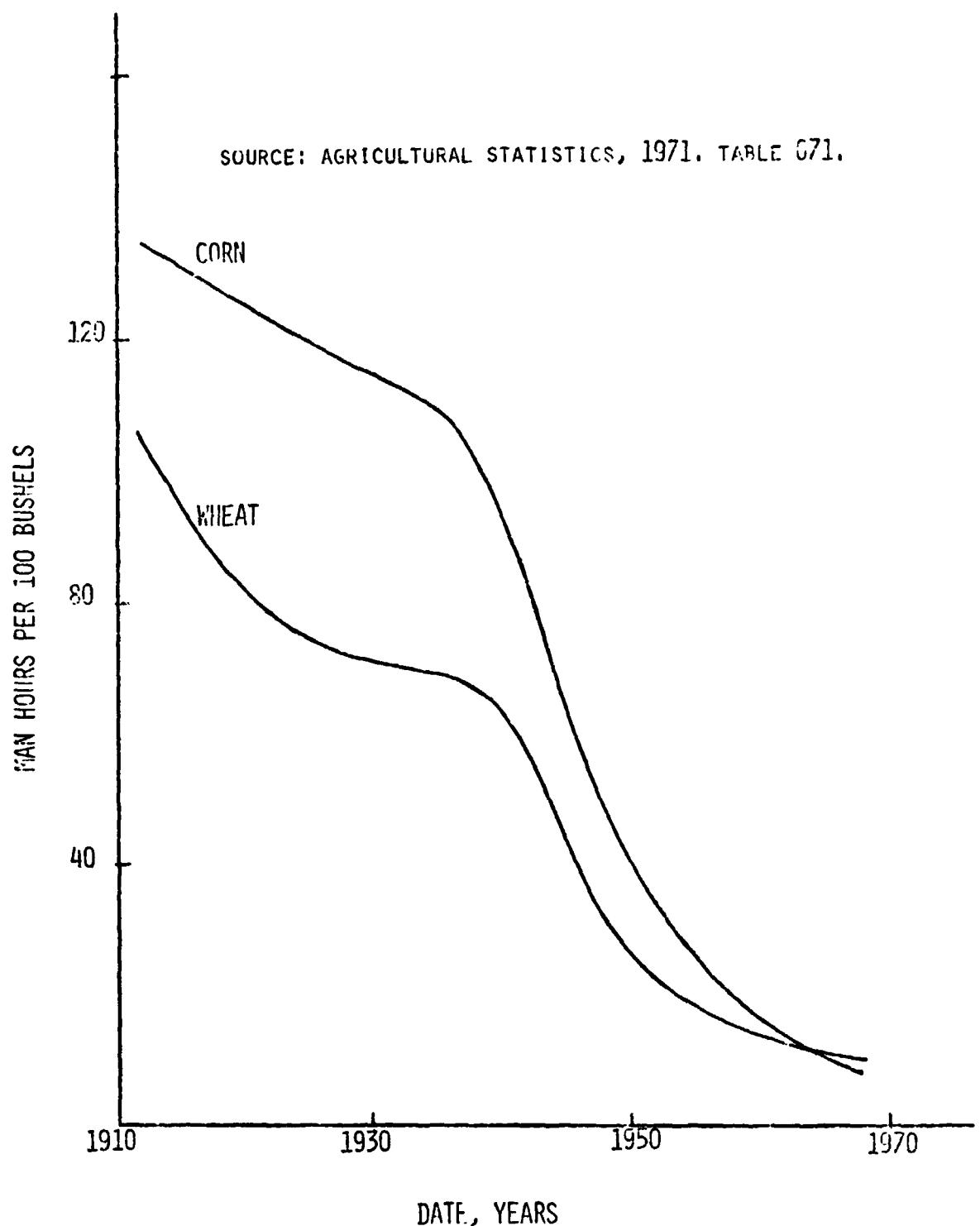
Figure 2.11. LABOR CONTENT: MAN HOURS PER 100 BUSHELS IN U.S. CORN AND
WHEAT PRODUCTION.

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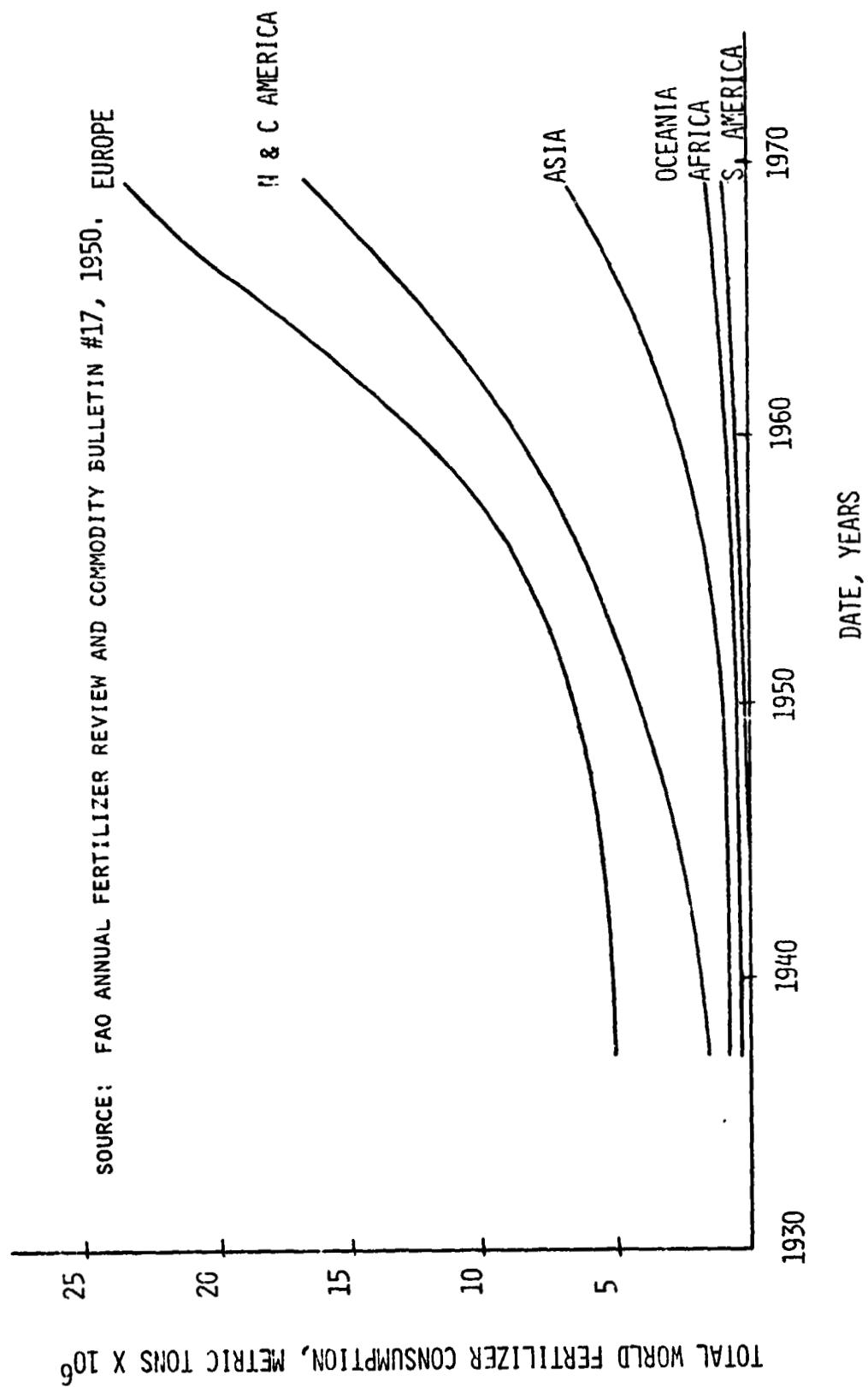
Figure 2.12. WORLD FERTILIZER CONSUMPTION.

SOURCE: FAO ANNUAL FERTILIZER REVIEW AND COMMODITY BULLETIN #17, 1950.

EUROPE

N & C AMERICA

ASIA

OCEANIA
AFRICA
S. AMERICA

TOTAL WORLD FERTILIZER CONSUMPTION, METRIC TONS X 10^6

DATE, YEARS

1930   1940   1950   1960   1970

5   10   15   20   25

Assuming an average tomato yield of 50 tons/acre the caloric output
becomes [21]

$$\frac{50 \text{ tons}}{\text{acre}} \times \frac{2000 \text{ lbs}}{1 \text{ ton}} \times \frac{85.2 \text{ kcals}}{1 \text{ lb}} = 8.5 \times 10^6 \frac{\text{kcals}}{\text{acre}}$$

This caloric output/acre of tomatoes is substantially higher than the
cereal crops which vary between 4 and 6 million kcals/acre.

However, of more importance is the cost of greenhouse
construction and operation. Table 2.13 gives the basic cost figures
for an average installation [22]. Labor content is very high. These
figures indicate this method of food production is both capital and
labor intensive and is not feasible from that viewpoint in spite of
the high caloric yield.

2.5.3 Nuplex Concept [23]

Another food production approach which has been suggested
is the nuplex. A nuplex consists of an integrated nuclear power plant,
desalination plant, and agro-industrial complex. Each subcomponent of
the nuplex is technologically proven at the present time. The innova-
tion lies in the integration both geographically and energetically of
the components to maximize the interrelationship of the sub-units. For
example, the agricultural facility receives fresh water, electrical
power, and fertilizer made adjacent to the fields.

The calorie productivity in a nuplex is summarized in Table
2.14. The projected yield figures are substantially optimistic compared
to the average Arizona yield from irrigated land. The economic summary
of the agro aspect of a nuplex directed to maximize the caloric output
of the agricultural sub-unit is shown in Table 2.15.

A projection of the impact of such complexes on world food
production has not been made in this report. However, it is clear that
orders of magnitude increase in yields are not achievable simply by the
integration of these facilities.

2.6 Synthetic Foods

2.6.1 History

An alternative to agricultural production of food is to
utilize chemical and/or enzymatic processes to produce materials which
could become a significant proportion of the diet. The rationale is
that food is a complex mixture of compounds, some of which might be
amenable to efficient synthesis. Thus, the possibility exists of as-
sembling "food factories" which would convert inexpensive starting
materials into safe and acceptable food components.

The advantages of such a food production system are many.
These include high efficiency of land utilization, ease of location,

Table 2.13

CAPITAL AND OPERATING COSTS OF CONTROLLED ENVIRONMENT (GREENHOUSE) FOOD PRODUCTION

(All figures on a per acre basis)

CAPITAL COST

STRUCTURE, INCLUDING ERECTION, AND ENVIRONMENTAL CONTROL	$ 87,000
SOIL PREPARATION, AUXILIARY STRUCTURES AND EQUIPMENT	$ 87,000
	$174,000

ANNUAL OPERATING COSTS

LABOR, 6-8 MEN	$ 35,000
SEEDS, FERTILIZER, MATERIALS, ENERGY	$ 30,000
	$ 65,000

Table 2.14

NUPLEX LAND USE AND YIELDS

	TOMATOES	SORGHUM	DRY BEANS	WHEAT	POTATOES
LAND USE, SUMMER WINTER ACRES	10,000	295,600	5,900	231,500	60,000
YIELD, LBS/ACRE	60,000	6,700	3,000	5,200	48,000
YIELD AS A PERCENTAGE OF ARIZONA IRR. AVERAGE YIELD		176	178	152	192
YIELD AS A PERCENT OF RECORD YIELD	50	61	86	50	48
FERTILIZER USAGE N/P$_2$O$_5$, LBS/ACRE	200/50	150/80	70/70	200/50	200/120

Source: Nuclear Energy Centers Industrial and Agro Industrial Complexes, ORNL Rept. 4290, 1968.

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Table 2.15

SUMMARY OF NUPLEX OUTPUT FOR HIGH CALORIE PRODUCTION

LAND USE, ACRES	301,500
CALORIES PRODUCED, BILLION KCALS	5,680
PERSONS FED/DAY, MILLIONS, @ 97 GRAM PROTEIN, 2500 KCALS	6.3
WATER CONSUMPTION, BGG.	1
LAND CAPITALIZATION, DOLLARS/ACRE	979
THERMAL POWER PRODUCTION, MW FAST BREEDER REACTOR, VERTICAL TUBE EVAPORATOR	11,590
SALABLE ELECTIRCAL POWER, MW FAST BREEDER REACTOR VERTICAL TUBE EVAPORATOR	2,724

independence from climate or type of soil, minimal water requirements, lack of agricultural waste and no environmental pollution from fertilizers or insecticides. This approach has some precedent.

2.6.1.1 Fat Synthesis

Of all the physicochemically derived materials considered for human consumption, the most practical experience has been with fats. This was due to the efforts of the Germans during World War II. In 1943-1944, considerable quantities of a synthetic margarine were produced. A waxy hydrocarbon by-product of the Fischer-Tropsch process was oxidized to fatty acids with air, extensively purified and then coupled with glycerol to form fat. After addition of water, salts, butter flavor and vitamins, the product was consumed by the German Armed Forces, hospital populations, and the general public. No digestive disturbances or ill effects were reported.

Just how safe this synthetic fat would be for prolonged human consumption can be questioned. Analysis revealed the presence of equal amounts of saturated odd- and even-chain fatty acids primarily from C_{10} to C_{20}. Variable amounts of dicarboxylic acids were present. Alkyl substituted fatty acids in some preparations amounted to as much as 35 percent of the total fatty acids. Most questionable was the presence of a small amount of nonsapnifiable material partially composed of polycyclic hydrocarbons. A study was performed more recently to determine whether advances in petrochemical technology since 1945 would permit the development of a better process. It appeared as though the Ziegler Process offered considerable promise. The postulated reaction sequence was (1) synthesis of ethylene from carbon monoxide, (2) polymerization of ethylene to α-olefins, (3) oxidative ozonolysis to monocarboxylic acids, and (4) condensation with glycerol to produce fats which would not contain branched chains, dicarboxylic acids, or polycyclic compounds. A first approximation engineering design was made for the process. The complexity of the automated system for potential use in the aerospace situation was so great as to preclude serious consideration for further development.

For several reasons, there has been some interest in the possibility of using the simplest even-chain triglyceride, triacetin, as food. Relatively simple schemes can be written for its synthesis. The literature concerning any toxicity associated with its long term ingestion is sparse. Although when fed as 77 percent of the calories for 60 days, it inhibited the growth of rats; good growth was observed when it was fed at lower levels.

2.6.1.2 Protein Synthesis

The physicochemical synthesis of the protein requirements of the body present difficulties not encountered with carbohydrates and fats in that the structures of the essential amino acids are relatively complex and different from one another. Further, all of them must be present for adequate nutrition, whereas a single molecule, for example,

glucose, can satisfy the body's carbohydrate requirement. Fortunately, there exist synthetic methods giving rise to complex mixtures of amino acids which may be useful.

When an electrical discharge is passed through a mixture of methane, ammonia, hydrogen, and water, significant amounts of a variety of amino acids are formed. An even wider variety of amino acids are found when methane saturated with aqueous ammonia is exposed to quartz or alumina at elevated temperatures. Thermal polymerization of mixtures of amino products have been examined for their nutritional properties. With the bacterium, L. plantarum, which has a complex amino acid requirement similar to man, thermally derived proteins were about 60 percent as effective as peptone in supporting growth. Replacement of half the protein in a rat diet with thermal protein and extra threonine did not alter weight gain.

2.6.1.3 Carbohydrates

Considering the difficulties in the physicochemical synthesis of edible fat and protein, it is not surprising that the major recent effort has been directed toward carbohydrate. The potential method that has received the most attention has been the formose reaction. In this reaction, certain alkalis and other substances catalyze the self condensation of formaldehyde to produce a mixture of monosaccharides. The resulting mixture has not been well characterized in most of the reported studies dealing with changes in the nature of the product with changes in catalyst or conditions. Relatively recent studies using gas-liquid chromatography have shown that the product is usually very complex with relatively small amounts of metabolizable carbohydrate. Efforts to obtain simpler mixtures have not been very successful. It should be noted that the formose reaction gives rise to both the D- and L-isomer of any given product and therefore it should be expected that even if it were possible to control the reaction to produce only hexoses, maximally only one-half the product could be metabolized.

The kinetics of the formose reaction have been the subject of a number of recent reports. Catalysts serve two major roles. First, some affect the lag period of initial condensation which produces the two-carbon compound. Second, some catalysts, including the products of the reaction, are able to alter the rate of subsequent reactions and cause the autocatalytic effects observed. The detailed mechanism for the reactions is not known although it appears as though the rate expression for reaction in homogeneous solutions is analogous to Langmuir-Hinshelwood relations for adsorption and desorption in neterogeneous systems.

There are few reports in the literature concerning the nutritive qualities of formose sugars. One would expect the results to be quite variable depending upon the catalysts and conditions used in their preparation. Mixed microorganisms can utilize a considerable proportion of the mixture for growth. When fed to rats as 40 percent of the diet, formose sugars caused diarrhea and death. At lower levels, the animals survived but suffered from diarrhea. As yet, the reason for this effect is not known.

Other carbohydrate-like materials such as glycerol have
been considered as potential nutrients. It is normally present in the
diet as a component of lipids and in the free form. It can be tolerated
in relatively large amounts in the human diet without obvious detrimental
effect.

2.6.1.4 Overall Comments

All of the preceding represent efforts to use physicochem-
ical processes for food synthesis. Recently, attempts have begun to ex-
ploit enzymatic processes. A major advantage of enzymatically mediated
reactions is the stereospecificity conferred on the products. Thus, in
the case of carbohydrates, all of the products could be in the D-form
and appreciably more might be suitable for human consumption. Potential
processes for carbohydrate production utilizing enzymes will be discussed
in detail in following sections of this report. These processes, in gen-
eral, are similar to those which occur in living organisms but involve the
use of isolated enzymes. Under some circumstances, these enzymes would
be immobilized and stabilized so as to permit their use in conventional
reactors as catalysts. The specialty of enzyme engineering is expanding
rapidly and the new tec   logy being developed shows high promise for
application to the problem of food synthesis. These raw materials for
the process would depend upon the location of the factory, available
starting materials, and type of product desired. The most desirable raw
material would be one requiring the least processing to convert it into
a food component. However, it is possible to conceptualize pathways
leading to food from materials as simple as carbon dioxide and water.
This latter process could mimic the photosynthetic production of carbo-
hydrate or alternatively, utilize chemical reactions quite dissimilar
from those occurring in nature. Various of these schemes involve a
complex sequence of individual reactions and have never been seriously
considered as practical methods for the large-scale production of food.

2.6.2 Economic, Social, and Political Implications of Synthetic Carbohydrates

Nonagricultural production of food is an attempt to increase
the availability of food which is only one aspect of a many faceted world
food problem. Thus an appraisal of the economic, political, or social
implications of the production of synthetic carbohydrates requires a
consideration of the broad context in which that impact will be made.

In previous sections of this chapter, an increased demand
for food is projected. However, for this latent demand to become effec-
tive demand, national economic development is necessary. For there is
extensive statistical evidence to document the fact that, in low income
countries, a large fraction of any increase in incomes goes into food
consumption. Indeed, we can reasonably estimate that total food demand
in the developing world will grow at a rate approximately equal to the
rate of population growth plus one-half to three-fourths of the growth
rate of per capita income. Therefore, decisions of public policy in

both the developed and developing worlds, which will determine whether
and the extent to which synthetic carbohydrates will be produced, will
probably rest on important considerations affecting the expansion of
balanced national economic development (an essential precondition for
dealing effectively with the world food problem) as well as on the
availability of food.

While it is not possible to anticipate the precise impact
of the synthetic production of carbohydrates on economic development of
the nations of the world, it would obviously have an impact upon world
trade in foods that presently supply the world with its carbohydrates.
Such trade has been and may continue to be an important factor in the
economic growth of the developing world, a projected major user of syn-
thetic carbohydrate production. An important constraint to the growth
of income in the developing world is the capacity to import. The ca-
pacity to import is restricted by the amount of foreign exchange held
by the importing nation which in developing countries is primarily lim-
ited by the value of its exports. For many developing countries agri-
cultural products constitute from 50 to 90 percent of their exports.
Thus the exportation of agricultural products is a prime factor in the
prospect for economic developmental success.

Will synthetic carbohydrates reduce the agricultural exports,
and hence the economic growth of the developing nations? The answer may
be found in the present structure of international trade. Developing
nations at present have serious difficulties in finding export markets.
These difficulties will only be intensified by synthetic carbohydrate
production unless such nations are the prime producers, since a high
percentage of their present exports consist of foods translatable into
carbohydrates. If these existing markets dry up, the impact upon the
economic growth of food-trade dependent nations is more likely than not
to be negative. Unless alternative sources of foreign exchange are
found, the effect of such a negative impact on economic development is
an absence of growth if not a reduction in effective food demand for
the affected nations.

On the more positive side, the proposal outlined in succeed-
ing chapters may be the answer to the problem of food distribution for
some states. It is well established by many previous studies that a
central problem of food availability is distribution, not production.
An attractive feature of the production method outlined herein is its
ability to be located at the heart of the area of need. Thus a country
could for the first time free food production from the historical limi-
tations to which it has been subject.

A second factor favoring synthetic production is the social
and economic effect of transforming agricultural workers into plant em-
ployees. Many noted authorities on economic development have insisted
that just such a transformation is essential to sound economic develop-
ment. Thus it could be argued that the proposal that follows offers to
the developing nations an aid to their development. But the value of
these possible advantages can only be assessed with reference to partic-
ular countries.

In a broader sense, it cannot be denied that the addition of
a new method of food production is a benefit to mankind. It helps to
assure that the problem of food availability will be met. Further, it
could free the means of production and energies of mankind in a general
sense for concentration on yet unsolved problems. On these matters one
can only speculate, but the challenge for a restructuring of interna-
tional trade and financial and monetary relations promises to remain.
It may be that the recognized potential problems for developing nations
could be resolved in the restructuring process. Such a result would
avoid the possible disadvantages for some so that the many may profit
from the new technology.

2.7 Summary

A thorough study of population, food needs, and food supplies in
the past, present, and future was made. During the course of this study,
we occasionally encountered deep pessimism (widespread famine and starva-
tion will inevitably occur) and high optimism (the food problem is eco-
nomic and development of economies will result in food supplies rising
to reach needs, by use of presently unused land, new seeds, and fertiliz-
ers). The uncertainties in the projections prevented us from reaching
either of these conclusions. Our conclusions are that there is a real
possibility of serious overall food shortage in the world by the turn
of the century and that nonagricultural food production will be a needed
and desirable augmentation to agriculture. We see synthetic food plants
serving the general world-wide need through achieving an economically
viable position and serving local food deficient zones on an emergency
basis even though they may not be competitive in the market place.

Investigations of the world-wide distribution of food were made in
order to estimate the most viable sizes for synthetic carbohydrate plants.
Once again, uncertainties in the projections made this task difficult.
However, the desirability of reasonably small plants in local areas be-
came apparent. Therefore, it was decided to study the feasibility of a
carbohydrate plant capable of producing 100 tons of carbohydrate per day.
Such a plant would provide approximately 50 percent of the carbohydrate
requirement for half a million people. This is considered compatible
with local area usage. It is also large enough to be efficient from a
process standpoint. Chapters 3, 4, and 5 contain specific information
on carbohydrate synthesis assuming such a plant size. Estimates of costs
for larger or smaller plants can be obtained by the usual plant-scaling
relations found in chemical engineering textbooks on plant design. Chap-
ter 3 is a short summary of the three processes. Chapter 4 goes into the
chemical pathways in detail. Chapter 5 is concerned with the design of
the plants themselves.

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Chapter 3

ESSENTIALS OF THE PRODUCTION OF SYNTHETIC CARBOHYDRATES

3.1 Introduction

This chapter is a general discussion of the three pathways to synthetic carbohydrate which were chosen to be examined in detail in this study. Chapter 4 will discuss these pathways in detail. Traditionally, food is provided for all forms of life by green plants, which use sunlight as a source of energy and carbon dioxide (CO_2) as a source of carbon. As photosynthetic organisms serve as food sources for animals, the energy and biologically important elements they contain are incorporated into the cells and tissues of the animals. When one animal consumes another, the biochemical compounds and energy stored in the first animal are partially transferred to the second animal. Photosynthesis is carried on in about equal quantity by the unicellular algae in the oceans and the seed plants on land. According to one estimate, the total annual fixation of carbon in the oceans amounts to approximately 1.2×10^{10} tons, whereas that on land is about 1.6×10^{10} tons [1].

The goal of this engineering systems design project is the development of processes for the production of carbohydrate from nonagricultural materials. Processes using cellulose and petrochemicals, materials which have in fact been produced by plants, but are unsuitable for consumption, have been developed. In addition, a process has been investigated for the conversion of inorganic carbon dioxide into starch by a series of complex reactions similar to the ones carried out by green plants during photosynthesis. A number of additional processes for the synthetic production of carbohydrates were considered which are discussed briefly at the end of Chapter 4.

The cellulose, fossil fuel, and CO_2 fixation processes all have the potential of producing the same end product, the sugar glucose. The basic features of these processes are outlined in Table 3.1. This glucose can be used directly as a food calorie source or polymerized to starch. The

Table 3.1

PATHWAYS FOR SYNTHETIC GLUCOSE

	Cellulose	Fossil Fuel	CO_2 Fixation
Starting Materials	Cellulose $(C_6H_{12}O_6)_x$	Petroleum (C_3H_6)	Carbon Dioxide (CO_2)
Chemical Energy Requirements	Low	Low	High
Complexity of Process	Simple	Moderate	Very Complex

following sections of this chapter briefly describe the three processes for the production of glucose and starch.

3.2 Cellulose Conversion to Glucose

Cellulose is a linear polymer of glucose units, and as such provides the most direct source for the production of glucose. Cellulose is the major constituent of all vegetation, comprising from one-third to one-half of all vegetable matter in the world. Thus, it is the world's most renewable resource. It is also widely dispersed and pentiful in both underdeveloped and developed countries. Large quantities of cellulose are used for the production of manufactured products such as textiles, paper, and building materials. In addition, enormous quantities of cellulose are consumed by livestock and serve as their principal source of energy. However, most vegetation is unused by man or animals and undergoes natural decay through the interaction of microorganisms. It is this unused cellulose that we think shows excellent potential as a food source through its conversion to glucose and/or starch.

3.2.1 Raw Materials

There is a wide range of available sources for vegetable cellulose, including wood, cotton, grasses, straws, agricultural residues (i.e., cereal straws, cornstalks, sugar cane bagasse), waste paper, weeds, and many others. The purity of the cellulose in these various vegetable sources range from a low of about 40 percent, as in some woods, to a high of 95 percent, as found in cotton. The remaining noncellulosic portion of vegetable matter is mainly hemicellulose (polymers made of sugar units other than glucose) and lignin (very complex nonsugar molecules). In the plant the hemicellulose and lignin compounds are found mixed with the cellulose. Many techniques are available for separating cellulose from these other plant components and subsequently depolymerizing it to glucose.

3.2.2 Nature of the Process

We know that a number of animals, insects, and microorganisms are able to digest cellulose to varying degrees. This digestion is accomplished by a group of enzymes loosely termed the "cellulase complex." One rapid cellulose decomposer is the fungus, Trichoderma viride. This microorganism produces a stable extracellular cellulase complex capable of converting cellulose quantitatively to glucose. It has been found that its cellulase complex contains at least three distinct enzymes, each playing an essential role in the overall process of converting cellulose to glucose. Since the fungus Trichoderma viride secretes its cellulase enzymes into the medium in which it is growing, they are easily separated from the cells by filtration. This cell filtrate containing the enzymes is then used directly to convert cellulose which has been partially purified to remove lignin and hemicelluloses to glucose.

3.2.3 Complexity

This is a relatively simple process compared to the fossil fuel or the carbon dioxide fixation processes since the starting material, green plant cellulose, is already in the form of a carbohydrate. This process, therefore, just involves the breakdown of this glucose polymer which cannot be used as a food by man because of the chemical nature of the glucose to glucose bonds in the polymer to its basic unit. This process is capable of efficiently converting cellulose into a useful food energy source at a reasonable cost.

3.3 Fossil Fuel Pathway

A second approach would use a pathway midway in complexity between the cellulose route and CO_2 fixation pathway. Petroleum is used as a source of simple compounds which can be altered in a series of 7 steps to form glucose. The energy requirements are minimal, since the petroleum would actually yield more energy on direct combustion than would carbohydrate. In essence, this would capitalize on past rather than present energy converting activities of the biosphere.

3.3.1 Raw Materials

Petroleum, the starting material, has been calculated to be in ample supply for this purpose. For example, a 100 ton per day carbohydrate factory would use only about 110,000 barrels of oil per year--about one ten-millionth of the supply of crude oil estimated to remain in the year 2000.* If other fossil fuel sources, such as coal (converted to hydrocarbon through gassification) or shale oil can be tapped, the potential resource is even greater. The remaining supplies of crude oil, coal, and shale oil in 2000 AD would provide life support for 15 billion people for many years, as shown in Table 3.2.

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*100 tons starch per day represents

$$100 \times \frac{2000 \text{ lb } CH_2O}{\text{day}} \times \frac{365 \text{ days}}{\text{yr}} \times \frac{14(CH_2)}{30(CH_2O)} \times \frac{1}{7.3 \text{ lb/gal}} \times \frac{1}{42 \text{ gal/barrel}}$$

$\cong$ 110,000 barrels per year

Total world supply is 2×10^{12} barrels of crude oil. At our current rate of consumption, 1×10^{12} barrels will be left in the year 2000. It is to be assumed that petroleum as an energy source will be replaced by nuclear energy, since otherwise we will run out anyhow.

Table 3.2

REQUIREMENT AND RESOURCES* OF FOSSIL FUEL

Total Resource	Estimated to Remain in 2000 A.D.	Years of Life Support for 15 billion people
Crude Oil 2.1×10^{12} barrels	1.0×10^{12} barrels	114
Coal 1.7×10^{13} short tons	1.6×10^{13} short tons	9,300
Shale Oil 2×10^{15} U.S. barrels	2×10^{15} barrels	220,000

*Resources & Man: Committee on Resources & Man, NAS-NRC.

Petroleum is a complex mixture of highly energetic compounds. It can be refined, as in the process of making gasoline, to propane (familiar as cooking gas) and to propylene, a similar compound containing less hydrogen. These two materials each contain three carbon atoms, exactly half the number required for glucose. If a few modifications are made to the basic three-carbon unit, it can be converted into a form suitable for condensation into the 6-carbon glucose.

3.3.2 Nature of Process

The conversion of the propane or propylene into glucose involves a series of steps, which are of two types. The first are chemical conversions to add oxygen to the molecule. Two steps are involved, and these are identical with those carried out presently on an industrial scale during the conversion of propylene to glycerin. Our design does not include these, but starts with the product glycidaldehyde. The sequence involves five reactions, not now done commercially, which are catalyzed by specific enzymes. The enzymes are to be isolated from microbial sources where possible, and stabilized for their function in catalyzing these steps. Since one of the steps requires the addition of phosphate under circumstances which demand an input of energy, a compound, ATP, found in living material, which can supply both phosphate and energy, will be used. This compound will, however, be regenerated by a combination of chemical and enzymatic techniques.

3.3.3 Complexity

The total process involves seven steps, four of which are enzyme catalyzed. Unlike the CO_2 fixation pathway, no recylcing of intermediates is required, and the energy requirements are less (by about a factor of ten). The process is straightforward and simple, but requires

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the preparation and stabilization of the enzymes, a factor which will contribute heavily to the cost of the end product. The starting material (propylene) is relatively cheap, amounting to about 1.5¢ per pound of finished carbohydrate at current prices, but the commercially available intermediate glycidaldehyde is estimated at about 18¢/lb of finished carbohydrate.

3.4 Carbon Dioxide Fixation Process

The most complex method to make synthetic carbohydrates is by way of the carbon dioxide fixation process.

3.4.1 Raw Materials

The pathway starts with a simple raw material, carbon dioxide, one of the ultimate products from the breakdown of organic compounds. This gas is found in the atmosphere in low concentrations, but can be more economically obtained as a by-product, such as in cement manufacture or power generation from fossil fuel. Because most processes that produce carbon dioxide release energy, energy must be used to make organic materials, glucose in this case, from carbon dioxide. The amount of energy required in this process is the greatest of any of the three pathways suggested here.

3.4.2 Nature of Process

The CO_2 fixation pathway is found in all green plants and is an integral part of photosynthesis. It is proposed to take this system out of the plant by separating the enzymes that catalyze its chemical reactions and use them to catalyze the same reactions in a chemical factory.

The cycle itself is composed of thirteen steps catalyzed by ten different enzymes. The first six steps attach carbon dioxide to an acceptor molecule and eventually change the resulting product to a six-carbon sugar containing phosphate. From here four enzymatic steps, not part of the cycle proper, convert 1/6 of this compound to starch. Another seven steps take the other 5/6 and reconstitute the acceptor for carbon dioxide.

3.4.3 Complexity

This recycling of a major part of the contents of the cycle means that this pathway is far more complex than either of the other two already discussed. Green plants have to use it because they must start with an extremely simple one-carbon molecule, carbon dioxide, that contains only carbon and oxygen, and build it up into starch, a complex polymer of the 6-carbon sugar, glucose, that contains hydrogen in addition to carbon and oxygen. We have found no better process to make starch from carbon dioxide than that used by the leaf. If it is possible to use

raw materials of greater complexity that have within themselves more chemical energy, simpler processes that require a lower energy input such as those above, are possible.

3.5 Glucose to Starch

Two of the three processes just discussed lead to the production of glucose. The capability to convert this compound to starch is highly desirable since starch is a more versatile foodstuff. (The third process, CO_2 fixation leads to starch directly.)

3.5.1 Nature of Starch

The term starch refers to an indigenous product isolated from food staples such as corn, wheat, sorghum, other cereal crops, and legumes. Actually, starch is a crystalline polymeric substance composed of amylose (a "straight-chained" glucose polymer) and amylopectin (a "branched" glucose polymer). The two forms are shown schematically as follows:

"Amylose"
(straight-chain glucose polymer)

"Amylopectin"
(branched-chain glucose polymer)
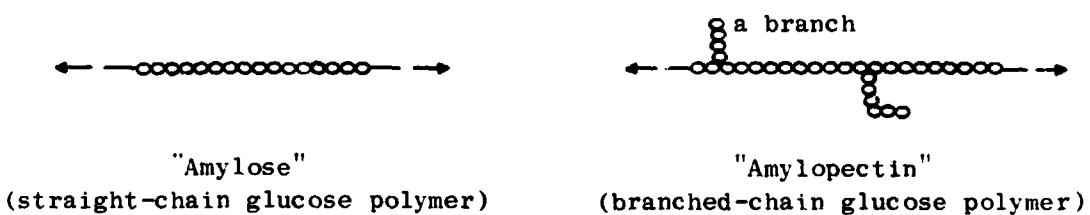
It differs from cellulose only in that the spatial orientation of the linkages is opposite. Starch from different sources contains varying amounts of the above two polymers. Common corns and cereal grains contain 17 to 28 percent amylose. Legumes yield starch with wider amylose ranges, e.g., peas vary from 34 to 70 percent amylose. Common dietary starch is derived from corn with about 27 percent amylose and 73 percent amylopectin. The nutritive value of the various starch compositions is essentially the same, but cellulose has no nutritive value as the reversed linkages cannot be digested.

Genetic variation in plants can yield very low percentages of amylose. Predominant among such low-amylose, high-amylopectin plants are waxy corn, waxy sorghum, and waxy, or glutinous, rice. These strains may contain as little as 2 percent amylose.

3.5.2 The Relation of Glucose to Starch

The individual building blocks of the polymer chains are a single sugar, glucose. In nature the construction of the polymeric chains can be thought of as the joining of one glucose to another with loss of water. The chain continues to lengthen by addition of further

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Step 1

    2 glucose   ──────────────▶   glucose-glucose + water
    ("reactants")   ("products")

Step 2

    glucose-glucose + glucose ──────────▶ glucose-clucose-glucose + water
                                             ("polymer chain")

glucose units. Chain lengths commonly exceed thousands of linked glucose units.

The branched polymer amylopectin has the same chemical composition, i.e., it is composed of glucose units, however, the amylopectin polymer appears to form by cleaving a short portion from the end of an amylose polymer and attaching it to the middle of the chain, thus forming a branch point. Each free end of the newly formed branched polymer can add glucose units.

3.5.3 Chemical Routes to Starch

The foregoing discussion has presented the formal synthesis of starch,* but a more circuitous route is followed by natural materials; and by the laboratory chemist. Several routes to starch synthesis are found in nature. All involve an activation of glucose either by the addition of phosphate or of a phosphate containing organic chemical followed by polymerization. Ironically, the least used route was discovered first and the most important route last. Each of these routes leads to the linear polymer amylose. One further step is necessary to produce amylopectin; this latter process is not well understood. When glucose is the starting material, we have elected to use ATP (a commonly used biochemical, needed anyway for both CO_2 fixation and fossil fuel processes) as a source of phosphate, and to polymerize the resulting phosphorylated glucose in a one-step enzymically catalyzed reaction.

REFERENCE

1. R. Y. Stainer, et al, The Microbial World, 3rd Ed., Prentice-Hall, Englewood Cliffs, N.J., 1970.

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Throughout the rest of this report the term starch will be used interchangeably with amylose as a matter of convenience.

Chapter 4

CHEMISTRY OF GLUCOSE AND STARCH PRODUCTION

This chapter considers in detail the chemistry of synthesizing carbohydrates. Sections 4.1, 4.2, and 4.3 discuss the pathways from cellulose, glycidaldehyde, and CO_2 to glucose. Section 4.4 is a comparison of these pathways. Section 4.5 discusses the conversion of glucose to starch. Section 4.6 discusses the regeneration of ATP, which is one of the more serious challenges in these processes. The final section discusses some of the pathways for carbohydrate synthesis which were rejected in the study for various reasons.

4.1 Chemistry of Cellulose Process--Cellulose to Glucose

The first pathway to be considered is the production of sugar from cellulose waste material. This process has claimed the attention of chemists since the early 19th century but it was not until 1898 that a commerical process using sulfuric acid under pressure was developed. This process gave a yield of about 6 percent sugar based on dry wood [1]. Subsequently, a plant using similar process was built at Georgetown, South Carolina, and operated from 1913 to 1923. Various improvements in this process were made through research efforts in the U.S., Germany and France from 1923 to 1945. At that time, the process yielded 45 to 55 percent sugar based on the weight of dry wood. Several acid hydrolysis plants were constructed in Germany during World War II, one such plant at Regensberg, Germany, having the capacity to process about 130 tons of wood per day. Sugar yields of about 60 percent were claimed. The sugars produced were used to produce yeast and alcohol [2]. Later plans by several companies in the U.S. to build plants for cellulose hydrolysis were abandoned because it was felt that sugar produced by the process could not compete with the low price of blackstrap molasses.

A large number of rumen and soil microorganisms possess the capacity to degrade cellulose rapidly and efficiently to glucose. In the last 10 years cellulolytic enzymes of these microorganisms have been considered as an alternative to acid hydrolysis. At the present time, there are no commercial processes or plants for enzymatically producing sugar from cellulose.

4.1.2 Raw Material

4.1.2.1 Structure and Organization of Wood and Plant Fibers

Plant tissue is composed of three main components: cellulose, hemicellulose, and lignin. Small amounts of resins, gums, proteins, fats, and mineral compounds are also present.

4.1.2.2 Cellulose

Cellulose, as it occurs in plant cell walls, is a polymolecular β-D-(1,4) glucan averaging about 10,000 glucose units.

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Its structure is shown in Figure 4.1. It is presen' in the cell wall in the form of elementary fibrils having a diameter of 35 Å, each containing approximately 40 cellulose chains. The elementary fibrils are aggregated to large microfibrils of various sizes [3]. The percentage of cellulose in plant material varies from a low of 40 percent as found in some woods to as high as 95 percent in cotton.

4.1.2.3 Hemicellulose

The term "hemicellulose" is used as a designation for the polysaccharides of low molecular weight which normally occur in plant tissues together with cellulose, and which can be isolated from the original material by extraction either with water, or more frequently, aqueous alkali [3]. Most of the hemicelluloses occurring in land plants are essentially linear polymers carrying numerous and varied short side chains. The principal sugar residues in hemicelluloses are D-xylose, D-mannose, D-glucose, D-galactose, L-arabinose, 4-^-methyl-D-glucoronic acid, D-galacturonic acid, D-glucuronic acid, and to a lesser extent L-rhamnose, L-fucose, and various O-methylated neutral sugars. The amount of hemicellulose in plant tissues ranges from 10 to 30 percent [3].

The first set of components, C_1 enzymes, hydrolyze the highly oriented crystalline solid cellulose into amorphous chains. There is uncertainty about what reaction is being catalyzed by C_1 components in general. One line of evidence indicates that C_1 may just be breaking intermolecular hydrogen bonds which destroys the crystal lattice of the cellulase. Other evidence shows that cellobiose is also produced by C_1 enzymes.

C_x enzymes c· (β-1 -.4 gluconases) are hydrolytic enzymes of clearly two types: (a) exo-β-1 -.4 glucanase, which successively removes single glucose units from the nonreducing end of the cellulose chains, and (b) endo-β-1 -.4 glucanase, which acts at random, the terminal linkages generally being less susceptible to hydrolysis than internal linkages.

Finally, β-glucosidases (cellobiase) are highly active on the β-dimer of glucose, cellobiose.
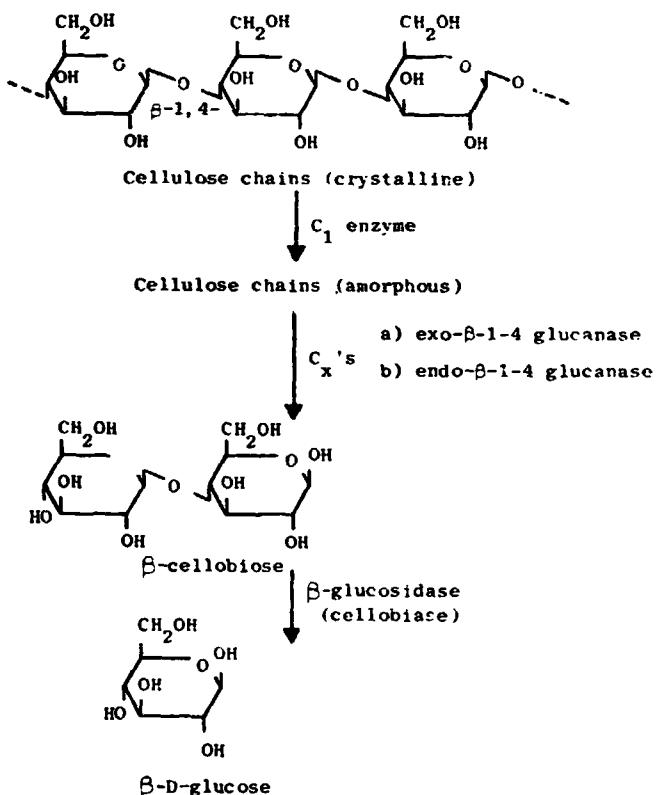
Figure 4.1. REACTIONS OF THE "CELLULASE COMPLEX."

4.1.2.4 Lignin

Lignin is a complex, three-dimensional polymer of phenyl-propane residues formed by dehydrogenation-polymerization of a precursor which probably of the coniferyl alcohol type. It is deposited in an amorphous state surrounding the cellulose microfibrils in the plant cell wall [4]. Its main function is to supply strength and rigidity to plant materials. The lignin content of plant tissues ranges from 15 to 30 percent [3].

4.1.3 Process

The digestion of cellulose involves two major steps: pretreatment of the plant material to remove the hemicellulose and disrupt the lignin surrounding the cellulose fibers, and the enzymatic digestion of the cellulose to glucose (Fig. 4.2).

PLANT TISSUE

ALKALI ⟶ HEMICELLULOSE

CELLULOSE-LIGNIN

ENZYMES ⟶ LIGNIN
(CELLULASE COMPLEX)

GLUCOSE
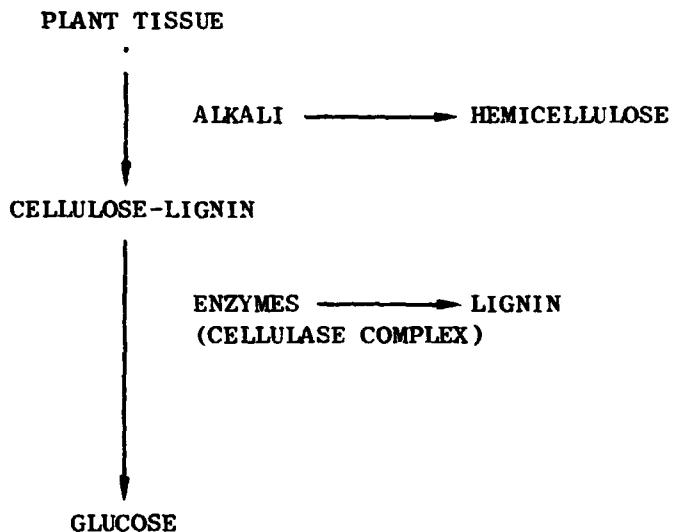
Figure 4.2. CELLULOSE DEGRADATION PROCESS.

4.1.3.1 Pretreatment of Cellulose

In order to render the cellulose fibers in plant tissues more susceptible to enzymatic hydrolysis it is necessary to decrease the relative crystallinity of the cellulose and to disrupt the physical structure of the lignin surrounding the cellulose fibers. To some extent this can be accomplished by physical techniques such as chopping, grinding and milling. It has been shown that milling of wood pulp cellulose increases hydrolysis rates and yields of glucose [5]. Chemical treatment with alkali also increase the digestibility of cellulose by microbial enzymes.

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Akali causes swelling of the cellulose which disrupts the lignin sheathing surrounding the cellulose fibers, allowing better access to the cellulose. The optimal conditions for alkali treatment vary with the variety of plant tissue, however, concentrations in the range of 1 to 10 percent sodium hydroxide appear to be sufficient [6]. An example of the effect of NaOH treatment on the in vitro digestibility of wheat straw and poplar wood by microorganisms is shown in Figure 4.3. In addition to disrupting the lignin polymer, the alkali treatment dissolves the hemicellulose fraction of the plant tissues [8]. This effect also affords the cellulose enzymes better access to the cellulose fibers and provides the additional benefit of preventing the hemicelluloses from entering the enzyme reactor whe. they could be partly depolymerized, thus contaminating the glucose product with other sugars such as xylose [9].

The lignin in the plant tissues is not decomposed by the action of the cellulose enzymes. It flows through the reactor and is separated from the glucose syrup after the cellulose has been digested.
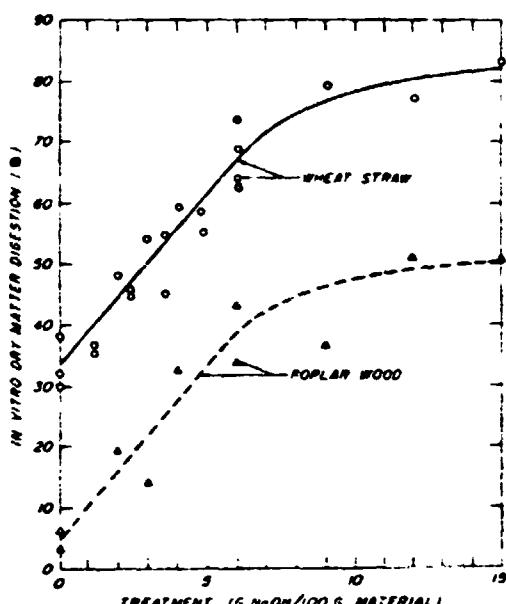
Figure 4.3. EFFECT OF NaOH PRE-TREATMENT ON THE PERCENTAGE OF DRY MATTER DIGESTION IN VITRO WITH RUMEN MICROORGANISMS.

4.1.3.2 Enzyme Reaction

Cellulase from Trichoderma viride strain QM 9123 has been used to catalyze the hydrolysis of cellulose pulp to glucose at conversions approaching 100 percent and yielding glucose syrups as concentrated as 15 percent [10,11]. A cellulose concentration in the reactor of about 10 percent will be used. This concentration was chosen since it has been shown that more than 90 percent of the cellulase protein will be adsorbed to the cellulose which assures that the enzymes are working at maximum capacity [12]. High amounts of adsorption also help prevent enzyme from being lost when the glucose syrup is removed from the reactor. One additional precaution that can be taken to prevent loss of enzyme is the use of a cellulose filter or prefilter when glucose is removed from the reactor, since the cellulase enzymes will adsorb to it and then the whole filter pad can be returned to the reactor for digestion.

The T. viride cellulase complex is a multi-enzyme system containing at least three different components. All three components play essential roles in the overall process of converting crystalline, amorphous, and chemically derived celluloses quantitatively to glucose, as shown in Figure 4.1.

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4.1.3.3 Factors Influencing the Activity of the Cellulase Complex Enzymes

Temperature. Optimum temperature for the enzyme from T. viride is 50 °C.

pH. A pH range of 4.5 to 5.5 appears to be the most effective for enzymatic hydrolysis of cellulose.

K_m Values. The Michaelis-Menton constant (K_m) of the cellulase enzymes with respect to cellulose appears to be independent of the pH and the particle size and displays a value of ca 0.3 M [12].

Product Inhibition. Cellobiose inhibits the hydrolysis of cellulose. Glucose inhibition is generally weak for T. viride cellulases (i.e., acting on heat treated cellulose, a concentration of 30 percent glucose gives only 40 percent inhibition). The inhibition by cellobiose can be reduced by keeping cellobiase concentrations high in the cellulose hydrolysates.

4.1.4 Enzyme Production

For simplicity and economy, the enzymes will be produced from the fungus Trichoderma viride (strain QM9123) as part of the continuous process for the saccharification of cellulose. The cellulolytic fungus will be grown on a simple mineral medium containing the cellulosic material to be used later as the substrate for the production of glucose. Using this substrate should assure that the proper complex of enzymes (the "cellulase complex") will be produced which contain all of the components necessary for attacking the particular cellulose substrate. The basic mineral medium should contain in grams/liter KH_2PO_4 2.0, $(NH_4)_2SO_4$ 1.4, urea 0.3, $MgSO_4 \cdot 7H_2O$ 0.3, $CaCl_2$ 0.3 and in mg/liter $FeSO_4 \cdot 7H_2O$ 5.0, $MnSO_4 \cdot H_2O$ 0.5, $ZnSO_4 \cdot 7H_2O$ 1.4, $CaCl_2$ 2.0. The culture is grown aerobically at 25 °C and pH 5.0 with a cellulose concentration of 1 percent [13]. The fungal mycelia and residual cellulose will be separated by filtration and the culture filtrate containing the enzyme transferred directly into the cellulose digester. In this was costly processing of the enzymes and losses in activity will be eliminated [13]. Growing under these experimental conditions, T. viride produced 1.25 mg protein/ml culture filtrate. This culture filtrate was able to produce 4.95 mg glucose/ml/hr at 50 °C and pH 4.8 [11].

4.2 Fossil Fuel Process--Glycidaldehyde to Glucose

4.2.1 Introduction

This fossil fuel process converts material derived from oil into edible carbohydrate. In the process suggested below, glucose is a

direct product, and can be tapped off, or converted to starch. It is possible to select alternatives in which starch is made directly, thereby reducing the amount of ATP required.

4.2.2 Raw Material

Glycidaldehyde, the starting material for this process, is derived from propylene (a product of petroleum refining). Details of this conversion were difficult to obtain, but it seems to involve two steps: treatment with oxygen to give acrolein, followed by peroxide to give glycidaldehyde.* In the industrial process, this would then be treated with hydrogen to give glycerol.

4.2.3 Process

Five steps are required to convert glycidaldehyde to glucose. Four of these are catalyzed by enzymes. The entire sequence requires the regeneration of 2 ATP from (2 ADP + 2 P_i) for each molecule of glucose formed. There are no oxidative or reductive steps involved--hence there are no electron carrier regenerations necessary. A chemical schematic is shown in Figure 4.4 and a list of reactions in Figure 4.5.

Step 1. Formation of DHA and GALD** from Glycidaldehyde

The glycidaldehyde is to be distilled into aqueous solution containing an acid ion exchange resin in suspension. This serves three purposes:

---

* According to the Kirk-Othmer Encyclopedia of Chemical Technology, 10, 619 (1966), the first step is done in the following way: a mixture of 10:1 to 4:1 by moles of propylene: and O_2, heated at 250 to 450°C using Cu_2O as a catalyst for 0.1 to 2 sec gives an 86 percent yield. We were unable to find any of the details of the second step, but its existence is al' ded to in the above reference, as well as in Chem. Engr., 67 (Sep 5, 1960), p. 68. Conversations by phone confirmed that glycidaldehyde is a recoverable intermediate which can be isolated by distillation.

** Abbreviations Used: GALD - glyceraldehyde
                          GALDP - glyceraldehyde phosphate
                            DHA - dihydroxyacetone
                          DHAP - dihydroxyacetone phosphate
                          FDP - fructose - 1,6-diphosphate
                          TPI - triose phosphate isomerase
                              P - organic phosphate
                             P_i - inorganic phosphate
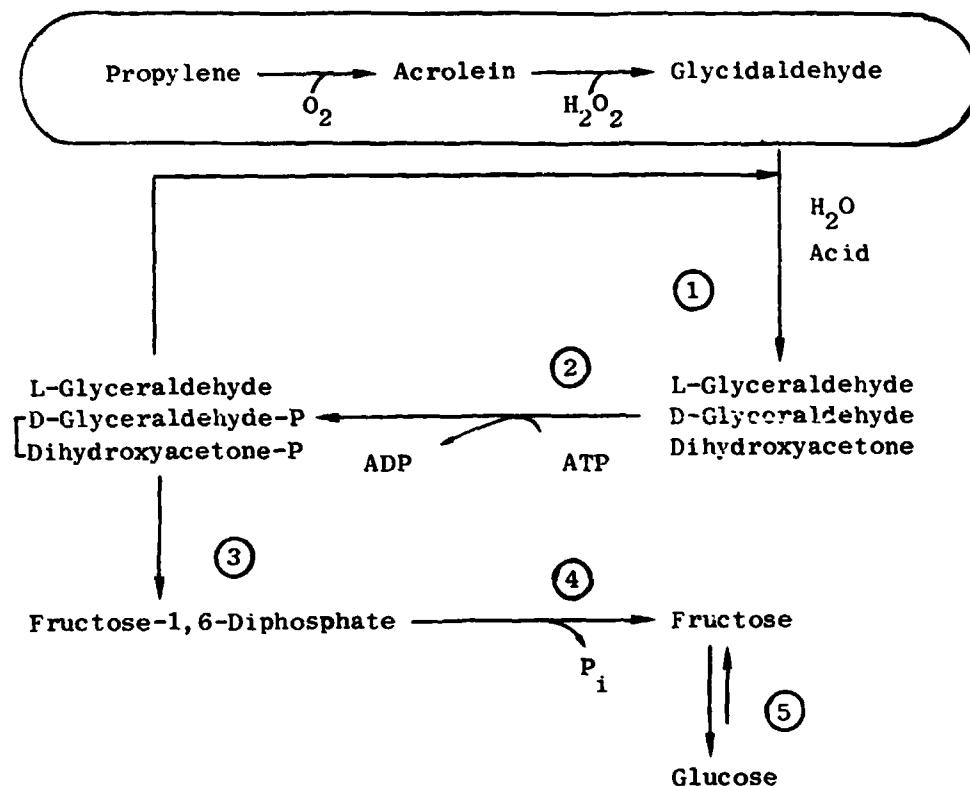
Figure 4.4. FLOW DIAGRAM--GLYCIDALDEHYDE → GLUCOSE.

(1) to prevent polymerization of the glycidaldehyde by diluting
     it down;

(2) to catalyze the ring opening by the acid from the resin:

$$
\begin{array}{c}
\text{CHO} \\
|\\
\text{CH} \\
|\\
\text{CH}_2
\end{array}
\!\!\!\!\searrow \!\! O
\;+\; H_2O \longrightarrow
\begin{array}{c}
\text{CHO} \\
|\\
\text{CHOH} \\
|\\
\text{CH}_2\text{OH}
\end{array}
$$
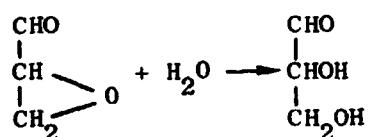
(3) to allow some isomerization to DHA:

$$
\begin{array}{c}
\text{CHO} \\
|\\
\text{CHOH} \\
|\\
\text{CH}_2\text{OH}
\end{array}
\;\rightleftarrows\;
\begin{array}{c}
\text{CH}_2\text{OH} \\
|\\
\text{C} = O \\
|\\
\text{CH}_2\text{OH}
\end{array}
$$
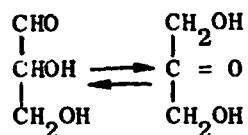
The ring opening is expected to be faster than the isomerization.
In view of this, the mixture could be tapped off at a point where the
DHA concentration is below that of D-GALD. (Doing this will aid in
regulating the aldolase reaction of step 3.) However, some isomeriza-
tion is desirable, as it converts the L-GALD to usable form.

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$$CH_3CH = CH_2 + \frac{1}{2} O_2 \xrightarrow{\text{①}} CH_2 = CH-CHO$$

$$CH_2 = CH-CHO + H_2O_2 \xrightarrow{\text{②}} CH_2\underset{O}{-}CH-CHO + H_2O$$

①

$$CH_2\underset{O}{-}CH-CHO + H_2O \xrightarrow{\text{③}} \begin{array}{c} CHO \\ | \\ CHOH \\ | \\ CH_2OH \end{array} \begin{bmatrix} CH_2OH \\ | \\ + \; C=O \\ | \\ CH_2OH \end{bmatrix}$$

(some)

②

$$\begin{array}{c} CHO \\ | \\ H-C-OH \\ | \\ CH_2OH \end{array} + ATP \xrightarrow{\text{④}} \begin{array}{c} CHO \\ | \\ H-C-OH \\ | \\ CH_2OP \end{array} + ADP$$

$$\begin{array}{c} CH_2OH \\ | \\ C=O \\ | \\ CH_2OH \end{array} + ATP \xrightarrow{\text{④}} \begin{array}{c} CH_2OH \\ | \\ C=O \\ | \\ CH_2OP \end{array} + ADP$$

③

$$\begin{array}{c} CH_2OP \\ | \\ C=O \\ | \\ CH_2OH \\ \\ CHO \\ | \\ H-C-OH \\ | \\ CH_2OP \end{array} \xrightarrow{\text{⑤}} \begin{array}{c} CH_2OP \\ | \\ C=O \\ | \\ HO-C-H \\ | \\ H-C-OH \\ | \\ H-C-OH \\ | \\ CH_2OP \end{array}$$

④

$$\begin{array}{c} CH_2OP \\ | \\ C=O \\ | \\ HO-C-H \\ | \\ H-C-OH \\ | \\ H-C-OH \\ | \\ CH_2OP \end{array} \xrightarrow{\text{⑥}} \begin{array}{c} CH_2OH \\ | \\ C=O \\ | \\ HO-C-H \\ | \\ H-C-OH \\ | \\ H-C-OH \\ | \\ CH_2OH \end{array} + 2HPO_4^=$$

⑤

$$\begin{array}{c} CH_2OH \\ | \\ C=O \\ | \\ HO-C-H \\ | \\ H-C-OH \\ | \\ H-C-OH \\ | \\ CH_2OH \end{array} \underset{\text{⑦}}{\overset{\longrightarrow}{\longleftarrow}} \begin{array}{c} CHO \\ | \\ H-C-OH \\ | \\ HO-C-H \\ | \\ H-C-OH \\ | \\ H-C-OH \\ | \\ CH_2OH \end{array}$$

Figure 4.5. CHEMICAL REACTIONS--GLYCIDALDEHYDE → GLUCOSE.

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An acid resin rather than an acid solution is to be used in order to keep the concentration low, thus minimizing side reactions. Use of resin also simplifies separation of acid and product. Ring opening is relatively easy, as it goes at low temperature in weak acid. The isomerization, on the other hand, goes rather slowly. Its rate constant in acetic acid is $\sim 10^{-5}$ M^{-1} min^{-1}. Acid rather than basic catalysis was chosen in order to avoid side reactions such as aldol condensation and the polymerization of the epoxide.

Step 2. Conversion of Trioses to Phosphorylated Sugars

The reactions to be carried out are:

$$
\begin{array}{ccccc}
\text{CHO} & & & \text{CHO} & \\
| & & & | & \\
\text{H–C–OH} & + \text{ATP} & \longrightarrow & \text{H–C–OH} & + \text{ADP} \\
| & & & | & \\
\text{CH}_2\text{OH} & & & \text{CH}_2\text{OP} &
\end{array}
$$

and

$$
\begin{array}{ccccc}
\text{CH}_2\text{OH} & & & \text{CH}_2\text{OH} & \\
| & & & | & \\
\text{C=0} & + \text{ATP} & \longrightarrow & \text{C=0} & + \text{ADP} \\
| & & & | & \\
\text{CH}_2\text{OH} & & & \text{CH}_2\text{OP} &
\end{array}
$$
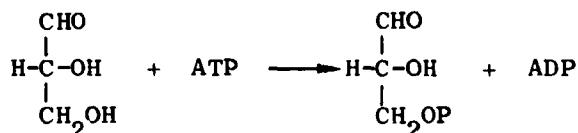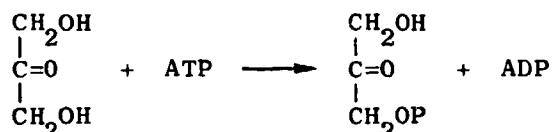
It is desirable that these proceed at reasonable rates, but that L-glyceraldehyde not react in this case. If the L-glyceraldehyde is phosphorylated, it becomes more difficult to separate and recycle. It may also be acted upon to give a nonmetabolizeable 6-carbon sugar. To avoid these complications, a method using triokinase was selected in preference to two alternative procedures.

In the first of these procedures, a nonenzymic conversion would be employed, but this has two drawbacks: a yield of only 24 percent, and the phosphorylation of the L-isomer. The second procedure would use the activity of glycerokinase, but this also phosphorylates the L-glyceraldehyde, at a rate higher than that for the D-isomer [15]. This was serious enough to overcome the advantage of the known ability to obtain this enzyme crystalline from Escherichia coli or in high yield from yeast in contrast to the scarcity of triokinase.

A preparation of triokinase free from glycerokinase has been described in the literature [16]. Crystalline enzyme was not, however, obtained. No reports of attempts to crystallize this enzyme could be found. Studies on its activity have usually been done with liver in connection with fructose metabolism [17] (Fructose-1-P is split by aldolase to give DHAP and glyceraldehyde, which is then phosphorylated by triokinase). An extensive study of the levels of triokinase in livers of various species, indicates a large quantity of raw material would be required [18]. The only microbial source found was Bacterium subtilis strain S_1 [19] and due to the nature of the data available, we were able to make only crude estimates of the weight of cells required.

Despite the drawbacks of no "off the shelf" procedure for preparation of pure enzyme from a convenient source, the above pathway appears to be the most reasonable since this enzyme is specific for D-glyceraldehyde and DHA, and does not phosphorylate the L-isomer [20].

Further studies should be undertaken to investigate the availability of this enzyme from yeast or other microbial sources, and the possibility of inducing its formation at higher levels by control of growth media. The probability of finding it in yeast is high due to the fact that yeast is able to metabolize fructose.

Step 3. Isomerization of the Phosphorylated Trioses and Condensation with Aldolase

After the phosphorylation step, a mixture of D-GALD and DHAP will be sent to a reactor containing triose phosphate isomerase. This enzyme catalyzes the interconversion of the two isomers and the equilibrium is 20/1 in favor of DHAP. Since the subsequent reaction requires a 50:50 mixture, we expect to control the timing of this reaction so that equilibrium is not reached. Entering the reactor, then, is a mixture preponderant in D-GALDP, and exiting is a mixture 50:50 in D-GALP and DHAP.

```
   CHO                  CHO           CH2OH
    |           TPI       |             |
  H-C-OH       ------>  H-C-OH    +    C=O
    |           <------    |             |
  CH2O-P                CH2O-P        CH2O-P
```
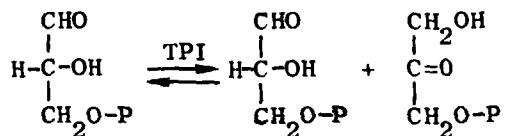
These two materials are then condensed using aldolase to give FDP:

```
                                        CH2O-P
                                          |
    CHO          CH2OH                   C=O
     |             |                      |
   H-C-OH    +    C=O      ------->    HO-C-H
     |             |                      |
   CH2O-P        CH2O-P                 H-C-OH
                                          |
                                        H-C-OH
                                          |
                                        CH2O-P
```
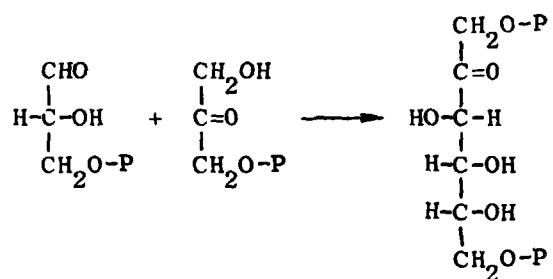
This procedure was used in preference to coupling the two enzymes since a simulation of the coupled reaction indicated a ten-fold increase in the time required to reach equilibrium (which is greatly in favor of FDP; K_{eq} = 12,000). This time lag is especially important, since apparently aldolase is substrate inhibited [21] and we intend to operate at 0.1 M concentrations because of engineering considerations. (Small molarities imply high flow rates.)

Both TPI and aldolase are available from yeast as well as from a variety of other sources [22]. Because of the availability of yeast and the high activities of the enzymes in this organism, yeast seems the best source.

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Step 4. Conversion of FDP to Fructose

The removal of phosphate groups from FDP could theoretically be accomplished in many ways. Two were seriously considered.

(1) Removal of both phosphates by alkaline phosphatase:

$$FDP \xrightarrow{\text{alk.Pase}} Fructose + 2P_i$$
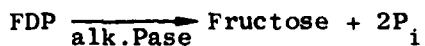
(2) Removal of the 1-P from fructose-1,6-diphosphate with FDPase, followed by isomerization to glucose-6-phosphate, followed by treatment with Glucose-6-Pase:

$$FDP \xrightarrow{\text{FDPase}} F6P \xrightarrow{\text{phosphohexoseisomerase}} G6P$$
$$\downarrow G6Pase$$
$$glucose$$
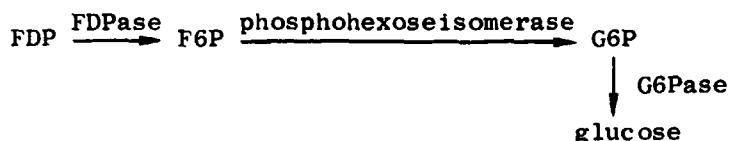
Of these, the first is the most direct, and we think, the most practical. Number 2 had the advantage of specificity: conceivably ADP could be carried along until the glucose was produced, making separation easy (compared to earlier separation from phosphorylated sugars). However, there were some evident drawbacks. First, increasing the number of steps is a disadvantage. Second, fructose as a possible tap off point is bypassed. (Since fructose is a desired sweetener, its market value would help offset some of the cost factors.) Third, the fructose-1,6-diphosphatase is substrate inhibited [22]. This is the most serious problem, since it would require operation of the process at levels of about 10^{-3} M, which in turn would require unacceptable amounts of fluid to be circulated. Although the rabbit liver enzyme exhibits this inhibition only at pH levels lower than 9 [23], the enzyme from Candida utilis, which would be more accessible for our purposes, was inhibited at low FDP concentrations at both pH's [24].

Should FDPase prepared from yeast have to be used, it is possible the problem might be overcome by previous incubation of the enzyme with Coenzyme A or with acyl carrier protein [25]. This activated preparation (from liver) does not exhibit substrate inhibition at pH 7.5, which it would have done without CoA treatment.

We have focused on reaction number 1 above because of its obvious brevity and the wealth of data available on the E. coli enzyme [26].

Step 5. Isomerization of Fructose to Glucose

Although fructose itself is a valuable material, it must be converted to glucose if starch is to be made. In order to do this, glucose isomerase, an enzyme prepared from Streptomyces albus, will be used to catalyze the reaction:

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```
      CH2OH                          CHO
       |                              |
      C=O                           H-C-OH
       |                              |
   HO-C-H          ──────▶        HO-C-H
       |           ◀──────            |
    H-C-OH                         H-C-OH
       |                              |
    H-C-OH                         H-C-OH
       |                              |
      CH2OH                         CH2OH

     D-fructose                    D-glucose
```
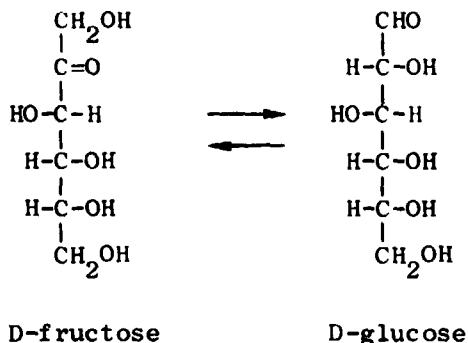
The equilibrium mixture contains substantial amounts of both fructose and glucose, so that the fructose should be recycled after the glucose is converted into starch.

We intend to use a stirred tank reactor, in view of the quantities that need to be processed, and to filter out the enzyme for reuse. The enzyme which catalyzes this reaction has been immobilized on porous glass (covalent binding) [27] and in polyacrylamide gel [28]. However, this treatment does not enhance the stability of the preparation except in column application. Therefore, it would be preferable to use heat treated whole cells, as suggested by Takasaki et al [29]. By using whole cells which had been treated for 10 min at 60 to 80°, the enzyme activity could be used repeatedly. This also eliminates the need or even desirability of purifying the enzyme.

4.2.4 Enzyme Production

All of the enzymes for this process are present in microbial sources. All have been crystallized and are present in considerable quantity with the exception of triokinase. A summary of characteristics relevant to their use in this process is given in Table 4.1. A more detailed account of their pertinent properties is in Appendix 4.1.

Methods of preparation have been scaled up from those appearing in the literature, and are described in Chapter 5. The quantities of source material required for sufficient enzyme in each case is given in Table 4.1 and is calculated from yields given in the literature by the following method.

In order to produce 100 tons carbohydrate per day, intermediate materials must be processed at the rate of 350 moles/min at the 6-Carbon stage, or 700 moles/min at the 3-Carbon stage. Therefore,

$$\text{quantity needed} = \frac{350 \text{ (or 700)} \times 10^6 \text{ } \mu\text{moles/min} \times \text{amount used in lit. prep.}}{\text{units of activity isolated in literature preparation}}$$

The appropriate conversion factors for grams to pounds to tons may also be included.

Table 4.1

ENZYMES FOR F. F. FROM GLYCIDALDEHYDE TO GLUCOSE

Enzyme	Status	Amount Starting Material	Needed Grams pure [a]	Sp. Act. [b]	Turnover Number [c]	K_m	pH Opt.
Triokinase 2.7.1.30 [1]	50 X pure from beef liver; present in B. subtilis (not crystalline)	70 tons B. subtilis	4675 g	75* est. based on ∿	7500* estimated based on liver enzyme	GALD: 1.2×10^{-4} DHA: 2×10^{-6}	7
TPI 5.3.1.1 [2]	Crystalline from yeast + other sources (250 X pure)	48 lb dried yeast based on GALDP	20 g based on GALDP	880 DHAP / 18,000 GALDP	49,000 DHAP / 1,000,000 GALDP	GALDP: 1.2×10^{-3} DHAP: 1.3×10^{-3}	7-8
Aldolase 4.1.2.13 [3]	Crystalline from yeast + other sources (25 X pure)	1,480 lb dried yeast	625 g	560	37,800	GALDP: 2×10^{-3} DHAP: 2.4×10^{-3}	7.3
Alkaline Pase 3.1.3.1 [4]	Crystalline from E. coli; also mammalian sources	3.5 tons wet (based on 6.2×10^5 ℓ)	103,000 g	3.4	2700	1.2×10^{-5}	8
Glucose Isomerase [5]	Crystalline from Strep. albus	1,100 lb wet (based on 100,000 ℓ)	50,800 g	6.9* For the	1070* reverse	0.16* reaction	7

Notes: Purification Procedures:
[1] J. Physiol. Chem. 324, 88 (1961)
    Meth. Enz. V, 362 (1962)
[2] Eur. J. Biochem. 14, 289 (1970)
[3] JBC 236, 3177 (1961)
[4] Meth. Enz. IX, 639 (1966)
[5] Fermentation Adv., p. 561

[a] calculation from sp. act. and 350 moles/min
[b] μmoles/min/mg
[c] moles/min/mole E

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In this process the enzymes are to be immobilized on solid supports to allow their repeated use. All except triokinase have, in fact, been placed on solid supports with varying degrees of success. It is assumed that improvements in technique with respect to any particular enzyme are forthcoming, so that the concept of enzyme use in this fashion would not be limited by preliminary attempts at immobilization. Further details on this problem are given in Appendix 4.6.

4.3 CO_2 Fixation Process--CO_2 to Glucose

4.3.1 Introduction

A schematic of the reductive pentose phosphate cycle to fix CO_2 and associated reactions is shown in Figure 4.6. Table 4.2 gives a summary of the reactions based on the production of one mole of glucose in the form of starch, and also free energy changes calculated on standard conditions ($\Delta G°$) [30].

The reduced pentose phosphate portion of the carbon dioxide fixation process is composed of thirteen reactions catalyzed by ten enzymes. (Three of the enzymes, aldolase, transketolase, and fructose-1, 6-diphosphatase, mediate two reactions each.) Since several are found only in photosynthetic tissue, it seems reasonable to obtain all ten from that source although the yields are low. An added advantage of this is that the enzymes should be present in roughly the relative amounts required for the proposed process, since it is the same as that carried out by the photosynthetic tissue. Spinach has been used commonly for preparation procedures.

Besides these ten enzymes, four others are required to effect the reactions on the pathway to starch. One is used to convert bicarbonate to CO_2. Carbon dioxide at the pH used in this process, approximately 7.5, is found mainly in the form of bicarbonate ion, while it is incorporated into the cycle in its native form. If this addition of carbon dioxide to ribulose-1,5-diphosphate occurs at a high rate, conversion of bicarbonate to carbon dioxide may not keep pace. To prevent this second reaction from being limiting, the enzyme carbonic anhydrase should be used to catalyze it. This may be obtained from bovine erythrocytes.

Three enzymes, glucose phosphate isomerase, phosphoglucomutase, and phosphorylase are required to convert fructose-6-phosphate to starch. These enzymes will undoubtedly be more economically isolated from yeast and E. coli rather than from corn, potato, or spinach.

It is proposed that all of the enzymes be stabilized on a soluble support such as dextran, and that the system be operated at approximately 30°C.

4.3.2 Regenerations Required

This process requires a higher energy input in the form of ATP and NADPH than any other proposed pathway. As may be noted from Table 4.2, 18 moles of ATP and 12 of NADPH are required for every mole

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ATP ADP + H$^+$

RIBULOSE-5-
PHOSPHATE   RIBULOSE-1,5-
    phospho- DIPHOSPHATE
    ribulokinase

CO_2 H_2O 2H$^+$

3-PHOSPHO-
GLYCERATE

ribulose-1,5-
diphosphate
carboxylase

ribulose
phosphate
3-epimerase

ATP

phosphoglycerate
kinase

ADP

PHOSPHORYL-3-
PHOSPHOGLYCERATE

ribose
phosphate
isomerase

NADPH + H$^+$

glyceraldehyde-
3-phosphate
dehydrogenase

NADP + HPO$_4^=$

XYULOSE-5
PHOSPHATE   RIBOSE-5
     PHOSPHATE

GLYCERALDEHYDE-
3-PHOSPHATE

transketolase

SEDOHEPTULOSE-7-
PHOSPHATE

triose
phosphate
isomerase

HPO$_4^=$

fructose-1,6-
diphosphatase

DIHYDROXY-
ACETONE-
PHOSPHATE

H_2O

SEDOHEPTULOSE-1,7 aldolase
DIPHOSPHATE   aldolase

FRUCTOSE-1,6-
DIPHOSPHATE

fructose-1,6- H_2O
diphosphatase HPO$_4^=$

ERYTHROSE-4-
PHOSPHATE

transketolase FRUCTOSE-6-PHOSPHATE

HPO$_4^=$ hexose phosphate
         isomerase

starch GLUCOSE-1-PHOSPHATE   GLUCOSE-6-PHOSPHATE
phosphorylase   phosphoglucomutase

Figure 4.6. REDUCTIVE PENTOSE PHOSPHATE CYCLE AND ASSOCIATED REACTIONS.

Table 4.2

REACTIONS OF THE CARBON DIOXIDE FIXATION PROCESS
AND ASSOCIATED REACTIONS OF ATP/ADP

Number	Reaction	$\Delta G°$ kcal/mole
1	$6(RuDP + CO_2 + H_2O) \rightleftharpoons 2\ 3\text{-}PGA + 2H^+)$	-8.4
2	$12(3\text{-}PGA + ATP \rightleftharpoons P\text{-}3PGA + ADP)$	+4.8
3	$12(P\text{-}3PGA + NADPH + H^+ \rightleftharpoons GALD3P + NADP^+ + HPO_4^=)$	-0.3
4	$5(GALD3P \rightleftharpoons DHAP)$	-1.8
5	$3(GALD3P + DHAP \rightleftharpoons FDP)$	-5.4
6	$3(FDP + H_2O) \rightleftharpoons F6P + H$	-3.4
7	$2(F6P + GALD3P \rightleftharpoons E4P + Xu5P)$	+1.5
8	$2(E4P + DHAP \rightleftharpoons SDP)$	-5.6
9	$2(SDP + H_2O \rightleftharpoons S7P + HPO_4^=)$	-3.4
10	$2(S7P + GALD3P \rightleftharpoons Xu5P + R5P)$	+0.1
11	$2(R5P \rightleftharpoons Ru5P)$	+0.2
12	$4(Xu5P \rightleftharpoons Ru5P)$	+0.5
13	$6(Ru5P + ATP \rightleftharpoons RuDP + ADP + H^+)$	-5.2
14	$F6P \rightleftharpoons G6P$	-0.5
15	$G6P \rightleftharpoons G1P$	+1.7
16	$G1P \rightleftharpoons starch\ (C_6H_5O_{10}) + HPO_4^=$	-0.5 (pH 7.4)
Net reaction:	$6CO_2 + 12NADPH + 18ATP + 11H_2O \rightarrow$ $Starch + 12NADP^+ + 18ADP + 18HPO_4^= + 6H^+$	
17 [4]	$ADP^{-3} + NH_2COOP^{-2} \rightarrow ATP^{-4} + NH_2COO^-$	-1.8 (pH 9.5)
18 [5]	$CNO^- + H_2PO_4^- \rightarrow NH_2COOP^{-2}$	-0.44 (pH 5.5)
19	$ADP^{-3} + CH_3CO_2P^{-2} \rightarrow ATP^{-4} + CH_3COO^-$	-3.0
20	$CH_3COOP^{-2} + PGA^{-3} + NADPH^{-4} + H^+$ $\xrightarrow{\text{(ADP)}} CH_3COO^- + GALDP + NADP^{-3} + P_i^{-2}$	+1.5

of glucose produced. ATP is best regenerated by the use of carbamyl phosphate, which is produced by a variant of the pathway proposed in Section 4.6.1. NADPH can be regenerated either by ethanol, as suggested in Section 4.6.2, or by elemental hydrogen, which can be transferred to NADP through ferredoxin:

$$H_2 + 2Fd^{+3} \xrightleftharpoons{ferredoxin\ hydrogenase} 2H^+ + 2Fd^{++}$$

$$2Fd^{++} + NADP^+ + H^+ \xrightleftharpoons{ferredoxin\text{-}NADP\ reductase} NADPH + 2Fd^{+3}$$
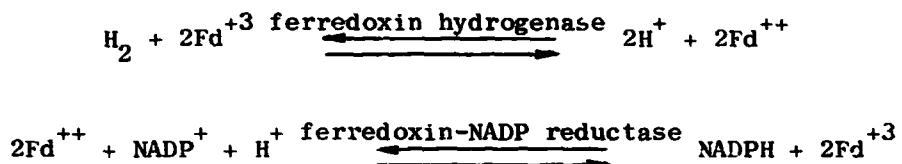
4.3.3 Design of the Process

There are at least two ways to produce glucose or starch by the carbon dioxide fixation process. One scheme contemplates incorporating all the reactions of the reductive pentose phosphate cycle, plus those that regenerate ATP and NADPH and produce starch, in one reactor. In the other scheme there would be five reactors for the reactions of the reductive pentose phosphate cycle, plus separate reactors for the regeneration of ATP and NADPH and two for the production of starch, a total of nine altogether. Because of the difficult separations involved, it does not seem desirable that there should be a reactor for each enzymatic reaction.

4.3.3.1 Single Reactor Scheme

The analogy to the single reactor scheme is the chloroplast, which carries out all the reactions proposed plus many others. In addition to converting CO_2 to starch by the pathway shown, it regulates concentrations of reactants and products, converts water to hydrogen and oxygen, phosphorylates ADP to make ATP using energy from sunlight, and synthesizes many of the enzymes involved. In the single reactor scheme proposed here, enzymes would catalyze the reactions converting CO_2 to starch. The entering materials would be hydrogen (to regenerate NADPH from $NADP^+$) and carbamyl phosphate (to regenerate ATP and provide the CO_2). Starch, $MgNH_4PO_4 \cdot 6H_2O$ magnesium ammonium phosphate and CO_2 would be taken out. These substances are easy to separate since starch and $MgNH_4PO_4 \cdot 6H_2O$ are solids, while carbon dioxide is gaseous. A summary of the reactions is given in Table 4.3.

The advantage of this setup is that it is mechanically simple and avoids many difficult separations. It obviously has the precedent of the cell itself. However, the question of localization of cyclic enzymes is still open. There is as yet no definite evidence that the enzymes in the chloroplast are organized [31,32], but the high concentrations of cyclic enzymes in the stroma region may imply that their movement is restricted and that there is some structure.

There are a number of disadvantages to a single reactor. The multiplicity of intermediates leads to the possibility of lower rates due to more inhibitors (though there may be more activators also).

Table 4.3

NET REACTIONS--PRODUCTION OF STARCH FROM $[CO_2 + H_2]$

$18 \ ATP^{-4} + 6CO_2 + 12 \ NADPH + 11 \ H_2O \rightarrow C_6H_{10}O_5 \ (starch)$

$$+ \ 18 \ ADP^{-3} + 18 \ HPO_4^{=} + 6 \ H^{+} + 12 \ NADP^{+}$$

$12 \ NADP^{+} + 12 \ H_2 \rightarrow 12 \ NADPH + 12 \ H^{+}$

$$18 \ NH_2\overset{O \ O}{\overset{\| \ \|}{COPO}}{}^{-}_{\underset{O-}{}} + 18 \ ADP^{-3} \rightarrow 18 \ NH_2\overset{O}{\overset{\|}{CO}}{}^{-} + 18 \ ATP^{-4}$$

$$18 \ NH_2\overset{O}{\overset{\|}{CO}}{}^{-} + 18 \ H_2O \rightarrow 18 \ NH_3 + 18 \ HCO_3^{-}$$

$18 \ NH_3 + 18 \ H^{+} \rightarrow 18 \ NH_4^{+}$

$18 \ HPO_4^{=} \rightarrow 18 \ PO_4^{\equiv} + 18 \ H^{+}$

$18 \ Mg^{++} + 18 \ NH_4^{+} + 18 \ PO_4^{=} + 108 \ H_2O \rightarrow 18 \ MgNH_4PO_4 \cdot 6 \ H_2O$

$18 \ HCO_3^{-} + 18 \ H^{+} \rightarrow 18 \ CO_2 + 18 \ H_2O$

$$Total: \quad 18 \ NH_2\overset{O \ O}{\overset{\| \ \|}{COPO}}{}^{-}_{\underset{O-}{}} + 12 \ H_2 + 119 \ H_2O + 18 \ Mg^{++} \rightarrow$$

$$12 \ CO_2 + C_6H_{10}O_5 + 18 \ MgNH_4PO_4 \cdot 6 \ H_2O$$
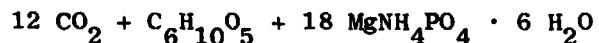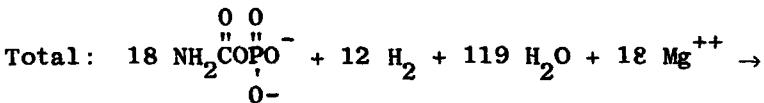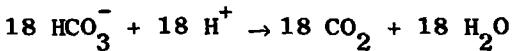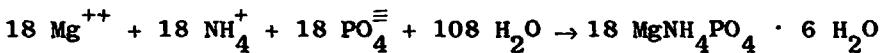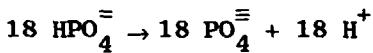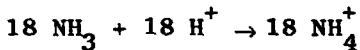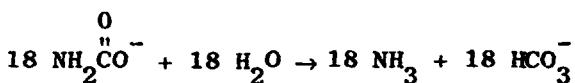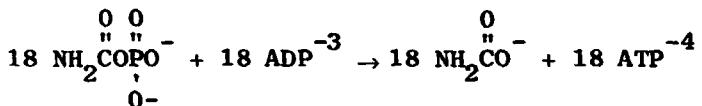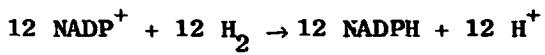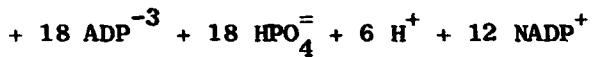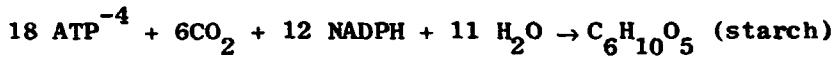
It would also be extremely difficult to design without a major experimental program, since very little is known at present of the reaction kinetics. There is also a problem in controlling each of the reactions as their individual enzymes decay with time. Since the many enzymes involved undoubtedly decay at different rates, in order to keep all at an adequate activity level, those that decay quickly will have to be separated from those that decay more slowly. Additionally, the question of nonspecificity (the case where one enzyme catalyzes more than one reaction) is still unresolved. There are two enzymes in the process,

transketolase and aldolase, which catalyze formation of extraneous products from intermediates present in the reactor that are part of the process. These products, if accumulated, would serve to drain intermediates from the cycle. They do not accumulate in a normally functioning cell [31,32] and it is unknown how much they would accumulate in the proposed reactor.

It is as yet difficult to carry out the reactions of the reductive pentose phosphate cycle by using enzymes obtained from broken chloroplasts. The cause of this seems to be in the regenerative part of the cycle, but the precise location of the sensitive step is yet to be discovered [32]. This implies the same difficulty when the enzymes of this cycle plus those that lead to starch are placed in a single reactor. It is quite obvious that further work remains to be accomplished in this area.

The single reactor scheme could employ stabilized but soluble enzymes homogeneously scattered throughout the reactor. If the enzymes in the chloroplast had some organization, while those in the synthetic reactor did not, it would be probable that the synthetic reactor would have slower rates, exclusive of changes caused by differences in enzyme concentration.

Design of the carbon dioxide fixation process has been severely hampered by the large gaps in knowledge of the mechanism and kinetics of the enzymes, either soluble or immobilized. Though many of the enzymes have been purified to a high degree, little is known about their inhibitors. Therefore, it is extremely difficult to formulate correct rate equations for their action on substrates.

In addition, very little experimental work has been performed on the functioning of this process all in one vessel.

Until further work is undertaken on the mechanisms and kinetics of these enzymes, it will be difficult to design a CO_2 fixation process plant with any degree of confidence.

4.3.3.2 Grouped Reactions

On the other hand, a system of multiple reactors, each reactor containing a series of appropriately coupled reactions, is appealing for several reasons: (1) inhibition problems would be minimal, (2) the desired carbohydrate products such as fructose, glucose and starch could be separated conveniently, (3) nonspecificity of the enzymes leading to undesired side-products would be prevented, (4) separation of ADP, $NADP^+$, and P_i would be simplified, (5) enzyme immobilization and replenishing would be simplified, (6) regulation and control of the operating factory would be simplified, and (7) trouble-shooting would be simplified.

The basis of this process is to group reactions in such a way that in a particular reactor, conversion of reactants to products is nearly complete. A standard free energy change of about -3 kcal/mole for an enzyme-promoted reaction will generally assure 98 to 99 percent conversion to product in an acceptable time span. Most reactions in the CO_2 fixation pathway have acceptable standard free energies (ΔG°);   those

that do not can usually be coupled with an energetically very favorable reaction to yield the desired results. In particular, the last reaction in the total sequence within the reactor must have a high negative ΔG° (see Appendix 4.2).

However, reaction 2 is unique in two aspects:

(1) the ΔG° is highly unfavorable

3-phosphoglyceric acid + ATP $\xrightarrow[\text{Rx. 2}]{}$ phosphoryl-3-phosphoglyceric acid + ADP
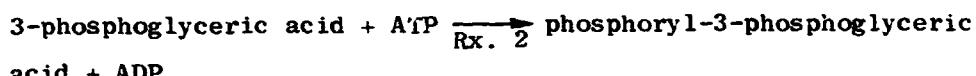
(2) the product of the reaction, P-3-PGA, is chemically very reactive.

As shown in Appendix 4.3, it is this combination of an unfavorable free energy coupled with a chemically unstable product, which makes any multiple-reactor design unworkable or overly cumbersome.

The scheme shown in Table 4.4 groups reactions in such a way that the energy requirements are fulfilled. It includes an associated reaction (19) to regenerate ATP. $\Delta G^{\circ}_{net} \leq -3$ kcal, and ΔG° of the last reaction of any sequence is < -3 kcal. Since reaction 1 is isolated, a known set of inhibitions is avoided since the enzyme is known to be inhibited by almost all the reactants in the total process. Undesirable aldolase catalyzed side reactions are also avoided.

The drawback in this scheme is that the large number of reactions in a single reactor, and the inclusion of reactions 2, 3, and 19 in three of the reactors lead to total potential inhibitions comparable to the single reactor scheme described in the preceding section.

4.3.4 Enzyme Preparation

From the literature survey, it is clear that much more work is needed on the large scale preparative method of purification and immobilization of enzymes that are involved in the production of carbohydrates. Highly purified preparations of some enzymes (i.e., phosphoglycerate kinase, ribulose phosphate epimerase, phosphoribulokinase) have not been made. Although some other enzymes (i.e., glyceraldehyde-3-phosphate dehydrogenase, transketolase, triose phosphate isomerase, aldolase, fructose diphosphatase) have been well characterized, they have been studied mostly as degradative enzymes participating in the glycolytic pathway but not as enzymes of the photosynthetic pathway. Most enzymes of the CO_2 fixation pathway have been purified from yeast or mammalian tissues rather than from green plants, and in many cases there is little or no data for reactions or assays performed in the direction of the photosynthetic reductive pentose phosphate cycle. The use of plant enzymes has the following advantages:

(1) Plants would contain all the enzymes needed for the photosynthetic pathway, including ribulose diphosphate carboxylase and phosphoribulokinase which are unique to the reductive pentose phosphate cycle.

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Table 4.4

A MULTIPLE REACTOR SCHEME FOR CO_2 FIXATION

Reactor Number	Reactions Carried Out In the Reactor*	Net Standard Free Energy Changes (kcal)
1. One Reaction	No. 1	-8.4
2. Five Reactions	2 (No. 19)	-6.0
	2 (No. 2)	+9.6
	2 (No. 3)	-0.6
	No. 4	-1.8
	No. 5	-5.4
	Total Energy Changes =	-4.2
3. Seven Reactions	No. 6	-3.4
	4 (No. 19)	-12.0
	4 (No. 2)	+19.2
	4 (No. 3)	-1.2
	2 (No. 4)	-3.6
	No. 7	+1.5
	No. 8	-5.6
	Total Energy Changes =	-5.1
4. Eight Reactions	No. 9	-3.4
	2 (No. 19)	-6.0
	No. 2	+4.8
	No. 3	-0.3
	No. 10	+0.1
	No. 11	+0.2
	No. 12	+0.5
	No. 13	-5.2
	Total Energy Changes =	-9.3

(2) We have no information on the in vitro compatibility of the
various enzymes needed, but at least the compatibility of
these enzymes in their natural environment (i.e., chloroplasts)
is known. This consideration is important for the design of a
process in which two or more reactions are performed in a com-
mon reactor.

(3) Spinach for example is ubiquitous, can be readily processed,
is relatively free of endogenous inhibitions, and its photo-
synthetic activity is high.

On the other hand, even at the in vivo value of 200 nanomoles
CO_2 fixed per mg protein per hour, over 700 tons of spinach would be re-
quired to obtain start-up quantities of enzymes for a 100 ton per day
plant. If laboratory scale preparations are used as a basis, this amount
goes up. For instance to obtain sufficient aldolase for the production
of 100 tons glucose per day, 84,000 tons would be needed.

The enzymes required for the proposed process are shown,
along with references to their preparation, in Table 4.5. Those which
have been immobilized show a reference for this also; where no reference
appears, the enzyme has not been studied in this way.

General methods for purification and immobilization of enzymes
is given in Appendix 4.6.

4.3.5 Enzyme Kinetics

Although this process was not carried out to a firm design,
its further study is still attractive for special applications. For
that reason, a list of properties for the enzymes employed in the carbon
dioxide fixation process may be found in the Appendix. Values are for
spinach unless otherwise indicated and are, unless noted, for pH's be-
tween 7 and 8.

Since in very few cases have complete mechanisms for enzyme
action been formulated, extremely simple rate equations have been pre-
sented. If used for modeling of the system, these equations will g.ve
maximum rates and therefore minimum reactor sizes, since inhibitions both
by products and by other substances have been left out. In addition, the
equations reflect the assumption that access of the substrate to the en-
zyme is random, again a situation leading to maximum rates.

Besides enzyme kinetics, rate and equilibrium data for chem-
ical reactions that occur in this process, as well as some solubility
data, are included in Appendix 4.3.

4.4 Comparison of Three Processes to Glucose

Of the various processes considered, only three were selected for
further consideration. Some were discarded because needed data were
lacking. For example, both the reverse oxidative pentose cycle and the

Table 4.5

ENZYMES CATALYZING $CO_2 \rightarrow$ STARCH

	Enzymes	E.C. Number	Reference Preparation	Reference Immobilization
1.	Ribulose Diphosphate Carboxylase	4.1.1.39	Wishnick & Lane [33]	Shapira, J. [46]
2.	Phosphoglycerate Kinase	2.7.2.3	Rao & Oesper [34]	Marshall, D. [47]
3.	Triose Phosphate Dehydrogenase	1.2.1.12	Rosenberg & Arnon [35]	Marshall, D. [47]
4.	Triose Phosphate Isomerase	5.3.1.1	Anderson [36]	Marshall, D. [47]
5.	Aldolase	4.1.2.13	Brooks & Criddle [37]	
6.	Fructose Diphosphatase	3.1.3.11	Preiss & Greenberg [38]	Marshall, D. [47]
7.	Transketolase	2.2.1.1	Horecker & Smyrniotis [39]	
8.	Pentose Phosphate Isomerase	5.3.1.6	Rutner [40]	
9.	Phosphoketopentose Epimerase	5.1.3.1	Williamson & Wood [41]	
10.	Phosphoribulokinase	2.7.1.19	Hurwitz, et al [42]	
11.	Glucose Phosphate Isomerase	5.3.1.9	Nakagawa & Noltmann [43]	
12.	Phosphoglucomutase	2.7.5.1	Joshi & Handler [44]	Marshall, D. [47]
13.	Glucan Phosphorylase	2.4.1.1	Lee [45]	

formaldehyde p·thway have one step which is not proven. Consequently, we could not .n our best judgment recommend designing a plant based on uncertain chemistry. Others were rejected as impractical, on the basis of data already known (see Section 4.6).

Of the three pathways we did study, the following comparisons can be made:

	Cellulose	Fossil Fuel	CO_2 Fixation
ATP req. per glucose formed	0	2	18
NADPH req. per glucose formed	0	0	12
Reactor size	smallest	moderate	largest
Number of steps	fewest (2)	moderate (5)	largest (19)
Cost of raw material	lowest	highest (glyc.)	low
Number of enzymes	smallest (1)	moderate (5)	largest (16)
Complexity of reaction	simplest	moderate	most complex
Energy requirement	lowest	moderate	highest

It is quite clear that the cellulose process has a distinct advantage on all counts, although it is not a strictly nonagricultural process (cellulose is from plants). The cost of ATP and NADPH regeneration and replenishment, enzymes, and the many steps and large reactors in the CO_2 fixation process make the fossil fuel process more feasible than the CO_2 process in spite of the higher raw material costs.

4.5   Starch Synthesis from Glucose

4.5.1   Introduction

Several alternate routes were available for the conversion of glucose to starch. A schematic which shows the available choices is shown in Figure 4.7.

There are two possible routes from glucose to G-1-P and two from G-1-P to starch. A selection on the basis of a variety of factors had to be made in each case. Criteria and factors leading to the decision for the pathway below are given in Appendix 4.4.

4.5.2   Characteristics of the Selected Scheme

As now proposed, the route from glucose to starch would involve a single reactor which converts glucose to glucose-6-phosphate, followed by a second reactor containing the two enzymes for the isomerization of G-6-P to G-1-P and the conversion of G-1-P to starch as shown in Figure 4.8.
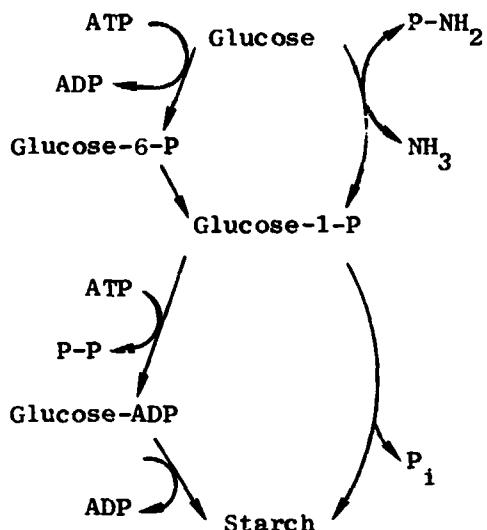
Figure 4.7. POSSIBLE ROUTES--
GLUCOSE → STARCH.
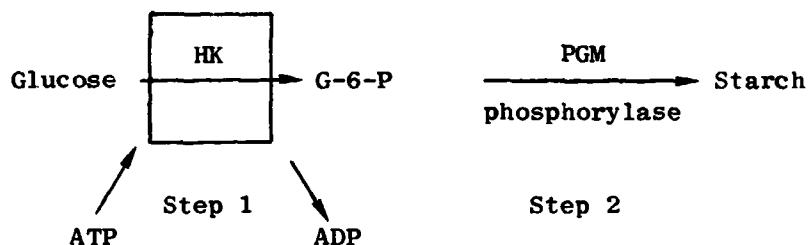
Figure 4.8. CONFIGURATION FOR GLUCOSE → STARCH.

The first reactor, containing hexokinase (HK), is fed equimolar amounts of ATP and glucose. It is important that glucose be freed of sizeable fructose contamination before entering the reactor, since hexokinase phosphorylates fructose 1.8 times as rapidly as glucose, and the resultant fructose-6-phosphate would need to be separated and recycled. The equilibrium constant for the phosphorylation is 386 [48]. If the components are given adequate time to react, conversion to products should be virtually complete. The emerging G-6-P can be separated from the ADP by ion exchange.

There is another enzyme, glucokinase which does not utilize fructose and if glucose-fructose separation were a serious problem, glucokinase could be substituted for hexokinase. The reason it is not used anyway is that it is less readily obtained. It is available from Aerobacter arogenes at levels which would require 605 tons wet weight of

starting material [49], whereas hexokinase is available from bakers yeast, requiring 27 tons of cakes.

In the second reactor, phosphoglucomutase and phosphorylase are combined to carry out the two steps $G-6-P \rightarrow G-1-P \rightarrow$ starch. These reactions are coupled because the equilibrium constant (K_{eq}) of the $G-6-P \rightarrow G-1-P$ isomerization is very low (0.05) and recycling is difficult because G-1-P and G-6-P are hard to separate (compared to separating starch and P_i from the G-1-P + G-6-P mix).

This coupling does cause some sacrifice, however, due to the discrepancy in pH optima for the two enzymes and the dependence of K_{eq} for the polymerization on pH. At pH 5.0, the K_{eq} is 10.8, but at pH 7.3, this is lowered to about 2. (This is due to the fact that the ionization constant of the liberated phosphate is different from that of G-1-P [50].)

Clearly it would be advantageous to run the reaction at as low a pH as possible. However, the pH optimum is 8.5 to 9.0 <u>E. coli</u> phosphoglucomutase and is well defined in the region between pH 7 and 10. At pH 6, virtually all activity is gone [51]. However, the yeast enzyme seems to have an optimum at pH 7.5 based on crude preparations. The phosphorylase from <u>E. coli</u> has an optimum of pH 7.2, and the activity is only about 1/3 optimal at pH 6. Potato phosphorylase has an optimum of pH 6.5.

This combination of factors necessitates running the reactor at a compromise pH of 6.9. At this pH, the K_{eq} for the phosphorylase reaction is 3 [52]. Thus the overall K_{eq} for the reactor is 0.15--necessitating the recycling of the products. In this recycling, the starch will be precipitated out, the phosphate removed, and the G-1-P + G-6-P mixture sent back to the reactor. Both enzymes in the reactor can tolerate a 45° running temperature, so that the precipitation of starch in the reactor can be eliminated.

4.5.3   Enzyme Production

The three enzymes required for this process are available from microbial sources in reasonable yields. Hexokinase would be prepared from yeast, and PGM and phosphorylase from E. coli. (Although phosphorylase is often prepared from potatoes, a larger quantity would be required, and the idea of using 500 tons of potatoes to obtain an enzyme to make starch seemed unappealing.)

The method for calculating quantities of starting materials required is given in Section 4.2.4.

Particulars on the enzymes in this process are summarized in Table 4.6 and details are given in Appendix 4.5.

4.5.4   An additional Alternative

In the cellulose process, glucose is a mandatory product and the appropriate process would be the one outlined above. Another alternative is available, however, in both the fossil fuel and CO_2 fixation

Table 4.6

ENZYMES--GLUCOSE TO STARCH

Enzyme	Inhibitors	Status	Starting Material Required	# pure Enzyme Needed	Specific Activity [a]	Turnover Number [b]	K_m Values	pH Opt.
Hexokinase 2.7.1.1 ①	ADP $K_1 = 1.2 \times 10^{-3}$ G6P $K_1 = 9.1 \times 10^{-3}$	Crystalline from yeast and muscle	27 tons yeast cakes	450	750	75,000	Glucose = 1.5×10^{-4} ATP = 1×10^{-4}	8.5
Phosphoglucomutase (PGM) 2.7.5.1 ②	G-1-P $K_1 = 1 \times 10^{-3}$ G-1,6-diP $K_1 = 7 \times 10^{-4}$	Crystalline from E.coli and muscle	55 tons wet E. coli	80,000	5.9 (pH 7.5)	370 (pH 7.5)	G-6-P = 3.5×10^{-4} G-1-P = 6×10^{-5}	8.8
Fructose-1,6-di Diphosphatase 3.1.3.11 ③	AMP substrate	Crystalline from liver and yeast	3800 tons yeast	4,200	83	8,300	FDP = 1×10^{-5}	9.5
Glucose Phosphate Isomerase 5.3.1.9 ④	P_i $K_1 = 1.7 \times 10^{-3}$	Crystalline from muscle and yeast	1.3 tons yeast [c]	520	675	98,500	G-6-P = 3×10^{-5} [d] F6P = 1×10^{-5}	8
Phosphorylase 2.4.1.1 E. coli ⑤		Homogeneous	36 tons	52,000	6.8 [e]	910 [e]	G-1P = ?	7.2
Potato ⑥		Crystalline	11,000	11,000	32	6400	G1P = 3.5×10^{-3}	6.3

① Lazarus: Biochem. 5, 4003 (1966)
② Joshi: JBC 239, 2741 (1964)
③ Trianello: ABB 146, 603 (1971)
④ Batch: JBC 235, 3130 (1960)
⑤ Lee: Meth. Enz. VIII, p. 550 (1966)
⑥ Schwartz: Eur. J. Biochem. 2, 133 (1967)

[a] μmoles/min/mg
[b] moles/min/mole
[c] estimated for 5% wet wt = protein, 100% yield
[d] values are for the muscle enzyme
[e] calculated from values for reverse reaction for r×n

proc ass; in fact, the preliminary design for the CO_2 fixation process actually adopts it. In this alternative, fructose-1,6-diphosphate would be reacted with fructose-1,5-diphosphatase to give F-6-P, which could then be isomerized to G-6-P with glucose phosphate isomerase. From there on, the process is identical with that given above (see Figure 4.9). The characteristics of the enzymes are included in Table 4.6.
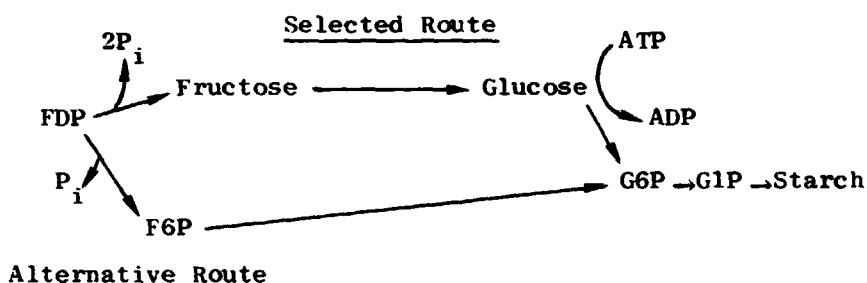
Figure 4.9. ALTERNATIVE TO THE SELECTED ROUTE TO STARCH.

The superiority of the selected route over the alternative for the fossil fuel process is not clear. Fructose-1,6-diphosphatase is severely substrate inhibited, which, if not circumvented, is a serious limitation. However, the use of one ATP per molecule of glucose is avoided. Further investigations of this alternate path were precluded by time limits rather than by theoretical ones.

4.6 Adenosine Triphosphate (ATP) Regeneration

4.6.1 Introduction

From both ecological and economic standpoints, it is most desirable that as many as possible of the reagents and intermediate compounds be regenerated and recycled. This is especially true for ATP which provides the high energy phosphate bond and costs $6/gm at the present time.[*] Without its reutilization the cost of producing 100 tons of glucose/day would be truly astronomical.

In the fossil-fuel process which requires two ATP molecules for each glucose molecule produced,[**] the regeneration of 564 tons of

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[*] Another compound, NADPH, which acts as a reducing agent, costs $250/gm. It is needed only in the CO_2 fixation process. Hence it is discussed in Section 4.3.

[**] Other process requirements for ATP: cellulose process to glucose, none; CO_2 fixation to glucose, 18; glucose to starch, 1 (all in ATP molecules per glucose molecule produced or consumed).

98

ATP per day would be required for the 100 ton/day plant. In dollar value
this would mean $3 billion down the drain if the ADP were let go as waste.
This also explains why ATP figured so prominently in the comparison of
the various processes.

There are two main pathways for the regeneration of ATP: the
cyanate (Battelle) process [53] and the acetate (Berkeley) process [54].
Because of

(a)   its relative simplicity,

(b)   the lower temperature required,

(c)   its enzyme not being as easily inhibitable, and

(d)   the potential for further innovation (see [c] below),

the cyanate process is chosen.

At the heart of this process is the enzymatic reaction be-
tween carbamyl phosphate, which provides the high-energy phosphate bond,
and ADP. Since the carbamyl phosphate is obtained by reacting potassium
cyanate with potassium dihydrogen phosphate, "cyanate" is designated the
identification for this process.

4.6.2   Chemical Schematic and Reaction Details

Figure 4.10 is a schematic diagram for all of the chemical
reaction involved, with the mass balance shown in Figure 4.11.
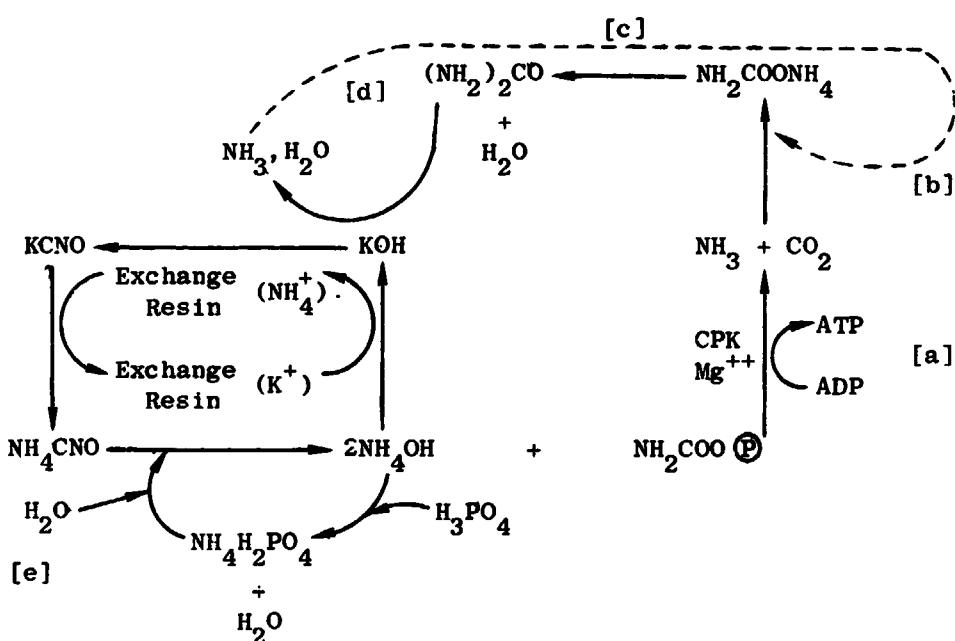
Figure 4.10.   ATP REGENERATION--CYANATE PROCESS.

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Basis: 100 tons glucose/day
2 ATP/glucose—fossil fuel process

[e] mol.wt.
H_3PO_4 + KOH ⟶ KH_2PO_4 + H_2O
(98)151.4   (56)86.5   (136)210   (18)27.8
lb/min

KH_2PO_4 + KCNO + $2H_2O$ ⟶ NH_2COO℗ + 2KOH
(136)210   (81)125   (18)55.6   (141)217.8   (56)173

[a] NH_2COO℗ + ADP ⟶ ATP + NH_3 + CO_2
(141)217.8   (427)660   (507)783   (17)26.3   (44)68

[b] $2NH_3$ + CO_2 ⟶ NH_2COONH_4
(17)52.5   (44)68   (78)120.4

[c] NH_2COONH_4 ⟶ $(NH_2)_2CO$ + H_2O
(78)120.4   (60)92.6   (18)27.8

[d] $(NH_2)_2CO$ + KOH ⟶ KCNO + NH_3 + H_2O
(60)92.6   (56)86.5   (81)125   (17)26.3   (18)27.8

Net Reaction: ADP + H_3PO_4 ⟶ ATP + H_2O
(427)660   (98)151.4   (507)783   (18)27.8

564 tons ATP/day!

Figure 4.11. MASS BALANCE FOR ATP REGENERATION.

Although the overall reaction (ADP + P_i →ATP + H_2O) seems
rather simple with both ADP and P_i being produced from the main process,
the various steps represent a combination of inorganic, organic, and
enzymatic reactions some of which are fairly complex. For example the
production of urea is a separate chemical plant by itself. These steps
will be discussed in some detail below with the subsections [a, b, c, ...]
being keyed to the reactions shown in Figures 4.10 and 4.11.

100

[a] $NH_2COO\text{-}P + ADP \rightarrow NH_3 + CO_2 + ATP$

This is the main reaction in the cycle $\llcorner$ which a "high energy" phosphate bond is attached to ADP to form ATP. The enzyme for this reaction is carbamate kinase (ATP: carbamate phosphotransferase; or carbamyl phosphokinase. While it can be prepared from S. faecalis, with a mol.wt. of 46,000 or from E. coli, with a mol.wt. of 70,000, the former is reported to be a much richer source [55].

The reaction needs magnesium (Mg^{+2}) as an activator. A temperature of 40°C and pH of 6.0 is recommended. More detailed kinetics and enzyme preparation will be given. The product accompanying ATP is carbamate which readily decomposes into NH_3 and CO_2.

[b] $2NH_3 + CO_2 \rightarrow NH_2COONH_4$
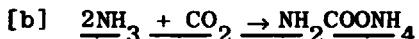
This is the first step toward urea formation prior to carbamyl phosphate regeneration. An additional NH_3 molecule supplied as a by-product of cyanate formation (reaction [d]) is required to supplement eacn pair of $NH_3 + CO_2$ evolved from reaction [a]. The reaction is highly exothermic with $\Delta H° = 38.00$ kcal/g-mole* and $\Delta G° = -7.26$ kcal/g-mole* [56, 57] and is enhanced by high pressure. In fact, the following ammonium carbamate vapor pressure must be exceeded at each of the opposing temperatures so as to prevent the redissociation of the carbamate:

40 °C	0.1	atm
60	1.1	
80	3.1	
100	8.5	
120	21	
140	46	
160	98	

Ammonium carbamate is a white crystalline solid soluble in water. In aqueous solution at room temperature, it slowly reverts to ammonium carbonate which can be avoided by raising the temperature to 60°-100°C. At the latter temperature the carbamate begins to lose a water molecule to form urea.

[c] $NH_2COONH_4 \rightarrow H_2O + (NH_2)_2CO$
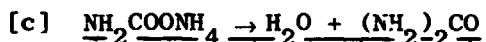
Normally in urea synthesis reactions [b] and [c] are combined together. We separate them here because of the possible short-cut from ammonium carbamate to carbamyl phosphate, as we will discuss in Section 4.6. This decomposition or dehydration does not assume an appreciable rate until the temperature reaches 150°C, half of the ammonium carbamate is converted to urea in about 30 minutes with maximum area conversion (53%).

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*Of carbamate formed.

The $\Delta H° = 7.50$ kcal/g-mole (endothermic) and $\Delta G° = -5.70$ kcal/g-mole of urea formed for reaction [c]. From the reaction equations, it is obvious that the entire sequence is favored by using excess ammonia in a dry environment.

The reactor is usually run at approximately 190°C and 220 atm. The commercial process ranges from a simple once through reaction to a total-recycle scheme, as depicted in Figures 4.12 and 4.13. Depending on our needs, they can be adapted readily to our overall process.

[d] $\underline{(NH_2)_2CO + KOH \rightarrow KCNO + NH_3 + H_2O}$

In this patented process [58] KOH and urea are reacted at 250° ~ 600°C: in a fluidized column of silica. The cyanate is formed on the surface of silica which leaves the column with H_2O and NH_3. The cyanate-laden silica is separated by a cyclone separator after which it is fed to a ball mill in which powderous KCNO is loosened from the silica. The two are then separated by sieving. The yield is claimed to be 90 percent complete with a purity of 98 percent.

[e] $\underline{KCNO + KH_2PO_4 \rightarrow NH_2COO\textcircled{P} + 2KOH}$
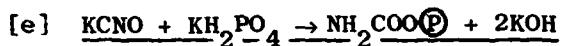
This reaction occurs spontaneously at room temperature and pH 5.5 with 40 percent conversion [53]. Two sets of thermodynamic constants have been found:

Soejima et al [59] (pH unknown)	Jones & Lipmann [60] (pH 6.0)
K_{eq}* (apparent) = 1.38 ± 0.1	K_{eq}* (avg.) = 3.1
$\Delta G° = -210$ cal/mole	$\Delta G° = -680$ cal/mole
$\Delta H° = 5000$ cal/mole	
$\Delta S° = 16$ cal/mole - °C	

No kinetic constants have been found although the reaction has been indicated to be the rate controlling step in the entire sequence [53]. However, Jones and Lipmann [60] reported that at the optimum between pH 5 and 6, equilibrium is reached "fairly rapidly."

The carbamyl phosphate thus formed decomposes spontaneously in aqueous solution at or above pH 7.0 into cyanate and inorganic phosphate [59,60,61]. To avoid this decomposition, the laboratory-type arrangement as shown in Figure 4.14 has been suggested [53]. In this scheme the carbamyl phosphate formed is given the immediate opportunity to react with

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*
Defined as K_{eq} = [carbamyl phosphate]/[cyanate][phosphate].
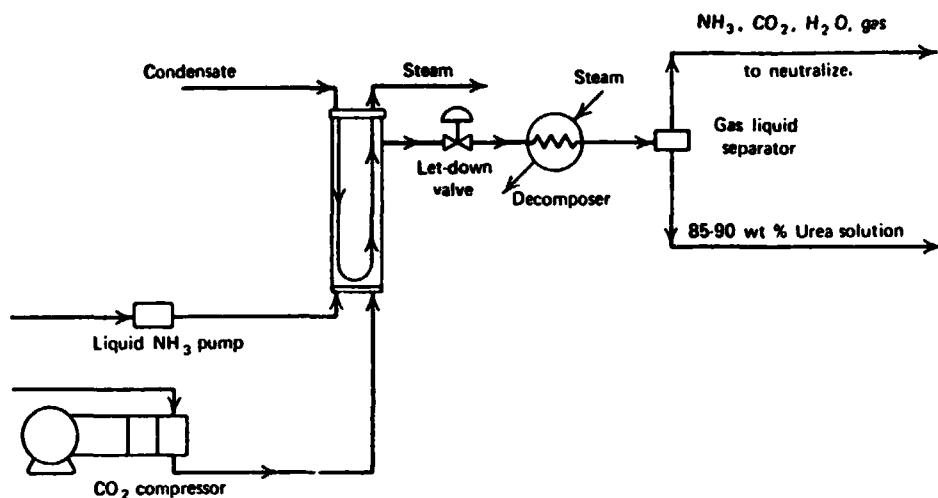
Fig. 4.12. ONCE-THROUGH UREA PROCESS.
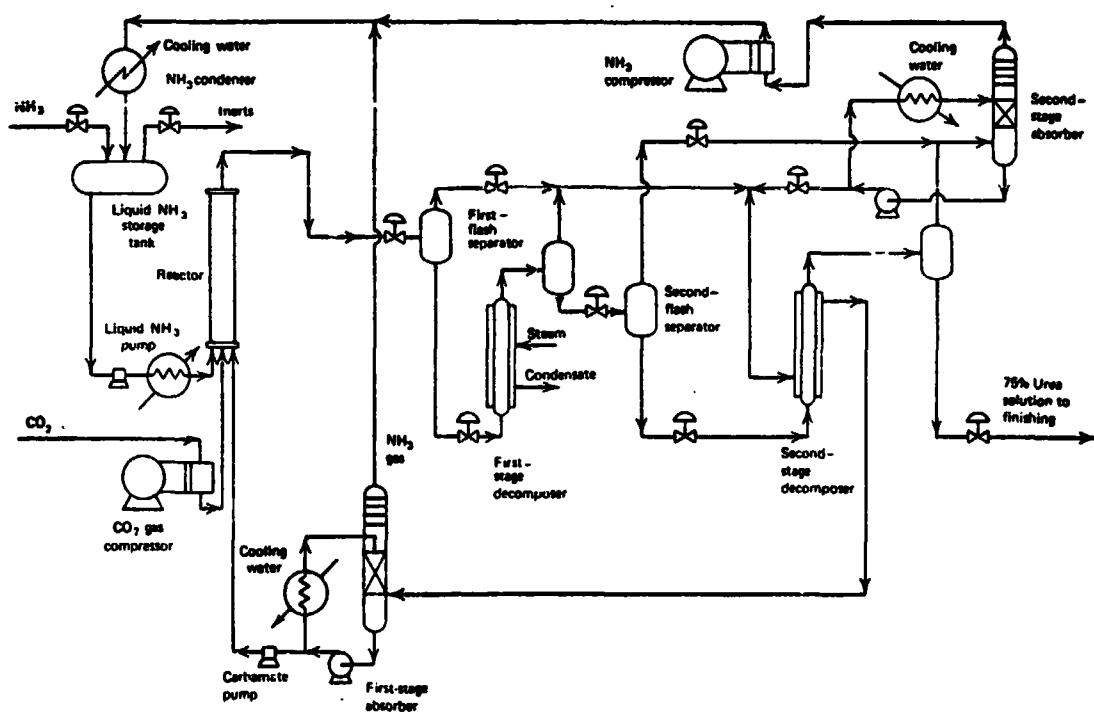
Fig. 4.13. TOTAL RECYCLE UREA PROCESS.

KH$_2$PO$_4$
+
ADP   KCNO

E ──►ATP, etc.
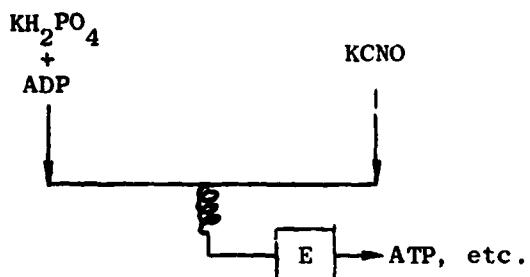
Figure 4.14. ATP REGENERATION--
LABORATORY SCHEME [53].

ADP enzymatically, thus allowing little chance for it to decompose non-
enzymatically. Our flow diagram proposed later reflects this scheme.

In actual operation, however, a sulfonic acid-type ion exchange
resin is used to replace the potassium by ammonium so that the intermedi-
ate byproduct in reaction [e] is NH$_4$OH instead of KOH. The ammonia can
be much more easily separated and after separation, half of it is used
to regenerate the resin, completing the ion exchange cycle. The other
half is used to react with phosphoric acid to provide the phosphate needed.

In the CO$_2$ fixation process, it is desired to separate the in-
termediate product by precipitation instead of ion exchange. A magnesium
salt is used in place of the potassium salt. as will be discussed in Sec-
tion 5.3.

4.6.3 Process Schematic

The entire proposed scheme is shown in Figure 4.15 in which
the two boxes for reactions [b] and [c] are contained in a larger box
sided with broken lines. It means an entire urea plant, as represented
by Figure 4.12 or 4.13, is fitted in.

4.6.4 Enzyme Preparation and Characteristics

Carbamyl phosphate kinase has been prepared on a laboratory
scale [58]. It has been immobilized on alkylanine glass [53].

(a) Kinetics

The equilibrium constant, defined as

$$K_{eq}: = \frac{(\text{carbamyl phosphate})(\text{ADP})}{(\text{ATP})(\text{carbamate})}$$

104
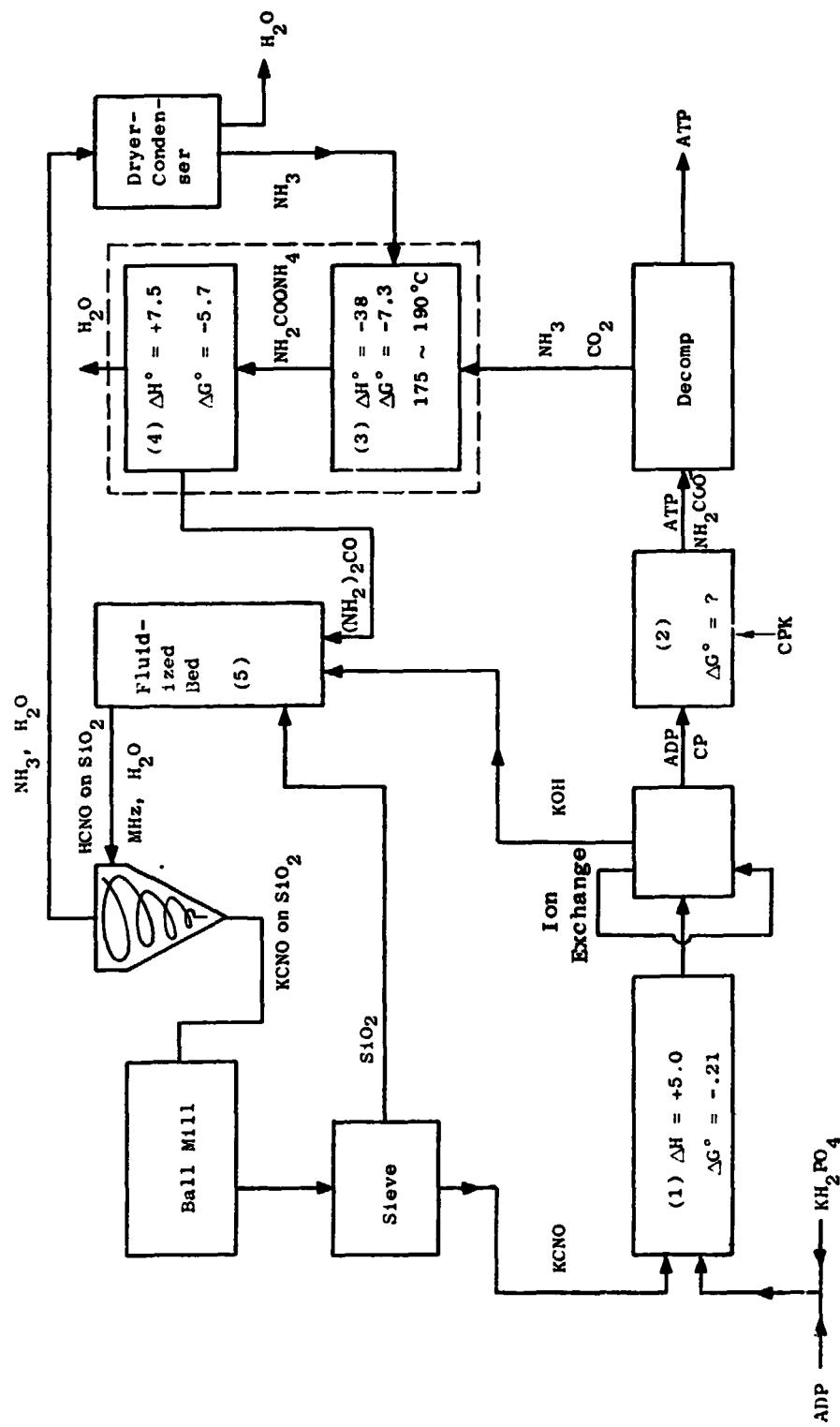
Figure 4.15. CYANATE PROCESS--SCHEMATIC FLOW SHEET.

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has been found to be 0.04 at 10°C and pH 9.5, and 0.027 at 25°C and pH 8.14 [62]. Based on simple Michaelis-Menten kinetics which yields the following rate equation:

$$V = \frac{V_{max,+1} \, E_0 \, [CP][Mg - ADP]}{(K_A + [CP])(K_B + Mg - ADP)} - \frac{V_{max,-1} \, E_0 \, [C][Mg - ATP]}{(K_C + [C])(K_D + [Mg - ATP])}$$

the following assumptions can be used

$V_{max,+1} = 7 \times 10^4$ moles/min-mole enzyme (max. forward reaction rate)

$V_{max,-1} = 1.5 \times 10^4$ moles/min-mole enzyme (max. reverse reaction rate)

$K_A = 1 \times 10^{-4}$ moles/ℓ (M-M constant for CP)

$K_B = 5 \times 10^{-5}$ moles/ℓ (M-M constant for Mg-ADP)

$K_C = 8 \times 10^{-5}$ moles/ℓ (M-M constant for C)

$K_D = 8 \times 10^{-6}$ moles/ℓ (M-M constant for Mg-ATP)

where CP = carbamyl phosphate, C = carbamate, Mg-ADP = magnesium ADP complex, Mg-ATP = magnesium ATP complex, E_0 = initial concentration of enzyme sites.

(b) pH Optimum

The reaction has a broad pH optimum between pH 4 and 9.5, while the reverse reaction (ATP + Carbamate → CP + ADP) has a narrower one between 8.5 and 10.5. For optimum rate as well as to prevent nonenzymatic decomposition of CP, a pH of 6.0 should be used.

(c) Stability

Since both ammonia and CO_2 are produced and subsequently evolved from the reaction mixture, the pH is not expected to go to either extreme. This, plus the fact that the pH optimum is very broad, indicates there should be no adverse effect from NH_3 on the reaction. In fact, no such effect has been observed or reported by any of the investigators cited. While a reaction temperature of 40°C is recommended, the inactivation time curve in Figure 4.16 should be noted.

4.6.5 Future Research

While each step of the process described seems to be well established, there is a potential shortcut for the overall cycle. That

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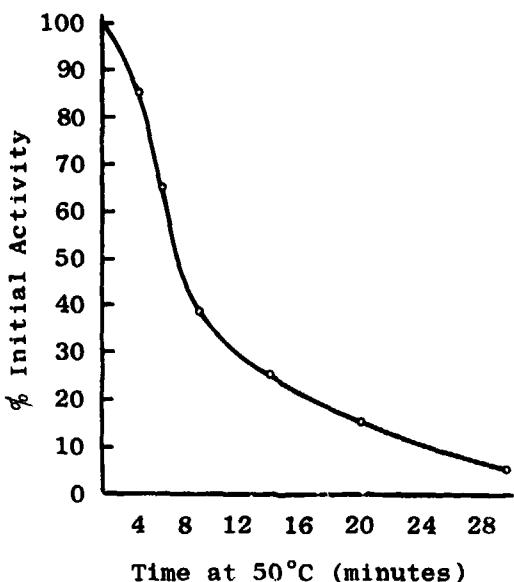
Figure 4.16. HEAT INACTIVATION OF
CARBAMYL PHOSPHOKINASE FROM S.
FAECALIS AT 50°C [63].

is, in the urea synthesis if we do not decompose or dehyirate the ammon-
ium carbamate but let it react with phosphoric acid, for instance, we
might be able to obtain carbamyl phosphate directly. In addition to
greatly reducing the urea plant, we would eliminate both the cyanation
and cyanate + phosphate steps.

However, we have not been able to find any literature refer-
ence to this reaction, even thermodynamic constants, such as ΔG°, are
hard to come by. We strongly recommend further research into this because
of the tremendous saving that would result if proven positively.

4.7 Rejected Pathways

In addition to the three processes to glucose considered in detail,
several others were given preliminary consideration. The nature of these
pathways, and the reasons they were not used as bases for design, are
given in the sections to follow.

4.7.1 Formose

Formaldehyde can be condensed using alkalis and other cata-
lysts to give mixtures of sugars. This type of process was considered
because of the simplicity of the starting material and the apparent ease
of conversion if, indeed, the right conditions could be found to give
digestible sugars.

There are, however, problems which caused this approach to be rejected.

(1) The product is a complex mixture of carbohydrate, only a small fraction of which can be metabolized. Six carbon linear sugars are not, in fact, obtained as a preponderant species under most conditions.

(2) Exclusively D-sugars could theoretically never be formed, since the reaction is inherently nonstereospecific.

4.7.2 Formaldehyde-Transketolase

This pathway was suggested by Bassham [64] and, as shown in Figure 4.17, a large part of it is identical to the CO_2 fixation pathway cycle. The overall reaction is

$$6HCHO + 6ATP + 5H_2O \rightarrow G6P + 6ADP + 5P_i$$
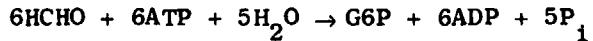
This pathway was considered because of the fewer and simpler steps and lower energy requirement (6 vs 18 ATP per G6P) than CO_2 fixation. The formaldehyde can be obtained by reduction of CO_2 or from other petroleum by-products. The carboxylation, phosphoglycerate kinase, triose phosphate dehydrogenase and phosphoribulokinase reactions are eliminated, while only transketolase and triokinase reactions are gained. There is no need for NADPH.

It was concluded that this was not the best pathway for the current project for the following reasons:

(a) The transketolase reaction

$$HCHO + Xu5P \xrightarrow{2.2.1.1} DHA + GALDP$$
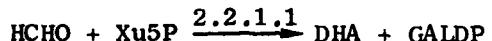
is an unproven reaction. In an extensive literature search, no report on it was found. Since this is the main step in the entire proposed scheme, this pathway was abandoned with the recommendation that it be studied in the future. In fact, based on the data provided by Bassham and Krause [65], the $\Delta G°$ for this reaction has been calculated to -5.6 kcal/g-mole, a favorable one.

(b) The CO_2 reduction tends to go all the way to methanol which has to be oxidized back to formaldehyde. The energetic economics of doing this are questionable. However, this has become only a minor reason as other hydrocarbon sources were later considered.
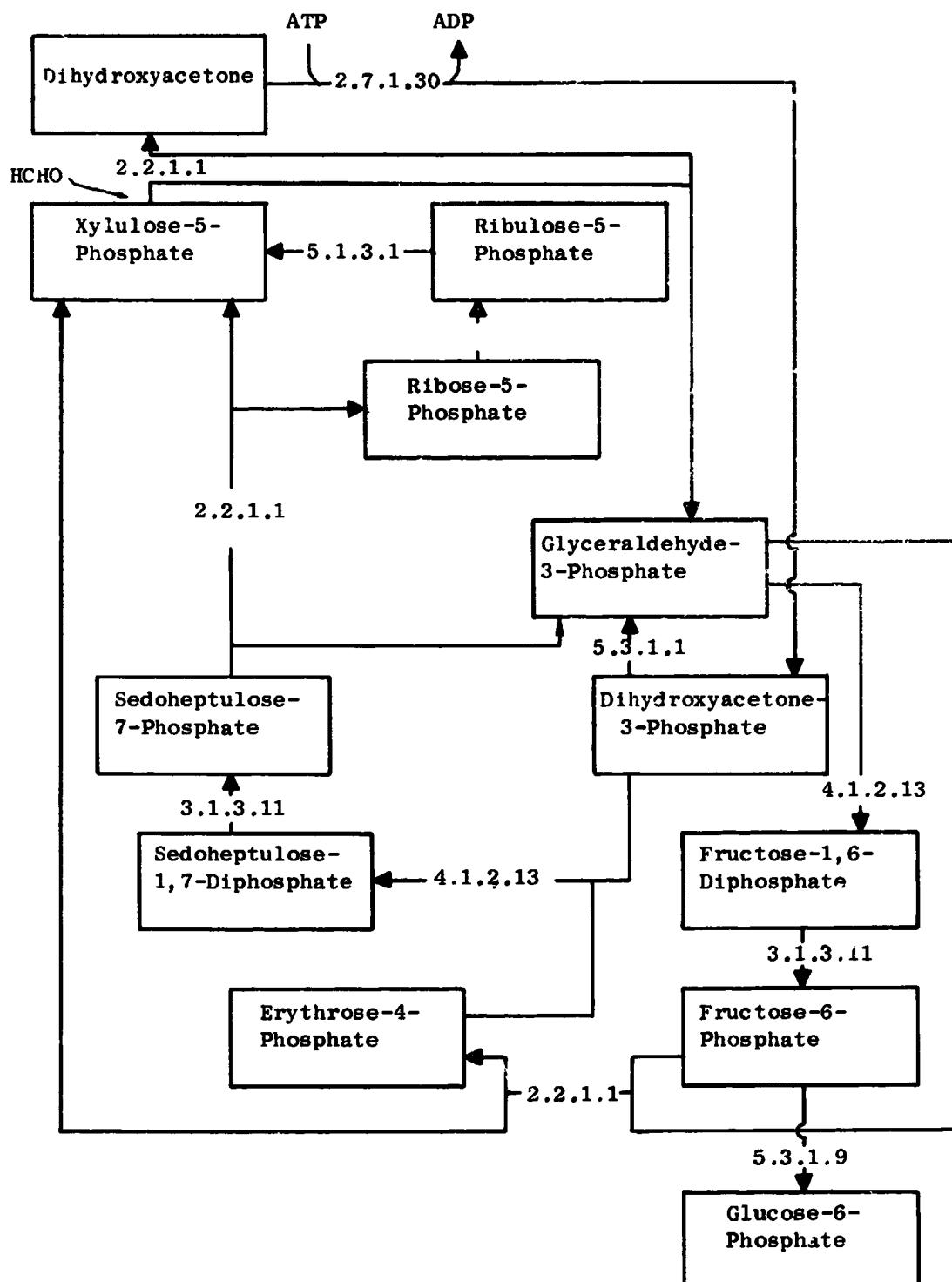
Figure 4.17.   THE FORMALDEHYDE-TRANSKETOLASE PROCESS FOR GLUCOSE SYNTHESIS.

(c) According to Bassham [64], formaldehyde might react with F6P,
S7P, and DHAP [66] if some of the steps in the proposed scheme
were to take place in the same reactor. This would complicate
the reaction control problem. Also, it was feared that formal-
dehyde might poison some of the enzymes [64]. However, these
problems could be at least partially alleviated if separe e
reactors were used for the different reactions as was later
considered.

4.7.3 Glycerolkinase

Since the glycerol used in the U.S. is produced about 40 per-
cent from petroleum sources, utilization of this product as a starting
material was explored. One pathway involved the steps shown in Figure
4.18. The glycerol is first phosphorylated by ATP to prepare it for
oxidation. The resulting triose phosphate would be condensed into glu-
cose by the same steps we are still proposing for the conversion of
propylene to glucose. This pathway was thought of seriously for two
reasons:

(1) The starting material is an established commercial product.
Initially, there was uncertainty as to the possibility of
tapping off the D,L-glyceraldehyde during the production of
glycerol [67].

(2) The two enzymes in the phosphorylation and oxidation steps
are well established and readily available from microbial
sources, whereas the triokinase is relatively unstudied
except in liver.

However, this route was discarded in favor of the one using
D,L-glyceraldehyde as a starting material since

(1) The path is two steps instead of one. When it appeared the
intermediate glycidaldehyde could be hydrolyzed in dilute acid
to D,L-glyceraldehyde, it seemed silly to convert this to glyc-
erol first, and then go back by two steps instead of one to
the triose phosphate level.

(2) Since there is an oxidation involved, an additional regenera-
tion is required. As a matter of fact, the enzyme catalyzed
oxidation with NAD^+ is thermodynamically unfavorable [68], and
an additional coupling enzyme, such as nitrate reductase would
have to be added to pull the reaction in the desired direction
[68]. This would, of course, generate all sorts of other prob-
lems, such as possible inhibition or toxicity due to nitrite,
etc., which we did not even explore (since we did not use the
pathway) [69].

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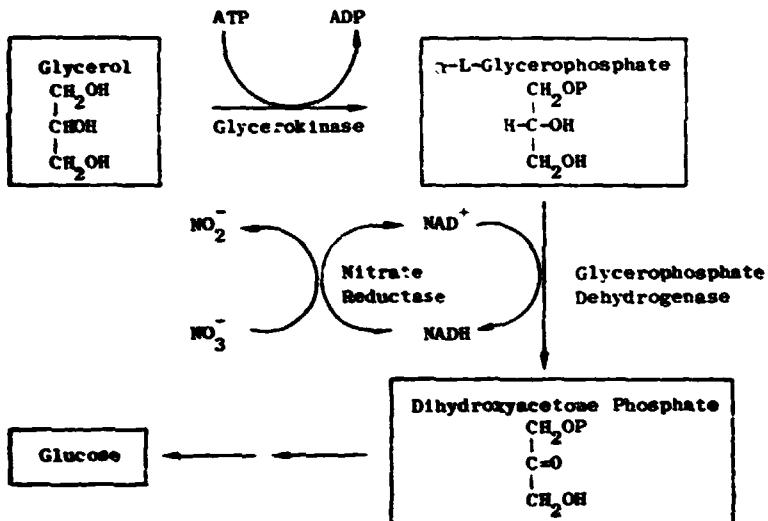
Figure 4.18. GLYCEROL KINASE PATHWAY.

4.7.4 Bacterial Fermentation to Fructose

An organism, Torulopsis versatilis, which condenses two glycerol units to mannitol has been reported [70]. In this sequence, the resulting mannitol can be oxidized by Acetobacter suboxydans to fructose. The scheme is shown in Figure 4.19.
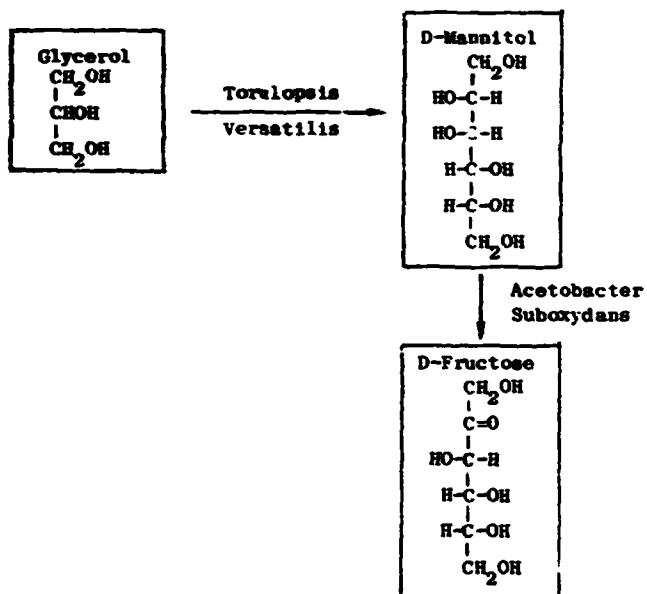
Figure 4.19. MANNITOL PATHWAY.

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This system was considered because of its obvious simplicity in leading directly to a desired product. It was discounted for two reasons:

(1) The conversion yield of glycerol to mannitol is only 50 percent. At the present cost of glycerol (22¢/lb), this would make the cost of the process high a priori.

(2) The rate of conversion under the previously studied conditions is rather low. For a 100 ton per day plant, at the listed conversion rates, a volume of about 5 million gallons would be required. Since the medium required 0.4 percent amino acids, or about 75 tons, this would constitute an unacceptable sacrifice of complex materials. Of course, it is to be assumed that the growth conditions were not optimized from the standpoint of economical use of medium constituents, but still this looked unpromising.

4.7.5 Atmospheric Carbon Dioxide

The use of atmospheric carbon dioxide as a feed for the carbon dioxide fixation pathway was briefly considered. The concept was discarded for the following reasons. First, the concentration of carbon dioxide in the atmosphere is 0.03 percent, and therefore a huge quantity of air would have to be processed to gather enough carbon dioxide for the process. If this air were directly introduced into the process, the absorber would be of inordinate size. An upgrading of its concentration, on the other nand, would be very expensive compared to its purification from a source of high concentration, such as from ammonia or cement manufacture or from a combustion process.

Since all carbon dioxide entering the carbon dioxide fixation process is incorporated into carbamyl phosphate by way of urea manufacture, it is desirable that this feed be close to 100 percent purity.

4.7.6 Reversed Oxidative Pentose Phosphate Cycle

Bassham's [64] second choice of pathways to make starch from carbon dioxide was the oxidative pentosephosphate cycle run in the reversed direction (Figure 4.20 and Table 4.7). There are fourteen steps catalyzed by twelve enzymes, transketolase mediating both reactions 8 and 10. The reductive pentose phosphate pathway was preferred for one major reason: the equilibrium of reaction 2 was so far to the left that it could not be accomplished enzymatically and would have to be conducted at high temperatures and low pH's. Not only had lactonization of 6-phosphogluconate not been experimentally demonstrated, but the isolation of 6-phosphogluconate from other phosphorylated compounds for this reaction promised to be very difficult.

We used the same reasoning to reject the reversed oxidative pentose phosphate cycle, not withstanding the fact that only one ATP, rather than 18, was required for the production of one mole of starch.

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ribose
phosphate
isomerase

GLYCERALDEHYDE-
3-PHOSPHATE

RIBOSE-5-
PHOSPHATE

CO_2

RIBULOSE-
5-PHOSPHATE

triose
phosphate
isomerase

ribulose-
phosphate
3-epimerase

XYLULOSE-
5-PHOSPHATE

DEHYDROXYACETONE-
3-PHOSPHATE

transketolase

NADPH
$+H^+$

SEDOHEPTULOSE-
7-PHOSPHATE

transaldolase

aldolase

FRUCTOSE-1,6-
DIPHOSPHATE

$NADP^+$

ADP

phospho-
fructokinase

ATP

phospho-
gluconate
dehydrogenase

ERYTHROSE-4
PHOSPHATE

transketolase

FRUCTOSE-6-
PHOSPHATE

hexose-
phosphate
isomerase

6-PHOSPHO-
GLUCONATE

Δ

acid

6-PHOSPHO-
GLUCONO-
LACTONE

glucose-6-phosphate
dehydrogenase

GLUCOSE-6-
PHOSPHATE

NADPH
$+H^+$

$NADP^+$

H_3PO_4

STARCH

phosphorylase

GLUCOSE-1-
PHOSPHATE

Figure 4.20.   REVERSED OXIDATIVE PENTOSE PHOSPHATE CYCLE AND ASSOCIATED
REACTIONS.

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Table 4.7

REVERSED OXIDATION PENTOSE PHOSPHATE CYCLE AND ASSOCIATED REACTIONS

Number	Reaction
1	$6(Ru\text{-}5\text{-}P + CO_2 + NADPH \rightleftarrows 6\text{-phosphogluconate} + NADP^+)$
2	$6(6\text{-phosphogluconate} + H^+ \rightleftarrows 6\text{-phosphogluconolactone} + H_2O)$
3	$6(6\text{-phosphogluconolactone} + NADPH + H^+ \rightleftarrows G\text{-}6\text{-}P + NADP^+)$
4	$5(G\text{-}6\text{-}P \rightleftarrows F\text{-}6\text{-}P)$
5	$F\text{-}6\text{-}P + ATP \rightleftarrows FDP + ADP + H^+$
6	$FDP \rightleftarrows DHAP + Gald3P$
7	$DHAP \rightleftarrows Gald3P$
8	$2(F\text{-}6\text{-}P + Gald3P \rightleftarrows Xu\text{-}5\text{-}P + E\text{-}4\text{-}P)$
9	$2(F\text{-}6\text{-}P + E4P \rightleftarrows S\text{-}7\text{-}P + GALD\text{-}3\text{-}P)$
10	$2(S7P + GALD\text{-}3\text{-}P \rightleftarrows Xu\text{-}5\text{-}P + R\text{-}5\text{-}P)$
11	$2(R\text{-}5\text{-}P \rightleftarrows Ru\text{-}5\text{-}P)$
12	$4(Xu\text{-}5\text{-}P \rightleftarrows Ru\text{-}5\text{-}P)$
13	$G\text{-}6\text{-}P \rightleftarrows G\text{-}1\text{-}P$
14	$G\text{-}1\text{-}P \rightleftarrows starcn + HPO_4^=$

Net Reaction: $6CO_2 + 12NADPH + 11H^+ + ATP \rightleftarrows starch + 12NADP^+ + ADP + HPO_4^= + 6H_2O$

However, since carbamyl phosphate for the regeneration of ATP is a major portion of the cost of starch produced by the reductive pentose phosphate cycle, and since its requirement drops to 1/18 of its previous value with the reversed oxidative pathway, a second look should perhaps be taken at this process if its use were desired for special applications. For this process to be successful, however, some method would have to be found to inexpensively separate 6-phosphoglucomate from the rest of the reaction mixture, and at this time no such method is readily apparent.

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Chapter 5

PROCESS AND PLANT DESIGN

This chapter contains detailed information leading to a better understanding of plant requirements (based on 100 tons/day output) for the synthetic carbohydrate processes under study. Section 5.1 contains a complete preliminary plant design for the cellulose-to-glucose process, including economic analysis. Section 5.2 is concerned with the fossil fuel process. Section 5.3 is a discussion of the CO_2 fixation process, which is carried to a lesser degree of completion because of the unknowns in the chemistry. Section 5.4 is concerned with the glucose-to-starch process. This process is considered in detail, as it is somewhat common to the other three and a necessary portion of the commercially attractive cellulose-to-starch process.

5.1 Cellulose to Glucose

5.1.1 Process Description

The flow diagram of the process for converting cellulose to glucose is shown in Figure 5.1. The mainstream indicated by the heavy line follows the processing of the cellulosic raw material (which in this design is bagasse) to the final product which is a 10 percent glucose solution. The basis used for plant design was 100 tons per day of product.

5.1.1.1 Preliminary Treatment

Raw bagasse from a sugar mill is sent via conveyor belt to a rotary knife cutter where it is reduced to 1/8" size particles to facilitate handling. The moisture content of the bagasse fed to the cutter will be kept in excess of 10 percent since there is a noticeable increase in abrasiveness at lower moisture content. Normally, the bagasse used will have a moisture content of 20 to 60 percent and a bulk density of about 6 lbs/cubic foot. The rotary knife cutter will require about 100 horsepower to operate.

The 1/8 inch bagasse will drop into a storage hopper that will allow (a) for any irregularity in flow from the storage area to the cutter, and (b) for minor repair and maintenance of the cutter. A vibratory metering screw feeder will stabilize the feed rate to the alkali reactor and the rest of the plant.

5.1.1.2 Alkali Reactor

In the alkali reactor the bagasse is diluted to a slurry of 10 percent by weight in solids and contacted with a 4 percent sodium hydroxide solution for one hour at 150°F. The reactor is equipped with
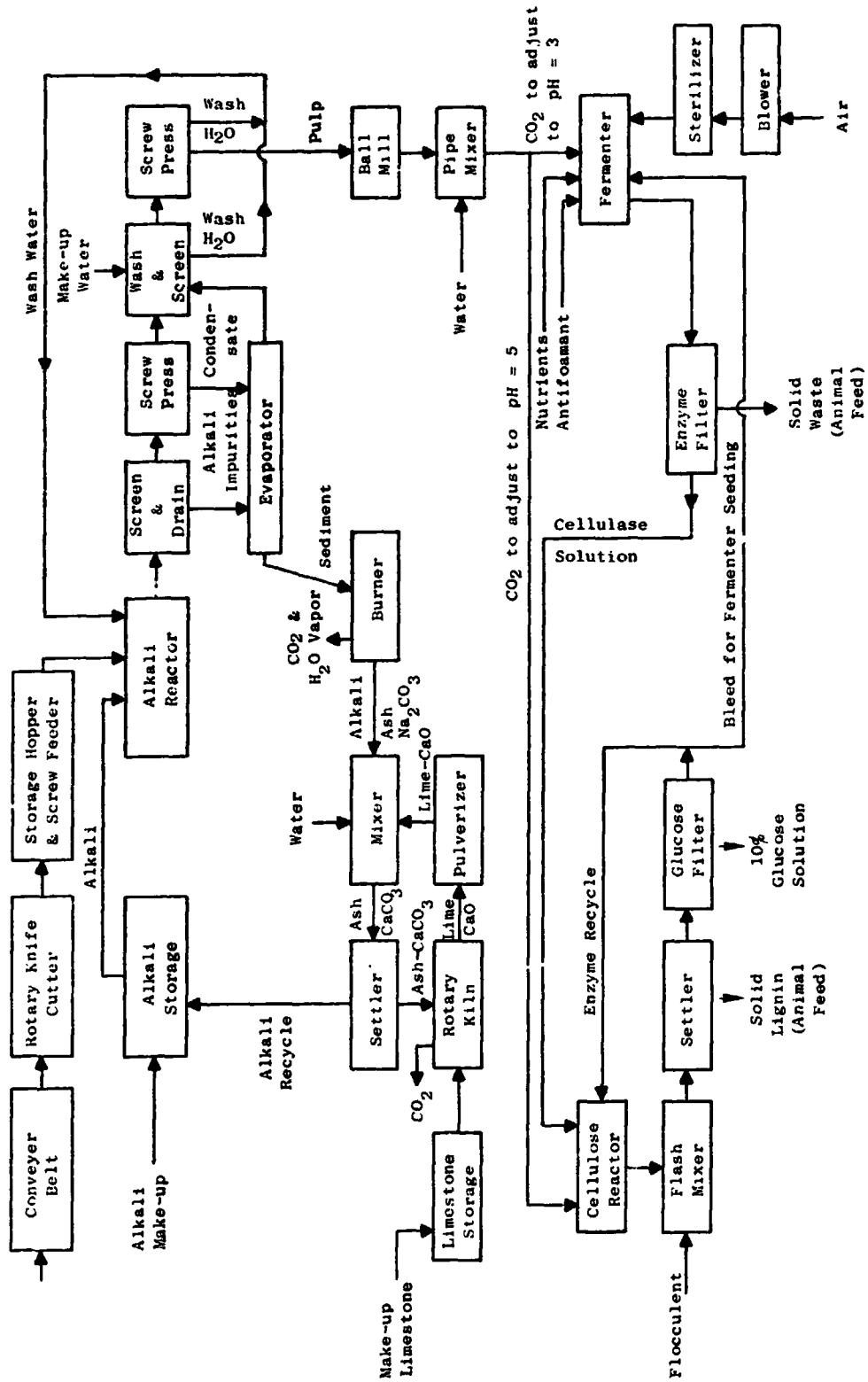
Figure 5.1. CELLULOSE TO GLUCOSE FLOW DIAGRAM.

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steam coils to maintain the temperature and with a pH control meter to maintain the required alkalinity of the mixture.

The output of the alkali reactor is about 2170 tons/day of liquid, containing approximately 138 tons equivalent dry weight of cellulose pulp which must be separated out. It first goes into a screener: a horizontal screen drum, 15 ft long, 3 ft in diameter, which is slowly rotated by a 3 hp motor. The mixture enters at one end; the liquor passes through the screen; the pulp exits at the other end, helped by helical baffles. A small amount of water is used to keep the screen clear by means of jets from the outside, but is neglected in the material balance. The pulp then goes into a screw press, which removes all but about 138 tons of the liquor, leaving a pulp containing about 50 percent liquid.

5.1.1.3 Recovery of Alkaline

The alkaline solution drained from the pulp contains the hemicellulose, pectins, fats, etc., plus small losses of cellulose and lignin. This solution is fed to a series of four multiple effect evaporators where the solution is evaporated to a dry solid. The evaporated water is recycled through a later washing step, but the solid sediment from the evaporator composed of alkali, hemicellulose and other constituents is sent to a burner. In the burner, the hemicellulose and other organic material is converted to carbon dioxide and water, which are used in pH adjustment of later streams or discharged to the atmosphere. The sodium carbonate ash remaining after combustion is mixed with water and treated with lime. The reaction which occurs in the mixer is

$$Na_2CO_3 + CaO + H_2O \rightarrow CaCO_3 + NaOH$$
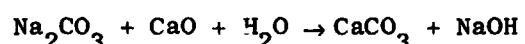
| sodium | lime | calcium | alkali |
| carbonate | | carbonate | |

The alkali is recycled to the alkali storage and the calcium carbonate which precipitated in the settler is charged to the rotary kiln. In the kiln, the calcium carbonate is heated by direct contact with hot combustion gases produced by burning fuel. The hot calcium carbonate decomposes to carbon dioxide and solid lime as follows:

$$CaCO_3 \rightarrow CO_2 + CaO$$
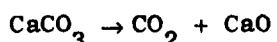
The solid lime clinker is pulverized and recycled to the mixer. The large quantity of carbon dioxide is available for adjusting the pH of various streams in the process or discharged to the atmosphere.

5.1.1.4 Further Treatment of the Akali-Treated Cellulose

The pulp, on the other hand, is washed in a screener-washer similar to the screener, but having external and internal water spray

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nozzles. About 1500 tons/day of water is needed, largely derived from the recycled water condensed in the alkali-recovery process. The pulp then goes into a second screw press, as before, so that the residual water may be reduced to the point where sodium hydroxide and sugars each pass at a rate of less than 1/2 percent.

The wash water from the preceding two steps is recycled by being used in the alkali reactor.

The cellulose after washing is sent to a ball mill where a further reduction to 150 mesh is achieved. This size reduction makes the cellulose more amenable to subsequent enzymatic attack in the cellulose reactor. The finely divided solids leaving the ball mill are mixed with water and divided into two portions. The major portion is adjusted to a pH ≈ 5 with acid and fed to the cellulose reactor. The minor portion is adjusted to a pH = 3 and sent to the fermenter.

5.1.1.5 Cellulase Catalyzed Breakdown of Treated Cellulose

The cellulose slurry sent to the fermenter, a stirred vessel, is mixed with a culture of the fungus, Trichoderma viride QM 9123, nutrients, and an antifoamant. A small bleed stream of unreacted cellulose is charged to the fermenter in order to continuously seed the fermentation reaction. The negligible quantity of this stream is neglected in the material balance. Sterilized air is also blown through the mixture. The flow rate of cellulase produced is 6 gal/min and must be filtered by continuous vacuum to remove fungal fibers. The solid cake which is removed from the filter by a knife, could be used for animal feed.

In the main reactor, which is a continuous stirred tank, the cellulose is contacted with the enzyme solution (cellulase) that has been prepared in the fermenter. The reaction proceeds until the concentration of glucose is 10 percent by weight. The glucose solution leaving the reactor is mixed with a flocculent in a flash mixer and allowed to settle. Lignin separates out as a solid, and the overflow from the settler is filtered using a pressure leaf filter. The filtrate is the desired product, 10 percent by weight glucose and the filter cake containing the remaining cellulose and the cellulase enzyme fixed on it is recycled to the cellulose reactor.

5.1.2 Details

5.1.2.1 Raw Material

Vast quantities of cellulose are available as waste products from food processing, lumbering, paper-making, grain harvesting, etc. At least two studies [1,2] have concluded that the most likely sources of waste cellulose for conversion to useful products such as protein and carbohydrates are (1) municipal organic wastes, (2) waste paper, and (3) bagasse (fibrous residue after the extraction of sugar from sugar cane).

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Municipal Organic Wastes

In the U.S. the annual production of municipal organic wastes is 200 to 300 million tons. Cellulose comprises 40 to 50 percent of this total. Currently, most municipal waste is disposed of by ᴗhe sanitary landfill method with relatively small amounts being disposed of by reclamation practices and incineration. Utilization of this waste will present a purposeful alternative to current disposal practices and various schemes are being currently considered by many people.

The cellulose fraction of municipal solid wastes will in all probability be only one output from a waste reclamation plant. Other output streams will be ferrous metals, glass, plastics, etc. The cost of each output, such as the cellulose fraction, will be a function of many things including reclamation plant capital and operating costs, the cost of municipal waste collection including transportation, output market considerations, etc. If such reclamation plants become a reality, there may be other demands for the cellulose fraction--the paper industry for example. Such sources of cellulose as reclamation plant output, however, are not currently available and therefore will not be considered further in this study.

Waste Paper

Wastepaper is generally collected on a local basis by junk dealers for resale as paper stock to the paper industry. The total distance from collector to consumer rarely exceeds 50 miles. The amount available in the United States each year exceeds 100 million tons, but the paper is widely dispersed and its utilization becomes mainly a matter of collection or concentration. At present, most of this paper is disposed of as part of the municipal solid waste discussed previously. Thus, the use of wastepaper is dependent upon development of waste reclamation plants. At present, wastepaper is not an attractive source of cellulose because of its greatly fluctuating price, its uncertain availability [1], and its scarcity in underdeveloped undernourished areas.

Bagasse

Bagasse is presently considered to be the most likely source of raw cellulose for glucose or starch production because of its availability in large quantities. The world's supply of bagasse is estimated to be over 100 million tons per year with over 10 million tons being produced in the U.S.

Comparative Raw Material Cost

The cost of the various materials that might serve as sources of cellulose for the production of glucose/starch has been estimated as follows [1]:

Source	Cost, ¢/lb
Municipal organic waste	0.125 - 0.225
Wastepaper (No. 1 grade-mixed)	0.20 - 0.60
Bagasse	0.25 - 0.75

While the cost of bagasse is estimated to be somewhat higher than municipal cellulose waste or wastepaper, bagasse is available in recoverable, dependable supply. This is essential for any continuous manufacturing operation. Therefore, we have selected bagasse as a suitable starting material for design purposes.

Other Major Raw Material Requirements

The major raw materials other than bagasse are alkali and crushed limestone. Both, however, constitute only makeup quantities to replace losses in the process since the overwhelming portion of these materials are regenerated in the processing scheme. Alkali and crushed limestone are cheap, commonly available materials. The amounts used and their costs are given in the economics section. Small amounts of nutrients and miscellaneous chemicals are also used in the process and their cost has been included in the economics.

5.1.2.2 Alkali Reactor

The primary reason for pretreating cellulose with an electrolytic solvent is that the cellulose is penetrated and swollen, thereby decreasing its crystallinity and increasing its reactivity for enzyme degradation. Many reagents are readily available for accomplishing this task, but those of most common industrial application are sodium hydroxide and sulfuric acid.

A decision had to be made as to what treatment would most efficiently purify the raw material, bagasse, for subsequent enzymatic reaction insuring a pure product without harmful toxicity levels. Four possibilities existed:

        (1) Acid treatment
        (2) Acid to alkali treatment
        (3) Alkali to acid treatment
        (4) Alkali treatment

Examination of the approximate analysis of bagasse gave much insight into solving the problem.

Sugarcane Bagasse Composition	Proximate Analysis
Component	% of Dry Weight
Cellulose	46
Hemicellulose	25
Lignin	20
Silica, Fat, Wax, Ash	9
	100%

The first three alternative treatments were rejected for the following reasons·

(1) Acid treatment alone would solubilize the cellulose along with hemicelluloses. About 10 percent of the hemicelluloses are composed of toxic materials such as xyloses which tend to build up in the human body and would therefore introduce a detrimental additive in the final product. No feasible separation was found which separates the cellulose from the hemicelluloses after they are both solubilized.

(2) Acid to alkali treatment would serve no useful purpose since the cellulose and hemicelluloses are solubilized in the acid treatment and subsequent addition of alkali has no effect.

(3) Alkali to acid treatment appeared to separate out all unwanted components of the bagasse material. The initial alkali treatment, about 4 percent NaOH, would solubilize only the hemicelluloses which could be separated from the cellulose and lignin. Subsequent treatment with 60 to 65 percent sulfuric acid would solubilize the cellulose leaving the solid lignin to be skimmed off and therefore separated. The detrimental feature of this scheme was the enormous amount of relatively high concentration acid which would be lost due to neutralizing of the cellulose solution before it was charged into the cellulose reactor. The acid makeup was calculated to be 2500 tons/day at approximately $30/ton--a most formidable daily cost consideration. The subsequent buildup in salts, the unresolved problems of separation and the probable inhibition of a high salt concentration on later enzyme fermentation made this approach very unattractive.

The fourth possibility, alkali treatment alone, was finally chosen. It eliminates the unwanted hemicelluloses but keeps the lignin with the cellulose. The assumption is that the lignin does not inhibit glucose formation and is later separated from the final product. This treatment proceeds most efficiently at 150°F for 1 hour using a 4 percent sodium hydroxide solution.

5.1.2.3 Cellulose Reactor and Fermentor Design

The conversion of cellulose to glucose is accomplished by the use of a single enzyme, cellulase. The cellulase enzyme required is

produced in significant quantities by T. viride, and excreted into the medium. Thus, it is conceptually possible to grow T. viride in a tank, filter the cellular material out, and use the resulting enzyme solution in another tank to break down cellulose.

The parameters for a plant design are, in part:

1. The quantity of enzyme activity produced per liter of broth per hour. (To compute size and cost of the enzyme production unit.)

2. Stability of the enzyme as a function of time.

3. Kinetics of the reaction. (To compute the holding time of the reaction vessel.)

4. Projected concentrations of cellulose and glucose.

For purposes of this design, an enzyme activity and production rate based on the work of Mandel et al, at Natick Laboratories, was used. Their numbers are 4.0 gm glucose/gm enzyme-hr for the enzyme activity and 1.0 gm E/liter-hr for the fermenter production rate. This implies an activity production of 4 g glucose/liter/hr. As discussed in Appendix 5.1, the design of the main reactor and the fermenter are tied together because enzyme lost due to degradation and separation inefficiencies must be replenished continuously. Obviously, the more rapidly enzyme decays and/or is lost in separation, the greater the enzyme generation capacity required. A recycle of enzyme is mandated by the high cost of enzyme. For purposes of designing the volume of reactor required, it is assumed that cellulase will degrade linearly with time, half of the activity being lost in 100 days. It has also been assumed that, at the outlet of the glucose reactor, the enzyme and the glucose can be separated from each other with an efficiency equivalent to the loss per day of 2 percent of the enzyme inventory in the glucose reactor. These values were chosen partly because they appear reasonable and partly because shorter half-lives or larger losses rapidly give uneconomic designs. It is expected, then, that the technology to achieve these levels will be developed.

For the above conditions (see Appendix 5.1) the reactor size required is nearly 50 percent greater than would be required if there were no degradation loss or separation loss of enzyme. A reaction volume of 270,000 gallons is required.

Implicit in the above calculation is that the residence time in the main reactor will be sufficient to produce 100 tons/day of glucose from the entering starch. No recycle of unreacted cellulose is shown because it is assumed that the enzyme attaches to the unreacted cellulose and returns to the reactor from the filter (the stream being a slurry containing little water). Considerations of pumping economy, minimizing reactor volume, and minimizing sterility problems indicated that a stream of 154 gpm entering the reactor and containing 10 percent cellulose could be expected to flow easily and would give reasonable reactor volumes for the requisite residence time. The exit concentration was chosen as 10 percent glucose because here is some evidence

that glucose concentrations of 10 percent or greater tend to inhibit growth of undesirable microorganisms. In a stirred tank reactor of the type proposed, the average concentration in the tank is essentially the effluent concentration.

For both the main reactor and the cellulase fermentor, operational problems such as flow upsets, contamination, etc., would require shutdown of the entire facility if single reactors were used. Thus, multiple reactors in parallel are proposed, the total volume being the design value plus some extra for mechanical reasons. This will permit reasonable continuity of operation and reduce the requirement for holding vessels with their associated problems of cost, contamination, pumps, manpower, and so on.

5.1.3 Enzyme Production

The only enzymes involved in the cellulose to glucose process are the "cellulase complex" enzymes, often simply referred to as cellulase. A typical laboratory production of cellulase is detailed in [3, 4].

In general, producing large quantities of enzymes first involves scaling-up laboratory and pilot-plant equipment to industrial production. Equipment such as fermenters, heat exchangers, crystallizers, filters, and so forth, can be designed and properly operated only if scale-up technology is fully appreciated.

This technology is discussed in [5] and essentially involves only elementary dimensional analysis. Hence, the typical scale-up problems encountered in going from laboratory to industrial production will not be dealt with here.

A full-scale production process is shown in Figure 5.2. The process would be started by innoculating 2000 gallons of medium in each of two 3000 gallon fermenter reactors with cultures of Trichoderma viride. The medium consists of 1.0 percent Solks Floc (cellulose), 0.1 percent proteose peptone, salts and water. The cultures are maintained at 28 to 29°C for six days to reach a high cellulase activity. The cells are then harvested at the rate of .8 gal/min, and simultaneously .8 gal/min of fresh nutrient, mainly cellulose from prehydrolization, is added to keep the fermenter volume and cellulase activity constant.

Following a suggestion of Mandels and Weber [4], a small amount of resistant cellulose residue, obtained from the glucose filter recycle stream is used as feed for the fermenter reactor. Apparently, use of the cellulose remaining after enzyme attack results in a cellulase preparation having a high activity on resistant cellulose.

To increase cell growth, the fermenter medium is stirred. An appropriate stirring speed is 10 rev/min with a 36 inch diameter four bladed impeller. Placement is low in the culture. A second impeller can be located just above the liquid-foam interface. Antifoam (Tween 80) is added to control foam. The aeration rate is about 250 cfm.

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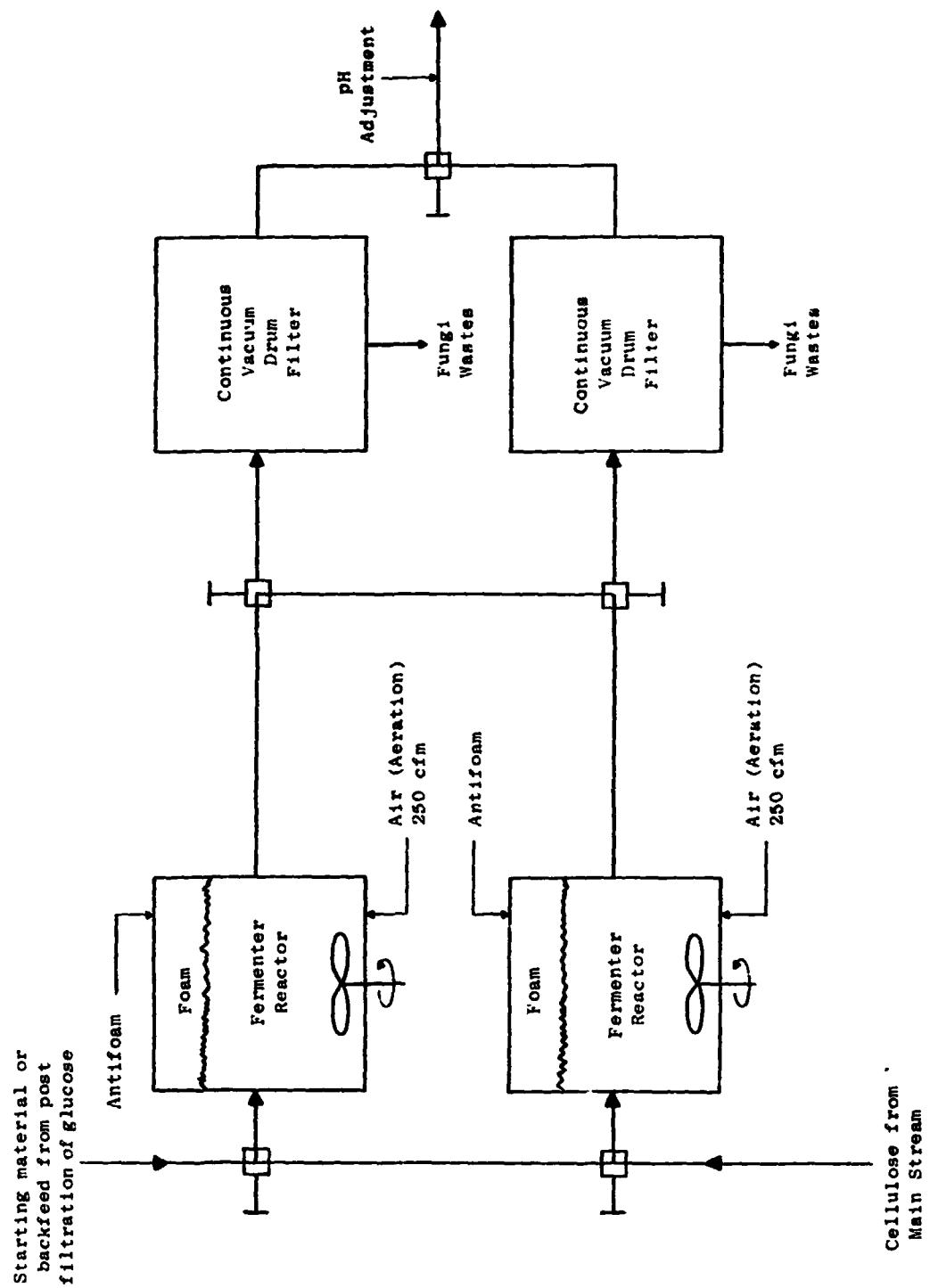
Figure 5.2. CELLULASE PRODUCTION.

The effluent (.8 gal/min) from the fermenter reactors is pumped to a system of two continuous vacuum drum filters of 10 square feet each. The filters remove the fungi wastes from the cellulase solution. Then a pH adjustment is made with NaOH or KOH, and the cellulase solution is pumped to the main reactor.

Typically, cultures of Trichoderma viride become contaminated after 100 days with Aspergellus niger [4], and the yield declines, but over 200,000 gallons of active cellulase can be harvested in a 100 day period.

5.1.4 Special Problems

5.1.4.1 Sterilization

One of the potential problems in the cellulase process is contamination of the fermenter or the cellulose reactor by undesirable organisms which metabolize glucose. In the reactor this would lead to consumption of glucose and to contamination of the product by the organisms and their excretions. In the fermenter there is the added problem of competition with the cellulase-producing fungus. Hence, the invasion of micro-organisms into either vessel must be prevented. The following steps should be taken:

- Construction of the entire process from the alkali reactor to the output end in such a manner that the plant can be sterilized before operation.

- Continuous sterilization of all entering streams: the alkaline pulp will be sterile, but incoming water and gases must be passed through micropore filters.

- Control of the glucose concentration in the cellulose reactor to a level high enough to slow down micro-organisms; possibly also oxygen level control.

- Splitting the process into two reactors so that one can be taken out of production and sterilized without closing down the entire plant if one becomes contaminated.

- Providing laboratory services for the monitoring of sterilization, for test cultures of the reactor media, and for control of the purity of the product.

5.1.4.2 Lignin

The alkaline process, while dissolving hemicellulose, does not effectively destroy the lignin. Hence, it passes into the cellulose reactor with the pulp, where it does not contribute anything, and may even capture some of the enzyme. Since it does not break down, a large part of it--plus some cellulose--may be separated out in a settler.

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However, some of it will stay with the cellulose and be recycled into the reactor. Hence, the lignin level will build up. For example, if lignin constitutes about 30 percent of the entering pulp, and the settler eliminates about 75 percent of the lignin in the recycle stream, it will build up to the point where it constitutes about 40 percent of the suspended solids. Since particularly small lignin particles will tend to recycle, the efficiency of the settler will fall off and the lignin level build up further with time. Hence it will be necessary to flush out the system from time to time, causing loss of cellulose and enzyme.

5.1.4.3 Solid Waste

The waste from the settler will contain lignin, some cellulose, and some cellulase adsorbed on the cellulose. When the system is blown down, lignin and substantial amounts of cellulose and cellulase will be rejected. Since cellulose and cellulase have nutritive value to some animals, it is intended to use this waste material as inexpensive feed, to be supplemented with more well-rounded feeds.

5.1.5 Major Items of Equipment and Fixed Capital Investment

A list of the major pieces of equipment in the cellulose process appears in Table 5.1. The table gives the specifications of each item, the number required, and the purchase price in 1972 dollars. The total purchase cost of the equipment is $1,450,000.

The fixed capital investment is shown in Table 5.2. It was evaluated by using the total purchase cost of the equipment as a base. Direct and indirect costs were calculated as appropriate percentages of the base (6). A contingency amounting to 10 percent of the total direct and indirect costs is included in the fixed capital investment. The fixed capital investment is $6,050,000 for a plant to produce 100 tons glucose per day.

5.1.5.1 Manufacturing Costs

The manufacturing cost amounts to 4 and 1/2 cents per pound glucose. This cost includes that for raw materials, labor, supplies, amortization, taxes, insurance, overhead and utilities. A detailed summary of the manufacturing cost per pound is given in Table 5.3.

(a) Raw Materials. The major raw material is bagasse. A cost of 1¢/lb dry cellulose is assumed which is based essentially on the fuel value of the bagasse and the cost of baling. The quantities of alkali and crushed limestone constitute make-up for the 5 percent loss that occurs in these materials during the process. Losses have been reduced by including equipment in the process for regeneration of alkali and lime.

Table 5.1

PURCHASE COST OF EQUIPMENT

Number Required	Item Description	Ref.	'72 $ Each	Total $
1	Belt Conveyor, 100' × 30", 40'/min, including motor and drive	[6a]	10,300	10,300
1	Rotary Knife Cutter, including motor drive and guard	[6b]	9,600	9,600
1	Storage Hopper, carbon steel	[6c]	3,200	3,200
1	Vibratory Screw Feeder including motor and drive	[6d]	1,900	1,900
1	Alkali Reactor, 30,000 gal with agitator, 304 stainless, includes drive unit	[6c]	42,500	42,500
1	Alkali Storage Tank, 30,000 gal, 304 stainless steel	[6c]	32,200	32,200
2	Screener-Washers		4,500	9,000
2	Screw Presses	[7]	55,000	110,000
4	Evaporators, long tube vertical, 4000 square feet heating surface	[6e]	64,500	258,000
1	Burner		10,000	10,000
1	Mixer, settler, 7500 gal, continuous, stainless steel shaft, motor, gear reducer with baffles for alkali regeneration	[8a]	4,400	4,400
1	Rotary Kiln, 8' diam × 320' long, including driver, burner and alumina brick lining	[8b]	574,000	574,000
1	Pulverizer, 2.7 tons/hr, including motor, drive and guard	[6b]	1,000	1,000
1	Storage vessel, 15,000 gal for crushed limestone	[6c]	7,100	7,100
1	Ball Mill Wet Grinder, closed circuit with air classifier, motor, drive, and balls included	[6b]	87,500	87,500

Table 5.1 (Cont)

Number Required	Item Description	Ref.	'72 $ Each	Total $
5	Cellulose Reactor, plastic coated carbon steel with agitator, drive unit included, 60,000 gal	[6c]	32,300	161,000
1	Flash mixer including agitator and motor, 15 gal capacity	[6f]	3,900	3,900
1	Settler, plastic coated carbon steel, 12,000 gallon with stirrer and drive unit	[6c]	15,000	15,000
3	Glucose Filter, vertical tank, pressure leaf, 304 stainless, 250 square feet	[8c]	10,300	30,900
2	Fermenter, 3000 gal, 304 stainless including drive unit and motor	[6c]	15,500	31,000
1	Air Blower, 10 psi maximum discharge, 500 cfm	[6g]	3,200	3,200
2	Enzyme Filter, continuous vacuum drum, 10 ft^2	[6h]	15,500	31,000
10	Pumps and Motors, 5 HP	[6i]	1,100	11,000
		Total		$1,448,200
		or approximately		$1,450,000

Each item was sized on the basis of the quantity of material handled. Purchase costs for the size or capacity required was obtained from several standard references [6,8,9]. These costs were updated to 1972 dollars on the basis of Chemical Engineering Cost Indices [6j].

For example, suppose a particular piece of equipment cost $10,000 in 1966. The cost index for 1966 is 107.2 while that for 1972 is 138.5. The increase in cost index reflects factors such as higher labor and material costs. Thus, the purchase cost of the equipment in 1972 is

$$\text{Purchase Cost in 1972} = \$10,000 \left[\frac{138.5}{107.2}\right] = \$12,900$$

Table 5.2

FIXED CAPITAL INVESTMENT

Direct Costs (DC)	% Equipment Cost	Cost, $
Equipment, purchased cost	100	1,450,000
Installation	45	652,000
Instruments and Controls	15	217,000
Piping, installed	50	725,000
Electrical, installed	10	145,000
Building, including services	15	217,000
Yard improvements	10	145,000
Service facilities, installed	40	580,000
Land	6	87,000
		Total DC = 4,218,000
Indirect Costs (IC)		
Engineering and supervision	32	463,000
Contractor expense	40	580,000
		Total IC ≈ 1,043,000
Other Costs (OC)		
Contractor's fee	5% DC + IC Totals	263,000
Contingency	10% DC + IC Totals	526,000
		Total OC = 789,000

Fixed Capital Investment (FCI) = Sum DC, IC, and OC Totals

FCI   = $6,050,000

Table 5.3

MANUFACTURING COST
Basis: 100 tons/day glucose

Raw Materials	Per Day
Bagasse containing 109 tons dry cellulose @ 1¢/lb cellulose	$2,180
Alkali, dry flake, 4.25 tons/day @ $150/ton	640
Crushed limestone, 6 tons/day @ $13.30/ton	80
Nutrients, miscellaneous chemicals	35
	$2,935
Labor and Supplies	
Operating labor, 288 man hours @ $4/man hr	1,150
Supervision, 15% operating labor	173
Maintenance and repair, 3% FCI	498
Operating supplies, 15% maint. and repair	75
Laboratory, 10% operating labor	115
	$2,011
Fixed Costs	
Depreciation, 10% FCI	1,660
Taxes and Insurance, 3% FCI	498
	$2,158
Plant Overhead	
50% operating labor, supervision and maintenance	$ 910
Utilities	
Water, 200 gal/min @ 15¢/1000 gal	45
Steam, 50 Mlbs/hr, 100 psi, @ 60¢/Mlb	720
Fuel, 11.2 M BTU/day @ $0.25/MM BTU	77
Electricity; 600 Kw-hr @ 0.01¢/Kw-hr	144
	$ 986

Overall Manufacturing Cost = $9,000/Day

$$\frac{\text{Mfg Cost}}{\text{lb gluc.}} = \frac{\$9,000/\text{Day}/100¢/\text{ton}}{\text{Day}/100 \ T/\$/2000 \ lb} = 4.5¢/\text{lb glucose}$$

(b) Operating Labor. The man-hours of operating labor were calculated using two different methods and good agreement was found. One method is based on 48 man-hours per day per processing step. It was assumed that there are 6 major processing steps: pretreatment, alkali reaction, evaporation, cellulose reaction, enzyme preparation in the fermenter, and regeneration of alkali and lime. The other method is based on processing steps, daily tonnage capacity, and the extent of continuous processing. The result is 288 man-hours per day or 12 men per 8 hour shift. An average cost of $4 per man-hour was used.

Other labor such as that for supervision, maintenance and repair, and laboratory analysis were taken as percentages of either the operating labor or the fixed capital investment.

(c) Amortization. Amortization or plant depreciation was calculated on the basis of straight-line depreciation. It was assumed that the plant service life was 10 years so that 10 percent of the fixed capital investment was depreciated annually.

(d) Utilities. The quantities of the various utilities needed (make-up, water, steam, fuel, and electricity) were calculated based on the process requirements.

5.1.5.2 Sensitivity Analysis of Manufacturing Cost

A summary of the sensitivity analysis is shown in Table 5.4 below.

Table 5.4

SENSITIVITY ANALYSIS--CELLULOSE TO GLUCOSE

Item			
Change	% Change	Absolute Change	Change in ¢/lb Glucose
Raw Material	100	1¢/lb	1.1
FCI	25	$1.5 Million	0.33
Utilities	50	$500/Day	0.25
Operating Labor	25	3 men/shift	0.26
Enzyme Mfg. FCI	100	$266,000	0.12

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(a) Increase in Raw Material Cost. An increase of 1¢/lb dry cellulose (50% by weight of dry bagasse) will raise the cost of the glucose by 1.1¢/lb to 5.6¢/lb. If municipal organic waste at 0.25¢/lb replaced the bagasse, the manufacturing cost would be reduced 0.8¢/lb to 3.7¢/lb. The substitution of waste paper for bagasse would probably not affect the manufacturing cost since de-inking would probably raise the raw material cost of waste paper to 1¢/lb.

(b) Effect of Changes in Fixed Capital Investment. An increase in fixed capital investment of 25 percent, or $1,500,000 to $7,500,000 would increase the manufacturing cost by 1/3¢/lb to 4.8¢/lb. A 50 percent increase in fixed capital investment would raise the cost by 0.7¢/lb to 5.2¢/lb.

(c) Effect of Changes in Utilities. Increases of 50 percent and 100 percent in the cost of utilities would raise the manufacturing cost by only 1/4¢/lb and 1/2¢/lb, respectively.

(d) Effect of Changes in Operating Labor. An increase of 25 percent in the operating labor required, or adding 3 men per shift, would increase the manufacturing cost by 1/4¢/lb to 4.75¢/lb. A 50 percent increase in labor would increase the manufacturing cost by 1/2¢/lb to 5.0¢/lb.

(e) Effect of Changes in Investment for Enzyme Production. The purchase cost of the equipment that is associated with producing the cellulase enzyme (fermenter, enzyme filter, and blower) is only $65,000 out of a total equipment purchase cost of $1,450,000. Assuming a 200 percent increase in this purchase, the total fixed capital investment would be increased to only $1,600,000. This would only increase the manufacturing cost by about 0.2¢/lb.

5.2 Fossil Fuel

5.2.1 Process Description

The flow diagram of the process for converting glycidaldehyde to glucose is presented in Figure 5.3. The standard processing presentation is used whereby the mainstream of the plant is indicated by a heavy line with major pieces of equipment and recycle streams indicated.

The glycidaldehyde enters into reactor R1 as a 33 percent by weight solution. In this reactor the glycidaldehyde as well as the recycled L-GALD come in contact with an acid resin which catalyzes the formation of D-GALD, L-GALD, and DHA. The process is controlled so that the products are formed in the following percentages:

D-GALD	37.5%
L-GALD	37.5%
DHA	25.0%
	100.0%

The central problem of obtaining this product stream was assumed solvable. The reaction gives equal amounts of D and L-GALD and the amount of DHA can be made less than or equal to the amount of D-GALD. In order to minimize the recycle stream of L-GALD, optimum choice of operating values of stream composition would dictate that L-GALD = D-GALD = DHA = 33% by weight. A margin of safety was used in assuming the aforementioned operating values.

This solution is charged into reactor R2 along with ATP which phosphorylates the DHA to DHAP and the D-GALD to D-GALD-3P. This reaction is catalyzed enzymatically by triokinase. The solution in R2 is 0.1 Molar with respect to substrate. The stream exiting R2 enters ion exchanger IE1A which contains a cationic resin which retards the ionic constituents of the stream, enabling the d-GALD-3P, the DHAP, and the ADP to be selectively eluted and the unionized L-GALD to be recycled to R1. The remainder of the stream enters ion exchanger IE1B which also contains a resin which selectively adsorbs the ADP which is eluted back to the ATP regeneration plant.

The d-GALD-3P and DHAP solution entering reactor R3 is 0.1 Molar in substrate. A controlled isomerization takes place aided by the enzyme triosephosphase isomerase. The resulting 50 percent d-GALD-3P - 50 percent DHAP mixture passes into R4 where it is converted to FDP by aldolase. The FDP enters as a .05 M solution into reactor R5 where the reactant is dephosphorylated to fructose. This reaction is catalyzed enzymatically by alkaline phosphatase. The products of R5 are charged into ion exchanger IE2 where the P_i is selectively eluted from the ion exchange anionic resin and recycled to the ATP regeneration plant. The remaining fructose is charged into R6 as a 0.1 M solution. Here the fructose is isomerized to a 50-50 glucose to fructose ratio, catalyzed enzymatically by a glucose-fructose isomerase. The glucose is separated out as a .05 M solution by passing through IE3, an ion exchanger containing a cationic resin, with the fructose being selectively eluted and recycled to the R6 reactor.

5.2.2 Details

5.2.2.1 Raw Materials

The source of glycidaldehyde, the starting material for the fossil fuel process, is presently dependent upon the processing of propylene--an oil refinery by-product. The procedure of oxidizing propylene to acrolein and then peroxidizing acrolein to glycidaldehyde is a relatively new commercial venture which is described briefly in Section 4.2. As demand increases for its use in the chemical industry, it seems conceivable that new technologies will develop which address themselves to the economics of glycidaldehyde production.

Figure 5.3. GLYCIDALDEHYDE TO GLUCOSE.

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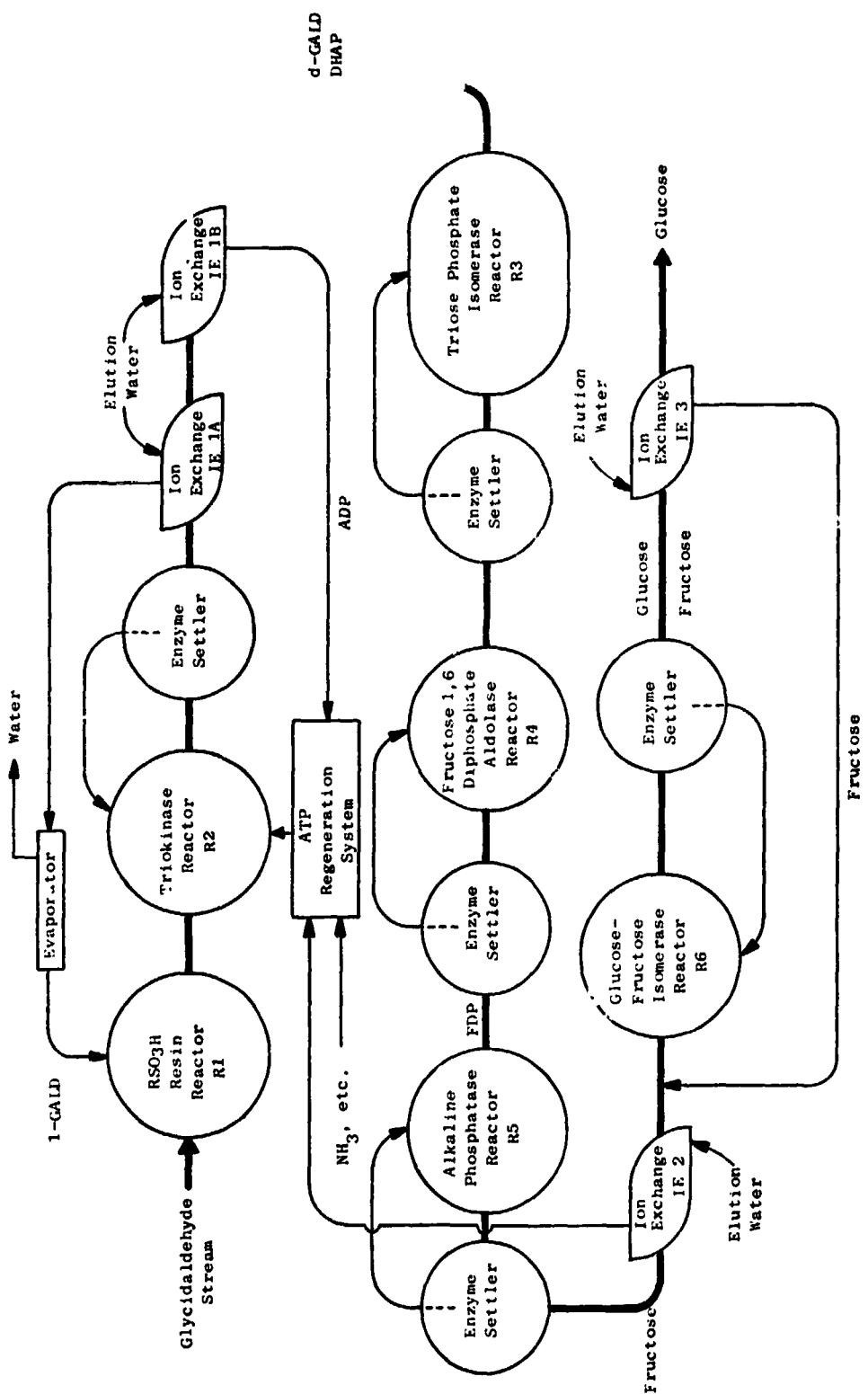
Other raw materials such as phosphoric acid, ammonia, carbamyl phosphate and potassium hydroxide are necessary primarily for ATP regeneration. These chemicals are readily available and are required in relatively small quantities for make-up to the process.

5.2.2.2   Reactors for Enzyme Reactions and Settlers for Recovery

The cost and availability of enzymes make it imperative that enzymes be conserved. Since the Continuous Stirred Tank Reactor (CSTR) was chosen for these processes (see Appendix 5.1), the enzyme must be in a form which can be easily dispersed through the reacting fluid and yet can be recovered at the outlet for recycle to the reactor. Soluble enzymes are easy to disperse in the fluid but present formidable separation problems at the reactor outlet due to their small absolute size. Filtration, membranes, ion exchangers--all have high operating and capital costs for such service. Immobilized enzymes (enzymes attached to a solid surface, on the other hand, present problems in dispersion but offer advantages in mechanical separation). On balance, calculations indicated that relatively high (10^{-4} M) effective enzyme concentrations could be achieved by putting the enzyme on very small glass particles (of the order of 0.01 cm) with bead concentrations in the fluid still low enough (perhaps 10 percent by weight) to give a reasonable overall viscosity for stirring. At the outlet, such particles can be removed by standard settling techniques so that the products leave in the clear fluid while the particle slurry containing the enzymes is returned to the tank. Settling times of the order of 20 ft/hr allow construction of settling tanks of reasonable capital cost while giving adequate enzyme concentrations, activities, and lifetimes.

The reactor design, then, is essentially that of a mixer-settler, the reactor size being chosen to provide the holding time required for the reaction desired, and the settler size chosen to permit separation of the immobilized enzymes.

The theory on which the settler design was based appears in Appendix 5.2. Consideration of the factors listed below led to the selection of one gallon per minute per square foot as the design overflow rate, one hour as the design detention time, and ten feet as the design basin depth.

(a) Allowance must be made for solid removal equipment in the bottom of the settling basin. This is generally taken to be on the order of two feet of basin depth.

(b) A freeboard allowance must be made to allow for flow surges that can occur from time to time. One foot of basin depth was estimated to be adequate for this purpose.

(c) Entrance and outlet zones in which essentially no settling will occur, due primarily to turbulence, consume about 30 percent of the basin volume.

(d) The particles upon which the enzymes are to be immobilized are
     expected to have a settling velocity of five feet per fifteen
     minutes.

(e) Basin shape effects the efficiency of a gravity-type settling
     basin. Circular basins are less efficient, on a unit volume
     basis, than are rectangular basins but are more economical
     because of construction and operating and maintenance costs.

5.2.2.3 Ion Exchangers

The performance of the reactors and of the separation pro-
cesses is crucial to the economic feasibility of the plant. Generally,
it is not adequate to scale up laboratory techniques. For example, one
of the separation processes could be done more specifically by ultrafil-
tration, but a cost analysis showed that it would require more than ten
times as much capital investment for membranes and supports, than is re-
quired for ion exchangers and their resin inventory. Hence, ion exchang-
ers take a central role in the process.

The first ion exchanger, R1, serves as a reactor. The
stream passes through a bed of resin granules. The resin has fixed sulfonic
acid radicals which provide acid catalysis as the solution passes through.

The second ion exchanger, IE1A, contains a resin which at-
tracts and holds negative ions. Initially, it is flushed out with a base
or a salt solution so that it holds OH^- or Cl^- ions. As the main stream
passes through the resin bed, it attracts all the valuable products of
the previous reaction except L-GALD and liberates the original base or
salt ions instead.

The L-GALD which is not adsorbed to the resin is recycled.
Long before the resin has adsorbed all the product ions it can hold, the
stream is switched to a parallel unit, and the products washed out of the
resin with an eluting stream. This stream is carefully controlled and
adjusted for pH, and the negative ions are eluted at slightly different
pH. The GALDP, DHAP, and ADP, which do not separate well in this way,
since they have electrically very similar ions, form the new main stream.

The next ion exchanger, IE1B, selectively absorbs ADP by
means of an intermediary metallic ion previously absorbed on a chelating
resin. The main stream, carrying the DHAP and GALDP continues through;
while the ADP is intermittently removed from the ion exchanger by an
eluting stream having a different pH, and returned to the plant which
converts ADP to ATP.

IE2 absorbs negative ions, while letting sugars go through
as the main stream. The $P_i \cdot Na$ is intermittently eluted and used in the
ADP conversion plant.

IE3 has the difficult job of separating different sugars
from each other. They can be complexed in certain resins through inter-
mediaries. After charging a long resin bed to only a small part of its
capacity, the eluting stream is applied. The two sugars progress through
the bed at different rates, and the eluting stream is directed to the

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water recovery when it is still relatively pure, to the main stream when the glucose is eluted, and to a recycling loop when the fructose is eluted.

5.2.3 Enzyme Production

The glycidaldehyde to glucose process utilizes five enzymes: triokinase, triosephosphate isomerase, aldolase, alkaline phosphatase, and glucose isomerase.

A review of the literature reveals that the five enzymes are obtained from a variety of sources and that similarties in the major steps in each enzyme preparation process leads, by inference, to a general method for preparing these enzymes.

There is reason to believe that all of the five enzymes mentioned above could be produced from the same or similar sources and that the same processing equipment could be used (see Figures 5.4, 5.5, and 5.6). It was therefore decided to compute the cost of producing the five enzymes by first determining the cost of producing a typical enzyme--aldolase. Then the cost of producing any other enzyme could be computed by assuming that, (1) cost is proportional to the amount of raw material processed, (2) enzymes have a half-life of 30 days, and (3) enzymes can be stored for two months without a loss in activity. For more details on the pricing method, see Section 5.2.5.

Loss of activity in the scaled-up plant, is assumed to be identical to that in the laboratory process. The amount of starting material needed has been adjusted accordingly. A list of references on preparation of the enzymes involved according to laboratory procedures appears at the end of this chapter (References 14 through 18).

Although it was assumed that aldolase would be needed in pure form, in certain cases pure enzymes are not needed and crude preparations of enzymes could be used. For these cases, the enzyme would obviously be less costly to produce.

5.2.3.1 Description of Process for Aldolase Preparation

The aldolase preparation process, shown in Figure 5.7 can be divided into six major steps: preparation, acetone fractionation, ammonium sulfate fractionation, gel eluting, dialysis, and chromatography. A typical batch process for 300 lbs of yeast which takes approximately 14 hours to complete might serve as a starting point. This process is based on published descriptions of both laboratory and pilot plant procedures.

Preparation

246 liters of distilled water and 27.6 liters of .5 M $NaHCO_3$ are added along with 300 lb_m of yeast (Candida utilis) to a stir tank (T-1) and the suspension is agitated for 15 minutes. Then a continuous flow centrifuge (C-1) is used to clarify the extract as it is pumped into another

Candida utilis

(50# Yeast)

4600 ml of .5 M NaHCO$_3$
and 41 liters distilled water

Autolysis
Stirred
Bath
15 minutes

1 liter of 5 N
acetic acid

Continuous
Centrifuge
12,000 × g

60 liters extract

Precipitate
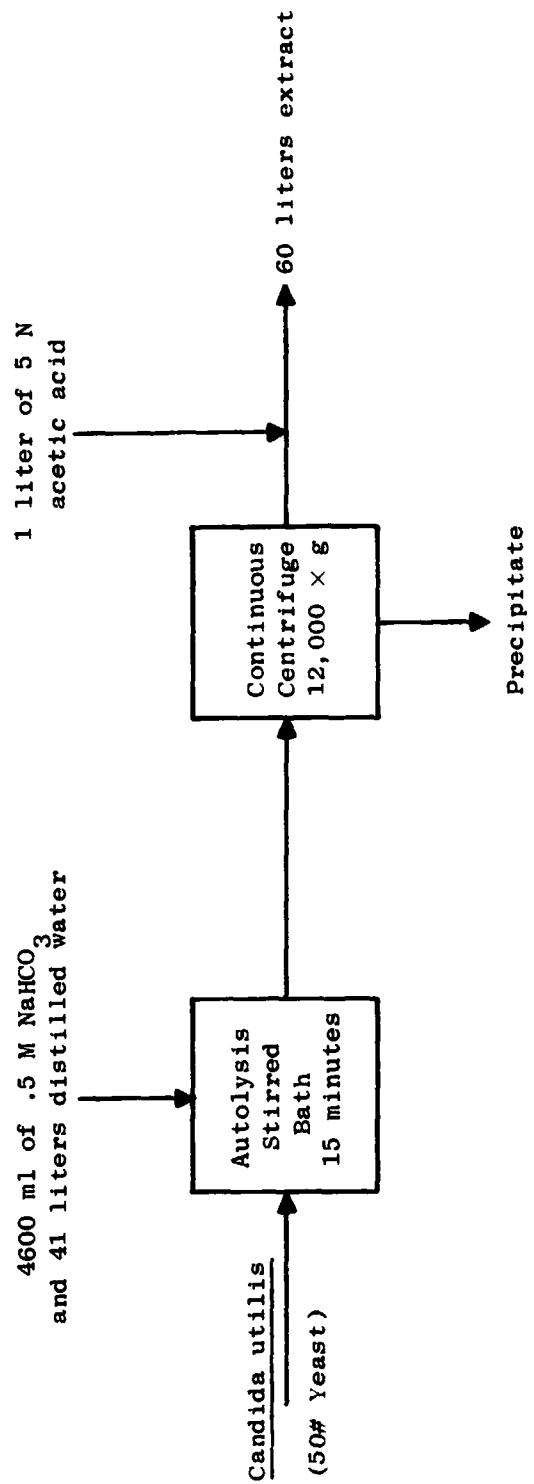
Note: Carry out all operations
at 23°C

Figure 5.4. PREPARATION OF YEAST EXTRACT.

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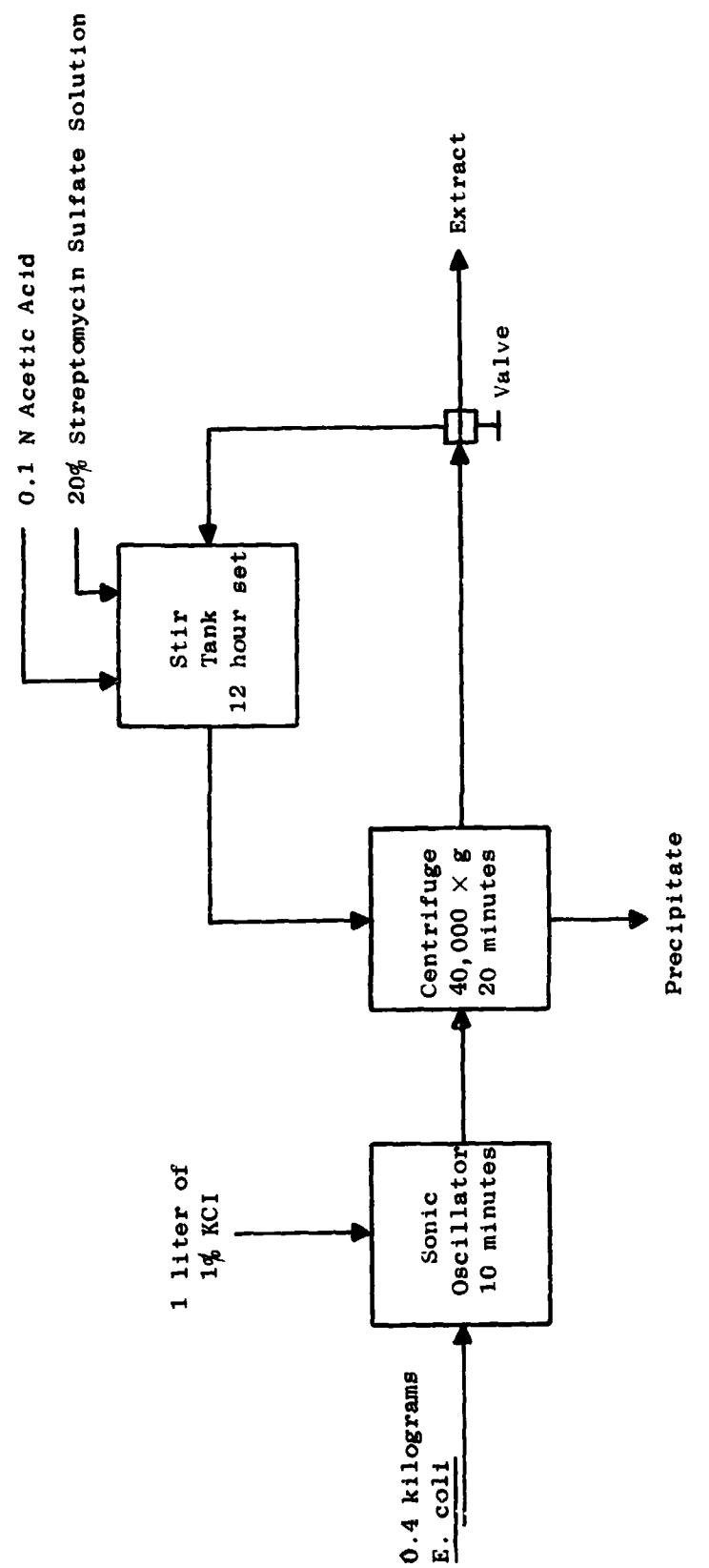
Figure 5.5. PREPARATION OF ESCHERICHIA COLI EXTRACT.

143
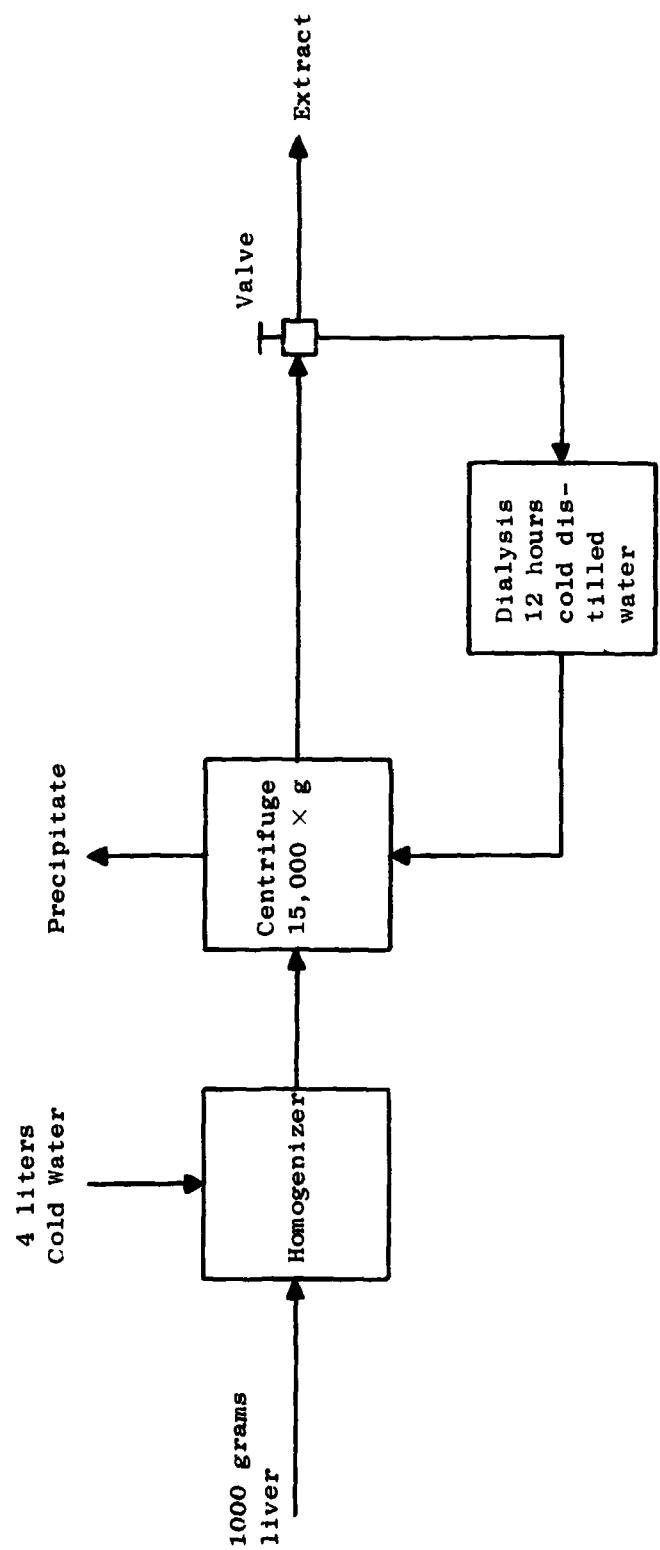
Figure 5.6. PREPARATION OF LIVER EXTRACT.
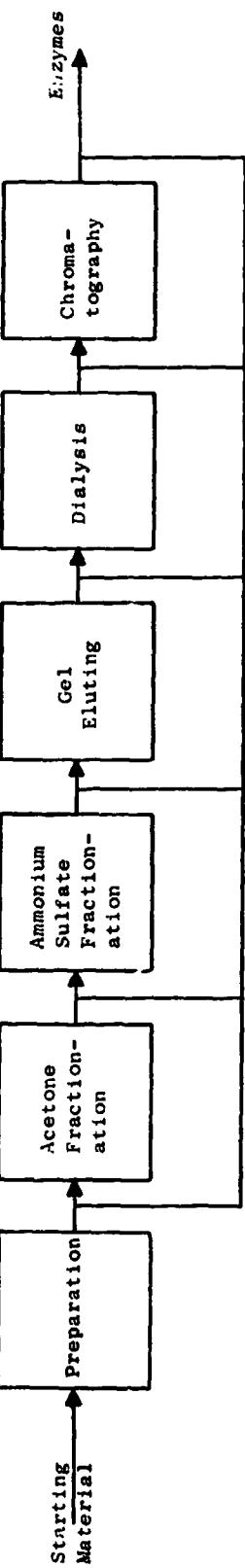
Figure 5.7.   SCHEMATIC OF ALDOLASE PRODUCTION.

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stir tank (T-2). Here, glycol at ~15°C is circulated through the jacket of the tank to cool the extract to 1°C, and the pH of the extract is adjusted to 4.8 with 6 liters of 5N acetic acid.

Acetone Fractionation

180 liters of acetone, which have been precooled to -70°C in a dry ice-acetone bath are added over a 20 minute period through a sixty hole distribution nozzle.

The glycol cooling is continued until the acetone fractionated suspension reaches -10°C. During this same time period the continuous centrifuge C-1 is precooled with a -15°C acetone-water solution.

The suspension is immediately centrifuged at a flow rate of 15 gal/min, and the supernatant is collected in a 300-gallon stainless steel jacketed tank (T-3). The entire centrifugation is completed in less than 20 minutes. The temperature of the supernatant is maintained below -5°C.

The precipitate from the centrifuge is discarded. The supernatant in stir tank T-3 is returned to tank T-2.

The second acetone addition, 180 liters, is started immediately. After addition, the suspension is stirred for 20 minutes at -12°C and centrifuged as above. At the end of the centrifugation the centrifuge is immediately stopped, by means of a mechanical brake. This prevents an excessive rise in the temperature of the precipitate. The enzyme-containing precipitate is then quickly suspended in 19 liters of cold distilled water in another tank (T-4). The pH of this suspension is adjusted to 7.0 with 72 ml of 1N NaOH.

During the precipitate suspending step, the supernatant (which is contained in T-3) from the centrifugation is pumped to the acetone recycle unit. The acetone is purified by distillation and condensation. This completes the acetone fractionation.

Ammonium Sulfate Step

The suspended precipitate in tank T-4 is clarified by centrifugation for 30 minutes at 10,000 $\times$ g in a large capacity batch-type centrifuge. The precipitate is discarded and the 18.6 liters of supernatant is pumped to another stirred tank (T-5). Here, 5.8 kilograms of solid ammonium sulfate are added to the supernatant. After stirring, the suspension is centrifuged. The precipitate is discarded, and the supernatant is returned to stirred tank T-5.

Then 3.6 kilograms of solid ammonium sulfate are added to the supernatant. The suspension is centrifuged. The supernatant is returned to stirred tank T-5 and discarded.

The precipitate is dissolved in 1.5 liters of cold 0.01 N NH_4OH contained in a stir tank (T-6). This solution is then centrifuged for 30 minutes. The precipitate is discarded, and the supernatant

solution is returned to stir tank T-6 where it is diluted with 18 liters
of 0°C water.

Alumina Gel

36 liters of 50 volumes percent alumina gel suspension is
added rapidly to the stirred solution contained in tank T-6. After stir-
ring for 30 minutes at 0°C, the suspension is centrifuged for 20 minutes.
The supernatant solution is discarded.

The precipitant gel is washed with 15 liters of water at
0°C and recollected by centrifugation for 20 minutes. The gel is then
placed in a homogenizer and eluted three times: First, the gel is mixed
with 2.5 liters of eluting solution in the homogenizer, and then the mix-
ture is centrifuged. The supernatant solution obtained is saved as the
first alumina eluate. Identical treatment of the gel yields a second and
third eluate. The elutes are placed in a stir tank (T-7) and ammonium
sulfate is added. The remaining gel is discarded, and this completes the
gel eluting process. If necessary, at this point the ammonium sulfate
suspension can be stored successfully at -15°C.

Dialysis

The ammonium sulfate suspension contained in tank T-7 is
centrifuged. The supernatant is discarded. The protein-containing pre-
cipitate is dissolved in 900 ml of cold 0.01 M histidine, pH = 6.5 at
5°C, and this solution is then dialyzed twice for 1 1/2 hours; each time
against 100 liters of 0.01 M histidine, pH = 6.5 at 5°C.

Although this process employs a typical dialysis sack pro-
cedure, countercurrent dialysis was also considered.

Chromatography

After dialysis, the protein solution is placed on a prop-
erly prepared DEAE-cellulose column and eluted with 5 liters of 0.01 M
histidine, pH 6.5. The appropriate tubes of high aldolase activity are
then combined and freeze-dried. Yeast aldolase prepared in this way
should contain no measurable hexokinase, glyceraldehyde-3-P dehydrogenase,
α-glycero-P-dehydrogenase, triosephosphate isomerase, or lactic dehydro-
genase activities.

5.2.4 Special Problems

5.2.4.1 ATP Regeneration

The conversion of glycidaldehyde to glucose requires the
consumption of 2 moles ATP per mole of glucose formed. Regeneration of
this amount of ATP from the ADP and P_i formed in the conversion is car-
ried out by reacting the ADP with carbamyl phosphate with the reaction
being catalyzed by the enzyme carbamate kinase as described in Chapter 4.

Process inputs needed for this segment of the plant include phosphoric acid, potassium hydroxide, ammonia, and carbamate kinase. Most of the potassium hydroxide and ammonia is regenerated within the confines of the ATP regeneration system. The carbamate kinase is generated in the enzyme production facility as described elsewhere in this report. Since the system is somewhat less than 100 percent efficient, some ATP input from outside sources is provided for in the process.

5.2.4.2 Desired Reactant Concentrations

The effectiveness of the fossil-fuel process depends upon the concentration of the organic chemicals in the process stream. Certain enzymatic reactions are inhibited by excessively high molarities; on the other hand, at low molarities the plant becomes so large for a given product quantity, that the capital investment and the operating costs become excessive. An attempt was made to estimate reasonable concentrations for the calculations in this study. However, all cost figures are quite sensitive to the molarities chosen. This problem acquires special significance in the final stages of glucose and of starch production where, if adequate concentrations are not achieved in the final elutions and separations, very large amounts of water must be evaporated from the stream. Until tests are run, these factors contribute the major economic uncertainties in the process.

5.2.4.3 Sterilization

Problems in keeping the process free from contamination by foreign bacteria are similar to those discussed in connection with the cellulose to glucose process (Section 5.1.4.1).

5.2.5 Economics and Cost Analysis

The process flow plan for converting glycidaldehyde to glucose may be divided into three sections: processing, enzyme production, and ATP regeneration.

5.2.5.1 List of Major Items of Equipment

Processing

The purchase price for each item of equipment in 1972 dollars, along with an item description appears in Table 5.5. Each item was sized on the basis of the quantity of material handled. The total purchase cost amounts to $606,000.

Enzyme Production

There are 5 enzymes involved in the production of glucose from glycidaldehyde. The steps involved in the enzyme preparation are

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similar for the various enzymes, so one enzyme, aldolase, was taken as
a basis for evaluating cost. The purchase cost of the equipment was
calculated. Assuming 300 lbs/day of starting material were to be pro-
cessed to make aldolase (Table 5.6). Analysis of the daily quantities
of starting material indicated that four of the enzymes could be made
alternately (see Table 5.7 for scheduling) in the same plant. The tri-
okinase enzyme requires such a large amount of starting material that
it was decided to prepare it in a separate enzyme plant.

ATP Regeneration

The manufacture of glucose from glycidaldehyde requires
the regeneration of two ADP molecules per molecule of glucose formed.
The regeneration requires a urea plant plus additional facilities to
manufacture the enzyme, carbamyl phosphatase. No equipment list is
included, since the fixed capital investment is calculated on the ba-
sis of a standard urea plant.

5.2.5.2 Fixed Capital Investment

Processing

The fixed capital investment was evaluated from the data
in Table 5.3 by taking direct and indirect costs as percentages of the
total purchase cost of the equipment. A contingency of 10 percent was
included in the fixed capital investment. This investment, which is
shown in Table 5.8, amounts to $2,330,000. In addition, the cost for
the initial resin loading of the ion exchangers, shown in Table 5.9,
is $2,470,000. Thus, the total fixed investment is $4,800,000 for
processing.

Enzyme Production

The fixed capital investments for both the four enzyme
and the triokinase plants were escalated on the basis of daily starting
materials using the aldolase investment in Table 5.10 as the basis.
Thus, the FCI for the four enzyme plant is $292,000, while that for
triokinase is $1,790,000. These values are shown in Table 5.11.

ATP Regeneration

The fixed capital investment for this combined facility
is $3,950,000 calculated on the basis of a standard urea plant. It is
shown in Table 5.13.

5.2.5.3 Manufacturing Costs

Manufacturing costs were calculated, in general, as percentages of FCI. However, the operating labor was estimated on the basis of the processing steps (including the cost of supervisory, maintenance, and laboratory labor). The plant would require 18 men/shift with 6 men assigned to each of the plant operational divisions: processing, enzyme production, and ATP regeneration. The maintenance labor was calculated as a percentage of the fixed capital investment, excluding from the latter the initial expense for resin and ATP loading.

Straight line depreciation of the total fixed capital investment was used for amortization. The total investment included that for initial resin and ATP loadings. A 10 year service life was assumed so that 10 percent of the total fixed capital investment was depreciated annually.

Utilities were estimated on the basis of process requirements.

Processing

Manufacturing costs involved in processing are outlined in Table 5.14. The total comes to 2.3¢/lb. The cost of the raw material, glycidaldehyde, is not included in the table, but is estimated here.

Glycidaldehyde, is an intermediate in the production of glycerol from propylene. Glycerol sells presently at 23¢/lb and we have assumed purchase of the glycidaldehyde at 17¢/lb. Since there are ample supplies of propane from which propylene may be made by dehydrogenation and since synthetic glycerol processes are currently in commercial operation, there should be no shortage of the starting material. However, at 17¢/lb, glycidaldehyde contributes 60 percent to the total manufacturing cost.

Enzyme Production

Table 5.15 summarizes the manufacturing cost of the enzymes at 1.8¢/lb of glucose produced.

ATP Regeneration

The cost of regeneration of ATP appears in Table 5.16 at 6.0¢/lb of glucose.

5.2.5.4 Summary

A summary of the fixed capital investments and the manufacturing costs per lb glucose is as follows:

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Glycidaldehyde to Glucose

Step	FCI	Mfg. Cost, ¢/lb Glucose
Processing	$4.80 million	2.3
Enzyme Production	2.08 million	1.8
ATP Regeneration	3.95 million	6.0
Glycidaldehyde	Purchased	17.0
	$10.83 million	27.1

It is seen that raw material purch··· contributes the largest amount to the total cost. A sensitivity analysis (showing the impact of percent variations in particular items on the total cost) is shown below.

	% Change	Absolute Change	Change in ¢/lb Glucose
Glycidaldehyde Cost	17.6	3¢/lb	3.0
FCI Total	25	$2.7 million	0.65
Operating Labor	20	4 men/shift	0.36
Utilities	50	$1600/day	0.8
ATP Replacement	33 1/3	$3000/day	1.5
Resin Replacement	100	300	0.15

Table 5.5

PURCHASE COST OF PROCESS EQUIPMENT--GLYCIDALDEHYDE TO GLUCOSE

Number Required	Item Description	'72 ? Each	Total
Ion Exchange Tanks (carbon steel, plastic coated)			
4	R-1 and IE-2 (3), 75,000 gal each	16,800	67,200
9	IE-1A (3) and IE-3 (6), 110,000 gal each	24,500	220,000
3	IE-1B, 50,000 gal each	14,200	42,600
		Total	$329,800
Reactors (carbon steel, plastic coated, with agitator and drive unit)			
2	R-2 and R-4, 300 gal each	3,000	6,000
1	R-3, 10 gal	650	650
1	R-5, 2500 gal	6,450	6,450
1	R-6, 20,000 gal	19,350	19,350
		Total	$32,450
Settlers			
3	S-2, S-4, and S-5, 18,500 cubic ft each	27,300	81,900
1	S-3, 11,250 cubic feet	19,300	19,300
1	S-6, 28,000 cubic feet	38,600	38,600
		Total	$139,800
1	Evaporator, long vertical tube, all steel	28,400	28,400
20	Pumps, 2000 gal/minute, including motor	3,750	75,000
	Total Purchase Cost of All Items =		$605,450
	or approximately		$606,000

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Table 5.6

ALDOLASE ENZYME PRODUCTION FOR GLYCIDALDEHYDE TO GLUCOSE
Purchase Cost of Equipment*

Number Required	Item Description	'72 $ Each	Total $
8	Reactor Tanks, 304 stainless, jacketed, stirred, assorted sizes, 20-300 gal	---	$50,000
2	Centrifuge, continuous, 12,000 × g, 15 gal/minute	13,850	27,700
2	Centrifuge, batch, 10,000 × g, 20 gal each	4,150	8,300
1	Homogenizer Blender, batch, jacketed, agitated, 304 stainless, 50 gal	3,900	3,900
2	Dialysis Unit Tanks, 304 stainless, jacketed, agitated	3,850	7,700
1	Refrigeration Unit, 10 ton	4,500	4,500
24	Pumps, 1/2 HP each, centrifugal including motor	300	7,200
		Total =	$109,300
		or approximately ≈	$110,000

*Will handle 300 lbs starting material/day. This is sufficient to produce enough aldolase to convert 20 tons glycidaldehyde to glucose.

Table 5.7

FIXED CAPITAL INVESTMENT (ENZYME PRODUCTION)--GLYCIDALDEHYDE TO GLUCOSE

The four enzymes listed below will be manufactured in the same facility with each enzyme produced for a certain number of days each month and stored until needed. Storage life of the enzyme is assumed to be two months with a 30 day half life when in use.

Enzyme	Starting* Material, (lbs)	Steady-** State, (lbs)	Producing Days/ Month	Daily Start- ing Material (lbs)
Aldolase	1,500	750	$5\frac{1}{2}$	~140
Triose Phosphate Isomerase	48	24	$\frac{1}{2}$	~ 50
Alkaline Phosphatase	6,840	3,420	20	~170
Glucose Isomerase	1,100	550	4	~140
	9,488	4,744	30	

* Amount needed to produce enzymes necessary for converting 100 tons glycidaldehyde to glucose.

** Steady quantity = 50% of starting material.

Table 5.8

FIXED CAPITAL INVESTMENT-PROCESSING--GLYCIDALDEHYDE TO GLUCOSE

Direct Costs (DC)	% Equipment Cost	Cost, $
Equipment, purchased cost	100	$606,000
Installation	35	212,000
Instruments and Controls	15	91,000
Piping, installed	40	243,000
Electrical, installed	8	48,000
Buildings, including services	15	91,000
Yard Improvements	9	54,500
Service Facilities, installed	30	182,000
Land	6	37,000
		Total DC = $1,564,500
Indicated Costs (IC)		
Engineering and Supervision	32	194,000
Contractor Expense	40	242,000
		Total IC = $436,000
Other Costs (OC)		
Contractor's Fee	5% DC + IC Totals	110,000
Contingency	10% DC + IC Totals	220,000
		Total OC = $330,000

Fixed Capital Investment (FCI) = Sum DC, IC, and OC Totals

FCI (Processing) = $2,330,000

Table 5.9

RESIN COST--GLYCIDALDEHYDE TO GLUCOSE

Ion Exchanger	Resin Volume, ft^3	$/ft^3$	Total $
R-1	3,100	20	62,200
IE-1A	22,200	20	444,000
IE-1B	8,800	20	176,000
IE-2	11,100	60	666,000
IE-3	56,100	20	1,120,000
		Total	$2,468,200
		$\approx$	$2,470,000

Table 5.10

FIXED CAPITAL INVESTMENT,* ALDOLASE ENZYME PRODUCTION--
GLYCIDALDEHYDE TO GLUCOSE

Direct Costs (DC)	% Equipment Cost	Cost $
Equipment, purchased cost	100	110,000
Installation	30	33,000
Instruments and Controls	20	22,000
Piping, installed	60	66,000
Electrical, installed	10	11,000
Buildings, including services	15	16,500
Yard Improvements	5	5,500
Service Facilities, installed	30	33,000
	Total DC =	$297,000
Indirect Costs (IC)		
Engineering and Supervision	32	35,000
Construction Expense	35	38,500
	Total IC =	$73,500
Other Costs (OC)		
Contractor's Fee	5% DC + IC Totals	18,500
Contingency	10% DC + IC Totals	37,000
	Total OC =	$55,500

Fixed Capital Investment (FCI) = Sum DC, IC, and OC Totals

FCI = $426,000

*Will handle 300 lbs starting material/day.

Table 5.11

FCI ENZYME PRODUCTION SCALED UP FROM ALDOLASE

All calculations are based on 426,000 FCI
shown in Table 5.10 for aldolase.

(1)   (4 enzyme plant) FCI $= 426,000 \left(\dfrac{175}{300}\right)^{0.7} = \$292,000$

(2)   The triokinase enzyme requires 140,000 lbs of starting
material, which is so large as to justify a separate
producing facility.

Daily triokinase starting material $= \dfrac{140000/2}{30} = 2330$ lbs

(triokinase plant) FCI $= 426,000 \left(\dfrac{2330}{300}\right)^{0.7} = \$1,790,000$

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Table 5.13

FIXED CAPITAL INVESTMENT (ATP REGENERATION)--
GLYCIDALDEHYDE TO GLUCOSE

The ATP regeneration facility requires a urea manufacturing
plant. A urea plant manufacturing 250 tons urea/day has a
fixed capital investment of $3,250,000* in 1966.

To manufacture 100 tons/day glucose from glycidaldehyde in
our facility will necessitate the production of 83.5 tons
urea/day. Hence, the fixed capital investment for the urea
plant to satisfy our requirement is

$$\text{FCI (urea plant only)} = 3,250,000 \left(\frac{83.5}{250}\right)^{0.67} = \$1,560,000$$
$$\text{(1966 cost)}$$

Not only will our urea plant operate at temperatures and
pressures above those used in normal urea manufacturing,
but it will also require three reactors plus separation
equipment to make carbamyl phosphatase which catalyzes the
regeneration of ADP to ATP. On this basis, the FCI for the
urea plant has been doubled.

Therefore, the FCI for the ATP regeneration facility to
convert glycidaldehyde to glucose is

$$\text{FCI} \begin{pmatrix} \text{ATP regeneration} \\ \text{for glucose mfg.} \end{pmatrix} = 2(1.56)(10^6)\left(\frac{138.5}{109.7}\right)^* = \$3,950,000$$
$$\text{(1972 cost)}$$

*Conversion to 1972 cost.

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Table 5.14

MANUFACTURING COST (PROCESSING)--GLYCIDALDEHYDE TO GLUCOSE

FCI

Processing	$2,330,000	(for repair & maint.)
Resin Loading	2,470,000	
	$4,800,000	(for depreciation)

Labor and Supplies	$/Day
Operating Labor, 144 man-hrs @ $4/man-hr	576
Supervision, 15% operating labor	86
Maintenance and Repair, 3% FCI	190
Operating Supplies, 15% maintenance	29
Laboratory, 15% operating labor	86
	$967

Fixed Costs	
Depreciation, 10% FCI	1,310
Taxes and Insurance, 3% FCI	394
	$1,704

Plant Overhead	
50% Operating Labor, Supervision and Maintenance	$426

Utilities	$1,000

Raw Materials (ex. glycidaldehyde)	
Resin Loss	292
Miscellaneous Chemicals	150
	$442

Total Daily Cost = $4,539

$$\frac{\text{Mfg. Cost (Processing)}}{\text{lb glucose}} = \frac{4539}{2000} = 2.26\text{¢/lb} \qquad \boxed{\approx 2.3\text{¢/lb glucose}}$$

Table 5.15

ENZYME MANUFACTURING COST--GLYCIDALDEHYDE TO GLUCOSE

	$/Day	
	4 Enzymes	Triokinase
Labor and Supplies		
Operating Labor, 72 man hours @ $4/man hr	288	288
Supervision, 15% operating labor	43	43
Maintenance and Repair, 5% FCI	40	245
Operating Supplies, 15% maint. and repair	6	37
Laboratory, 20% operating labor	58	58
	435	671
Fixed Costs		
Depreciation, 10% FCI	80	490
Taxes and Insurance, 3% FCI	24	147
	104	637
Plant Overhead		
50% Operating Labor, supervision, maint. and repair	186	288
Raw Materials	125	175
Utilities	80	120
Total	$930	$1,891

$$\frac{\text{Enzyme Mfg. Cost}}{\text{lb glucose}} = \frac{0.465\cancel{c}}{\text{lb}} \qquad \frac{0.95\cancel{c}}{\text{lb}}$$

Immobilization Cost = 30% Enzyme Mfg. Cost

$$\frac{\text{Total Enzyme Cost}}{\text{lb Glucose}} = 1.3(1.42) = \boxed{\approx 1.8\cancel{c}/\text{lb}}$$

Table 5.16

MANUFACTURING COST (ATP REGENERATION)--GLYCIDALDEHYDE TO GLUCOSE

ATP Regenerating Plant FCI	3,950,000	(for repair and maint.)
ATP Initial Loading	600,000	(for depreciation)
Total FCI	$4,550,000	

Labor and Supplies	$/Day
Operating Labor, 144 man hrs @ $4/.ran hr	576
Supervision (15% operating labor)	86
Maintenance and Repair (5% FCI)	540
Operating Supplies (15% maintenance)	81
Laboratory (15% operating labor)	86
	1,369

Fixed Costs	
Depreciation (10% FCI)	1,245
Taxes and Insurance (3% FCI)	374
	1,619

Plant Overhead	
50% Operating Labor, supervision and maintenance	601

Utilities	1,400

Raw Materials	
ATP Make-up	6,000
Miscellaneous Chemicals	1,000
	7,000

Total Daily Cost = $11,989

$$\frac{\text{Manufacturing Cost}}{\text{lb Glucose}} = \frac{11,989}{2,000} = 6.0\text{¢/lb}$$

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5.3 Carbon Dioxide Fixation Process

5.3.1 Flow Diagram and Description

A flow diagram of the entire carbon dioxide fixation process, including production of carbamyl phosphate, is shown in Figure 5.8. A rough material balance, based on one mole of starch, is presented in Table 5.17. Table 5.18 contains a list of net reactions taking place in the process.

The reactions that occur in the starch reactor (A) have already been discussed in Chapter 4. The advantages and unknown factors of the single reactor scheme proposed here have also been presented in Chapter 4. Contents of the starch reactor (A) are passed to a filter (B) where solid starch and $MgNH_4PO_4 \cdot H_2O$ are removed. The liquid (2) is reduced in pressure and stripped of product carbon dioxide in a gas-liquid separator (C). The remaining reaction mixture (3) is pumped back to the reactor.

Solid starch and $MgNH_4PO_4 \cdot 6H_2O$ (5) are washed with a small part of stream 18 to remove adhering enzyme and reaction mixture, and this wash is returned to the reactor (A). The solid is treated with 80°C water (C), which redissolves the starch. The $MgNH_4PO_4 \cdot 6H_2O$ remains as solid, since it is less soluble at high temperatures than at low [18]. Starch can then be reprecipitated by cooling (D1) and filtered (D2). The supernatant water is heated and reused for wash. $MgNH_4PO_4 \cdot 6H_2O$ is conveyed to a furnace (E) where it is heated to roughly 200°C to drive off water and ammonia (8) [19,20]. This gas stream is compressed (F), and together with a recycle stream of NH_2, CO_2, and water (9) from the ammonium carbamate decomposer (J) enters a gas-liquid separator (G) which is held at 100°C to decompose NH_4HCO_3. Two streams of water are taken off as liquid. The larger one (18) is returned to the starch reactor to make up the water removed in the $MgNH_4PO_4 \cdot 6H_2O$ precipitate. The smaller stream (19) is removed as product.

Gas from the separator (G) is compressed together with streams 11 and 12 from gas-liquid separators C and L, makeup CO_2 (13), and recycle from the carbamyl phosphate precipitation process (32) to provide a 200 atm feed for the production of urea.

Urea is produced at approximately 185°C by several well-known processes [20]. If the once-through process (which obtains approximately a 50 percent conversion in CO_2 when NH_3 is fed in excess) is used, the unreacted ammonium carbamate is decomposed by reducing the pressure to approximately 4 atm (J). Ammonia and carbon dioxide are recycled (9), while molten urea passes to a 400°C reactor (K), where it is decomposed to cyanic acid and ammonia [21,22,23]. This reactor, which is generally in the form of a fluidized quartz bed, has a hold time in the order of seconds. Reaction is complete because the products come off as gases, while the urea reactant is molten.

The products are separated by partial condensation (L), with the gas stream (12) which is predominantly NH_3, being recycled to the compressor and the liquid stream (21) which is mainly HOCN, being sent to the carbamyl phosphate reactor (M).
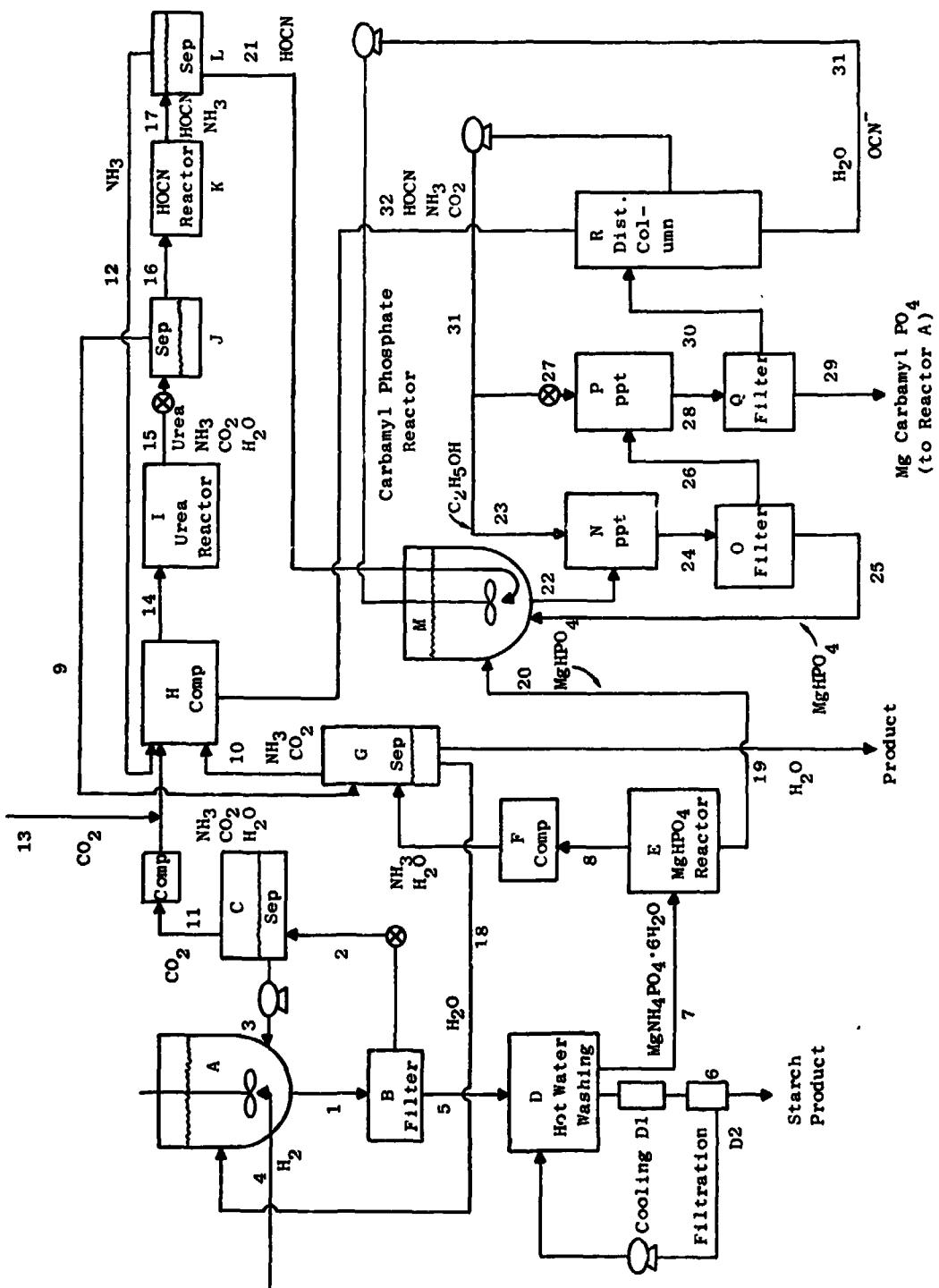
Figure 5.8. FLOW DIAGRAM OF CO_2 FIXATION PATHWAY.

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Table 5.17

MATERIAL BALANCES--CARBON DIOXIDE FIXATION PROCESS

Position in Figure 5.8

| Stream Component	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	16	19	20	21	22	23	24	25	26	27	28	29	30	31	32
H_2				12																												
NH_3, NH_4^+	12+	12+	+					18	74	90	18	18		110	74		18	+			2	2	+	2+		2+	+	2+		2+		2
CO_2, HCO_3^-			+						18	18	12		6	36	18			+					+	+	18	+	+	+		+	+	+
H_2O	++	++	++		1H			108	18+		+			+	18+			119	7			++	+	++		++	++	++		++	++	
Starch	1				1	1																										
$Na_2HPO_4\cdot6H_2O$	1R				1H		1R																									
$MgHPO_4$															18	18				18		18		18	18	18+		18+		18+		
$O=... NH_4OCNH_2$															11	11	20												16			
$OCH..., OCH^-$			+											12							27	18	+	1+		18+	+	18+		18+	9	9
C_2H_5OH																				1			++	++		++	++	++		+		
$N_2(CPO_3)_3^{=}...$	+	+	+																			18		18		18		18	16			
Sugar Phosphates	+	+	+																													
Additional information																																
Phase	s+z	s	z	g	g	s	s	g	g	g	g	g	g	g	z	z	g	g	z	s	z	z	z	s+z	z	z	z	z+z	z	z	z	z
T, °C	30	30	30	30	30	30	30	200	185	r100	30	r5C	30	185	185	185	400	r100	r100	200	r50	30	30	30	30	30	30	30	30	30	r100	r10
P, atm	r?	r? <0.1 0.1	<0,1 r?	r?	1	1	1	0.1	4	4	<0.1	4	4	200	200	4	4	4 r3	4	1 4	4 30	4	4	4	3	3	3	3	3	2	2 3	2

The numbers in the body of the table indicate the number of mols of each component at the listed location, all relative to 1 mole glucose as starch.

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Table 5.18

NET REACTIONS--CARBON DIOXIDE FIXATION PROCESS

Reactor A

$$18NH_2\overset{\overset{O}{\|}}{C}\overset{\overset{O}{\|}}{P}\underset{\underset{O^-}{|}}{O}^- + 18Mg^{++} + 12H_2 + 119H_2O \rightarrow$$

$$12CO_2 + 6C_6H_{10}O_5 + 18MgNH_4PO_4 \cdot 6H_2O$$

Reactor E

$$18MgNH_4PO_4 \cdot 6H_2O \rightarrow 18MgHPO_4 + 18NH_3 + 108H_2O$$

Reactor I

$$36NH_3 + 18CO_2 \rightarrow 18NH_2\overset{\overset{O}{\|}}{C}NH_2 + 18H_2O$$

Reactor K

$$18NH_2\overset{\overset{O}{\|}}{C}NH_2 \rightarrow 18HOCN + 18NH_3$$

Reactor M

$$18HOCN + 18MgHPO_4 \rightarrow 18NH_2\overset{\overset{O}{\|}}{C}\overset{\overset{O}{\|}}{P}\underset{\underset{O^-}{|}}{O}^- + 18Mg^{++}$$


Total

$$12H_2 + 6CO_2 \rightarrow C_6H_{10}O_5 + 7H_2O$$

Joining stream 21 at the carbamyl phosphate reactor are two streams of solid $MgHPO_4$, one from the $MgHPO_4$ reactor and one from filter O, and stream 31, which is composed of water with some $Mg(OCN)_2$ from the bottom of distillation column R. When Mg^{++} is replaced by K^+, OCN^- and $H_2PO_4^-$ react in liquid phase to give carbamyl phosphate in approximately 50 percent yield at pH 5.5 and approximately room temperature [24,25]. Assuming that the same behavior holds with Mg^{++}, we can separate the products and reactants by differential precipitation with ethanol [25]. Though no data are available, it is expected that $MgHPO_4$ would precipitate with the smallest amount of ethanol added, followed by Mg carbamyl phosphate. If this is the case, $MgHPO_4$ would be precipitated by adding ethanol (M), filtered (O), and sent back to the carbamyl phosphate reactor. The supernatant (26) would be contacted with more ethanol (P) and filtered (Q) to remove Mg carbamyl phosphate, which would be recycled to the starch reactor. The supernatant from this step, which would contain mainly water, ethanol, and magnesium cyanate along with some cyanic acid and ammonia and carbon dioxide from the breakdown of cyanate ion [26,27, 28] is sent to a distillation column (R). Here the vapor stream from the top of the column (32), which is mainly HOCN, NH_3, and CO_2, is recycled to the urea process, while a middle cut, containing mainly ethanol, goes for precipitation. The bottoms, which is water with $Mg(OCN)_2$, is recycled to the carbamyl phosphate reactor.

This process, including the production of Mg carbamyl phosphate, has been explained in detail because several steps are carried out differently than elsewhere in this report. For instance, because cyanate ion is exceedingly inhibitory to several enzymes found in the starch reactor [29,30] it could not be allowed to enter there. This forces the requirement that carbamyl phosphate be purified and added to the starch reactor so that its concentration in the reactor is sufficiently low that it has no chance to decompose to cyanate [24]. It was not possible, therefore, to react OCN^- and $H_2PO_4^=$ in the presence of ADP as suggested by Marshall [25].

In addition, the use of Mg^{++} as a precipitating agent dictates that other cations not enter the starch reactor since they would not easily be removed from it. This forced the abandonment of KOCN and KH_2PO_4 as reactants in the formation of carbamyl phosphate in favor of $MgHPO_4$ and HOCN.

5.3.2   Enzyme Production

An approach, similar in general to that described in the fossil fuel process (Figure 5.5) would be used in the production of enzymes here. A general process based on common features of enzyme purification techniques would result in a flow diagram similar to the one shown in the previous section (Figure 5.7) with various source possibilities accomodated as in Figures 5.4, 5.5, and 5.6. An additional source, chloroplasts, which would contain all of the necessary enzymes with the exception of those needed for ATP regeneration, is illustrated in Figure 5.9.

700 gm
Spinach

800 ml
distilled water

Waring
Blender
5 qt
Capacity

pH = 7.5

Fibrous Material

Cheese-
cloth
Filter

Extract

Glass
Wool
Filter

Unbroken Cells
and Cellular
Debris

Filtrate

Recentrifuge

Centrifuge
400 × g for
5 minutes

Supernatant
(discard)

Sediment

Suspend
1200 ml
water
5 minutes

Disintegrated
Chloroplasts
0.1 to 2 μ

700 mg

Note: Carr. out all operations at
temperatures close to 0°C

Figure 5.9.   PREPARATION OF CHLOROPLASTS.

Although chloroplasts would represent a convenient source from the standpoint of completeness, the level of some of the activities appears low. As an example, to produce enough aldolase for a 100 ton/day glucose factory would require processing 84,000 tons of spinach. This amount of spinach is believed to be typical for several enzymes obtained from chloroplasts; and unfortunately, several of the enzymes needed in the CO_2 fixation pathway have been obtained only from this source. References 31 through 35 at the end of this chapter give procedures for several photosynthetic enzymes.

5.3.3 Special Problems

Several difficulties exist which are unique to the total scheme proposed in Section 5.3. First, it is possible at pH 7.5 that product CO_2 will be removed from the reaction mixture by separator C only at sufficiently high bicarbonate concentrations that $MgCO_3$ will be precipitated in the reactor. This would be deleterious, for $MgCO_3$ under the present scheme would be passed predominantly to the carbamyl phosphate reactor, where carbonate has no role. This precipitation of $MgCO_3$ can be prevented by addition of more phosphate to the reactor or by acidification of the reaction mixture at the separator, followed by restoration of the pH to 7.5. The first expedient causes a very high and perhaps inhibitory concentration of inorganic phosphate, while the second adds by-products not easily removed and probably causes hydrolysis of the sugar phosphates in the reaction mixture.

Secondly, the carbamyl phosphate reactor has never been run with magnesium ions as is proposed here. While this is not expected to change the reaction to any extent, very little is known of the kinetics in any case, except that equilibrium is reached in approximately 150 minutes at room temperature at pH 5 or 6 [22].

Also, lithium carbamyl phosphate has been precipitated after lithium phosphate by ethanol in Marshall's lab [25]. While it is assumed that the magnesium salts will behave in the same manner with $Mg(OCN)_2$ remaining in solution as LiOCN does, there is no experimental evidence that this is so, since the solubility of these compounds in mixtures of ethanol and water is not known.

Until the rates of formation and precipitation of carbamyl phosphate are known better so that the hold time can be calculated, it is difficult to determine how much of the cyanate ion decomposes to CO_2 and NH_3.

5.3. Cost Factors

Because of the uncertainties already explained as to compatibilities and inhibition, and because the dependencies on concentration levels and separations are difficult from those in the cellulose and fossil fuel process, it is extremely difficult to design the carbon dioxide fixation process accurately and really impossible to calculate cost accurately. We do know, however, that there are approximately 17

enzymes involved in this process plus ATP and NADPH, and that they all have to be produced for every mole of starch.

If we do extrapolate the values for processing, enzyme production, and carbamyl phosphate production from the cellulose and fossil fuel processes (including conversion of glucose to starch) and make some alterations when the CO_2 fixation process has significant differences, we can arrive at a very rough order of magnitude manufacturing cost of $.77/lb for starch produced by the carbon dioxide fixation process (as shown below).

Processing	3¢/lb
Enzyme Production	10
ATP Regeneration	60 [36]
Raw Material	4
	77¢/lb

Though the carbon dioxide fixation process is currently too expensive for large-scale food production, it might be used to produce starch in one special case, that of an extended spacecraft mission. Since weight put into space costs approximately $100/lb [32], it becomes desirable to regenerate raw materials. This is not easily done in the cellulose or fossil fuel processes, but the carbon dioxide fixation process can be perfectly balanced with other processes in the spacecraft:

$$12H_2 + 6CO_2 \rightarrow C_6H_{10}O_5 + 7H_2O \qquad \text{carbon dioxide fixation process}$$

$$C_6H_{10}O_5 + 6O_2 \rightarrow 6CO_2 + 5H_2O \qquad \text{astronaut respiration}$$

$$12H_2O \rightarrow 12H_2 + 6O_2 \qquad \text{electrolysis}$$
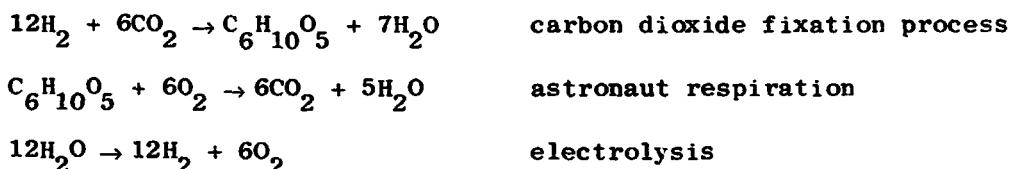
Enzymes, ATP and NADP, for makeup can be brought along, since they weigh very little. If total plant weight were sufficiently low and the mission sufficiently long so that required food weight were high, it is quite possible that a small carbon dioxide fixation plant would be economical on board a spacecraft.

5.4 Glucose to Starch

5.4.1 Flow Chart and Summary Description

The flow diagram of the glucose to starch process is shown in Figure 5.10. The mainstream indicated by the heavy line follows the processing of the glucose to the final product which is solid (moist) starch. The basis used for plant design was 100 tons per day of product.
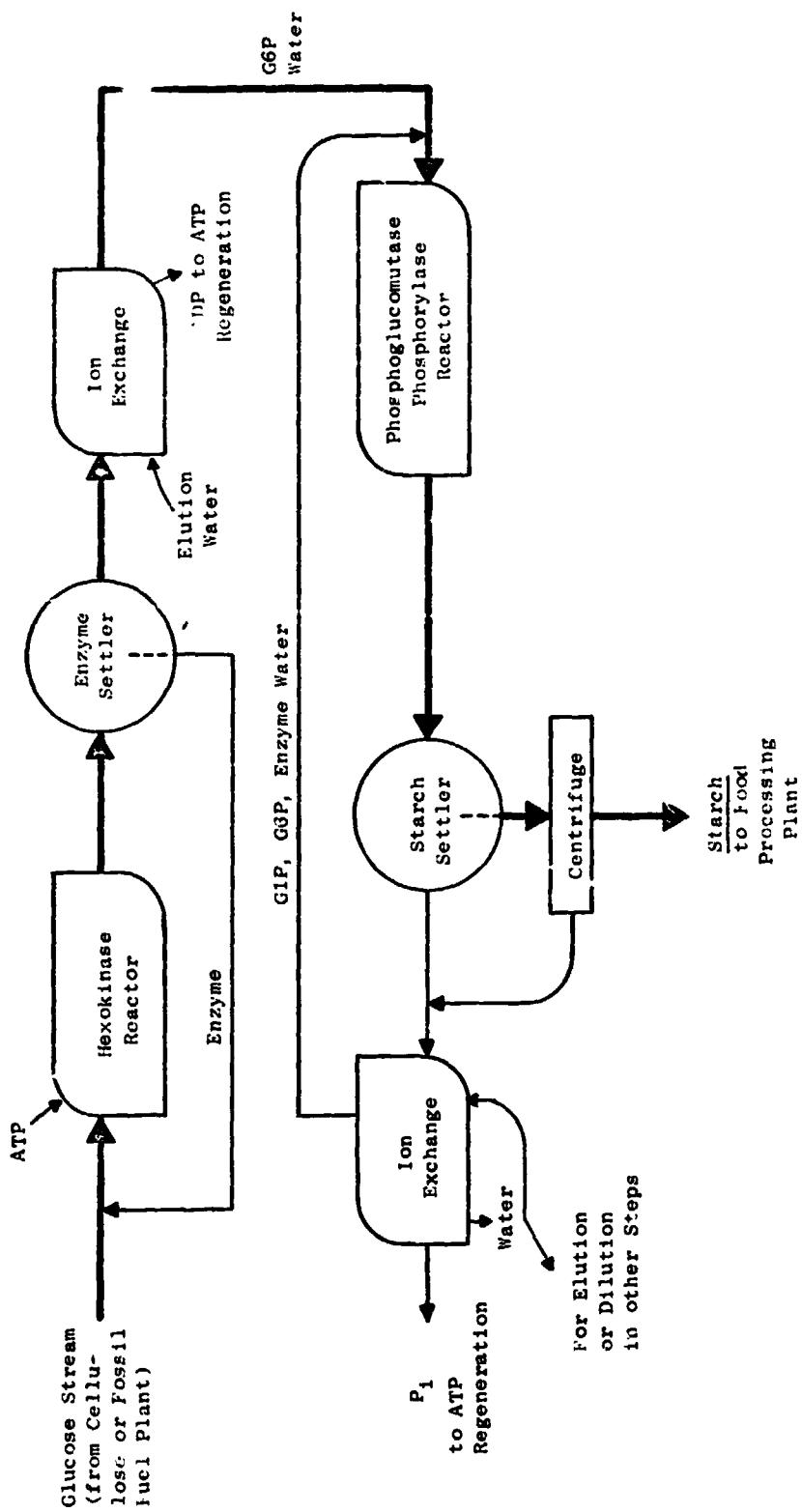
Figure 5.10. GLUCOSE TO STARCH FLOW DIAGRAM.

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5.4.1.1 Process Description

Process Input

The process input is a water-carried glucose stream from either the cellulose or fossil fuel plant. The glucose concentration in this stream is approximately 0.05 M if from the fossil fuel plant, and about 10 percent by weight if from the cellulose plant.

Hexokinase Reactor

ATP is added to the glucose stream as it enters this stirred tank reactor. Water is also added, if needed, such that a 0.10 molar concentration of glucose plus ATP is maintained. The enzymes in the reactor are immobilized on glass beads or other suitable material. The output flow stream from this reactor is water containing glucose-6-P, ADP, and a portion of the immobilized enzymes.

The reactor output enters a gravity type clarification or settling basin. Here the immobilized enzymes are settled out and continuously recycled back to the reactor in a concentrated slurry, thus conserving the hexokinase concentration in the reactor. The settler overflow is routed on to an ion exchange unit.

This ion exchange unit selectively adsorbs the ADP which, when eluted from the column is recycled through the ATP regeneration plant. The glucose-6-P and all the mainstream process water is passed through the exchanger and is routed to the phosphoglucomutase-phosphorylase reactor. This ion exchanger unit is actually a three tank unit sized such that a one hour duty cycle calls for a one-on-line and two-off-line operation.

Phosphoglucomutase-Phosphorylase Reactor

The phosphoglucomutase-phosphorylase reactor, as the name implies, is a two enzyme reactor. The basic reactor input, glucose-6-P, is converted by the phosphoglucomutase enzyme to glucose-1-P which in turn is converted to starch by the phosphorylase enzyme. The enzymes in the reactor are solubilized rather than immobilized to circumvent problems associated with immobilized enzyme/insoluble starch separation.

Conversion of glucose-6-P to glucose-1-P proceeds at such a slow rate that only 17 percent conversion is affected by a single pass through the reactor. After starch separation is accomplished, the reactor output of glucose-6-P and glucose-1-P are recycled back to the reactor input stream in such a way that a 0.10 molar concentration is maintained in the total reactor input stream.

The reactor output stream, containing glucose-6-P, glucose-1-P, starch, enzymes, and water, is routed through a starch separation unit. This unit is a gravity type settler designed so that the starch settles to the bottom where it can be removed from the unit as a heavy slurry. The starch slurry is then passed through a continuous centrifuge

where the starch is exited as a moist solid ready for transporting to a food processing plant. The centrifuge supernatant is mixed with the starch settler overflow and routed to an ion exchange unit.

The ion exchange unit acts as a retarding column whereby all inputs other than water are adsorbed. The water is recycled around the unit effecting selective elution of the various adsorbed materials. As the inorganic phosphate is eluted, it is recycled to the ATP regeneration plant. The glucose-1-P, glucose-6-P, and enzymes are recycled to the reactor as they are eluted. The "excess" water at this point is then available for use at other points in the plant; e.g., elution water for ion exchange columns or dilution water to maintain desired reactor molarity.

5.4.2 Preparation of Enzymes

Three enzymes are used in the glucose to starch process: hexokinase, phosphoglucomutase (PGM), and phosphorylase. Hexokinase can be obtained from yeast and phosphorylase from Escherichia coli or potatoes [31,32]. PGM has been identified in yeast, potato, Neurospora crassa, rabbit muscle, E. coli, and other sources [33].

The general design of an enzyme plant and factors used in extrapolating this to more than one enzyme have been given elsewhere (5.2.3). Briefly, a facility designed for aldolase is used as a model, with scaling factors based on the amounts of starting material needed.

The amount of starting material needed in this case was estimated from laboratory production results. For the 100 ton/day glucose-starch plant, 96,000 lb_m of E. coli are needed for producing PGM, and 54,000 lb_m of yeast are needed for producing hexokinase. If phosphorylase can be extracted along with PGM from E. coli, no additional starting material is required. However, if this is not possible, an additional 72,000 lb_m of E. coli is needed.

5.4.3 Economics and Cost Analysis

As before, the glucose to starch conversion is divided into three major areas for cost analysis purposes; processing, enzyme production, and ATP regeneration.

5.4.3.1 Major Items of Equipment

Lists of equipment are based on considerations already outlined in Sections 5.1.5 and 5.2.5. Table 5.19 gives the data for processing. Enzyme production is based on the equipment list for aldolase (Table 5.6). ATP regeneration is based on urea plant costs, as explained in Section 5.2.5.1.

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5.4.3.2 Fixed Capital Investment

The basis for calculations has been explained in Section 5.2.5.2. Tables 5.20, 5.21, and 5.22 show the results for processing, enzyme production, and ATP regeneration.

5.4.3.3 Manufacturing Costs

Tables 5.23, 5.24, and 5.25 give the estimated manufacturing costs involved in processing enzyme production, and ATP regeneration. Estimates were made in a similar way to that in Section 5.2.5.3. No raw material costs have been included, as the input is assumed to be the effluent from the glucose production plant.

5.4.3.4 Summary

A summary of the costs for glucose to starch appears below.

Step	FCI	Mfg. Cost, ¢/lb Starch
Processing	$2.03 million	1.0
Enzyme Production	2.73 million	1.4
ATP Regeneration	2.73 million	3.4
	$7.48 million	5.8

A sensitivity analysis is shown below with respect to FCI, and to major items in the calculation of manufacturing cost for the sum of all three phases.

	% Change	Absolute Change	Change in ¢/lb Starch
FCI	25	$1.87 million	0.46
Operating Labor	25	3 men/shift	0.3
Utilities	50	$665/day	0.33
ATP Replacement	33 1/3	$1000/day	0.5
Resin Replacement	200	$70/day	0.04

Table 5.19

PURCHASE COST OF EQUIPMENT--GLUCOSE TO STARCH

Number Required	Item Description	'72 $ Each	Total $
Ion Exchanger Tanks (carbon steel, plastic coated)			
3	IE-4, 25,000 gal each	9,000	27,000
3	IE-5, 106,000 gal eacn	28,000	84,000
			111,000
Reactors (carbon steel, with agitator and drive unit)			
1	R-7, 500 gal	3,000	3,000
1	R-8, 150,000 gal	51,500	51,500
			54,500
Settlers			
1	S-7, 18,500 cubic feet	27,300	27,300
2	S-8 and Starch Separator, 31,500 ft^3 each	57,900	115,800
			143,100
1	Centrifuge, 32" diameter, 304 stainless steel	34,800	34,800
5	Pumps, 2000 gal/minute, including motor	3,750	18,750

Total Purchase Cost = $362,150

≈ $362,000

Table 5.20

FIXED CAPITAL INVESTMENT (PROCESSING)--GLUCOSE TO STARCH

Direct Costs (DC)	% Equipment Cost	Cost, $
Equipment, purchased cost	100	362,000
Installation	45	163,000
Instruments and Controls	15	54,300
Piping, installed	50	181,000
Electrical, installed	10	36,200
Buildings, including services	15	54,300
Yard Improvements	10	36,200
Service Facilities, installed	40	145,000
Land	6	21,700
	Total DC =	$1,053,700

Indirect Costs (IC)		
Engineering and Supervision	32	116,000
Contractor Expense	40	145,000
	Total IC =	$261,000

Other Costs (OC)		
Contractor's Fee	5% DC + IC Totals	65,700
Contingency	10% DC + IC Totals	131,500
	Total OC =	$197,200

Fixed Capital Investment (FCI) = Sum DC + IC + OC Totals

FCI (Processing) = $1,511,900   say $1,512,000

RESIN COST

Ion Exchanger	Resin Volume, ft^3	$/ft^3	Total $
IE-4	5,570	20	112,000
IE-5	20,200	20	405,000
			$517,000

Table 5.21

ENZYME PRODUCTION (FCI)--GLUCOSE TO STARCH

Enzyme	Starting* Material (lbs)	Steady** State (lbs)	Producing Days/ Month	Daily Start- ing Material (lbs)
Hexokinase	54,000	27,000	7	3,860
Phosphoglucomutase	110,000	55,000	13	4,230
Phosphorylase	72,000	36,000	10	3,600
	236,000	118,000	30	say 4,250

$$\text{FCI (starch enzymes)} = 426,000 \left(\frac{4250}{300}\right)^{0.7} = \$2,730,000$$

The three enzymes listed above will be manufactured in the same fa-
cility with each enzyme produced for a certain number of days each
month and stored until needed. Storage life of the enzyme is as-
sumed to be two months with a 30 day half life when in use.

Table 5.22

FIXED CAPITAL INVESTMENT (ATP REGENERATION)--GLUCOSE TO STARCH

The FCI for an ATP regeneration facility to convert glycidaldehyde
to glucose is $3,950,000. It will regenerate two ADP units. How-
ever, the glucose to starch facility requires regeneration of only
one ATP unit. Therefore, conversion of glucose to starch requires
an ATP facility that is half that required for glycidaldehyde to
glucose. Hence,

$$\text{FCI} \begin{pmatrix} \text{ATP regeneration for} \\ \text{glucose to starch} \end{pmatrix} = 3.95 (10^6)\left(\frac{1}{2}\right)^{0.7} = \$2,430,000$$

The investment for the initial amount of ATP is

$$\frac{\text{ATP loading}}{\text{investment}} = \frac{500,000 \text{ grams}}{\$0.60 / \text{gram}} = \$300,000$$

Table 5.23

MANUFACTURING COST (PROCESSING)--GLUCOSE TO STARCH

```
                         FCI
     Processing       $1,512,000   (for repair and maintenance)
     Resin Loading       517,000

                      $2,029,000   (for depreciation)
```

Labor and Supplies	$/Day
Operating Labor, 72 man-hrs @ $4/man-hr	288
Supervision, 15% operating labor	43
Maintenance and Repair, 3% FCI	124
Operating Supplies, 15% maintenance	18
Laboratory, 15% operating labor	43
	$516

Fixed Costs	
Depreciation, 10% FCI	556
Taxes and Insurance, 3% FCI	1C7
	$723

Plant Overhead	
50% Operating Labor, Supervision and Maintenance	$228

Utilities	$400

Raw Materials	
Resin Loss	35
Miscellaneous Chemicals	100
	$135

Total Daily Cost = $2,002

$$\frac{\text{Mfg. Cost (Processing)}}{\text{lb Starch}} = \frac{2,002}{2,000} = 1\cancel{c}/\text{lb}$$

Table 5.24

ENZYME MANUFACTURING COST--GLUCOSE TO STARCH

Labor and Supplies	$/Day
Operating Labor, 96 man hours @ $4/man-hr	384
Supervision, 15% operating labor	58
Maintenance and Repair, 4% FCI	300
Operating Supplies, 15% maintenance	45
Laboratory, 20% operating labor	77
	$864
Fixed Costs	
Depreciation, 10% FCI	750
Taxes and Insurance, 3% FCI	225
	$975
Plant Overhead	
50% Operating Labor, Supervision and Maintenance	$371
Raw Materials	$175
Utilities	$180
	$2,565

Immobilization Cost = 10% Enzyme Cost (2 enzymes not immobilized)

$$\frac{\text{Total Enzyme Cost}}{\text{lb Starch}} = \frac{2565}{2000} \Big/ 1.1 \approx 1.4 \text{¢/lb starch}$$

Table 5.25

MANUFACTURING COST (ATP REGENERATION)--GLUCOSE TO STARCH

```
                              FCI

     ATP Regeneration    $2,430,000   (for repair and maintenance)
     ATP Loading            300,000

                         $2,730,000   (for depreciation)
```

Labor and Supplies	$/Day
Operating Labor, 120 man-hrs @ $4/man-hr	480
Supervision, 15% operating labor	72
Maintenance and Repairs, 5% FCI	333
Operating Supplies, 15% maintenance	50
Laboratory, 15% operating labor	72
	$1,007

Fixed Costs	
Depreciation, 10% FCI	748
Taxes and Insurance, 3% FCI	249
	$997

Plant Overhead	
50% Operating Labor, Supervision and Maintenance	$442

Utilities	
	$750

Raw Materials	
ATP Make-up	3,000
Miscellaneous Chemicals	500
	$3,500

Total Daily Cost = $6,696

$$\frac{\text{Mfg. Cost (ATP Regeneration)}}{\text{lb Glucose}} = \frac{\$6,696}{\$2,000} \approx 3.4\text{¢}/\text{lb glucose}$$

Modification for Fossil Fuel to Starch

The conversion of glycidaldehyde to glucose required two moles of ATP per glucose formed. Since the cost in a facility is not directly proportional to the amount of material processed, the use of a single ATP regeneration plant for both the formation and polymerization of glucose modifies the cost factors for these processes. The FCI for the ATP regeneration facility for the total glycidaldehyde to starch process was calculated to be $5,250,000, and the initial loading investment to be $900,000.*

On this basis a total manufacturing cost for ATP regeneration of 8.6¢ per lb of starch is calculated in Table 5.26. A summary of total costs for glycidaldehyde to starch including this modification is shown below.

Enzyme	FCI	¢/lb Starch
Processing	$6.83 million	3.3
Enzyme Production	4.81 million	3.2
ATP Regeneration	6.15 million	8.6
Glycidaldehyde	Purchased	17.0
	$17.79 million	32.1

*Calculated in a similar manner to that given in Table 5.13.

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Table 5.26

MANUFACTURING COST (ATP REGENERATION)--GLYCIDALDEHYDE TO STARCH

FCI

ATP Regeneration	$5,250.000	(for repair and maintenance)
ATP Loading	900,000	
	$6,150,000	(for depreciation)

Labor and Supplies	$/Day
Operating Labor, 168 man-hrs @ $4/man-hr	672
Supervision, 15% operating labor	101
Maintenance and Repairs, 5% FCI	720
Operating Supplies, 15% maintenance	108
Laboratory, 15% operating labor	101
	$1,702

Fixed Costs	
Depreciation, 10% FCI	1,685
Taxes and Insurance, 3% FCI	561
	$2,246

Plant Overhead	
50% Operating Labor, Supervision and Maintenance	$746

Utilities	$2,000

Raw Materials	
ATP Make-up	9,000
Miscellaneous Chemicals	1,500
	$10,500

Total Daily Cost = $17,194

$$\frac{\text{Mfg. Cost (ATP Regeneration)}}{\text{lb Starch}} = \frac{17,194}{2,000} = 8.59 \qquad \boxed{\approx 8.6\cancel{c}/\text{lb starch}}$$

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Chapter 6

FOOD TECHNOLOGY

6.1 Introduction

It is the purpose of this chapter to define and characterize the
potential outputs available from a synthetic carbohydrate plant and to
indicate the ways they may be utilized in a general balanced diet. The
nutritional requirements for a balanced diet have already been discussed
in Section 2.1.3.

Food contributes to physical, mental and emotional health. In addi-
tion to nourishing the body, food eaten in a favorable setting contributes
to one's sense of well being. Eating habits are influenced by social,
moral, and psychological factors to a much greater extent than by the
explicit nutritional needs of a person. A given food may be eaten or
considered unacceptable for a variety of reasons. For example, certain
foods are consumed as status symbols while others are rejected because
of religious taboos. In general, it appears that the less developed a
community is, the more conservative it is likely to be in its choice of
food. It is the highly developed economies that show the greatest degree
of adventure in their approach to new foods [1].

6.2 Factory Products: Current and Potential Utilization

There are a number of processes and required reactions necessary
for the production of synthetic carbohydrates. At the end of each pro-
cess a potentially useful product is produced. In order of production
complexity the products are fructose, glucose, and starch. Each product
has its nonsynthetic counterpart which is presently utilized by the food
processing industry in a variety of ways. It is the purpose of this sec-
tion to describe and characterize each carbohydrate form and indicate by
current standards how it is or might be used. Because of the general na-
ture of the carbohydrates, the list of applications can never hope to be
completely exhaustive. For that reason only a limited number of specific
applications will be presented.

6.2.1 Fructose

Fructose, a six-carbon sugar, is the free sugar found in
honey, some fruits and combined with glucose in sucrose [2]. It is
frequently called fruit sugar or levulose and is 173 percent as sweet
as sucrose. One of the primary uses of fructose is in the candy indus-
try as a sweetener and as an additive to prevent crystallization of su-
crose in soft candies.

6.2.2 Glucose

Glucose like fructose is also a six-carbon sugar which occurs naturally in fruits, honey, and plant tissues. It is also called dextrose and is approximately 75 percent as sweet as sucrose.

Glucose is the most efficient form of energy source for the body, since, in the process of digestion, it is absorbed directly by the intestine and passed into the blood. For this reason it is sometimes called blood sugar, and when necessary, can be fed intravenously.

Glucose is used in the food processing industry in a number of ways. In the baking industry, one of its largest consumers, it is used as a fermentable sugar, flavor and aroma former, and crust color enhancer. In the beverage and canning industries large quantities are used to supply sweetness, body, osmotic pressure, and contribute to the color retention of certain products. It is used as a sweetener and consistency regulator in the candy industry and in frozen desserts, by the dairy industry, to improve flavor and prevent oversweetness [4,5,6].

6.2.3 Starch

Starch can be chemically classified into two broad classes called amylose and amylopectin. (Amylose is a linear polymer consisting of about 400 glucose units whereas amylopectin is a branched polymer having a higher molecular weight.) Most starches derived from conventional products such as wheat, rice, potato, and so on are composed of approximately 25 percent amylose and 75 percent amylopectin [7,8]. The synthetic carbohydrate factory would ordinarily output amylose but can also produce amylopectin by the addition of another enzymatic step.

Starch is the principle carbohydrate of the diet and the major source of energy for man and animals. Its uses are broad and diverse with abundant applications in many food processing industries. A principle consumer is the bakery industry where large amounts are used both independently and in conjunction with flours to make breads, cakes, crackers, cookies, and to act as thickening agents for pie fillings. Other bakery related applications include its use in the production of noodles, pastas, and snack foods such as ice cream cones [9].

Starch is used in the confectionary industry in a number of applications, notably that of providing consistency to gum type candies.

Cross-linked starches are particularly useful in applications such as instant puddings, batters for deep frying, salad dressings, and thickeners for gravies, sauces, cream soups, baby foods, and fruit puddings.

Because of its ability to form strong flexible films, amylose starch is used extensively in the food industry as a binding and coating agent [10].

6.3 Augmentation

6.3.1 Nutritional Factors

There are several strategies that can be adopted to implement a synthetic carbohydrate into a diet. To a great measure the particular strategy adopted will depend on which of the carbohydrate forms discussed in Section 6.2 above is considered. In the discussion which follows consideration will be directed toward three possible alternative strategies.

6.3.1.1 Substitution of Synthetic for Natural Starch in Standard Recipes

Each of the three products (fructose, glucose, and starch) could be used as a substitute for its nonsynthetic counterpart in familiar foods of a contemporary nature, such as breads, cakes, puddings, batters, noodles, pastes, cereal substitutes, beverages, and so on. With no new or novel food forms considered, the price, quality, and availability of the synthetic carbohydrate, relative to that of natural carbohydrate would be the limiting factor. Supplementation with other essential nutrients would be a matter of judgment relating to the role such products in fact play in peoples' diets, much as this is done today in cereal products, for example.

6.3.1.2 Development of a "Complete" Food

Under situations where an acute food shortage exists, it may be necessary to formulate a complete food to meet all the nutritional requirements outlined in Chapter 2. The most useful carbohydrate forms will be glucose and starch which can become the building blocks of staple foods. Where possible the product configuration should be similar to familiar products so that rejection is minimized. In some cases it may be necessary to introduce the synthetic food into the diet in a novel way. In any event, regardless of the method used, great care in its introduction will be necessary, since many problems can arise. For example, a poor substitute of a natural food is more likely to be rejected than a novel commodity.

6.3.1.3 Synthetic Foods for Animals

This strategy is viable for both an abundant or scarce natural food supply. Its importance when food supplies are scarce is obvious. That is, if animals can be fed synthetically produced feed this frees the natural carbohydrate food formerly committed to their production to humans. The reward of this strategy is two-fold since not only does the animal-committed natural carbohydrate become diverted to human use but so does the animal. Thus, in one broad stroke the system, when optimized, is capable of providing not only the carbohydrate needs but the protein needs of the human diet as well.

When food is abundant, this strategy still remains viable provided that synthetically produced carbohydrates can be produced more economically than natural ones.

All is not utopia, however, since the animal must be made to eat the product. It is true that animals may not be as discriminating as humans, but they must still be provided with a nutritious diet on which they can grow and thrive. Also, in the sense of fair play, they should at least be provided with a feed that is reasonably palatable.

6.3.2 Fabrication

Food types ranging from breads to beverages can be fabricated from synthetic carbohydrates. Each type w'll require a particular handling technique depending on the carbohydrate employed, the desired product configuration, and, if used, the nature of enrichment additives. Some products will require little or no special handling over that which is ordinarily done when using naturally produced carbohydrates. Other products will require significantly more handling to make them attractive and acceptable. For example, synthetically produced fructose or glucose can be substituted for their natural counterparts in ex'sting recipes with no additional handling processing On the other h? 'd, if glucose is to be utilized as the major energy source in a substantial portion of the diet, then special recipes will have to be devised to produce foods which will be acceptable in large quantities over long periods of time.

A s'milar situation exists if synthetic starches and flours are used in bread products. The familiar consistency, crumb texture and cell structure of breads made from wheat flour is produced primarily by the gluten or wheat proteins. In order to duplicate those qualities or produce a reasonable facimile, binders and other additives will be necessary when synthetic starch is used.

If a universal food is to be devised which will meet not only the energy requirements but also all of the nutritional requirements of a diet, then proteins (balanced essential amino acids), fats, vitamins, and minerals will have to be included in the product. When this is done, the final product will still have to meet the requisite of being attractive in appearance, acceptable in aroma, texture and taste, and yet not be toxic when large quantities are injested.

In the remainder of this section several types of potential food products will be discussed. Some will be fashioned after existing products already on the market and in some cases suggestions for enrichment will be presented.

6.3.2.1 Beverages

Gatorade, manufactured by Stokely-Van Camp, Inc. and Cal-Power, a product of General Mills, are two beverages currently available on the market which were designed to supply energy calories and fluids to the body.

Gatorade thirst quencher is essentially a citrus flavored
5 percent glucose solution with additions of sodium, potassium, chloride
and phosphate to achieve appropriate electrolyte ionic concentrations.
Its caloric value is approximately seven calories per ounce [11].

Cal-Power is a ready-to-drink high calorie beverage made
from mono and polysaccharides. The approximate composition is 10 percent
glucose, 40 percent maltose (di, tri, tetra-saccharides and higher), and
50 percent flavored water with small traces of minerals. Its caloric
value is 71.8 calories per ounce [12].

Similar products can be produced using synthetic glucose.
As suggested by the two examples above, functions can range from low cal-
orie thirst quenchers to relatively high calorie energy sources. In the
case of Cal-Power, where polysaccharides are utilized, they can be ob-
tained by hydrolyzing amylose [13], one of the alternative outputs of a
synthetic carbohydrate plant. If desired, the beverages can be enriched
with proteins, fats, vitamins, and minerals to produce a more complete
food or food substitute. Some precedent has been set for the production
of protein enriched beverages which are currently used as nourishment
aids in protein deficient communities. One such beverage is ProNutro,
which was developed by the University of Natal for use in South Africa.
It is composed of maize, peanuts, soya, skim milk powder, wheat germ,
protein concentrates, vitamins, sugar, and iodized salt. Its protein
content is 22 percent and is presently being manufactured by Hinds Food
Company [14].

It is conceivable that a total combined product can be
formed by using protein sources, vitamins, and minerals in conjunction
with synthetic glucose to produce a nutritious energy food. Some prob-
lems of ingredient solubility, flavor retention, and storage may arise
but there seems to be sufficient technology and precedent established
for overcoming them.

There are a number of ways in which such a product might
be served:

(1) as a noncarbonated beverage with or without refrigeration,

(2) as a chilled carbonated beverage,

(3) as a beverage sweetener,

(4) in frozen form as a popsicle or frozen dessert

(5) as a syrup sweetener for fruits.

Several different flavors ranging from colas to fruit
could be offered to provide variety and/or suit regional preferences.

6.3.2.2 Puddings

A number of canned dessert puddings have become available
in the past few years. One example is Hunt's Snack Pack produced by
Hunt Wesson Foods, Inc. The product is composed of approximately 90%

carbohydrates in the form of sugars and starches and 10 percent fats with small traces of minerals, proteins, and flavoring agents [15]. Its caloric value is 35 calories per ounce. A similar product could readily be produced using synthetic glucose and starch, which as in the case of the beverages discussed above, can be protein enriched and flavored for variety and/or regional taste preferences.

Alternate marketing configurations could include frozen or refrigerated dessert puddings. Since handling and storage problems are much more severe for frozen and refrigerated products, it is likely that canned forms requiring no refrigeration would be more easily introduced and used in underdeveloped areas suffering from food shortages.

6.3.2.3 Pastas

A carbohydrate form having a long history of acceptance by many cultures is that of noodles or pastas. Traditionally, noodles and pastas have been made from various grain flours which, in addition to starch, include fats, fibers, proteins, and minerals. On a dry weight basis, they consist typically of approximately [16] 85 percent carbohydrates, 7 percent proteins, and 3 percent fats. Moisture, vitamins, and minerals constitute the remaining proportion. Some precedent has been set for producing low protein content macaroni for persons on restricted diets requiring a limited intake of protein, gluten, phenyl-alamine, or other amino acids. A product called Cellu Lo/Pro Pasta [17] is composed of wheat starch, vegetable gums, calcium phosphate, hydrogenated vegetable fat, titanium dioxide, and artificial coloring. The nutrient distribution is approximately 83 percent carbohydrate, 0.4 percent protein, 0.6 percent fat, 3 percent fiber, 1 percent minerals, and 10 percent mositure. Its caloric value is 89 calories per ounce.

By using synthetic starch with appropriate additives, similar products could be produced. If desired, they could be augmented with additional nutrients (vitamins, minerals, and proteins) for use in areas where nutrient deficiencies exist. It is anticipated that similar handling and processing techniques involving mixing, extruding, drying, and packaging currently practiced by the industry in the production of traditional noodles and pastas could equally well be used in the production of artificial macaroni and noodle products.

6.3.2.4 Bread

Like noodles, bread and bread-like products traditionally have been made from grain flours. For example, the familiar consistency and texture of wheat breads is derived primarily from the gluten contained in the wheat flour. If a pure starch containing little or no gluten is used to make breads, cakes, muffins, and so on, a binding agent such as glyceryl monosteorate must be added [18] to provide coherence among the starch granules so that the finished product will have an acceptable texture and cell structure.

Some precedent has been set in producing breads and cakes from wheat and nonwheat starches and flours. Reasonable texture has been achieved using mixtures of cassava, wheat and/or soya flours and starches [19]. Furthermore, two companies, General Mills and Chicago Dietetic Supply, are presently producing low gluten wheat starches for use in the production of breads and cakes for restricted protein diets. Their products, Cellu [20] and Paygel-P are similar and contain approximately 87 percent carbohydrate, 0.3 percent protein, 1 percent fat, 11 percent moisture, and provide about 90 calories per ounce. Both companies provide recipes [22,23] for breads, cakes, cookies, muffins, and biscuits. It is reasonable to assume that synthetic starches, with minor augmentation, will function equally as well in each recipe. If desired, the product can be enriched for use in areas where protein or other deficiencies exist.

The list of applications for synthetic carbohydrates is by no means meant to be exhaustive. Several examples have been presented to show how the potential products available from a synthetic carbohydrate plant can be included in a diet. Further applications are limited only by a food designer's imagination and the regional preferences of a population. For comparison of composition, the commercially available products described above are listed in Table 6.1

Table 6.1

COMPOSITION OF COMMERCIALLY AVAILABLE CARBOHYDRATE PRODUCTS

Product Precedent	Type	Composition	Cal/Oz
Gatorade (Stokely-Van Camp)	Beverage	5% Glucose Salts, Flavoring No Fat or Protein	~7
Cal-Power (General Mills)	Beverage	10% Glucose 40% Maltose Salts, Flavoring No Fat or Protein	~70
Snack Pack	Pudding	~90% Carbohydrate ~10% Fats + Flavoring & Minerals	~120
Cellu Lo/Pro (Chicago Dietetic Supply)	Pasta	~80% Starch 3% Fiber 0.6% Fat 0.4% Protein 10% Moisture	~100
Paygel-P (General Mills)	Wheat Starch	87% Carbohydrate 1% Fat 0.3% Protein 11% Moisture 0.15% Fiber	~100

6.3.3 Acceptability

The acceptability of a food depends on a great many factors ranging from taste and appearance to simply that of available alternatives. Perhaps the single most important factor is that of cultural customs. For example, in certain parts of the world, puppies are considered a delicacy whereas in other parts the thought of eating dog meat is abhorrent.

In recent years, a number of attempts have been made to introduce protein enriched foods into populations suffering from protein malnutrition. In some cases, a reasonable degree of success has been achieved, while others have met with failure. A notable example is the successful introduction of Incaparina [24], a protein enriched blend of maize and cotton seed flour, into Guatemalan diets. It is generally felt that the success was primarily due to good advertising aimed at all segments of the populace not merely those families having low purchasing power and the fact that it was supplied as a bland flour which could be cooked and flavored at home [25]. Attempts to market a similar product in El Salvador failed. In that case, it is felt that the failure was due to the fact that the product was poorly packaged and had been promoted more as a medicine than as a food [26]. A second attempt was made to reintroduce the product in a sweetened cinnamon flavored form, but that too failed.

From these examples, one can conclude that a number of important criteria must be met if a new and perhaps novel food is to be introduced. First of all, the product must be culturally acceptable. In addition, it must be desired on its own merits and be priced at a figure that people can and will pay. It must have advantage over its competitors in quality, price, and nutrition and must be identifiably packaged.

Furthermore, there must be a clear marketing concept and advertising strategy which informs the potential consumer of the products' existence and shows them how and why it can and should be used.

In the case of carbohydrates, specifically glucose and starch, it would appear that staple foods could readily be produced which would not be in conflict with cultural preferences. As in the case of Incaparina in Guatemala, synthetic starch with appropriate additives could equally well serve as a flour type produce which could be cooked and flavored by the consumer to meet individual tastes. Of course, if an acute natural food shortage existed, there would be less hesitancy to use synthetic starches and it is likely that food in most any form would be accepted.

If a novel food is being anticipated, it clearly must not be in conflict with cultural customs, preferences, or taboos. Market surveys and test samplings of sufficient duration should be made to insure that the product will not only be accepted but will be used by a significant percentage of the population. Great care must be taken to insure that the product is in a desirable form when initially introduced. If it is not, and it is subsequently rejected, it becomes very difficult if not impossible to reintroduce it and expect it to

be accepted. A notable example of slow acceptance was that of margarine which had to live down the memories of early war-time poor quality [27].

Peoples food habits can be changed if a desirable, inexpensive, nutritional product is made available. There are many instances where foods formerly not esteemed now make significant contribution to the diet. In Japan, thousands of tons of fish sausage are eaten annually though it was almost unknown a few years ago. In Hong Kong, a soft drink made from soya-beans has captured a quarter of the market and in India significant quantities of ground-nut flour is being eaten. In Uganda, 14 percent of the babies were bottle fed in 1950 and 42 percent in 1959. In France, Parisians have taken to drinking whiskey [28].

In summary, in order to introduce a new food and expect it to be acceptable, there are several factors which must be considered. A few are:

(1) The food must not be in conflict with cultural preferences.

(2) It must not be associated with any taboos, religious, or otherwise.

(3) It must have desirable characteristics of taste, aroma, eye appeal, texture, mouth feel, storage life, and flavor retention.

(4) Sufficient market surveying and testing must be done to insure that the product is in the most desirable form.

(5) Its cost must be competitive with alternative foods.

(6) It must be healthful if it is to fulfill the dual requisite of supplying energy and nutrition.

(7) It must not be toxic when large quantities are consumed.

(8) It must be easily prepared.

(9) It must be properly advertised and promoted.

(10) It must not be construed to be a poor peoples' food.

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Chapter 7

SPECIFIC APPLICATIONS--INDIA, AS AN EXAMPLE

7.1 Introduction

An important objective of this study was to design a system of food
production of use in meeting the world's food need. To accomplish this
aim, it is necessary to study the applicability of the system to specific
countries, for future food shortage if solved at all, will be solved with-
in the context of and by the actions of specific countries. This assump-
tion is reasonable for at least two reasons. First, the world food needs
are presently defined on a country by country basis. Second, the politi-
cal realities of the modern world anticipate the solution of problems on
a nation-state basis consistent with the accepted organization of the
world community.

It is a fact that nations vary in their form of government, economic
system, culture, and stage of modernization. To be useful generally,
therefore, a system of food production such as proposed herein should be
adaptable to the peculiar and particular conditions of each country which
may need the food producing system.

The baseline economic figures in the report are for the United States.
These U.S. figures may be converted by appropriate factors for any country
to obtain a reasonable assessment of the feasibility of the process for
the selected country.

To test the versatility of the synthetic carbohydrate production sys-
tem discussed in previous chapters, India has been selected for special
study because of the availability of pertinent data.

It should be remembered that in addition to the conversion of cost
estimates into the currency of the selected country, there are questions
of capital and technology availability which must be answered. It may be
that the costs associated with the importation of capital and technology
may outweigh the value of domestic production. Thus, it is conceivable
that the best utilization of the process, for some countries, may be the
importation of the product rather than the production process. However,
each country should be individually evaluated and the final judgment on
that country's use should be based on the merits of that particular case.
The analogies which such a country might share with the United States or
India should not be considered any more than analogies. Under no circum-
stances should such analogies become the basis of governmental action.

7.2 Demand for Food

The first step in the assessme᾽ of the food requirements of a coun-
try is the determination of its popᵤₐₜᵢon size and projected population
growth.

Table 7.1 shows India's 1970 and projected 1980 and year 2000 popu-
lation figures together with the assumed population growth rate. Food

Table 7.1

U.N. WORLD POPULATION PROJECTIONS "HIGH"--1970 TO 2000

	Estimated Annual Rate of Growth (%)	1970	1980	2000
India*	2.6	543.2	696.3	1,121.7

*
U.N. Dept. of Soc. & Econ. Affairs, World Population Prospects as As-
sessed in 1963. Population Studies No. 41, N.Y., 1966.

demand on the basis of these figures would double by the year 2000 and
would increase at the rate of 2.6 percent per year. Food demand is not
only a function of population size, it is also a function of family or
per capita income. There is no effective demand until one has the money
with which to purchase available food. Population charts reveal the
growth of "real" demand, but it is both population and income that de-
termine effective demand. What is the picture on India's income per
capita, hence its effective food demand?

In 1971 the per capita income in India was $100.00 per annum. By
contrast, the United States per capita income was $3,980.00 per annum.
Thus the real problem for India in terms of food demand is to increase
its overall wealth and more equitably distribute its existing resources.
A plan to meet this demand must be compatible with the demand creating
objectives.

7.3 Agricultural Production of Food

7.3.1 Low Productivity of Traditional Agriculture

Until recently, India had one of the lowest rates of produc-
tivity in the world because of its primitive agricultural technology.
During the first two 5-year Plans, 1951-1961, food production was able
to keep slightly ahead of population growth largely because it was still
possible to bring new arable land into cultivation. However, by the be-
ginning of the Third-Year Plan in 1961, India was already cropping 402
million acres out of a potential 410 million. Mere extension of culti-
vated land had ceased to be the answer for feeding its rapidly expanding
population.

Between 1960 and 1965, increases in agricultural production
began to taper off as population began to press on the land. By 1964,
the alarming fact emerged that increases in food supplies were actually
falling behind population growth (Figure 7.1) and that it was no longer
possible for India to rely on traditional agriculture to support a grow-
ing population on a limited supply of land. It became apparent that,

```
120 ┌   INDIA (0.16)
110 ┤
100 ┤
 90 ┤
 80 ┴  ┴ ┴ ┴ ┴ ┴ ┴ ┴ ┴ ┴
    1952  54   56   58   60   62   64   66   68   70
```

---- Per capita food production
    (observed values)
─── Per capita food production
    (trend values)
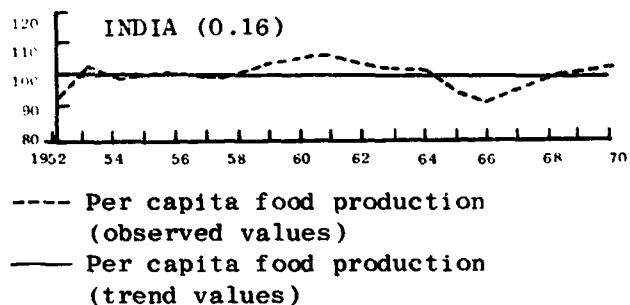
Source: FOA, The State of Food and Ag-
         riculture, 1971. Rome, 1971.

Figure 7.1. INDIA: TREND OF PER CAPITA
FOOD PRODUCTION, 1952 TO 1970.

unless there was a radical change in farming methods, the gap between
food supplies and food needs would not only persist, but actually would
deepen.

7.3.2 Failure of the Monsoons, 1965 and 1966

        Beginning in 1965 to 1966, the food situation in India took
a sharp turn for the worse, when the summer monsoon, upon which Indian
agriculture was heavily dependent, failed for two years in a row. Be-
cause of the severe drought, food grain production fell sharply below
the food needs of the Indian population (Figure 7.1 above). The actual
grain output of 72 million tons in 1965 and 1966, and 76 million tons in
1966 and 1967, was not only far below the 88.4 million tons in 1964 and
1965 (see Table 7.2) but about 25 percent below the originally scheduled
100 million tons under the Third Five-Year Plan. Mass starvation was
averted only by heavy food-grain imports, most of which were supplied
by the United States under Public Law 480 (the "Food for Peace" program).
During these years, food grain imports rose from 1.4 million tons in
1956 to 7.4 million in 1964, and then to 10.4 million tons during the
drought year of 1966 (see Table 7.3).

        The severe droughts of 1965 and 1966 awakened the Indian
Government to the precariousness of its food supply and the need for a
reassessment of its agricultural policy. Up to this time, agriculture,
while not actually neglected, had not been given major emphasis in In-
dia's development strategy. For example, in the First Three Plans, pub-
lic development expenditure accorded agriculture (including irrigation
and flood control) was not only considerably less than for industry (in-
cluding transport and communications) but actually was reduced during
the 15 years covered by the three plans. Moreover, little attention
was paid to providing price incentives for farmers, to providing such
agricultural inputs as fertilizers, improved seeds, and farm machinery,
or to the problems of farm credit. Thus progress in agricultural tech-
nology was slow indeed.

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Table 7.2

INDIA: FOOD GRAIN PRODUCTION, 1961/1962 AND 1971/1972

Year	In Millions of Tons	Average Yearly Rate of Growth
1950-1951	50.8	
1961-1962	81.0	
1963-1964	80.2	
1964-1965	88.4	2.5%
1965-1966	72.0	
1966-1967	76.0	
1967-1968	95.4	
1968-1969	94.0	
1969-1970	100.0	5.0%
1970-1971	108.0	
1971-1972	113.0	

Sources: "Republic of India," Background Notes.
          Department of State, October 1969, p. 5.

          "World Bank Group Assistance for Indian
          Agriculture," Finance and Development,
          June 1972, p. 57.

          Kasturi Rangan, "Surplus Grain Reported
          in India," New York Times, April 30, 1972,
          p. 4.

          U.N. Department of Economic and Social
          Affairs, World Economic Survey (New York:
          1967), p. 164.

Table 7.3

INDIA: IMPORTS OF FOOD GRAIN, 1957-1971
SELECTED YEARS
(in millions of tons)

Year	Imports
1956	1.4
1965	7.4
1966	10.4
1967	8.7
1968	5.7
1969	3.9
1970	2.1
1971	0.0[*]

[*] According to the New York Times, April
30, 1972, India became a surplus nation
in wheat and rice in 1971. Not only did
India stop importing American wheat un-
der the Food-For-Peace program in 1971,
but she started her own Food-For-Peace
program with 700,000 tons of grain pro-
vided to Bangla-desh. Officials are
reported to be looking for export mar-
kets for a grain surplus of 8 million
tons that they expect to have at hand
at the 1971 and 1972 year's end.

Sources: Commission on International
Development, Partners in De-
velopment, Lester P. Pearson,
Chairman (New York: Praeger
Publishers, 1969), p. 288.

Kasturi Rangan, "Surplus of
Grains Reported in India,"
New York Times, April 30,
1972, p. 4.

Table 7.4

INPIA: PERCENTAGE DISTRIBUTION OF PLAN OUTLAYS IN THE PUBLIC SECTOR

	1st Plan (1951-1956)	2nd Plan (1956-1961)	3rd Plan (Est. 1961-1966)
Agriculture and community development	15%	11%	14%
Irrigation	16%	9%	9%
Power	13%	10%	13%
Village and small industries	2%	4%	4%
Industry and minerals	4%	20%	20%
Transport and communications	27%	28%	20%
Social services and miscellaneous	23%	18%	20%
Total	100%	100%	100%

Source: Government of India, Third Five Year Plan (1961), p. 59.

In 1965, the Indian Government reversed its previous policy toward agriculture and in its development plan, gave top priority to agriculture. An all-out effort was to be made to raise yields per acre in order to assure that India would be self-sufficient in food by 1971. In its Fourth Five-Year Plan, the share of public investment going into agriculture was increased 2 1/2 times over the share alloted in the Third Plan.[*] At the same time, India outlined a bold new strategy to improve agricultural productivity. This included sharply increased use of fertilizer and pesticides, increased research in high-yielding seed varieties, increased irrigation, price incentives, and improved agricultural education.

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[*] The following table compares the Fourth Plan public sector outlay for agricultural improvement with the expenditure in the Third Plan and Annual Plans (1966 to 1969) under each sector related to agriculture.

(Footnote continued on following page.)

7.3.3 The Green Revolution

          Simultaneously with its new emphasis on agriculture, a
breakthrough occurred in agricultural productivity, the so-called
"Green Revolution." Twenty years of research went into producing new,
carefully selected hybrid strains of rice and wheat which could safely
absorb up to 120 pounds of nitrogen per acre. Traditional strains could
do so but the resulting heads of grain were simply too heavy for the thin
stems and fell over or "lodged" if more than 40 pounds of fertilizer was
applied to the acre. This increased tolerance for fertilizer, combined
with a quicker period for maturing--only 120 days compared with 150 to
180 with older species--made the new hybrids two or three times more pro-
ductive, provided they received enough water, fertilizer, and pesticides.

          Because the new hybrid varieties needed adequate water sup-
plies, it was decided to concentrate planting the new varieties on 32
million acres of irrigated land in the Punjab region. It was estimated
that planting on such land would produce a 25 percent increase, as op-
posed to a 10 percent increase on unirrigated land.

          The new seed was quickly adopted by Indian farmers, espe-
cially as to wheat. For example, the area planted with high-yielding
wheat increased from 7,400 acres in 1966 to 15,100,000 acres in 1971 as
shown in Table 7.5. The rapid adoption of new seeds demonstrated that
the peasants, contrary to expectations, were not hopelessly bound to
tradition. Given reasonable government support prices and reasonable
assurance of a large profit on his investment, the Indian farmer proved
willing to adopt new technology with amazing rapidity.

          New technology plus the new government strategy led to a
dramatic increase in grain output over the next four years. Aided by
good weather, grain production rose 30 percent in 1967 and 1968 to 95.4
million tons, though it still was only 7 percent higher than the previ-
ous record reached three years earlier. In 1968 and 1969, production

Sector	Third Plan	1966-1969	Fourth Plan
	(in Rs. crores)		
Agriculture and allied sectors	1,088.9	1,166.6	2,728.2
Irrigation and flood control	664.7	457.1	1,086.6
Power*	1,252.3	1,182.2	2,447.6
Total	3,005.9	2,805.9	6,262.4

*
Although agriculture is not the sole beneficiary of this outlay, a
large part of the investment for power construction directly bene-
fits the farming population and is therefore, included as part of
the total efforts of the Indian Government to improve India's agri-
cultural potential.

Source: Government of India, Fourth Five Year Plan, 1969-1974,
          Summary, January 1971, p. 20.

Table 7.5

AREA PLANTED WITH HIGH-YIELDING
MEXICAN WHEATS IN INDIA
1966-1970

Crop Year	(in acres)
1966	7,400
1967	1,278,000
1968	6,681,000
1969	10,000,000
1970 (est)	15,100,000

Source: Lester R. Brown, "The Social
Revolution," International
Conciliation, No. 581, January
1971, p. 6.

fell slightly to 94.0 million tons because of unfavorable weather in some parts of the country. However, with three years of good weather, food grain production climbed to 113 million tons by 1971. At that time, India announced that she had reached her goal of self-sufficiency in grain and that she would no longer need to import food grains from the developed countries. In fact, she even spoke of a possible 8 million ton grain surplus.

Since the breakthrough in agricultural technology, India has boosted her overall growth in food production to 5 percent a year--double the previous rate and almost double the present population growth of 2.6 percent. Wheat production, in fact, actually increased at an annual rate of 21 percent between 1967 and 1970. Despite the overall improvement in food production, there has been little or no expanded production of other crops than grains. During the same period, pulses barely increased, and the same was true of meats, eggs, and milk--all important sources of protein.

Nevertheless, great optimism has been expressed by many Indian agriculturists that if the new hybrid grains were planted on sufficient acreage, India would have good prospects of feeding her population well into the eighties, despite an expected population growth of 153 million during the decade 1970 to 1980.

7.3.4 Can India Sustain a 5 Percent Growth in Food Production?

Many experts do not share India's confidence that the Green Revolution will be able to sustain its growth trend. A number of reasons

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are cited. First, thus far increases in crop yields have been made where they are easiest, among the most progressive farmers who are eager to innovate and who have better land and access to water and capital. These farmers can apply the fertilizer, drill the wells for irrigation, and have access to transportation to move their crops to market [1]. It is unlikely the Green Revolution will spread as rapidly among the small subsistence farmers and sharecroppers, who have fewer resources, unless there are fundamental changes in land tenure and credit policies.

Second, the new high-yielding seeds require irrigation, but over 75 percent of India's arable land is without assured water supplies. At the present time, the new seeds have been planted for the most part in East Punjab, where irrigated land already exists. To benefit the majority of farmers, the new grain varieties would have to spread to the remaining cropland. This would require either greater investment in irrigation projects or a breakthrough in new varieties suitable for nonirrigated agriculture.

Third, only a wheat revolution has taken place in India. The "miracle rice" has touched only a fringe of rice cultivation and has barely taken root in the rice fields of West Bengal, Oressa, and other major rice-growing areas [2]. Since wheat accounts for 15 percent of the total acreage in food grains as against 31 percent in rice, the latter is more important in determining the overall rate of agricultural growth.

Why the problem in rice? The new hybrids are highly susceptible to pests and disease, because they are presently unsuited to India's climate. Also because the new grains are coarse and turn sticky on cooking, they lack consumer appeal and sell at a discount. Furthermore, the lack of irrigation and absence of drainage facilities in some of the major rice-growing areas are limiting factors [3]. The spread of rice hybrids to more parts of India awaits substantial investment in water supplies and drainage comparable to that made in Japan and Taiwan over the past several decades.

Fourth, agriculture in India is heavily dependent on the monsoon rains. With barely a fourth of the country's 325 million arable acres properly irrigated, agricultural performance in India still continues to be highly subject to the vagaries of weather. One reason for the improvement of grain output in India since 1969 has been the unusually good weather it has enjoyed for three years in a row. There is no guarantee that the spell will not break at any time.

Some critics of the Green Revolution point out that it is really difficult to know how much of the increase in grain output in the past three years can be attributed to the new technology and how much to the good weather in India. A drought followed by rain, they argue, will cause a spurt in production with or without new technology. In the three years period 1969 to 1971, India, for instance, increased her production of barley, chickpeas, tea, jute, cotton, and tobacco by 20 to 30 percent, and did so with no new high-yielding varieties [4].

Lastly, the Green Revolution is presently being monopolized only by wealthier farmers. So far the more advantaged farmers have obtained disproportionate shares of irrigation water, fertilizers, seeds, and credit. Unwise financial policies have sometimes encouraged these

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farmers to carry out excessive mechanization, which has led to an increase in rural unemployment. In India, agricultural incomes are largely exempt from direct taxation and large farmers have used their windfall profits to enlarge their farms even further. What is forgotten is that small farmers often work their holdings more intensively than larger farmers, and often achieve a higher output per acre. All this suggests that a reasonable redistribution of land, currently held in excessively large blocks, to the landless or to small farmers would be desirable not only on grounds of equity, but on grounds of efficiency as well [5].

Mere land redistribution by itself, however, is not likely to lead to more output unless those who receive it are also given the necessary assistance to finance and improve farming techniques. This will require a change in the structure of credit institutions and extension services, which typically serve large farmers. If the poorer farmers do not benefit from the Green Revolution's increase in output, they cannot increase their own food consumption, and the whole drive toward greater productivity will be diminished by a sluggish market.

7.3.5 Summary and Conclusion

Although Indian food production has attained a rapid growth of 5 percent a year since the introduction of the so-called Green Revolution, there is no clear-cut assurance that this rate will continue over the next few decades unless a number of technological, economic, and financial problems are solved. For example, India must find high-yielding rice seeds suitable to its climate. It must maintain price incentives and adequate supplies of the necessary inputs, such as seeds, fertilizers, and pesticides. It must reorient its irrigation investment and practices so as to bring assured water supplies to the "dry farming" areas. It must reorganize its credit system to permit the majority of India.) farmers who lack resources to take advantage of the new agricultural technology. Moreover, it must devote more funds to agricultural research to cope with a number of second generation problems such as new plant disease, difficulties in marketing, storage, and crop diversification.

Finally, unless there are basic improvements in per capita income, there could well be a lack of effective demand because of the low income of the Indian masses. The recent announcement by India that she is now self-sufficient in food, meaning independent of food imports, is highly misleading. Self-sufficiency in this instance is defined in terms of effective demand--what people can afford--not in terms of what they need. The bulk of Indians live in poverty and "per-capita food consumption is at least 25 percent below health requirements" [6]. Starvation and malnutrition are major problems that the government has been unable to surmount. For India to attain self-sufficiency in the broader sense, the per capita income of the mass of people--presently under $200 per year--would have to grow considerably to afford both caloric and protein sufficiency. "Self-sufficiency" in food would then require a much higher level of grain production than India presently has.

All that can be said is that the Green Revolution has purchased time. It has given India a momentary respite in which (1) further agricultural progress can be made, (2) alternative processes such as artificial foods can be developed to supplement agriculture, and (3) some start can be made, hopefully, in the control of population itself.

7.4 Synthetic Carbohydrates

7.4.1 Feasibility

The proposal developed for the synthetic production of carbohydrates presents two alternate plans or pathways. First, is the cellulose process, named after its input material. Second, is the fossil fuel process.

India is a fossil fuel importer, whereas it is the world's largest producer of bagasses, a prime input to the cellulose process. Fossil fuel imports are under strict government quotas and constitute a scarce resource valued for many purposes. Bagasse on the other hand is available in large quantities from more than 120 plants located in eight areas of the country which include sites in Uttar Pradesh, the major source of bagasse, Bihar, East Punjab, West Bengal, Maharashtra, Andra Pradesh, Madras, and Mysore. Because of the facts outlined above, the cellulose process seems immediately preferable to a fossil fuel process in India.

An adequate technology exists in India for both construction and operation of an artificial carbohydrate facility. Similar conveyers, feeders, motors, mixers, stainless steel vessels, and other necessary equipment as needed by the facility are presently being manufactured for the countries' sugar and milk processing industries.

The major chemical requirement in the process is for caustic soda. Since alkali products are manufactured in large quantities in India for domestic demands, it is presumed that sufficient quantities of dry flake alkali would be available for the process.

7.4.2 Costs and Financing

Construction costs of a complete chemical process plant in India are estimated to be 30 to 40 percent above U.S. Gulf Coast figures. These costs are on the basis of an outside contractor erecting a complete plant. They include allowances for problems in obtaining licenses and other governmental approvals, differences in material standards, import duties, delivery delays, labor costs, and many other factors which influence the cost of doing business. The cost of capital is also high. Even under the assumption that construction and operation would be entirely indigenous, it is likely that the cost of the product would be at least equal to that for U.S. production. All costs, with the exception of labor, are substantially high. Low labor costs are offset by low efficiency.

India has indicated its strong preference for internal financing of her projects for development. At page 8 of the Fourth Five Year Plan it is stated that:

"In regard to the financing of the Plan, emphasis is being placed on additional mobilization of internal resources in a manner which will not give rise to inflationary pressures."

The plan further specifies (page 21) that money for particular geographical regions is a major factor in the allocation of national resources. Among the recipient regions, those which have been projected as potential sites for synthetic carbohydrate plants have been designated as major recipient regions. Thus money for new projects could probably be bound into these funds. Further, at page 23, the criteria for central financing is set forth. These projects should:

(1) relate to pilot programs

(2) have a regional character

(3) require lump sum provision

(4) have overall Indian significance

Under these criteria, the proposal herein envisioned would qualify or stand a good chance of qualifying for central financial support.

7.4.3 Compatibility with National Policy

Synthetic production of food is not an alternative considered by the Indian planners in the development of their strategy to meet the emerging food shortage crisis. Yet the projected budget outlays under the Fourth Five Year Plan do anticipate considerable sums being expended on agricultural research. Thus it should be possible to find financial support for the planning of the implementation of such a synthetic production plant in these funds. Moreover, such a suggestion would probably constitute a significant and innovative alternative to the Indians in their efforts to meet their obvious challenge to produce more food domestically.

The Indian Central Planning body is created pursuant to obligation of the Indian national government created by the Indian constitution. That document requires governmental participation in social and economic planning and development. Thus the Fourth Five Year Plan and other similar documents, though not juridicial in that they may not become the cause of litigation, do have a primary legal basis. Hence, they carry the force of law in general implementation and administration. An evaluation of the acceptability of any new scheme for the fulfillment of recognized needs within India must come within the restraints set forth in these various policy documents.

Indian foreign trade and domestic development policies would probably have the most important impacts upon its prospective utilization of the carbohydrate production system found herein. That policy anticipates that the major industries will be locally controlled, and that further industrialization should be encouraged with governmental aid and participation. These policies direct that any projected Indian utilization of the system anticipate participation by the Indian government and that the production plant be located within India and be primarily if not exclusively under Indian ownership and control [7].

7.4.4 Product Acceptability and Utilization

The utilization of synthetic starch in India offers two attractive possibilities: human foods and animal feeds. Human use can be considered as part of a normal diet, in which case it would need to be a nutritionally balanced product in itself, or as an emergency food, where energy to sustain life would be the prime consideration. Use of starch as a major component in animal feeds is appealing from the standpoint of improving diet quality with meat protein, most likely chicken. At the same time, cereal grain for human consumption would be made more freely available. Glucose, an intermediate product in starch synthesis, can be used to a limited extent in either case to improve palatability and to provide a relatively inexpensive energy source.

7.4.4.1 Human Use

Macaroni products are particularly suited to easy mass production and can be fortified to meet nutritional needs. As dry products, they can be easily stored and transported and offer consumer appeal for convenience in preparation as well as permitting flavoring to suit regional tastes. Among the macaroni products, vermicelli is the only one that has been traditionally produced in India for a long time. It is well accepted, particularly for dessert use, which also lends a prestige factor. This, and the success of similar products in that region of the world, appear to indicate that pasta products would be a reasonable choice for starch utilization as human food in India.

7.4.4.2 Animal Feeds

The utilization of synthetic starch in animal feeds is attractive as a means of improving diet quality in a protein deficiency situation. Chicken is the most widely acceptable meat form in India, and the poultry industry is growing rapidly. Chickens are also an extremely efficient convertor of feed to meat, and laying hens are efficient "factories" that use feed to turn out economical eggs. Synthetic starch as a major poultry feed component offers promise in assisting the development of the Indian poultry industry without absorbing a disproportionate share of the food grains needed for human use.

The efficiency possible in the poultry industry is well illustrated by a common measure--the pounds of feed required to produce 1 lb of meat. For broilers, the U.S. ratio, which was 4 in 1948, has dropped to around 2.2. In addition, this industry can be developed in areas where the land is not suited for other agricultural purposes. Clearly, there is a place for large quantities of synthetic starch and glucose in poultry feeds, particularly if they can be produced at prices competitive with traditional feed materials.

Poultry farming being labor intensive, would create new jobs, rather than destroy existing ones, would increase the woefully inadequate supply of protein, and would keep population in rural areas by strengthening the rural economy. Not only could starch plants be located in these areas but also new industries for processing and marketing poultry. Furthermore, the poultry industry would help distribute the gains of economic development more equitably since its relatively low capital costs lends itself to small-sized family operations rather than large-scaled corporate agri-businesses.

Finally, it would protect the environment compared with, for example, increasing the cultivation of hybrid wheat and rice. Poultry waste is biodegradable and less dangerous than chemical fertilizers, herbicides, and pesticides needed for grain production. Equally important, it would release land in India, already in short supply, since far less acreage would be needed for an equal amount of protein produced on a poultry farm using inputs of synthetic starch compared with feed grains grown by conventional agriculture.

In short, a synthetic starch-poultry operation has high social value, low social cost, and permits an immediate response to immediate social and economic problems. Moreover, it is in complete harmony with the basic goals of India--a rapid increase in the standard of living, and an emphasis on the common man, the weaker sectors of the economy, and the less privileged.

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Appendix 4.1

CHARACTERISTICS OF ENZYMES: GLYCIDALDEHYDE → GLUCOSE

1. Triokinase E.C. 2.7.1.30

Detailed studies of the kinetics of triokinase apparently have not been made, due to the lack of purity of the preparation. However, K_m values for D-glyceraldehyde and DHA have been determined [2] for the beef liver enzyme as 1.2×10^{-4} and 2.0×10^{-6}, respectively. The pH optimum is around 7 [1]. Some typical levels in crude extracts of liver from various vertebrate sources are as follows (in μmoles/min/g wet weight of liver at 25°C) [3]:

man	2.07
rat	1.65
chicken	0.86
hedge lizard	0.36
land toad	0
guinea pig	1.5 (has been purified 30 times) [4]

Taking 1.5 μmoles/min/g as a reasonable estimate for mammalian liver, it would appear that 10^6 lbs of liver would be required to obtain sufficient enzyme, or about 0.4 percent of the beef liver harvested annually in the U.S.

$$\#lb = \frac{700 \text{ moles/min}}{1.5 \times 10^{-6} \text{ moles/min/g} \times 453 \text{ g/lb}} \approx 1 \times 10^6 \text{ lb}$$

On the other hand, based on rather sketchy data [4] about 140,000 lbs of B. subtilis would be required. This is based on an estimate of 12 μmoles/min per gram of cells. This may be quite unduly pessimistic since the assay system was fairly crude, and no attempt at optimizing enzyme production was made.

2. TPI E.C. 5.3.1.1 [5]

Specific activity and turnover number: (MW = 56,000)

4.9×10^4 moles/min/mole enzyme - DHAP

1.0×10^6 moles/min/mole enzyme - GALD-P

or

0.88 moles/min/g enzyme - DHAP

18 moles/min/g enzyme - GALD-P

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K_m values

GALD-P - 1.3×10^{-3}

DHAP - 1.2×10^{-3}

pH optimum

Appears fairly sharp: between pH 7.0 and 8.0, with a rapid decrease in activity to nearly zero at pH 5.5 and pH 10.

Inhibitions

Compounds relevant to this process (ADP or ATP nonphosphory-lated trioses) were not listed as inhibitors.

Level of activity

If recoveries of activity similar to those obtained on a lab-oratory scale were possible, in order to obtain the required amount of crystalline enzyme, only 48 lbs of dried yeast would be necessary. (The enzyme was purified 250 fold from crude homogenates. One kg of dried yeast yielded 1600 mg of crys-talline TPI of specific activity 10,000 μmoles/min/mg. On this basis

$$\#lb = \frac{350 \text{ moles/min required} \times 2.2 \text{ lb/kg}}{.010 \text{ moles/min/mg} \times 1600 \text{ mg enzymes/kg yeast}} = 48 \text{ lbs})$$

3. **Aldolase** E.C. 4.1.2.13 [6]

Specific activity or turnover number (MW $\cong$ 70,000)

3.78×10^4 moles/min/mole enzyme

or

0.56 moles/min/g enzyme

K_m values

DHAP - 2.4×10^{-3}

GALDP - 2×10^{-3}

pH optimum

About pH 7.3 appears optimal for the aldol synthetic reaction
we are considering. The activity falls off rather sharply on
either side. This differs from the optimum of pH 6 for the
catalysis of the H^3 exchange at C-1 of DHAP.

Inhibitions

The enzyme is inhibited by substrate at levels higher than
twice the K_m value but no data as to the extent of inhibition
are given. Since the enzyme has a fairly high turnover number,
it may be possible to overcome this problem by increasing the
concentration of enzyme in the reactor by a factor of 10 or
100. For example, fructose-1,6-diphosphatase is substrate in-
hibited such that a five-fold increase in concentration over
K_m causes the rate to decrease by a factor of two. If we were
to run at 0.1 M, this would represent a 15 fold increase over
the K_m for aldolase.

Tris, imidazole, and phosphate buffer systems are inhibitory,
and glycyiglycine buffer is, therefore, routinely used. EDTA
also inhibits, but this can be reversed by Zn^{++}.

Activation

0.1 M K^+ ions give maximal activity (about ten times that in
the absence of K^+).

Level of activity

Assuming laboratory recoveries are achieved, about 0.8 tons of
yeast would be necessary to achieve the desired activity. Al-
dolase was purified 25 fold from yeast homogenates. 1.5 kg of
dried yeast yielded 116,500 units (by FDP cleavage assay). This
would amount to 349,500 units by the aldol synthesis assay.

$$\#lb = \frac{350 \text{ moles/min required} \times 2.2 \text{ lb/kg}}{0.35 \text{ moles/min/1.5 kg yeast}} = 1,480 \text{ lbs}$$

$\#tons = 0.8$ tons

If a cruder extract could be used, the amount of yeast needed
could diminish since only a 22 percent recovery of aldolase
was achieved. Although this is a substantial amount of yeast,
it is a vast improvement over the situation with spinach, which
would require 38,600 tons of raw material [7].

4. Alkaline Phosphatase E.C. 3.1.3.1

Kinetics and Inhibition

Inorganic phosphate, a product of the reaction, is a powerful competitive inhibitor of the reaction [8]. At pH 8 and depending on the ionic strength of the solution, the K_m for P_i is 0.6 to 10×10^{-6} [9]. (Increasing salt concentration to 1M NaCl seems to raise the K_i for P_i maximally.)

A reasonable expression for the forward reaction using the competitive inhibition of P_i as a factor seems to be [10]

$$v = \frac{kE_0}{1 + \dfrac{K_m}{(FDP)} + \dfrac{K_m}{(FDP)} \cdot \dfrac{P_i}{K_i}}$$

and this was used for simulation purposes. The reverse reaction was not considered.

Specific activity

3.4 μmoles/min/mg

Turnover number

2,700

K_m value

FDP $= 1.2 \times 10^{-5}$

pH optimum

The enzyme is assayed at pH 8.0, which ties up the intermediate phosphate to a minimal extent [10]. The pH optimum range is complex and dependent on substrate and concentration.

Substrate specificity

This enzyme hydrolyzes a wide variety of esters at roughly the same rate [9]. Fructose-1,6-diphosphate is hydrolyzed at a rate 0.6 of that for the standard p-nitrophenyl phosphate [11]. Unfortunately, ADP is also hydrolyzed at roughly this rate [8] although earlier reports had indicated it was not hydrolyzed [12]. Had ADP not been attacked, it might have been carried

through the several reactors in the fossil fuel process on to the stage of fructose, where its separation and regeneration would have been simpler.

Sources and levels

Alkaline phosphatase has been prepared from mammalian and other microbial sources, but its preparation from E. coli is well documented.

On the basis of the purification table given in the literature [13], 250 liters of cells would produce sufficient enzyme in the crude extract for a 100 ton per day plant. The yields of crystals, however, appear to be sufficiently low so that 620,000 liters of cells would be needed if the procedure could not be improved:

(1) Crude extract from crystalline enzyme:

$$\frac{\text{Total}}{\text{units}} = 234,000 \ \mu\text{moles/hr/ml} \times 1000 \ \text{ml} = 2.34 \times 10^8 \ \mu\text{moles/hr}$$

or

$$2.34 \times 10^8 \frac{\mu\text{moles}}{\text{hr}} \times \frac{1}{60 \ \text{min/hr}} \times 10^{-6} \ \text{moles}/\mu\text{mole} = 3.7 \ \text{moles/min}$$

$$\text{Liters req} = 350 \ \text{moles/min} \times \frac{2.5 \ \text{liters}}{3.7 \ \text{moles/min}} = 250 \ \text{liters}$$

(2) Crystals from crystalline enzyme:

$$\text{Total units} = 4.7 \times 10^3 \ \mu\text{moles/hr/ml} \times 18 \ \text{ml} \times \frac{1}{60 \ \text{min/hr}}$$
$$\times 10^{-6} \ \text{mole/min} = 1.4 \times 10^{-3} \ \text{moles/min}$$

$$\text{Liters req} = 350 \ \text{moles/min} \times \frac{2.5 \ell}{1.4 \times 10^{-3} \ \text{moles/min}}$$
$$= 620,000 \ \text{liters}$$

5. Glucose Isomerase [14]

The characteristics of the glucose isomerase have been determined chiefly with respect to the reverse reaction. Its relevant properties are:

Turnover number and K_m

The turnover number (or V_{max}) for the glucose $\rightarrow$ fructose transformation is 1070 moles/min/mole enzyme. The K_m value for glucose is 0.16 M. Since the reverse reaction has not been studied, we have assumed similar data in view of the fact that the K_{eq} is close to 1.

pH optimum

This was dependent on the buffer used. For phosphate buffer pH had a broad range between 7 and 10.

Inhibitions and activations

No compounds likely to be present in this system (other than heavy metal ions) were found to inhibit the reaction; e.g., phosphate compounds. A combination of 0.001 M Co^{+2} and 0.1 M Mg^{+2} enhanced the rate of reaction. When these were completely removed, the enzyme was inactive.

Levels

Since it is suggested to use heat treated whole cells, the amounts obtainable were calculated on this basis, rather than for crystalline enzyme. Apparently the cells from about 100,000 liters of medium would be required. This was calculated in two ways:

(1) The highest obtainable activity was 2.8 μmoles/min/ml broth.

$$\#liters = \frac{3.5 \times 10^8 \ \mu moles/min \ needed}{2.8 \ \mu moles/min/ml \times 1000 \ ml/l}$$

(2) In a pilot plant process, the cells from 5000 liters brought 15 tons of glucose to equilibrium in 3 days.

$$\# \ liters = \frac{100 \ tons/day \times 5 \times 10^3 \ liters}{15 \ tons/3 \ days}$$

The broth contained about 6 g/l of protein, so that the estimated wet cell weight would be about 70 tons.

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Appendix 4.2

IMPORTANCE OF SEQUENCE IN COUPLED REACTIONS

In proposing a reactor containing a sequence of coupled reactions, the last reaction in the sequence plays a unique part in success in converting reactants completely to products; i.e., the last reaction in a sequence must have $\Delta G° \leq -3$ kcal/mole. To illustrate what will happen if this is not the case, assume reactions 9 and 10 (of Table 4.2) are coupled

$$\text{SDP} + \text{H}_2\text{O} \xrightleftharpoons{\text{Rx. 9}} \text{S7P} + \text{P}_i \qquad \Delta G°_{30} = -3.4 \text{ kcal/mole}$$

$$\text{S7P} + \text{GALD3P} \xrightleftharpoons{\text{Rx. 10}} \text{R5P} + \text{Xu5P} \qquad \Delta G°_{31} = +0.1$$

Net Reaction $\left\{ \text{SDP} + \text{GALD3P} + \text{H}_2\text{O} \underset{\leftarrow}{\rightarrow} \text{R5P} + \text{Xu5P} + \text{P}_i \right.$ $\left\{ \begin{array}{l} \Delta G°_{net} = -3.3 \text{ kcal/mole} \\ K_{eq} = 300 \end{array} \right.$
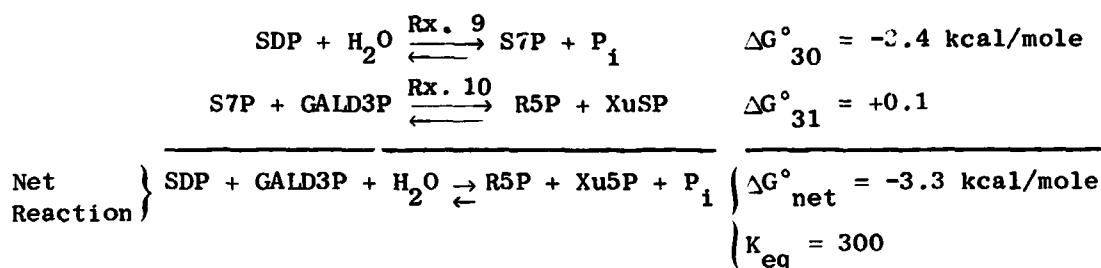
The net energy change (-3.3 kcal/mole) is very favorable. This free energy change corresponds to an equilibrium constant of about 300 for the coupled reactions. However, all reactants and products of each reaction are in equilibrium with reactants and products of the other reaction. Further, $\Delta G°_9 = -3.4$ kcal and $\Delta G°_{10} = +0.1$ kcal must also hold. These free energy changes indicate that, although the product "R5P - Xu5P - P$_i$" is much more stable than reactant "SDP - GALD3P - H$_2$O," "S7P - P$_i$" is more stable than either. The situation is shown schematically below.
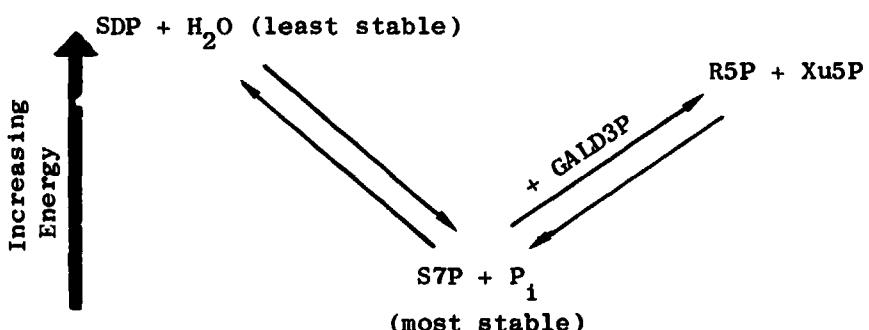
Consequently, the coupled reactions 9 and 10 will produce some R5P and Xu5P, but the major product at equilibrium will be S7P + P$_i$.

Thus, the requirement that the last reaction of a series of linked reactions be highly favored places severe restriction on any multiple reactor design. Nevertheless, there are a number of highly favorable reactions in the CO_2 fixation pathway. The important ones are reactions 1, 5, 6, 8, 9, and 13.

1. Alternative Groupings for Multiple Reactors in the CO_2 Fixation Process

Configuration 1

Suppose reactions 4 and 5 are coupled. Here the net free energy change is very favorable [$\Delta G°_{net}$ = (-1.8) + (-5.6) = -7.4 kcal/mole], and the last reaction has $\Delta G°_{net}$ = -5.4 kcal/mole. But, the reactant GALD3P must come from reaction 3. Furthermore, two GALD3P are needed: one each for reactions 4 and 5. Still no problem:

$$2 \times \Delta G°_3 = -0.54 \text{ kcal/mole}$$

$$\Delta G°_4 = -1.8 \text{ kcal/mole}$$

$$\Delta G°_5 = -5.4 \text{ kcal/mole}$$

$$\Delta G°_{net} = -7.7 \text{ kcal/mole}$$

But there is a hitch. The reactant for 3, PPGA, must come from 2. We need two moles of PPGA, and two moles of PPGA from reaction 2 are going to cost +9.54 kcal. The result now is that reactions 2, 1, 4, and 5 must be coupled to obtain FDP, but when this is done, $\Delta G°_{net}$ = +9.54 - 7.7 = +1.8 kcal/mole FDP.

This problem could almost be solved by adding reaction 6. Now $\Delta G°_{net}$ = -1.6 kcal/mole F6P. The energy is such that with a single recycle perhaps a fair yield of F6P could be obtained. However, recycling means a tough separation first. Besides there are worse problems. A quick inspection of the reactions in Table 4.2 shows that GALD3P, or its direct product DHAP, appears in reactions 4, 5, 7, 8, and 10. That means any multiple reactor scheme must combine reactions 2 and 3 with each of the reactions using GALD3P or DHAP. This requirement makes each multiple reactor a fairly complex set of coupled reactions.

It can now be seen that the major difficulty lies in reaction 2. It is very appealing, then, to attempt to force this reaction to completion. The only way to achieve this would be separation of the products ADP - PPGA and recycling of the starting materials ATP-3-PGA. Unfortunately, no obvious method for separation presents itself. The products would be present in very small amounts, and they have properties similar to the reactants. Furthermore, as mentioned above, PPGA is highly reactive. It is doubtful that it could survive even mild separation procedures.

Configuration 2

Alternatively, reactions 2, 3, and 4 could be coupled. The free energy change would be about +2.5 kcal/mole DHAP. At equilibrium a few percent of DHAP could be expected. ADP (along with

ATP) could be separated by ion exchange on a copper-containing resin. The ADP could be regenerated and after many recycle passes, perhaps, a fair yield of DHAP could be built up. DHAP could then be converted to GALD3P via reaction 4 when needed. This method, although feasible chemically, is more an academic exercise than one of any practical utility.

Configuration 3

Still another scheme would be to couple ATP synthesis (reactions 17 and 18) with reactions 2, 3, and 4 to produce DHAP. Energetically, this gives:

$$\Delta G^{\circ}_2 = +4.77 \text{ kcal}$$

$$\Delta G^{\circ}_{18} = -0.44 \text{ kcal}$$

$$\Delta G^{\circ}_{17} = -1.8 \text{ kcal}$$

$$\Delta G^{\circ}_3 = -0.27 \text{ kcal}$$

$$\underline{\Delta G^{\circ}_4 = -1.82 \text{ kcal}}$$

$$\Delta G^{\circ}_{net} = +0.44 \text{ kcal}$$

The overall reaction would be an equilibrium mixture of comparable amounts of an array of reactants and products. The separation problem would be immense on a massive scale as contemplated for a 100 ton glucose plant.

Configuration 4

The transformation of ADP to ATP using acetylphosphate (reaction 19) is catalyzed by the enzyme Acetate kinase (E.C. 2.7.2.1). The standard free energy change for this reaction is about -3 kcal favoring ATP production. If reactions 2, 3, and 19 are coupled, the following net reaction (reaction 20) obtains:

$$CH_3\overset{O}{\overset{\|}{C}}OP^{-2} + 3PGA^{-3} + NADPH^{-4} + H^+$$
$$(ADP^{-2}) \quad \uparrow\downarrow \quad \text{(reaction 20)}$$
$$CH_3CO_2 + GALD3P^{-2} + NADP^{-3} + P_i^{-2}$$
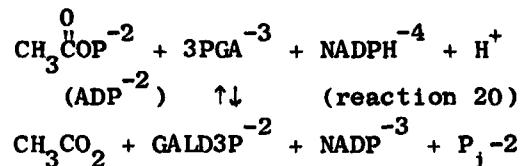
$$\Delta G^{\circ}_{20} = \Delta G^{\circ}_{net} = \Delta G^{\circ}_2 + \Delta G^{\circ}_3 + \Delta G^{\circ}_{19} = +1.5 \text{ kcal}$$

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The net free energy change is not favorable, but it is the most favorable energy change available for producing $GALD3P^{-2}$. Note that ADP/ATP does not appear explicitly in reaction 20, but is required by virtue of reactions 2 and 3. Hence, in using reaction 20 to couple with other reactions, the presence of ADP and the enzymes for reactions 2, 3, and 19 must be recognized.

The main problems with this scheme are: (1) the large number of potential inhibition problems due to coupling from 6 to 8 reactions within single reactors, and (2) the required separations of NADP; ATP, acetic acid, and P_i from F6P and RuDP.

Appendix 4.3

ENZYME KINETICS FOR THE CARBON DIOXIDE FIXATION PROCESS

1. Ribulose Diphosphate Carboxylan (4.1.1.39)   Mw = 550,000   [1]

$$V = \frac{V_{max} E_o (RuDP)(CO_2)(Mg^{++})}{\left[(RuDP) + K_A\right]\left[(CO_2) + K_B\right]\left[(Mg^{++}) + K_C\right]}$$

where

V_{max} = 1.34 × 10^3 moles/min-mole enzyme   [1]

K_A = 1.5 × 10^{-4} mole/liter   [1-6]

K_B ≈ 1.3 × 10^{-4} mole/liter (soybean)   [6]

K_C = 1.1 × 1C^{-3} mole/liter   [1]

Major Inhibitors

RuDP   (noncompetitive abcve 5 × 10^{-4} moles/liter)   [1,6]

FDP   (K_i = 8.8 × 10^{-4};   competitive with RuDP)   [6]

3-PGA (K_i = 8.3 × 10^{-3};   noncompetitive with RuDP)   [1]

       (K_i = 9.5 × 10^{-3};   competitive with CO_2)

P_i (K_i = 4.2 × 10^{-3};   competitive with RuDP)   [1]

Ru5P   (38 percent inhibition at 1 mM)   [6]

NADPH (46 percent inhbition at 5 mM)   [6]

ATP   (52 percent inhibition at 5 mM)   [6]

2. Phosphoglycerate Kinase (2.7.2.3)   Mw = 34,000   [7]

All tabulated values except those noted are yeast enzyme.

$$K = 3.2 × 10^{-4}$$ [8]

$$V = \frac{V_{max\ 1}E_o(3\text{-PGA})(MgATP)}{\left[(3\text{-PGA}) + K_A\right]\left[(Mg\text{-ATP}) + K_B\right]} - \frac{V_{max\ 2}E_o(P\text{-3PGA})(MgADP)}{\left[(P\text{-3PGA}) + K_C\right]\left[(MgADP) + K_D\right]}$$

where

$$V_{max\ 1} = 1.224 \times 10^4 \text{ moles/min-mole enzyme} \tag{8}$$

$$V_{max\ 2} = 1.09 \times 10^5 \text{ moles/min-mole enzyme} \tag{8}$$

$$K_A = 6.3 \times 10^{-4} \text{ mole/liter} \tag{9}$$

$$K_B = 4 \times 10^{-4} \text{ mole/liter} \tag{9}$$

$$K_C = 2 \times 10^{-6} \text{ mole/liter} \tag{8,10}$$

$$K_D = 2 \times 10^{-4} \text{ mole/liter} \tag{8}$$

Major Inhibitors

Mg^{++} (inhibitory above 2×10^{-3} moles/liter) (pea seed) [11]

3. <u>Glyceraldehyde-3-Phosphate Dehydrogenase</u> (1.2.1.13) Mw = 150,000 [12]

$$K = 2.0 \frac{mole^*}{liter} \text{ (pH 7.0)}$$

$$K = \frac{(GALD3P)(P_i)(NAD)}{(P\text{-3PGA})(NADP^+)} \tag{13}$$

$$V = \frac{V_{max\ 1}E_o(P\text{-3PGA})(NADPH)}{\left[(P\text{-3PGA}) + K_A\right]\left[(NADPH) + K_B\right]} - \frac{V_{max\ 2}E_o(GALD3P)(NADP^+)(HPO_4^=)}{\left[(GALD3P) + K_C\right]\left[(NADP^+) + K_D\right]\left[(HPO_4^=) + K_E\right]}$$

where

$$V_{max\ 1} = 1.49 \times 10^4 \text{ mole/min-mole enzyme}^* \Big\} \text{ rabbit} \tag{14}$$

$$V_{max\ 2} = 1.25 \times 10^3 \text{ mole/min-mole enzyme}^* \Big\} \text{ muscle} \tag{14}$$

$$K_A = 1.14 \times 10^{-6} \text{ moles/liter (pH 8.5 pea)} \tag{15}$$

$$K_B = 4 \times 10^{-6} \text{ moles/liter} \quad\quad\quad\quad\quad\quad [15]$$

$$K_C = 2.57 \times 10^{-4} \text{ mole/liter} \Big\rangle \text{ pH 8.5 pea} \quad [15]$$

$$K_D = 1.12 \times 10^{-4} \text{ mole/liter} \quad\quad\quad\quad\quad [15]$$

$$K_E = 1.3 \times 10^{-4} \text{ mole/liter}^* \quad \text{(rabbit muscle)} \quad [14]$$

Major Inhibitors

NADPH   ($K_i = 3 \times 10^{-7}$; competitive with both NADP and GALD3P)*[14]

GALD3P   ($K_i = 6 \times 10^{-8}$; competitive with both NADPH and 3PGA)*
[14]

SDP   ($K_i = 6 \times 10^{-4}$; competitive with GALD3P)
[15]

4.   Aldolase (4.1.2.13) Mw = 150,000 (Class I)

$$K_{eq\ A} = 1.24 \times 10^4 \text{ liter/mole} \quad\quad\quad [16]$$

$$V_A = \frac{V_{max\ 1} E_o (GALD3P)(DHAP)}{\left[(GALD3P) + K_A\right]\left[(DHAP) + K_B\right]} - \frac{V_{max\ 2} E_o (FDP)}{\left[(FDP) + K_C\right]}$$

$$V_B = \frac{V_{max\ 3} E_o (E4P)(DHAP)}{\left[(E4P) + K_D\right]\left[(DHAP) + K_B\right]} - \frac{V_{max\ 4} E_o (SDP)}{\left[(SDP) + K_E\right]}$$

where

$$V_{max\ 1} = 1 \times 10^4 \text{ moles/min-mole enzyme}^* \quad\quad [17]$$

$$V_{max\ 2} = 3.9 \times 10^3 \text{ moles/min-mole enzyme} \quad\quad [18]$$

$$V_{max\ 3} = \text{No value reported}$$

$$V_{max\ 4} = 2.3 \times 10^3 \text{ moles/min-mole enzyme} \quad\quad [18]$$

$$K_A = 1 \times 10^{-3} \text{ moles/liter}^* \quad\quad\quad\quad [19]$$

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*For NAD-linked enzyme (1.2.1.12) which is quite similar.

$$K_B = 2 \times 10^{-3} \text{ moles/liter}^* \tag{[19]}$$

$$K_C = 6.8 \times 10^{-5} \text{ moles/liter} \tag{[18]}$$

$$K_D = \text{No value reported}$$

$$K_E = 1.7 \times 10^{-5} \text{ moles/liter} \tag{[18]}$$

5. <u>Fructose-1,6-Diphosphatase</u> (3.1.3.11) Mw = 143,000*   [20]

$$V_A = \frac{V_{max\ 1} E_o (FDP)}{\left[(FDP) + K_A \right]}$$

$$V_B = \frac{V_{max\ 2} E_o (SDP)}{\left[(SDP) + K_B \right]}$$

where

$$V_{max\ 1} = 2.1 \times 10^{3} \text{ mole/min-mole enzyme}^* \tag{[20]}$$

$$V_{max\ 2} = \text{No value reported for neutral enzyme}$$

$$K_A = 2 \times 10^{-6} \text{ moles/liter}^* \tag{[20]}$$

$$K_B = \text{No value reported for neutral enzyme}$$

<u>Major Inhibitors</u>

FDP (inhibitory above approximately 10^{-4} moles/liter)   [20]

6. <u>Transketolase</u> (2.2.1.1) Mw = 140,000   [21]

All values are for yeast enzyme.

$$K_{eq\ A} = 0.1 \tag{[22]}$$

$$K_{eq\ B} = 0.95 \tag{[22]}$$

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*Rabbit liver enzyme.

$$v_A = \frac{V_{max\,1}E_o(F6P)(GALD3P)}{\left[(F6P) + K_A\right]\left[(GALD3P) + K_B\right]} - \frac{V_{max\,2}E_o(E4P)(Xu5P)}{\left[(E4P) + K_C\right]\left[(Xu5P) + K_D\right]}$$

$$v_B = \frac{V_{max\,3}E_o(S7P)(GALD3P)}{\left[(S7P) + K_E\right]\left[(GALD3P) + K_B\right]} \quad \frac{V_{max\,4}(R5P)(Xu5P)}{\left[(R5P) + K_F\right]\left[(Xu5P) + K_D\right]}$$

where

$V_{max\,1}$ = No value given

$V_{max\,2}$ = No value given

$V_{max\,3}$ = No value given

$V_{max\,4}$ = 3.4×10^3 moles/min-mole enzyme   [22]

$\quad K_A$ = 1.8×10^{-3} moles/liter   [22]

$\quad K_B$ = No value given

$\quad K_C$ = No value given

$\quad K_D$ = 2.1×10^{-4} moles/liter   [22]

$\quad K_E$ = No value given

$\quad K_F$ = 4.0×10^{-4} moles/liter   [22]

7. **Ribose Phosphate Isomerase** (5.3.1.6) Mw = 53,000   [23]

$$K = 0.30 \qquad [24\text{-}26]$$

$$V = \frac{V_{max\,1}E_o(R5P)}{\left[(R5P) + K_A\right]} - \frac{V_{max\,2}E_o(Ru5P)}{\left[(Ru5P) + K_B\right]}$$

where

$V_{max\,1}$ = 1.2×10^5 moles/min-mole enzyme   [23,24,27]

$V_{max\,2}$ = No value given

$\quad K_A$ = 2.5×10^{-3} moles/liter   [26-30]

$\quad K_B$ = No value given

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<u>Major Inhibitors</u>

RuDP $(K_i = 3.3 \times 10^{-5})$ (<u>R. rubrum</u>)   [31]

AMP $(K_i = 1.2 \times 10^{-3})$ (<u>R. rubrum</u>)   [31]

P_i $(K_i = 1.5 \times 10^{-2})$ (<u>R. rubrum</u> and pea)   [30,31]

8. <u>Ribulose Phosphate 3-Epimerase</u> (5.1.3.1) Mw = 46,000 (yeast) [32]

$$K = 0.67 \quad (\underline{Lactobacillus\ pentosus})$$ [33]

$$V = \frac{V_{max\ 1}E_o(Xu5P)}{\left[(Xu5P) + K_A\right]} - \frac{V_{max\ 2}E_o(Ru5P)}{\left[(Ru5P) + K_B\right]}$$

where

$V_{max\ 1} = 1.2 \times 10^4$ mole/min-mole enzyme (yeast)   [32]

$V_{max\ 2}$ = No value given

$K_A = 5 \times 10^{-4}$ mole/liter $\Big\}$ [33]

$K_B = 1 \times 10^{-3}$ mole/liter $\Big\}$ (<u>L. pentosus</u>)   [33]

9. <u>Phosphoribulokinase</u> (2.7.1.19) Mw = 240,000 (Chromatium) [34]

$$V = \frac{V_{max}E_o(Ru5P)(MgATP)}{\left[(Ru5P) + K_A\right]\left[(MgATP) + K_B\right]}$$

where

$V_{max} = 2.16 \times 10^4$ moles/min-mole enzyme   [35]

$K_A = 2.2 \times 10^{-4}$ moles/liter   [25]

$K_B = 2.8 \times 10^{-4}$ moles/liter   [25]

10. Phosphoglucose Isomerase (5.3.1.9) Mw = 125,000   [36-38]

$$K = 3.35 \qquad [39]$$

$$V = \frac{V_{max\ 1} E_o (F6P)}{(F6P) + K_A} - \frac{V_{max\ 2} E_o (G6P)}{(G6P) + K_B}$$

where

$V_{max\ 1}$ = 2 × 10^5 moles/min-mole enzyme (human erythrocytes)   [37]

$V_{max\ 2}$ = 1.875 × 10^5 moles/min-mole enzyme (pea)   [38]

K_A = 2 × 10^{-5} mols/liter (human erythrocytes)   [37]

K_B = 2.7 × 10^{-4} mole/liter (pea)   [38]

Major Inhibitors

ATP (K_i = 4 × 10^{-4}; competitive inhibitor of F6P)   [39]

P_i (K_i = 1.7 × 10^{-3}; competitive inhibitor of F6P)   [39]

11. Phosphoglucomutase (2.7.5.1) Mw = 65,000   [40]

$$K = .058 \qquad [41]$$

$$V = \frac{V_{max\ 1} E_o (G6P)}{(G6P) + K_A} - \frac{V_{max\ 2} E_o (G1P)}{(G1P) + K_B}$$

where

$V_{max\ 1}$ - No value reported

$V_{max\ 2}$ = 4.85 × 10^4 mole/min-mole enzyme (bovine mammory gland) [42]

K_A = No value reported

K_B = 8.5 × 10^{-6} mole/liter (bovine mammory gland)   [43]
      or
      4 × 10^{-4} mole/liter (shark or flounder muscle)   [44]

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12. Carbamyl Phosphokinase (2.7.2.2) Mw = 66,000   [45]

All values for enzyme from Streptococcus faecalis.

$$K = 40 \qquad [45]$$

$$V = \frac{V_{max\ 1} E_o (CP)(MgADP)}{\left[(CP) + K_A\right]\left[(MgADP) + K_B\right]} - \frac{V_{max\ 2} E_o (carbamate)(MgATP)}{\left[(carbamate) + K_C\right]\left[(MgATP) + K_D\right]}$$

where

$$V_{max\ 1} = 8.76 \times 10^4 \text{ moles/min-mole enzyme} \qquad [45]$$

$$V_{max\ 2} = 1.1 \times 10^4 \text{ moles/min-mole enzyme} \qquad [45]$$

$$K_A = 1 \times 10^{-4} \text{ moles/liter} \qquad [45]$$

$$K_B = 5 \times 10^{-5} \text{ moles/liter} \qquad [45]$$

$$K_C = 8 \times 10^{-5} \text{ moles/liter} \qquad [45]$$

$$K_D = 8 \times 10^{-6} \text{ moles/liter} \qquad [45]$$

13. Other Reactions

(1) $CO_2 + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} H^+ + HCO_3^-$
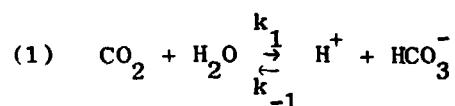
$$V = k_1 (CO_2) - k_{-1}(H^+)(HCO_3^-)$$

$$k_1 = 2.16 \text{ min}^{-1} \qquad [46, 47]$$

$$k_{-1} = 4.8 \times 10^6 \text{ liter/mole-min} \qquad [46, 47]$$
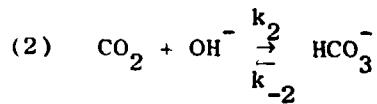
(2) $\quad CO_2 + OH^- \overset{k_2}{\underset{k_{-2}}{\rightleftharpoons}} HCO_3^-$

$$V = k_2(CO_2)(OH^-) - k_{-2}(HCO_3^-)$$

$$k_2 = 5.25 \times 10^5 \text{ liter/mole-min} \qquad\qquad [48,49]$$

$$k_{-2} = 1.17 \times 10^{-2} \text{ min}^{-1} \text{ (calculated by } k_{-2} = 10^{-14} \times$$
$$k_2 k_{-1}/k_1)$$
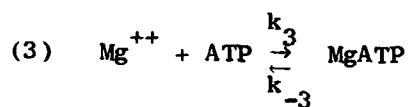
(3) $\quad Mg^{++} + ATP \overset{k_3}{\underset{k_{-3}}{\rightleftharpoons}} MgATP$

$$V = k_3(Mg^{++})(ATP) - k_{-3}(MgATP)$$

$$k_3 = 7.2 \times 10^8 \text{ liter/mole-min} \qquad\qquad [50]$$

$$k_{-3} = 7.2 \times 10^4 \text{ min}^{-1} \qquad\qquad [50]$$
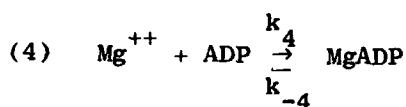
(4) $\quad Mg^{++} + ADP \overset{k_4}{\underset{k_{-4}}{\rightleftharpoons}} MgADP$

$$V = k_4(Mg^{++})(ADP) - k_{-4}(MgADP)$$

$$k_4 = 1.8 \times 10^8 \text{ liter/mole-min} \qquad\qquad [50]$$

$$k_{-4} = 1.5 \times 10^5 \text{ min}^{-1} \qquad\qquad [50]$$

14. Equilibrium

(1) $\quad [H^+][OH^-] = 10^{-14}$

(2) $\quad \dfrac{[ATPH_2]}{[H^+][ATPH]} = 2.24 \times 10^4 \text{ liters/mole} \qquad\qquad [50]$

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(3) $\dfrac{[ATPH]}{[H^+][ATP]} = 8.52 \times 10^6$ liters/mole [50]

(4) $\dfrac{[MgATPH]}{[Mg^{++}][ATPH]} = 1.26 \times 10^2$ liters/mole [50]

(5) $\dfrac{[ADPH]}{[H^+][ADP]} = 4.5 \times 10^6$ liters/mole [50]

(6) $\dfrac{[MgG6P]}{[Mg^{++}][G6P]} = 34$ liter/mole [50]

(7) $\dfrac{[MgG1P]}{[Mg^{++}][G1P]} = 50$ liter/mole [42]

(8) $\dfrac{[NH_3][HCO_3^-]}{[NH_2\overset{O}{C}O^-]} = 0.53$ mole/liter [45]

(9) $\dfrac{[H^+][NH_3]}{[NH_4^+]} = 10^{-0.24}$ mole/liter [51]

(10) $\dfrac{[H^+][HPO_4^=]}{[H_2PO_4^-]} = 6.23 \times 10^{-8}$ mole/liter [51]

(11) $\dfrac{[H^+][PO_4^\equiv]}{[HPO_4^=]} = 2.2 \times 10^{-13}$ mole/liter [51]

15. Solubility Data

(1) K_s $MgNH_4PO_4 \cdot 6H_2O = [Mg^{++}][NH_4^+][PO_4^\equiv] = 10^{-12.3}$ [52]

(above pH = 6.87)

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(2) $K_s \, Mg_3(PO_4)_2 = [Mg^{++}]^3 \, [PO_4^{\equiv}]^2 = 10^{-23.5}$ [52]

          (above pH = 6.87) (This species generally remains
          supersaturated as $MgNH_4PO_4 \cdot 6H_2O$ precipitates)

(3) $K_s \, MgHPO_4 = [Mg^{++}][HPO_4^{\equiv}] = 10^{-4.95}$ [52]

          (below pH = 6.87)

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Appendix 4.4

CRITERIA FOR SELECTION OF GLUCOSE → STARCH PATHWAY

There are two possible routes from glucose to G-1-P, and two from G-1-P to starch, so a little arithmetic leads to four possible routes. For simplicity, we will discuss the two choices from G→G-1-P first, and then the two choices from G-1-P to starch. This is an arbitrary choice since the total process is interdependent, but an attempt at integration will be made in the discussion.

Glucose → G-6-P → G-1-P → G-ADP → Starch

Glucose → G-6-P → G-1-P → Starch

Glucose → G-1-P → ₋-ADP → Starch

Glucose → G-1-P → Starch

1. G → G-1-P

Phosphorylation of glucose using ATP and hexokinase (HK) followed by isomerization with phosphoglucomutase (PGM) was used to size and cost the proposed plant, rather than the phosphoramidate hexose transphosphorylase (PHT) alternative, because its disadvantages seemed to be somewhat fewer and less serious. There were difficulties with either alternative, however.

The positive attributes of the HK-PGM process follow.

(1) ATP is already required in the fossil fuel process, so facilities to recycle and replace it would have to be on hand in that instance. Such facilities have been designed, based on a fair amount of data regarding the chemistry of the process. Information on phosphoramidate synthesis, however, is relatively scarce. There are two recent patents giving directions for its synthesis from either P_2O_5 [1] or $Na(PO_3)_3$ [2], but it appears to be unavailable as a commercial product [3]. Workers studying the enzyme that utilizes this compound ordinarily synthesize it themselves from $POCl_3$ and ammonia. If the commercial cost of $POCl_3$ were used as a very rough estimate of the cost involved in recycling, this process would add about 60¢ per pound to the cost of the finished carbohydrate.* This

---

* A 100 ton/day factory using this approach would require 1.7×10^5 lb per day of $POCl_3$ to be involved in the recycling process. The present cost of this chemical is roughly 70¢ per pound (P & B catalog $9.00/6 kg). The total cost per 100 tons carbohydrate, then, is 1.2×10^5 or 60¢ per pound of starch.

figure may, in fact be low, since the process would not yield phosphorous in its most suitable form for the production of $POCl_3$. Also, it does not take into account the fact that the <u>actual</u> amount of PNH_2 needing recycling is double the theoretical amount.

(2) The reaction catalyzed by the enzyme phosphoramidate-hexose transphosphorylase (PHT) is not as simple as desirable. Besides the desired reaction: PNH_2 + glucose $\rightarrow$ G-1-P + NH_3, the homogeneous enzyme also catalyzes the breakdown of PNH_2 in aqueous solution at a comparable rate [4]. Thus, twice as much PNH_2 is required as G-1-P is produced, raising its recycling costs by a factor of two. In addition, this enzyme transfers the phosphate from G-1-P to fresh glucose to give G-6-P at about 0.2 times this rate, and has phosphatase activity for both G-1-P and G-6-P at about this same level. This combination of factors leads to the requirement that conditions be regulated so as to minimize these side reactions.

The length of time in the reactor must be controlled carefully requiring monitoring of the reaction. The pH must be kept at 8.7, or at least in a high range, since the phosphoramidase activity becomes much more serious as the pH is lowered. This makes the reaction incompatable with the G-1-P $\rightarrow$ starch reaction, which runs best at pH 6.3 or 7.2 depending on the enzyme source. Also, the ratio of glucose to PNH_2 must be kept high (4:1) to get maximum yields. (Glucose inhibits several of the side reactions.) Constant recycling of the unused glucose would be required, and most of the glucose is unused, since under optimum conditions only half the PNH_2 goes to four G-1-P, the rest being lost due to the phosphoramidase activity.

A decided drawback of the HK-PGM scheme, however, is the equilibrium constant for the isomerization of G-6-P to G-1-P. Its value is only 0.05, so that even when coupled to the phosphorylase reaction to make starch, the ratio of G-6-P:G-1-P: starch is roughly 6:0.3:1. This requires extensive recycling. On the other hand, the PHT catalyzed reaction is energetically favorable. (Ordinarily this would mean that an uncomplicated mixture of products--no reactants--would result, but this is modified in this particular case by the vagaries of the enzyme. As explained above, the glucose:PNH_2 ratio is high, so the large fraction of unphosphorylated glucose must also be recycled.)

An additional drawback is the cofactor requirement of PGM, which needs G-16-diP to be effective. The continuous recycling of this reaction mixture will make it difficult to keep the level of this cofactor up to its required value, and it is fairly expensive to replace.

On the basis of this rather inconclusive balance sheet, the HK-PGM route was selected. The enzymes can be obtained from yeast and from <u>E. coli</u>.

2. G-1-P $\rightarrow$ Starch

Again, there was no clear preference for one route or the other.
The phosphorylase catalyzed direct conversion of G-1-P to starch has the
advantage of requiring only one enzyme, and no additional substrate which
would need regeneration, while the path involving the conversion to G-ADP
as a first step requires two enzymes and ATP or an equimolar basis. This
would immediately add the cost of recycling and replacement for ATP, as
well as the separation problems attending the ultimate release of ADP and
pyrophosphate. However, the phosphorylase route has a much lower equili-
brium constant, so that recyc'ing is inevitable. (The K_{eq} varies from
10.8 to 2 in the pH range of ɔ.0 to 7.2, and is about 3 at pH 6.9.) This
is particularly serious given the chosen route to G-1-P, which needs a
tug in the desired direction.

A decision based on the relative ease of acquiring the enzymes tends
also to go in the direction of the phosphorylase path. Calculations based
on preparations in the literature indicated that probably large amounts of
starting materials would be required in either case, but much more for the
two-enzyme system. In tabular form:

Enzyme	Tons	Starting Material[**]
Phosphorylase	500	potatoes [5]
	36	E. coli [6]
ADP-PPylase	12,800	corn [7]
	400	Athrobacter [8]
ADP-starch glucocyl transferase	88,000	corn [9]

[*]
$$\text{All based on tons} = \frac{350 \times 10^6 \ \mu\text{moles/min} \times \text{lb extracted}}{\mu\text{moles/min obtained}}$$

On these bases, then, the pathway chosen was the direct conversion
of C-1-P to starch using phosphorylase.

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Appendix 4.5

CHARACTERISTICS OF ENZYMES--GLUCOSE $\rightarrow$ STARCH

1. **Hexokinase E.C. 2.7.11**

    Turnover number and V_{max}

    750 moles/min/mg protein

    or

    (For glucokinase the sp. act. is 59 moles/min/mg)

    75,000 moles/min/mole enzyme

    K_m values

glucose	1.5×10^{-4}	Average of $1.67 \times 10^{-4} + 1.45 \times 10^{-4}$
ATP	1×10^{-4}	Average of $2.00 \times 10^{-4} + 9.5 \times 10^{-5}$

    pH optimum

    The optimum is broad between pH 8 and pH 9 [2]. The assay has been run at pH 8.5 [3] but also at 7.6 [1] when coupled to another enzyme.

    Inhibitors

    ADP is a noncompetitive inhibitor of glucose and the K_i is 1.14×10^{-3} M. Glucose-6-P also inhibits the reaction at a concentration $K_i = 9.1 \times 10^{-3}$ as do high concentrations of Mg^{++}.

    Activators

    Mg^{++} is essential for the activity of the enzyme.

    Activity level and other sources

    Crude extracts from 8 lb yeast cakes contained 360,000 units of hexokinase activity. The crystallized enzyme has been prepared in 14 percent yield. Other, less convenient sources are brain and liver, as well as microbial systems.

<u>Stability</u>

Soluble hexokinase (in solution) loses almost all its activity after a day at 25°C, and 80 percent of its activity after nine days at 0°C [4]. However, insolubilization on PEI silica improves its stability somewhat.

The kinetics of this enzyme have been studied extensively [1,5].

2. <u>Phosphoglucomutase</u> E.C. 2.7.5.1 [6]

<u>Turnover number and sp. act.</u>

5.9 μmoles/min/mg   or   370 moles/min/mole*

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*
Calculated on the basis of:

$$G\text{-}6\text{-}P \rightarrow G\text{-}1\text{-}P \qquad K_{eq} = 0.05$$
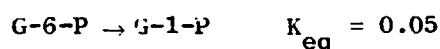
$$K_{eq} = 0.05 = \frac{V_{max}(f) \cdot K_m(r)}{V_{max}(r) \cdot K_m(f)}$$

$$0.05 = V_{max}(f) \ (6 \times 10^{-5})$$

K_m G-6-P estimated by assuming the same ratio for <u>E. coli</u> enzyme as for bovine mammary gland enzyme:

$$\frac{8.5 \times 10^{-6} \ G1P}{5 \times 10^{-5} \ G6P} = \frac{6 \times 10^{-5} \ G1P}{3.5 \times 10^{-4}}$$

$$0.05 = \frac{V_{max} \ (6 \times 10^{-5})}{20 \ \mu moles/min/mg \cdot 3.5 \times 10^{-4}}$$

$$V_{max} = \frac{0.05 \times 20 \times 3.5 \times 10^{-4}}{6 \times 10^{-5}} = 5.9 \ \mu moles/min/mg$$

T.N. = 5.9 μmoles/min/mg × 63,000 mg/m mole

= 3.7 × 10^2 moles/min/mole

This is calculated assuming a pH of 7.5 and a (G16diP) = 8.5 × 10^{-8}. At (G-16diP) = 1.3 × 10^{-6}, the K_m values are different. At pH 9, the specific activity of the enzyme is twice as high.

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K_m values

$G\text{-}6\text{-}P = 3.5 \times 10^{-4}$ (pH 7.5, $G16diP = 8 \times 10^{-8}$)

(see previous footnote)

$G\text{-}1\text{-}P = 6 \times 10^{-5}$ same conditions

pH optimum

Between 8.5 and 9. About half maximal at pH 7.5.

Inhibitors

$G\text{-}1\text{-}P$ at concentrations 8×10^{-3} inhibit the reaction G-1-P
G-6-P. The effect on the desired direction has not been tested.

Activation

Mg^{++} at 1×10^{-3} M is necessary for activity, as well as a
sulfhydryl compound. Cysteine is the most effective at
2×10^{-2} M. Catalytic amounts of G16diP are also necessary.

Level of activity and sources

PGM is found in a variety of tissues, animal and microbial.
It is present in yeast and in muscle. In E. coli, a crude
extract of 300 g of cells contained 2100 units (μmoles/min)
of activity (based on the assumptions implicit in the specif-
ic activity calculation above). This means 55 tons of E. coli
would be required to obtain the desired activity.

Stability

The enzyme can be stored at 4° at pH 6 for 3 to 4 weeks, but
60 percent of the activity is gone after 10 weeks.

3.   Phosphorylase--E. Coli 2.4.1.1

Turnover number and sp. act.

Data are given for the reverse reaction [7] (starch breakdown)
as follows: 4.8 μmoles/min/mg protein, or 650 moles/min/mole
enzyme. Based on the Haldane assumption, and assuming a K_m
ratio for $G\text{-}1\text{-}P/P_i$ similar to potato phosphorylase, the calcu-
lated values for the forward reaction are 6.8 μmoles/min/mg
enzyme and 910 moles/min/mole (at pH 7.3).

K_m value

Given for $P_i = 8 \times 10^{-4}$, dextrin $= 7 \times 10^{-4}$. Assumed for
G-1-P $= 4 \times 10^{-4}$.

Inhibitors

Glucose and maltose were tested and found not to inhibit.

Activators

1 mole of pyridoxal phosphate is required per mole of enzyme.

pH optimum

Sharp between 6 and 8, with a maximum at 7.2.

Stability

Can be stored for at least a year at $-20°$, and can survive at
$25°$ for appreciable periods. Begins to be inactivated at 50 °C
and above.

Level

About 36 tons of E. coli would be required for the amount
needed in a 100 ton/day plant. This is based on the yield
obtained by Schwartz [7].

4.   Phosphorylase--Potato 2.4.1.1

Turnover number

Given [8] as 6700 moles/min/mole protein for the forward
(starch synthesizing) reaction at pH 6.3, 30°C.

K_m values

G-1-P $= 3.5 \times 10^{-3}$ M

$P_i = 7.5 \times 10^{-3}$ M

dextrin $= .13$ mg/ml

Inhibitors

No inhibition by relevant materials were listed.

Activators

Requires 2 moles pyridoxal phosphate per mole enzyme.

pH optimum

6.3

Stability

Stable at 30° for at least 17 hours, but inactivated at 65°.

Level

About 500 to 600 tons of potatoes would be required for this process [9].

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Appendix 4.6

GENERAL METHODS OF ENZYME PREPARATION AND IMMOBILIZATION
AND SPECIFIC EXAMPLES OF SEPARATION AND PURIFICATION

1. Separation and Preparation

    There are common preparative methods that can be applied to separa-
tion and purification of most enzymes, if not all, that are required for
the production of carbohydrate. These common techniques are listed in
Table 1. The physico-chemical properties of enzymes which the separation
methods are based on are also shown in Table 2. (Detailed purification
procedures for ribulose diphosphate carboxylase and phosphoribulokinase
are described. These two enzymes are unique to the photosynthetic path-
way.)

    Ribulose diphosphate carboxylase   (E.C. 4.1.1.39)

    (1) Ammonium sulfate fractionation (37%):
             226 g ammonium sulfate = 1 liter of extract
             1 hour standing and precipitation centrifuge as 9000 g
             for 30 min; precipitate is discarded.

    (2) Ammonium sulfate (50%):
             The supernatant is brought to 50 percent saturation with
             ammonium sulfate. Centrifuged. The precipitate is dis-
             solved in 0.1 M potassium phosphate buffer, pH 7.6, con-
             taining 0.1 mM EDTA and 10 mM mercaptoethanol. Centri-
             fuged. The precipitate is discarded.

    (3) Sephadex G-25 column chromatography:
             with 5 mM potassium phosphate buffer, pH 7.6, containing
             0.1 mM EDTA and 10 mM 2-mercaptoethanol.

    (4) DEAE-cellulose chromatography:
             with 0.5 M potassium phosphate buffer pH 7.6, then washed
             with 5 mM potassium phosphate buffer, pH 7.6. The enzyme
             is precipitated by bringing the saturation to 55 percent
             with saturated ammonium sulfate. Centrifuged. The pre-
             cipitate is dissolved in 5 mM potassium phosphate buffer,
             pH 7.6.

    (5) Sephadex G-25
             This solution is subjected to gel filtration on a sephadex
             G-25 column.

    (6) Hydroxyapatite chromatography:
             The gel filtered enzyme is applied to a hydroxyapatite
             column. The ribulose diphosphate carboxylase is eluted
             with 25 mM potassium phosphate buffer, pH 7.6, containing
             0.1 mM EDTA and 10 mM 2-mercaptoethanol.

Table 1

ENZYME SEPARATION METHODS

1. Separation Based on Solubility
   A. Solvent System
   B. Crystallization

2. Separation by Molecular Size
   A. Gel Filtration
   B. Chromatography

3. Separation Based on Immuno Adsorbent
   A. Affinity Chromatography
      (Including Enzyme-Substrate Affinity)

4. Separation Based on Charge Characteristics
   A. Ion Exchange Chromatography

5. Separation Based on Isoelectric Characteristics
   A. Isoelectric Focussing
   B. Electro Decantation

6. Separation Based on Both Charge and Size
   A. Electrophoresis

7. Separation Based on Molecular Weight and Size
   A. Ultracentrifuge
   B. Ultrafiltration
   C. Zonal Centrifuge

Table 2

PHYSICO-CHEMICAL PROPERTIES IMPORTANT
TO SEPARATION AND PURIFICATION

1. Molecular Weight and Molecular Size

2. Charge Characteristics

3. Isoelectric Point

4. Structure and Composition

5. Dielectric Constant

6. Affinity to Substrate and Other Protein

(7) The enzyme is brought to 55 percent saturation with ammonium sulfate that contains EDTA and 2-mercaptoethanol (0.1 mM, and 10 mM, respectively). The enzyme is stored as a suspension at 0° ~ 2°C.

(8) The specific activity of the purified enzyme is about 1.4 ~ 1.6 units per mg.

Phosphoribulokinase (E.C. 2.7.1.19)

(1) Crude extract from spinach is treated with ammonium sulfate. The precipitate is discarded.

(2) Subsequently, two ammonium sulfate fractions (I, 40%; II, 55%) were used. In each treatment the precipitate, first centrifuged, is dissolved in 2 mM triethanolamine buffer, pH 8.5.

(3) Acetone (-20°C) is added to the solution and centrifuged. The precipitate is extracted with tris buffer pH 7.7.

(4) Ammonium sulfate fraction III:
The solution is treated with third ammonium sulfate fraction. The precipitate is collected and dissolved in 0.1 M tris buffer, pH 7.7. The enzyme activity of this preparation is about 310 units/mg, and 40,000 units were obtained from 475 g spinach.

2. Immobilization

Immobilizing isolated enzymes is advantageous when they are to be used as production catalysts: the enzyme can be filtered out of the reaction mixture and used repeatedly, and stability can be improved.

So far very few photosynthetic enzymes have been immobilized and the properties of immobilized photosynthetic enzymes have been characterized only sketchily.

Knowledge of the structure of the enzyme (especially the primary structure: amino acid sequence) and of the active site are very helpful in selecting the support material for the enzyme and the method of immobilization. Information on these properties of the photosynthetic enzymes is scarce. Thus, the choice of the enzyme carrier and the methods of immobilizing these particular enzymes, appears to be largely empirical.

General methods for immobilization include:

(a) Adsorption

Enzymes can be adsorbed onto both charged resins and neutral surfaces. A suitable adsorbent should possess high affinity for the enzyme and cause minimal denaturation. The enzyme

is readily desorbed by the following factors: change in pH, high concentration of salt, ionic strength, and change in temperature. Thus, caution must be taken to minimize desorption of the enzyme. Desorption of enzymes from the adsorbent can be overcome, in some instances, by cross-linking the enzyme subsequent to its adsorption. Fixation of the adsorbed enzyme could be accomplished by intermolecular cross-linking with the use of such cross-linking agents as diazobenzidine-2, 2'-disulfornic acid, and glutaraldehyde.

DEAE-cellulose, DEAE-sephadex, CM-cellulose are some examples of ion-exchange resins that have been used as the adsorbent. Glass beads, quartz, charcoal particles, dialysis tubing, millipore filters, silica gels, collodion membranes, are other examples of adsorbents.

(b) Entrapment

Enzymes can be entrapped within a cross-linked gel matrix by carrying out the polymerization reaction in an aqueous solution containing the enzyme. Polyacrylamide is widely used for this purpose with N,N-methylene bis (acrylamide) as the cross-linking agent. The resulting enzyme entrapped in the polymer gel can be dispersed into particles of defined size.

Some disadvantages of entrapment method are: (1) leakage of enzyme occurs due to uncontrolled and nonuniform pore size of polymer gels, (2) mass transfer of substrate and product in and out of polymer matrix could become the rate limiting factor and the resistance to mass transfer could create a microenvironment of the enzyme that is significantly different from the bulk environment of the reaction mixture.

Microencapsulation of enzymes within a thin semipermeable membrane (i.e., nylon, collodion) is another method of entrapping enzyme. The entrapped enzymes have been shown to have good activity when low molecular weight substrate is used.

(c) Covalent Binding

Certain functional groups of enzymes are suitable for covalent binding to support material as long as the functional groups of the enzyme are not essential for its catalytic activity. Some examples of the support materials that have been used for immobilization of enzymes by covalent binding are: (1) carboxylic polymers with amino groups of enzymes, (2) cellulose with amino groups of enzyme by activating cellulose by trichlorotriazine, (3) sephadex and sepharose, activated by cyanogen bromide, and to this the amino groups of enzymes can be bound, (4) Copolymer, ethylene-maleic anhydride (1:1), can be used as an acylating agent of enzymes, (5) Aminoethyl cellulose or other resins containing primary aliphatic amines can be bound with enzymes by the carboxyl group of enzymes, (6) Polydiazonium salts of p-aminobenzyl cellulose and poly-p-amino

styrene can be used to bind enzymes possessing triosine residues that are not essential for catalytic activity, (7) glass is activated by coupling with 8-aminopropyl triethoxysilane and used as enzyme support material. This method of covalent binding is preferred since the covalently bound enzyme does not leak out of the support material. The sensitivity of enzymes to chemical modification, however, in some cases limits the use of this method.

(d)   Intermolecular Cross-Linking

Enzymes can be immobilized making use of bifunctional reagents. These are glutaraldehyde, bisdiazobenzidine-2,2'-disulfonic acid, trichloro-s-triazine, bisdiazobenzidine-2,2'-disulfonic acid, etc.

Appendix 5.1

REACTOR DESIGN

The purpose of this appendix is to present in some detail the methods used for determining the reactor size and operating characteristics used in the design analysis. Due to limited availability of firm chemical information and time for the project, no attempt has been made at a sophisticated and complete reactor description. Rather, the broad outlines of volumes, flows, and concentrations have been looked at parametrically in an effort to define problem areas and to point the way toward optimal design.

1. Types of Reactors

Chemical processing can be discussed in terms of the time-behavior of the reactors (i.e., discontinuous or batch, and continuous or steady-state) and the nature of the mixing process (i.e., no mixing or "plug flow," and complete mixing). These variables give four kinds of possible reactor configurations and these represent limiting cases. Real systems fall somewhere between the various extremes. (This discussion is limited to processes which might be used for large chemical plants; these are numerous laboratory-scale techniques which can be devised which do not readily lend themselves to large scale processing for reasons of energy cost, precision of construction, or unavailability in large quantities of exotic materials.)

A. Batch-Plug Flow

An ion exchange or chromatography column is typical of this form. The fluids enter one end of the column, which is usually packed with beads, rings, or other structures, and pass through the column with little back-mixing. The reaction progresses until either (1) the packing material is exhausted and the device is shut down for regeneration or refilling, or (2) the requisite amount of product has been made and a new cycle is begun at a later time. In this device, the length of the column is directly proportional to the time required for the reaction, the parameters being the volumetric flow rate and the cross-sectional flow area of the column.

B. Batch-Complete Mixing

This is the classical laboratory process in which the reactants are added to a vessel, the contents stirred, and time is allowed to pass until the reaction reaches the required degree of completion. The vessel is emptied and the process is repeated as often as is required. In this system, the concentrations of reactants and products are changing with time and approaching thermodynamic equilibrium asymptotically.

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C. Continuous-Plug Flow

Packed columns for catalyzed reactions are an example of this type, since the fluid entering passes over the catalyst particles and leaves the bottom of the column without causing any loss of capacity of the catalyst. The important parameters are, typically, the amount of surface area which can be packed into a given volume of column and the pressure drop or energy required to force the fluid through the packing. In laboratory systems, these parameters are secondary or tertiary considerations; in full-scale chemical plants, however, what is done readily on the bench may be economically impossible.

D. Continuous-Complete Mixing (CSTR)

This is similar to Section B except that there is a steady flow of reactant into the vessel and a mixture of reactants and products out of the vessel. The flows are adjusted so that the reaction volume remains constant and a stirring device is used to maintain essentially uniform concentrations throughout the volume. Because of the complete mixing, it is possible to show[*] that the reactant concentration is that of the outgoing stream. Thus, CSTR's typically are larger in volume than plug flow reactors, since the reaction rate depends on the reactant concentration.

2. Assumptions in Design Equations for Reactors

For final design, preparatory to actual construction, careful and detailed analyses of all nonidealities and pilot-plant data are required. However, due to the limited scope and time available for this study, simplifying assumptions were made at appropriate points in an effort to determine the general dimensions of reactors and their operations. The assumptions comprise those in the kinetics (chemistry) and those in engineering (technological). In some cases, the assumptions could be removed by additional mathematical effort (analytic or computer). In other cases, only laboratory and pilot plant studies would serve.

The following is a listing of major assumptions, in no special order:

(1) Mixing in CSTR's is perfect.

(2) Packing particles in columns are spheres.

(3) The fluid properties are those of water.

(4) Particles in CSTR's do not agglomerate to form large, surface area-deficient aggregates.

(5) Diffusion effects in CSTR and plug-flow are minor.[**]

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[*] Levenspiel, O., "Chemical Reactor Engineering," Wiley.
[**] July 1972 issue, "Biotech. & Bioeng."

(6) Reaction kinetics for insolubilized enzymes are similar to those for soluble species.

(7) Inhibitions and competitive reactions can be minimized at operating concentrations.

(8) CSTR's operate at atmospheric pressure, or near it, and the temperatures and pH's are those required by the enzymes.

(9) Degradation losses of enzymes are linear with time or nonexistent and separation processes are highly efficient for enzyme conservation.

(10) Thermodynamic equilibrium is adequately defined by published values of free energy changes for the given reaction, even if these published values are measured indirectly and in complex environments.

The impact of these assumptions will become apparent in the following sections on design.

3. Comparison of CSTR's and Plug-Flow Reactors

CSTR's and Plug-Flow reactors offer individual advantages which predominate in particular designs.

Generally speaking, plug-flow reactors require that only the fluid reactants be processed, since the catalyst or enzyme can be immobilized on solid particles which remain inside the reactor until regeneration or replacement become necessary. Thus, there is no requirement to separate enzyme from product or substracts. This can be very important since enzyme and product molecular sizes, while quite different from each other, are very small on an absolute scale and may require extremely precise membrane or ion exchange separations. Low specific flow rates and high pressures required for membranes can make such processes prohibitively expensive. Ion exchange columns can present large pressure drops at high flows and initial cost, regeneration, and loss of resins can be very serious economic factors.

CSTR's, on the other hand, typically have small pressure drop requirements and can be operated at pressures which allow simple and inexpensive vessel design. However, it is necessary to separate the enzyme from the reactants and recycle the enzyme. In addition, the mixing of reactants and products together produces, generally, a lower concentration of reactants, thus reducing the reaction rate and increasing the required volume. (In some cases, however, this can be an advantage. For example, in enzyme reactions which can be substrate inhibited, the immediate reduction in substrate concentration caused by the mixing can cause an increase in effective reaction rate and a decrease in required volume.)

For the particular processes considered here, the packed columns require very high pressure energies and present other problems in cost. Therefore, the CSTR-type was chosen early in the study and all designs and models are based on the latter. If substantially lower flow rates

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and higher enzyme activities can be achieved due to advances in chemical technology in the future, a re-examination of this decision may become necessary.

5. Mathematical Expressions for Reactor Sizing

    A. Single Enzyme, Single Substrate Reaction with High Free Energy

        The basic equation for developing the reactor design is the mass balance on substrate or product entering the reactor. In general:

$$input-output = accumulation + reaction$$

        For this study, constant volume, steady-state reactors were chosen. Thus, the accumulation term vanishes and the expression becomes

$$input-output = reaction$$
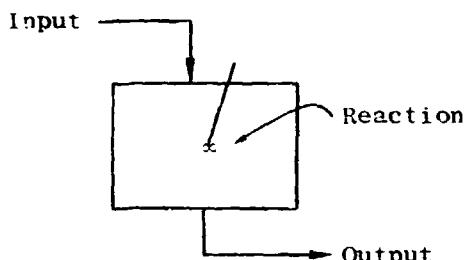
        If the reaction occurs infinitely rapidly, there is no reactor volume required since no time is required for the reaction. Furthermore, if the reaction is irreversible and highly energetic (in the chemical sense), it is possible to have complete conversion of reactant to product. Thus, one limiting case is that of a very rapid, highly irreversible reaction. Such a system requires only very small reactor volumes.

        At the other extreme, a reaction which proceeds very slowly and in which the entering stream is nearly at thermodynamic equilibrium between reactant and product, requires holding times approaching infinity (or infinite volume).

        Furthermore, conservation of expensive reactant requires that a recycle stream be provided to reprocess unreacted material. For some conditions, the recycle stream can be very large, thus increasing the reactor size further and also requiring additional pumps, piping, controls, etc.

        In this study, the highly variable nature of the enzymes used, the great range of thermodynamic equilibrium of the reactions, and chemical considerations vis-a-vis reactant concentrations lead to a very large range in reactor sizes for different parts of the processes.

Returning to the computation, define the input and output concentrations in molarity and the flow, F, in liters/sec. If the substrate concentration is designated as [S], or more simply, S, the input and output terms are:

$$\text{Input} = FS_i, \text{ Mols/sec}$$

$$\text{Output} = FS_o, \text{ Mols/sec}$$

The reaction term depends on kinetics and is defined to give moles/liter-sec. This is the reaction rate per unit volume of CSTR. The total reaction rate is the volumetric rate times the reactor volume.

$$\text{reaction} = (\text{rate})(\text{volume}) = rV_t$$

The rate term can take on many forms. If the reaction is independent of concentrations,

$$r = \text{constant}$$

For a 1st order reaction $(S \rightarrow P)$

$$r = kS$$

For a 2nd order reaction $(S + S \rightarrow P)$

$$r = kS^2$$

and so forth.

For enzyme reactions, the simplest form of the reaction term is the Michaelis-Menton form:

$$r = \frac{V_{max} S}{Km + S} = \frac{kES}{Km + S}$$

where

  r = maximum reaction rate, moles/liter-sec

  S = substrate concentration, mols/liter

 Km = Michaelis constant, mols/liter

  k = turnover number, mols/sec/mol Enzyme

  E = Enzyme concentration, mols/liter

The mass balance equation is thus:

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$$FS_i - FS_o = \left(\frac{kES_o}{Km + S_o} \right) V_t$$

$$F(S_i - S_o) = \frac{kES_o}{Km + S_o} V_t$$

Figure 1 is taken from these relations. Several qualitative conclusions can be made:

(1) Increases of enzyme concentration have inverse effect on reactor volume.

(2) Increases in inlet concentrations reduce reactor volume.

(3) Increases in conversion required increase reactor volume.

(4) For reactions with a low Km and low substrate concentrations, the reactor size does not depend greatly on conversion desired. Put another way, such systems give high conversion (subject to thermodynamic limitations, discussed below).

B. Single Enzyme, Single Substrate Reaction with Low Free Energy

The equilibrium constant for a reaction can be related to the free energy of reaction by the following:

$$-\Delta G^\circ = RT \, \ell n \, Ke$$

ΔG° = free energy of reaction, calories/mol

Ke = equilibrium constant $= P_o/S_o$ at infinite time in a batch reactor (P_o = molarity of product at output)

Expressing $-\Delta G^\circ$ in Kcal/mol and taking a temperature of ~50°C, the equation can be written:

$$Ke = e^{-1.55 \, \Delta G^\circ}$$

This expression places a constraint on the maximum conversion possible in a reactor with no recycle and defines the recycle necessary to achieve a steady production rate of a chosen value.

An examination of the Michaelis-Menton expression shows no limitations on the reaction, yet it must be true that, as the reaction proceeds and the substrate concentration decreases, the product concentration
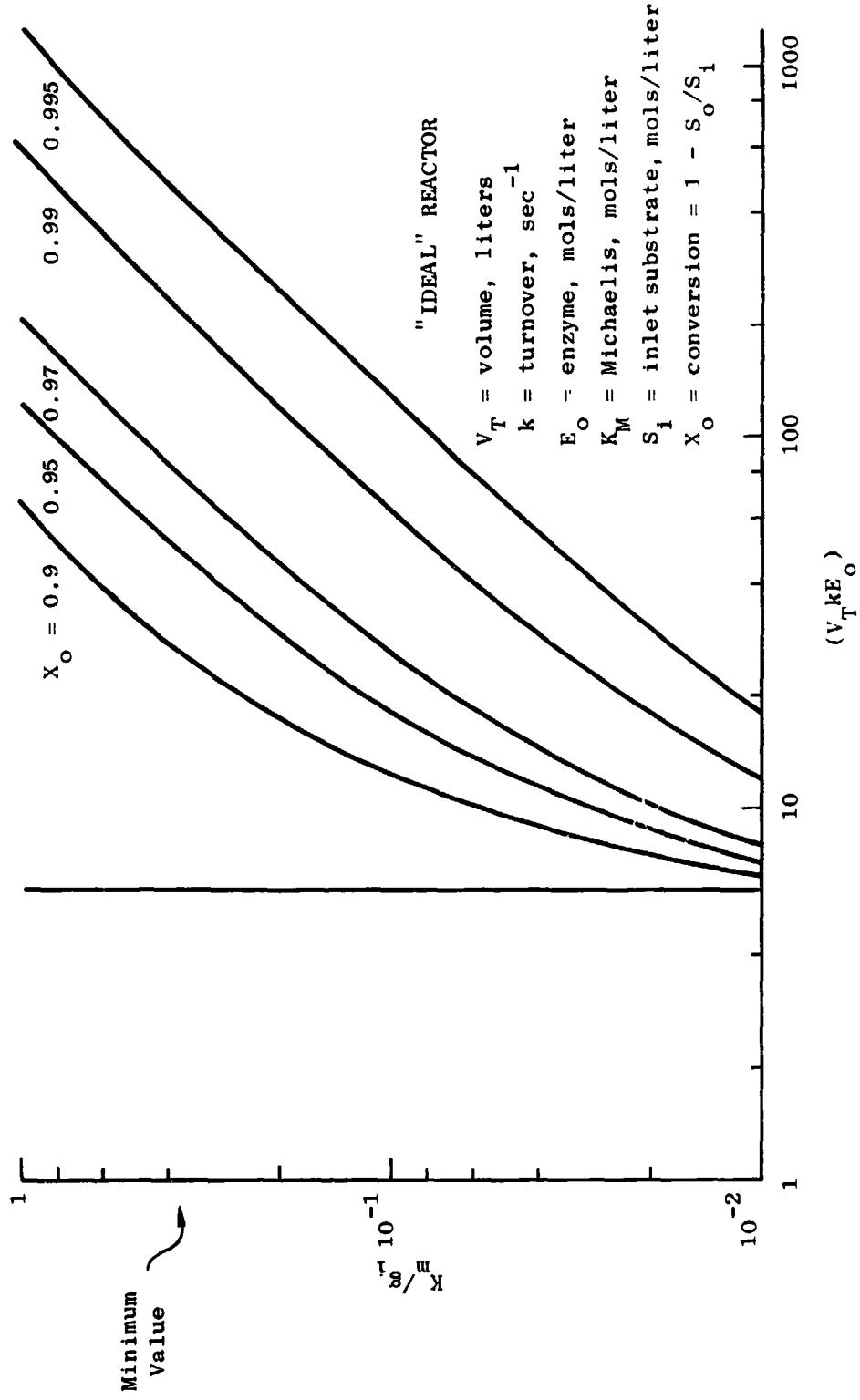
Figure 1.   "IDEAL" REACTOR.

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increases until the equilibrium relation above is satisfied and the reaction stops. For purposes of this calculation, an altered form of the Michaelis-Menton equation is proposed:

$$r = \frac{kE(S_o - S_e)}{Km + S_o} \geq 0$$

where S_e is the equilibrium concentration under the given conditions. The denominator is left unchanged since it is presumed that $Km = S_o$ when the reaction ceases. Now we can write:

$$Ke = \frac{P_{oe}}{S_{oe}} = \frac{\Delta S}{S_{oe}} = \frac{X_m}{1 - X_m}$$

where X_m is the maximum conversion which can be achieved in a once-through operation.

Proceeding from here we can derive an expression for the limiting conversion

$$X_m = \frac{e^{-1.55\ \Delta G^\circ}}{1 + e^{-1.55\ \Delta G^\circ}}$$

A plot of this expression is shown in Figure 2. Note that 99 percent is the maximum conversion in once-through when $-\Delta G^\circ = 3$ Kcal/mol. This result has significant consequences for some of the reactions in the CO_2-fixation and fossil fuel processes and in the glucose $\rightarrow$ starch system.

Even for conversions less than maximum, the reactor volume may become large because the reaction rate decreases rapidly close to equilibrium. This effect can be examined by considering the volume ratio, for the same production and inlet conditions, of the actual reactor compared to one for which the free energy was ignored in the calculation. This volume ratio can be found to be

$$\tau = \frac{(1 - X_o)(1 + e^{-1.55\ \Delta G^\circ})}{(1 - X_o)(1 + e^{-1.55\ \Delta G^\circ}) - 1}$$

A plot of this expression is shown in Figure 3. The figure demonstrates that the increase in reactor volume for reactions with $-\Delta G^\circ < 3$ can be very serious when high conversions are desired. For example, at 87.5 percent conversion for a reaction with $-\Delta G = 2$ Kcal/mole, the actual reactor must be more than 50 percent larger than a simple calculation

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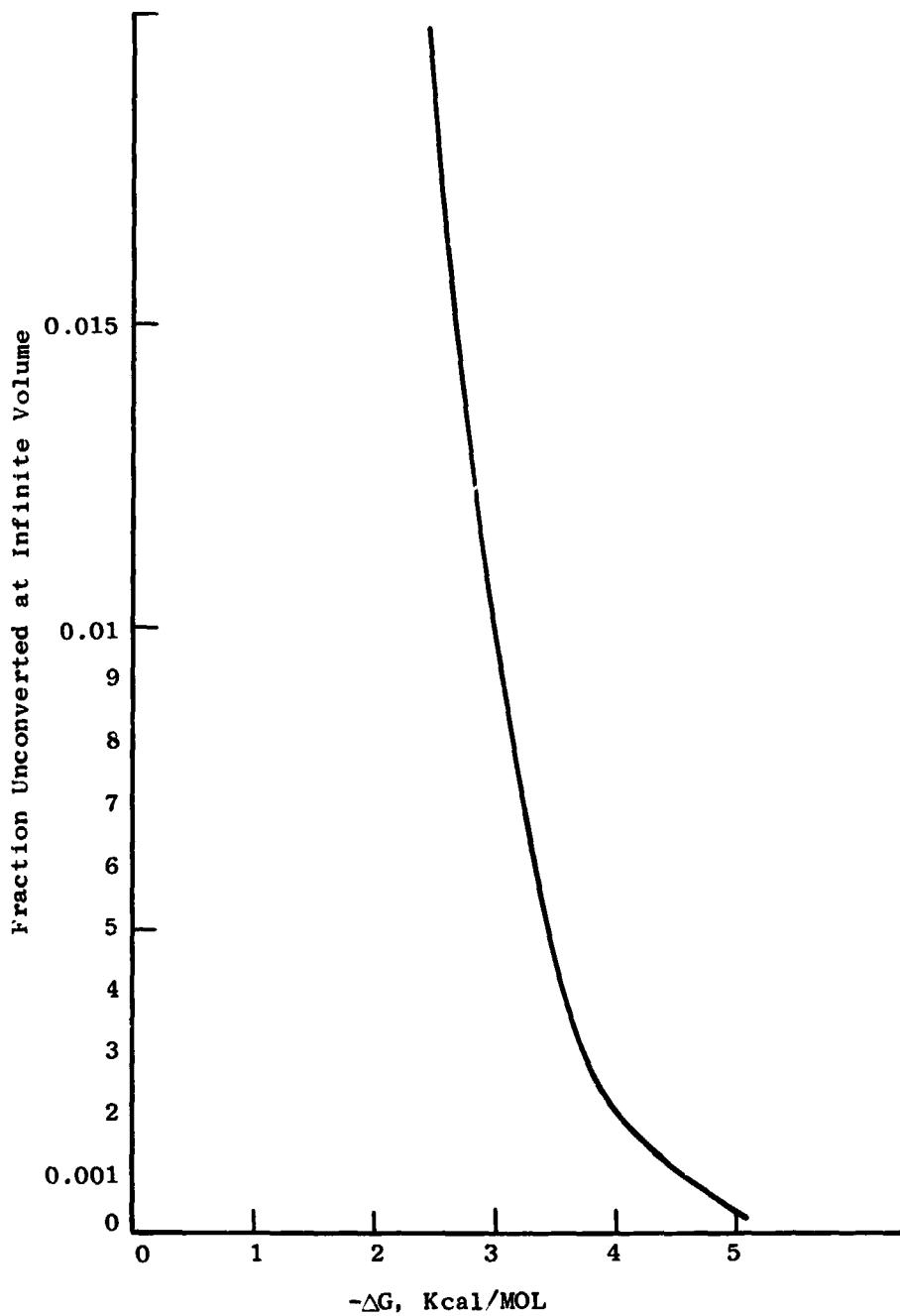
Figure 2. EQUILIBRIUM LIMITS ON CONVERSION.
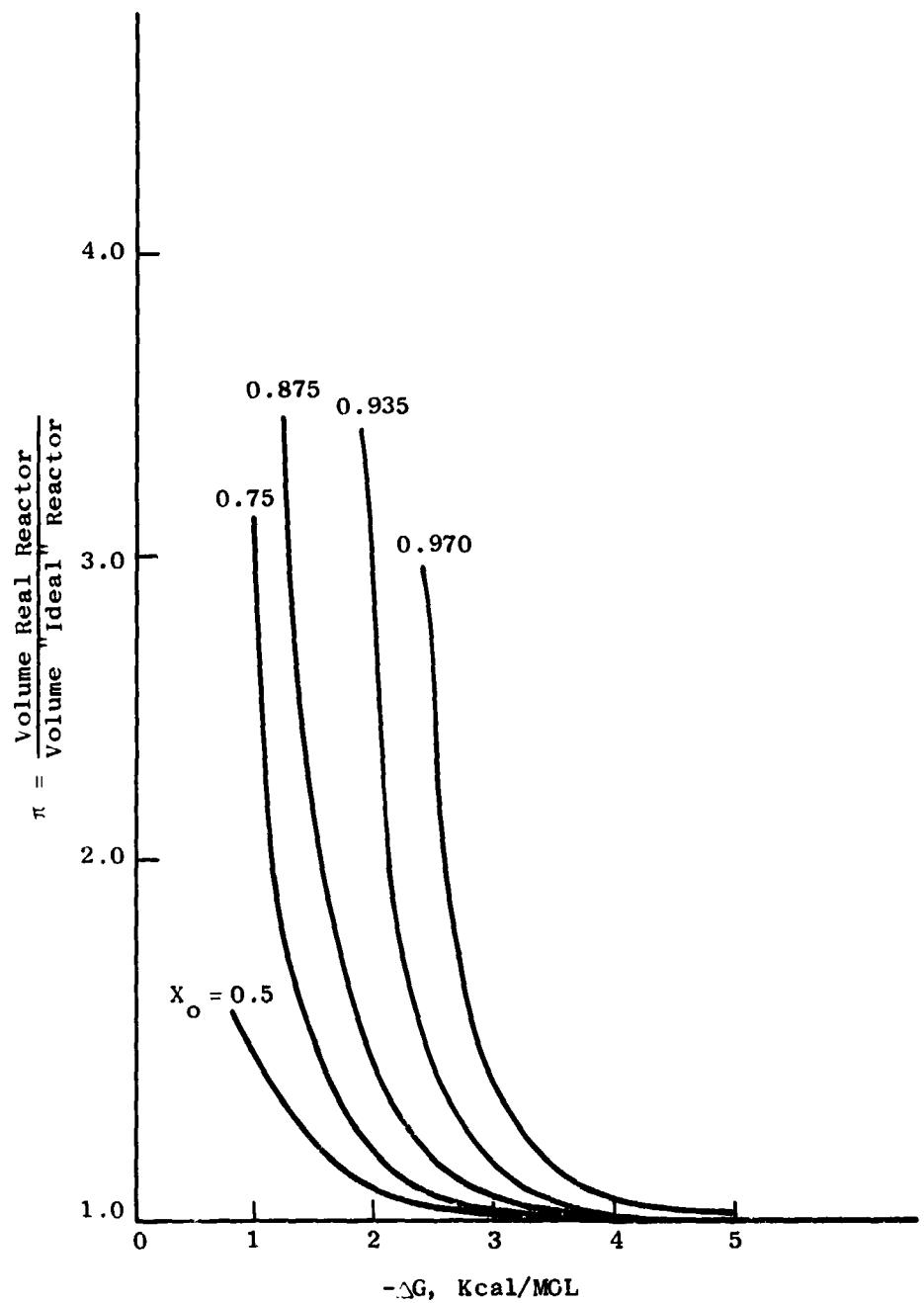
Figure 3. EFFECT OF EQUILIBRIUM ON REACTOR VOLUME.

might indicate. Indeed, ignoring the equilibrium conditions could result in specifying a reactor which could never achieve the production or conversion desired.

Using the data in Figure 2 and the value for a simple or "ideal" reactor, the actual absolute size can be computed. For the case of a total production of product (H) of 6 mol/sec (roughly that of a 100 ton/day starch plant), the "ideal" reactor equation is shown in Figure 1. Note that as K_m/S_i decreases, the "ideal" reactor size approaches a fixed value for any conversion allowed by the thermodynamics.

C.   Single Enzyme, Two Substrate Reaction with High Free Energy

Many enzyme systems catalyze reactions in which two substrates react to form the product. An example of a reaction with unit stoichometry is the conversion of glucose to glucose-6-phosphate in the presence of ATP and the enzyme hexokinase:

$$G + ATP \overset{E}{\underset{\leftarrow}{\rightarrow}} G\text{-}6\text{-}P + ADP - \Delta G^{\circ} \simeq 3.8 \frac{Kcal}{MOLE}$$

$$A + B \overset{\rightarrow}{\underset{\leftarrow}{}} C + D$$
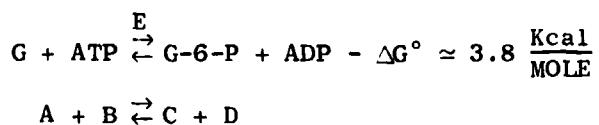
The reaction rate can be written:

$$\gamma = \frac{kEAB}{(K_{m_A} + A)(K_{m_B} + B)}$$

Assume that the reactants are added stoichometrically so that

$$A_i = B_i \quad , \qquad A_o = B_o \quad , \qquad \text{generally} \quad A = B \quad .$$

Mass balance

$$A_i + B_i = C_o + D_o + A_o + B_o$$

$$C_o = A_i - A_o$$

Define

$$X_o = C_o/A_i$$

$$\therefore X_o = 1 - A_o/A_i \quad \text{or} \quad A_o/A_i = 1 - X_o$$

Similarly,

$$r_o/B_i = 1 - X_o$$

$$\therefore FA_1 - FA_o = \frac{kEA_o B_o}{(K_{m_A} + A_o)(K_{m_B} + B_o)} V_T$$

Substituting and manipulating

$$V_T = \frac{H}{kE} \left\{ \left(\frac{K_{m_A}}{A_i(1 - X_o)} + 1 \right) \left(\frac{K_{m_B}}{A_i(1 - X_o)} + 1 \right) \right\}$$

For the special case where $K_{m_A} = K_{m_B}$,

$$V_T = \frac{H}{kE} \left(\frac{K_m}{A_i(1 - X_o)} + 1 \right)^2$$

D. __Two Enzyme Reactions Occurring in Same Tank, Product of One__
   __is Substrate of the Other__

The "coupling" of enzyme reactions is attractive from the
viewpoint of minimizing the number of reactors and separations required.
Furthermore, for reactions which have highly unfavorable equilibrium,
the "attachment" of a reaction which removes the product as rapidly as
it is formed causes the coupled system to achieve conversion rates un-
attainable with the single reaction alone.

An example pertinent to this study illustrates the concept:
consider the process of taking a glucose solution and producing starch.
One proposed scheme requires the phosphorylation of the glucose to make
G-6-P. The next step is to convert the G-6-P to G-1-P so that polymeri-
zation to starch can proceed. However, this conversion is highly unfav-
orable to G-1-P. The conversion is only about 5 percent at equilibrium.
Thus, a reactor using this reaction would have very high recycle and
large volume requirements. However, the next reaction, G-1-P → starch,
is highly energetic toward starch formation. The result is that G-1-P
is formed into starch very completely, thereby unbalancing the first
reaction and causing more G-1-P formation. In the steady-state contin-
uous flow condition, then, the concentrations of G-6-P and starch are
relatively high in the reactor while G-1-P concentration is low. By
removing G-6-P and starch from the reactor, separating the two and re-
turning the G-6-P to the reactor, the production rate is raised to the
desired level and the __effective__ conversion of entering glucose is 100

percent barring losses in separation. A reactor with a poor equilibrium reaction, then, can produce as much product as a reactor with a good equilibrium and high energy system, the difference being that the former reactor is much larger and has a much higher inventory of reactant present. If the initial reactant is costly, the amortization of this inventory may be a significant item in the final product cost.

Consider the following reaction sequence:

$$S_1 \underset{\longleftarrow}{\overset{E_1}{\longrightarrow}} S_2 \underset{\longleftarrow}{\overset{E_2}{\longrightarrow}} P$$
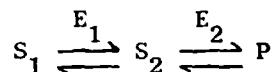
Assume that there are no molecules S_2 or P in the inlet to the reactor (either the reactions proceed rapidly to completion, or the recycle separatio⁻ step is essentially perfect and only S_1 and minute quantities of S_2 are returned). A mass balance gives:

$$S_{1_i} = S_{10} + S_{20} + P_o$$

At steady operation, the concentrations of all components are invariant with time. Therefore, the rate of production of S_2 must equal the rate of production of P. If we use the Michaelis-Menton expression for each reaction, we get:

$$\frac{k_1 E_1 S_{10}}{K_{m_1} + S_{10}} = \frac{k_2 E_2 S_{20}}{K_{m_2} + S_{20}}$$

or

$$S_{10} = \frac{k_2 E_2 S_{20} K_{m_1}}{k_1 E_1 (K_{m_2} + S_{20}) - k_2 E_2 S_{20}}$$

But the mass balance requires that $S_{10} + S_{20} = S_{1_i} - P_o$

$$\therefore S_{20} + \frac{k_2 E_2 K_{m1} S_{20}}{k_1 E_1 K_{m_2} + S_{20}(k_1 E_1 - k_2 E_2)} = S_{1_i} - P_o$$

An overall "conversion" of $S_1 \rightarrow P$ can be defined as:

$$Y_o = \text{conversion} = P_o / S_{1_i}$$

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Thus,

$$S_{1_i} - P_o = S_{1_i}(1 - Y_o)$$

Further manipulation gives:

$$S_{20} = \frac{-\alpha + \sqrt{\alpha^2 + 4\beta}}{2}$$

$$S_{10} = S_{1_i}(1 - Y_o) + \frac{\alpha + \sqrt{\alpha^2 + 4\beta}}{2}$$

where

$$\alpha = \frac{K_{m_2}/K_{m_1} + \dfrac{k_2 E_2}{k_1 E_1} - \dfrac{S_{1_i}}{K_{m_1}}(1 - Y_o)\left(1 - \dfrac{k_2 E_2}{k_1 E_1}\right)}{\dfrac{1}{K_{m_1}}(1 - k_2 E_2/k_1 E_1)}$$

$$\beta = \frac{S_{1_i}(1 - Y_o)K_{m_2}}{1 - k_2 E_2/k_1 E_1}$$

The results of these equations can be calculated for any given pair of reactions for which the kinetics are available. Furthermore, if the equilibrium constants or the free energies are also available, the maximum conversion without recycle can be computed. If in addition the desired inlet concentration to the reactor is fixed and is a value greater than the feed stream supplied, the recycle stream is fixed with respect to concentration and flow. The volume of the reactor comes directly from the rate expression:

$$V_T = \frac{H}{k_1 E_1}\left\{K_{m_1}/S_{1_i}(1 - Y_o) + 1\right\}$$

For example, returning to the glucose to starch system, and using production, kinetic, and thermodynamic values appropriate to this study (see main body of the report), we have the following:

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Equilibrium Constants: G-6-P $\rightarrow$ G-1-P,   keq $\cong$ 1.17

G-1-P $\rightarrow$ starch, keq $\simeq$ 5.7
(based on phosphate)

Kinetics: G-6-P $\rightarrow$ G-1-P, k_1 = 370 mols/min/mol E

K_{m_1} = 3 $\times$ 10^{-4} moles/liter

k_2 = 6400

K_{m_2} = 6 $\times$ 10^{-5} moles/liter

Let   A = G-6-P   Flows: Inlet 0.694 TM/day
     B = G-1-P   G-6-P
     C = starch (or phosphate)   775 TM/day
 H_2O
                             Molarity = 0.05 M

Keq_1 = B/A , Keq_2 = C/B , $K_{overall}$ = $K_1 K_2$ = C/A .

Assume that the reactor is run to very close to equilibrium. Concentrations can then be approximated as equilibrium values. The flow sheet is as follows:

775 TM H_2O F
       X = 0.05 M
0.694 TM G-6-P

       X = 0.1 M   R
                                                    X_R

                          Reactor
                          V_T

                          X_B (F + R)
                                        Starch
                                + 775 TM H_2O
                                - 0.694 TM starch
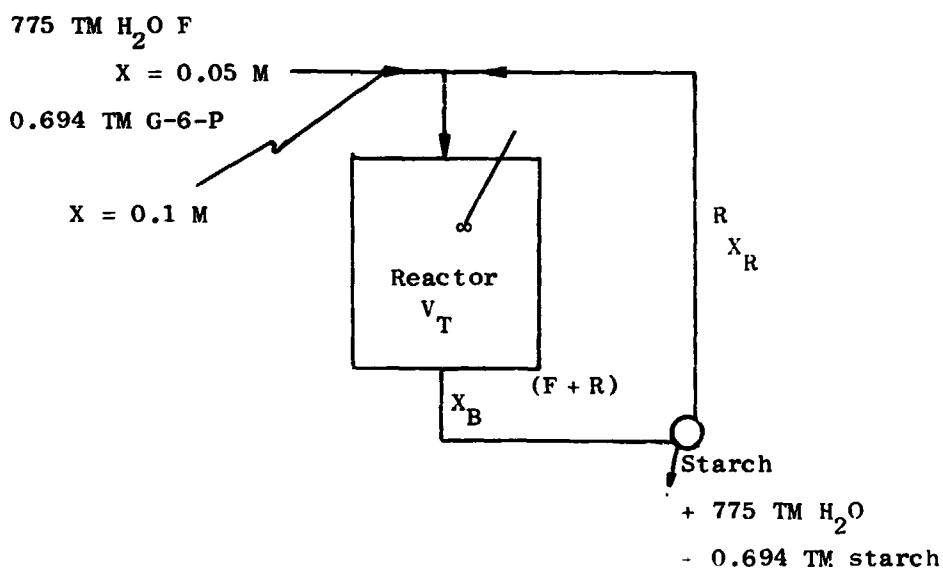
$$K_o \quad \frac{\text{Starch Concentration at Outlet of Reactor}}{\text{G-6-P Concentration at Outlet}}$$

$$\text{Starch concentration at outlet} = \left(\frac{0.694}{F + R}\right)\left(\frac{0.1\ M}{18 \times 10^{-4}}\right)$$

$$\left[\left(\frac{0.1\ M}{18 \times 10^{-4}}\right) = \text{factor to convert to molarity}\right]$$

$$\text{G-6-P Concentration at Outlet} = \frac{(X_F F + X_R R) - 0.694 - G\text{-}1\text{-}P}{(F + R)} \left(\frac{0.1}{18 \times 10^{-4}}\right)$$

In words, G-6-P is either unreacted, in the form of starch, or in the form of G-1-P. Thus,

$$K_o = \frac{0.694}{(X_F F + X_R R) - 0.694 - (G\text{-}1\text{-}P)_o}$$

$$(G\text{-}1\text{-}P)_o = \frac{(G\text{-}6\text{-}P)_o}{1/K_1} = \frac{(X_F F + X_R R) - 0.694 - (G\text{-}1\text{-}P)_o}{1/K_1}$$

Rearranging

$$(G\text{-}1\text{-}P)_o \frac{1}{K_1} + (G\text{-}1\text{-}P)_o = (X_F F + X_R R) - 0.694$$

$$(G\text{-}1\text{-}P)_o = \frac{(X_F F + X_R R) - 0.694}{1 + 1/K_1}$$

Substituting into K_o:

$$K_o = \left[\frac{0.694}{(X_F F + X_R R) - 0.694 - \dfrac{(X_F F + X_R R) - 0.694}{1 + 1/K_1}}\right]$$

$$K_o = \frac{0.694}{(X_F F + X_R R)\left(1 - \dfrac{1}{1 + 1/K_1}\right) - 0.694\left(1 - \dfrac{1}{1 + 1/K_1}\right)}$$

$$K_o = \frac{0.694}{(X_FF + X_RR - 0.694)\left(1 - \frac{1}{1 + 1/K_1}\right)}$$

but

$$X_XF = 0.694$$

$$\therefore K_o = \frac{0.694}{(X_RR)\left(1 + \frac{1}{K_1} - 1\big/1 + 1/K_1\right)}$$

or

$$X_RR = \frac{0.694}{K_o\left\{\dfrac{1/K_1}{1 + 1/K_1}\right\}}$$

$$K_o = K_1K_2$$

$$X_RR = \frac{0.694}{\dfrac{K_2}{1 + 1/K_1}} = \frac{0.694\ (1 + 1/K_1)}{K_2}$$

$$X_RR = \frac{0.694\ (1 + K_1)}{K_1K_2}$$

$$K_1 = 1/17, \quad K_2 = 5.7$$

$$\therefore X_RR = \frac{0.694\ (1 + 1/17)}{5.7/17} = 2.18$$

Thus, the G-6-P entering the reactor is:

$$\underset{\text{process}}{0.694} + \underset{\text{recycle}}{2.18} = 2.874$$

The entering molarity is chosen as 0.1 M. Thus, the water in the recycle stream can be calculated:

$$\frac{2.874}{(775 + R)} \times \left(\frac{0.1}{18 \times 10^{-4}}\right) = 0.1$$

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$$(775 + R) = \frac{2.874}{18 \times 10^{-4}} = 1600$$

$$\therefore R = 1600 - 775 = 825 \text{ TM/day}$$

The recycle stream molarity is thus,

$$\frac{2.18}{825} \times \frac{0.1}{18 \times 10^{-4}} = 0.147 \text{ M}$$

The reactor volume can be estimated from the kinetic parameters of the slowest and least favorable reaction, the first

$$rV_T = \text{production}$$

$$V_T = \frac{H}{r} = \frac{H(K_m + S_{10})}{k, E, S_{10}}$$

In the calculations,

$$S_{10} = 0.147 \times \frac{F}{(F + R)} = 0.147 \left(\frac{775}{1600}\right)$$

$$S_{10} = 0.071 \text{ M}$$

$$H = 350 \text{ moles/min}, \quad k = 370, \quad E \cong 3 \times 10^{-6}$$

$$\therefore V_T = \frac{350 \ (3 \cdot 10^{-4} + 0.071)}{370 \times 3 \times 10^{-6} \times 0.071} = 320,000 \text{ liters}$$

$$V_T = 84,000 \text{ gallons}$$

A reactor of 150,000 gallons capacity was specified to allow flexibility and safety factors, as well as head space for internal equipment, foaming, etc.

E.   Single Enzyme Reaction with Product Inhibition

There are frequently systems in which one or more products and/
or nonreacting species inhibit the enzymatic process. The net effect is
to slow down the reaction rate, sometimes, severely, particularly with
product inhibitions at high conversions. The reaction equation is modi-
fied to include an additional term as follows:

$$r = \frac{kES}{S + K_m + K_m \; Pi/Ki}$$

The same algebraic analysis as used previously can be applied to such
systems. For example, suppose a reaction is:

$$F \xrightleftharpoons{E} G + P_i$$
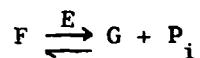
Pi is a product, perhaps phosphate, and inhibits the reaction. The reac-
tor design equation becomes:

$$V_T = \frac{H}{kE} \left\{ 1 + \frac{K_m}{S_i (1 - X_o)} + \frac{K_m}{K_i} S_i X_o \right\}$$

Thus, the net effect is to make the required reactor larger by an addi-
tive term:

$$\Delta V_T = \frac{H}{kE} \left\{ \frac{K_m}{K_i} S_i X_o \right\}$$

The ratio of reactor volume with inhibition of this sort to the reactor
volume without inhibition is then:

$$\frac{V_{inh}}{V_T} = 1 + \frac{(K_m/K_i) \; S_i X_o}{1 + \dfrac{K_m}{S_i (1 - X_o)}}$$

Increases in S_i, X_o, or K_m tend to make the problem worse. Decreases
in K_i have a similar effect.

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For the case of $K_m/S_i = 0$, $K_i/S_i = \infty$, Figure 4 gives the CSTR volume. This volume must be multiplied with the factor ᐤ given in Figure 5.

F.   Single Enzyme, Single Product, Substrate Inhibition

Again, the algebraic manipulations are straightforward. Assume a reaction rate equation:

$$r = \frac{kES}{K_m + S + S/K_i}$$

The resulting reactor design expression is:

$$V_T = \frac{H}{kE} \left\{ \frac{K_m}{S_i(1 - X_o)} + 1 + 1/K_i \right\}$$

The volume is, thus, larger by the following amount:

$$\Delta V_T = \frac{H}{kE} \left(\frac{1}{K_i} \right)$$

G.   Reactors and Fermenters for Cellulose Process

The design of the main reactor in the cellulose process was carried out differently than the techniques discussed here, partly because this reactor was the first one examined during the study but mostly because kinetic and production data were available only in a form not suitable for methods of this appendix.

The reactor and fermenter were considered to form a coupled system since it is expected that the enzyme activity will decrease with time, requiring makeup, and that losses in the end separation will also occur. In addition, as in all proposed enzyme processes, it is important to conserve enzyme, so recycle is mandatory and further couples the reactor and the fermenter.

The variables examined here include the production of enzyme, the degradation losses, the separation losses, and the enzyme activity. The results of Mandel, et al at Natick Laboratories have been used as the basic expressions for enzyme activity and production.
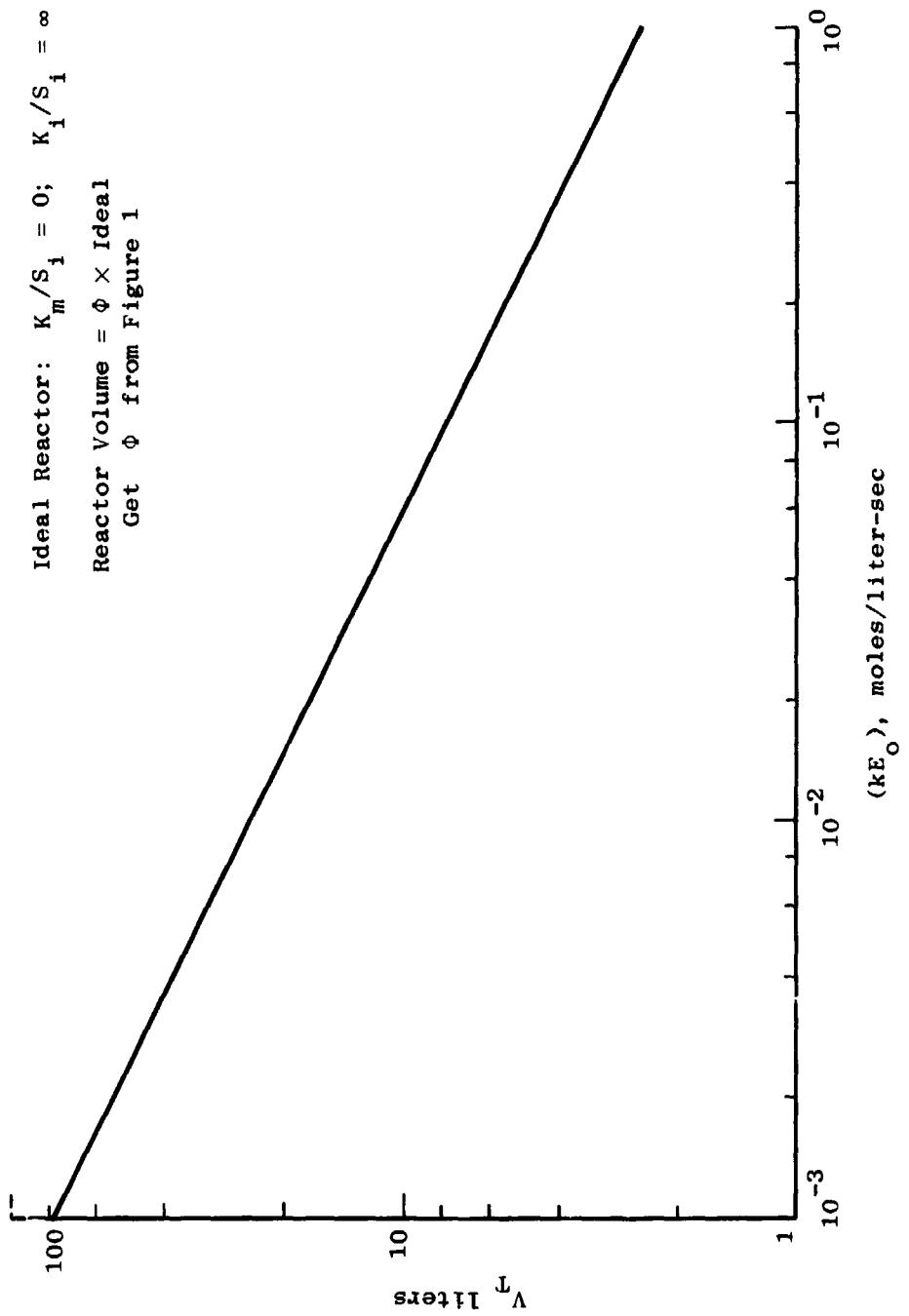
Figure 4. CSTR VOLUME FOR "IDEAL" REACTOR.

Ideal Reactor: $K_m/S_i = 0$; $K_i/S_i = \infty$

Reactor Volume = $\Phi \times$ Ideal
Get Φ from Figure 1

(kE_o), moles/liter-sec

V_T liters

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Assumptions: $X_o \approx 0.99$

$$\text{rate} = \frac{kE_o S_o}{K_m + S_o + (K_m/K_i)P_i}$$

$P_i =$ phosphate

$S \to P + 2P_i$

$$\zeta = \frac{\text{Reactor Volume}}{\text{Ideal Volume}}$$

Ideal Volume is when

$K_m/S_i = 0; \quad K_i/S_i = \infty$

10^{-3}

10^{-2}

$K_i/S_i = \infty$

K_m/S_i

ζ
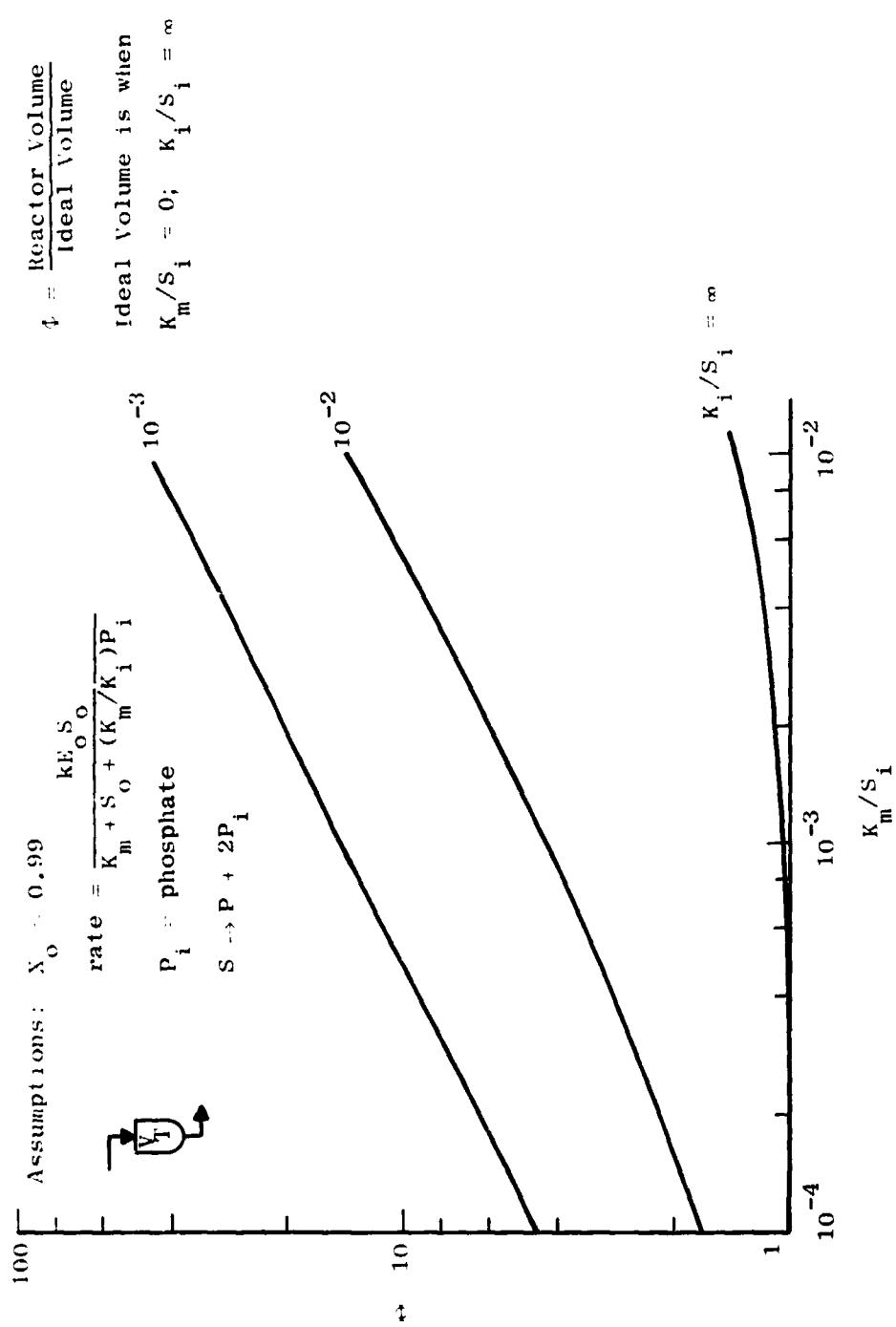
Figure 5. EFFECT OF PHOSPHATE INHIBITION ON 1-16DP.

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These values are:

$$\frac{1 \text{ gm enzyme}}{\text{liter-day}} = \text{production of fermenter}$$

$$\frac{4 \text{ gm-Glucose}}{\text{gm-Enzyme-He}} = \text{activity of fresh enzyme}$$

$$1.25 \text{ gm E/liter} = \text{concentration in tank}$$

For a 100 ton/day plant, the flow rate is about 154 gallons/minute of a 10 percent by weight cellulose.

Assume the enzyme activity decreases linearly with its "age" and that half of the activity is lost in θ_1 days.

$$A_t = A_o \left[1 - \frac{\theta}{2\theta_1} \right]$$

Writing a balance on "activity":
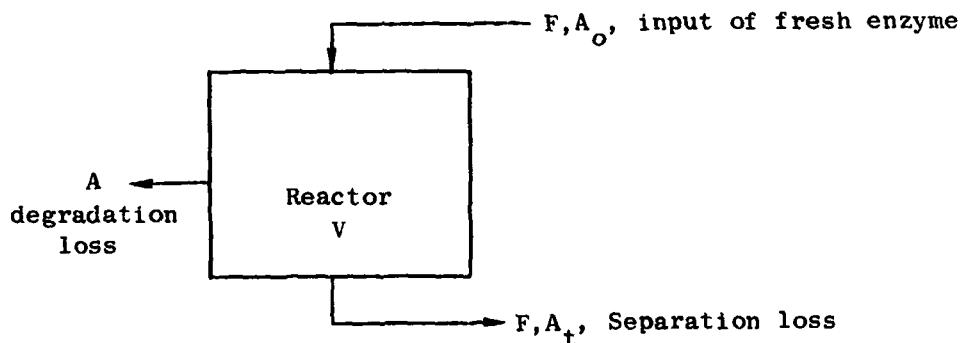
$$FA_o - FA_t + V \frac{dA}{d\theta} = 0$$

input output "reaction"

$$\therefore A_t/A_o = 1 - \left(\frac{V}{F} \right) \left(\frac{1}{2\theta_1} \right)$$

FA_t = loss in separation

VA_t = activity in tank

$$\frac{FA_t}{VA_t} = \frac{F}{V} = \text{fraction lost in separation}$$

As is seen later, the reactor volume and flow is such that the reaidence time of the cellulose stream is approximately 1 day. Thus, $F/V = L$ represents the fraction of tank enzyme inventory lost per day in the separation step (removing enzyme from glucose and cellulose). Let $F/V = L$.

$$\frac{A_t}{A_o} = 1 = \frac{1}{L}\left(\frac{1}{2\theta_1}\right)$$

The volume of reactor required is directly proportional to the actual enzyme activity. Thus, as A_t/A_o decreases due to losses or to degradation, the main reactor volume increases.

The ratio is:

$$\frac{V}{V_o} = \frac{A_o}{A_t}$$

where

V_o = reactor volume if enzyme lasts indefinitely and none is lost

V = actual volume required

V_o can be estimated from the known enzyme activity.

100 tons/day Glucose = 3.78×10^6 gm/hr

$\therefore$ gm-Enzyme = $3.78 \times 10^6 \frac{\text{gm-G}}{\text{HR}} \times \frac{1 \text{ gm-enzyme/hr}}{4 \text{ gm Glucose}}$

$= 9.5 \times 10^5$ gm enzyme

with an enzyme rate of 1.25 gm enzyme/liter

$\therefore \frac{9.5 \times 10^5 \text{ gm E}}{1.25 \text{ gm E/liter}} = 7.6 \times 10^5$ liters

$V_o \cong \underline{2(\!)0,000 \text{ gallons}}$

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Thus

$$V = 200,000 \left(\frac{A_o}{A_t}\right) = 200,000 \left(\cfrac{1}{1 - \cfrac{1}{L}\cfrac{1}{2\theta_1}}\right)$$

$$V = 200,000 \; \frac{2L\theta_1}{2L\theta_1 - 1} = 4 \times 10^5 \; \frac{L\theta_1}{2L\theta - 1}$$

A plot of this expression is shown in Figure 6. For the conditions estimated for this plant $(\theta_1 = 700 \text{ days}, \; L = 0.003)$ we get a reactor size of 270,000 gallons, a significantly larger number than that computed for the case of no enzyme degradation or loss. Without enzyme recycle, the age of the enzyme remains near zero, so the main reactor can be smaller. However, the enzyme producer becomes very large and may dominate the cost of product. The next step, then, is to size the fermenter required.

The fermenter is directly related to the enzyme production required to supply the main reactor. The fermenter can make:

1 gm E/5 liters-day

The makeup rate is related to the reactor size, the separation losses, and the enzyme life-time. The loss due to degradation is $1/2\theta_1$ fraction of the tank activity per day. The separation loss is L fraction of the tank enzyme holdup per day. Thus, the enzyme makeup required is:

$$\left(\frac{1}{2\theta_1} + L\right)\left(\frac{V}{V_o}\right)(E)$$

where

$$\frac{V}{V_o} = \text{tank volume/"ideal" volume}$$

E = enzyme holdup in equivalent grams of fresh enzyme

$$\frac{\text{tank volume}}{\text{ideal volume}} = \frac{2 \times 10^5}{2 \times 10^5}\left(\frac{2L\theta_1}{2L\theta_1 - 1}\right) = \frac{2L\theta_1}{2L\theta_1 - 1}$$

and the enzyme load equivalent is 9.5×10^5 gm fresh enzyme.
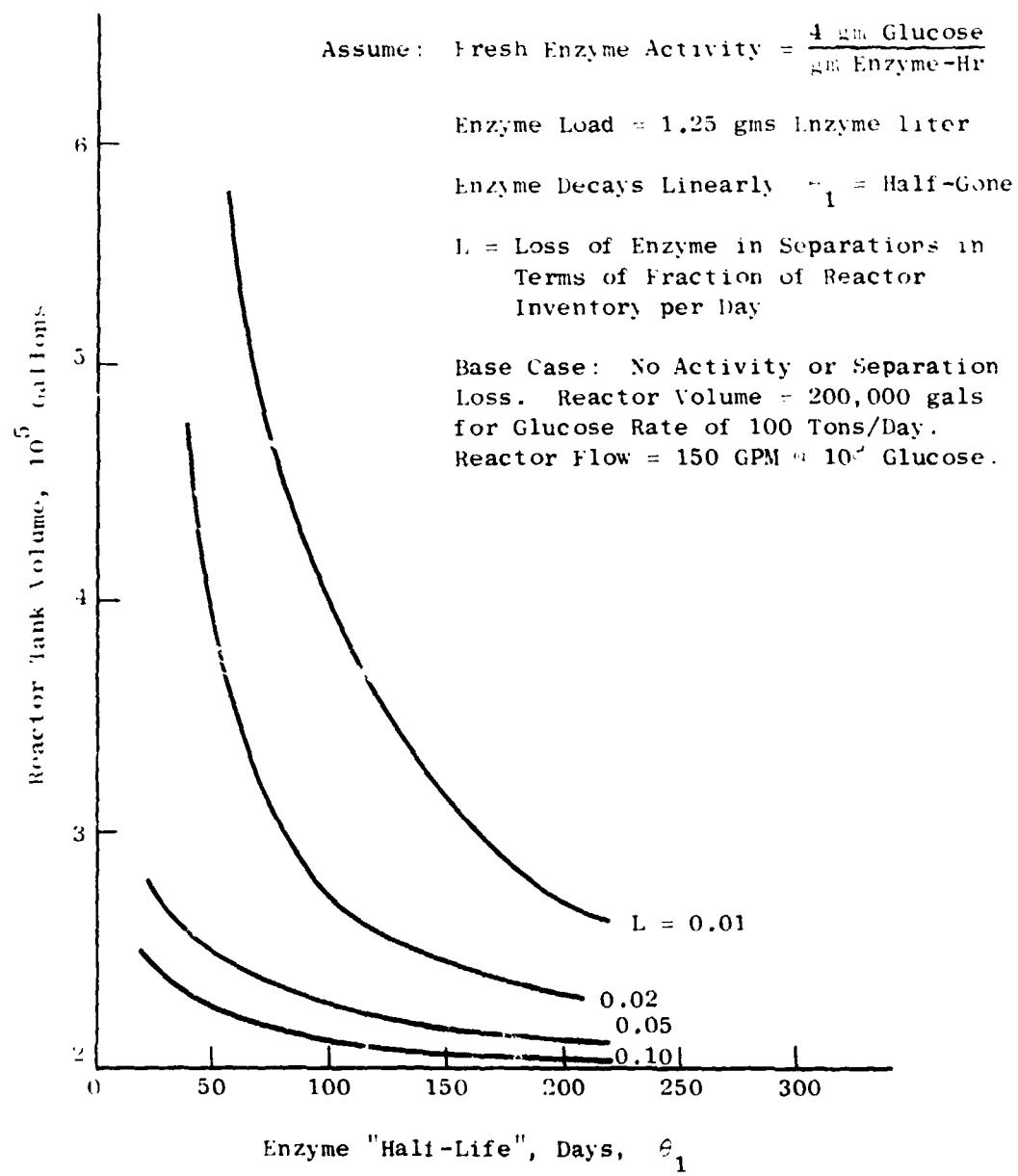
Figure 6. CELLULOSE PROCESS REACTOR SIZE.

$$\text{Makeup Rate} = (9.5 \times 10^5)\left(\frac{2\theta_1 L + 1}{2\theta_1 L - 1}\right) L$$

For $L = 0.003$, $\theta_1 = 700$, $L\theta_1 = 2.1$,

$$\text{Makeup Rate} = 4700 \text{ gms/day}$$

The fermentor can deliver about 1 gm Enzyme/5 day-liter

$$\text{volume} = 5 \times 4700 = 23,500 \text{ liters}$$

$$= \underline{6200} \text{ gallons}$$

The size of 6000 gallons was chosen on the basis that optimization of design can effect at least modest increases in productivity of cellulose fermentation.

The volume of fermenter required in general is:

$$V_F = 1.39 \times 10^6 \; L\left(\frac{2\theta_1 L + 1}{2\theta_1 L - 1}\right) \text{ gallons}$$

H. Comparison of Plug Flow and CSTR's Volume and Energy

It is possible to show that volume savings can be realized by use of packed towers compared to CSTR's if

(1) the enzyme can be immobilized on a support suitable for tower packing,

(2) the enzyme "concentration," moles E/volume reactor can be made the same.

We proceed as follows: (ignoring thermodynamic and inhibition limitations)

$$V_{CSTR} = \frac{H}{kE}\left\{\frac{K_m}{S_i(1 - K_o)} + 1\right\}$$

For packed column:

F ——

S

L

Stds

d_z

tower has diameter
of D and a linear
flow velocity of
the fluid of U_o

Cross-sectional
area $= A_o$
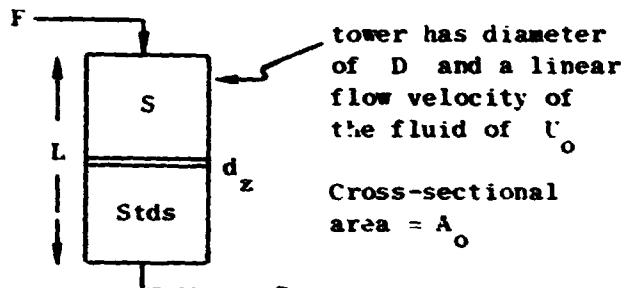
Balance on element dz:

$$\text{Input of } S = FS$$

$$\text{Output} - F(S + dS)$$

$$\text{Accumulation} = 0$$

$$\text{Reaction} \cong A_o dz \frac{kES}{K_m + S}$$

$$FS - F(S + ds) = A_o dz \frac{kES}{K_m + S}$$

$$-FdS = A_o dz \frac{kES}{K_m + S}$$

$$\frac{dS(K_m + S)}{S} = - \frac{A_o}{F} dz \, kE$$

Integrating from $S_i \to S_o$ and $z = 0$ to $z = L$:

$$K_m \ln \frac{S_o}{S_i} + (S_o - S_i) = -V_p \frac{kE}{F}$$

$$S_o = S_i - \Delta S \quad \text{and} \quad X_o = \frac{\Delta S}{S_i}$$

$$\therefore \frac{S_o}{S_i} = 1 - X_o$$

Substituting:

$$K_m \, \ell n \, (1 - X_o) - \Delta S = -V_p \frac{kE}{F}$$

but $FS_i X_o = H$, as before

$$\therefore \; F = \frac{H}{S_i X_o}$$

$$K_m \, \ell n \, (1 - X_o) - \Delta S = - \frac{V_p}{H} S_i X_o kE$$

Divide by S_i:

$$\frac{K_m}{S_i} \ell n \, (1 - X_o) - X_o = - \frac{V_p X_o kE}{H}$$

Solving for V_p:

$$V_p = \frac{H}{kE} \left\{ 1 - \frac{K_m}{S_i X_o} \ell n \, (1 - X_o) \right\}$$

The ratio of volumes is:

$$\frac{V_p}{V_{CSTR}} = \frac{\dfrac{H}{kE} \left\{ 1 - \dfrac{K_m}{S_i X_o} \ell n \, (1 - X_o) \right\}}{\dfrac{H}{kE} \left\{ 1 + \dfrac{K_m}{S_i (1 - X_o)} \right\}}$$

This ratio is shown in Figure 7. It can be seen that, for a given value of K_m, the plug-flow reactor seems advantageous at low inlet concentrations and high conversions.

Unfortunately, packed bed reactors can have very high pressure drops for flows encountered in high production plants when the allowable reactant concentrations are low, as in enzyme reactors with inhibited reactions. The volume of carrier fluid, in this case water, which must be processed can be very large. For example, at concentrations of 0.001 M substrate in the fossil fuel or CO_2-fixation processes, a 100 ton/day
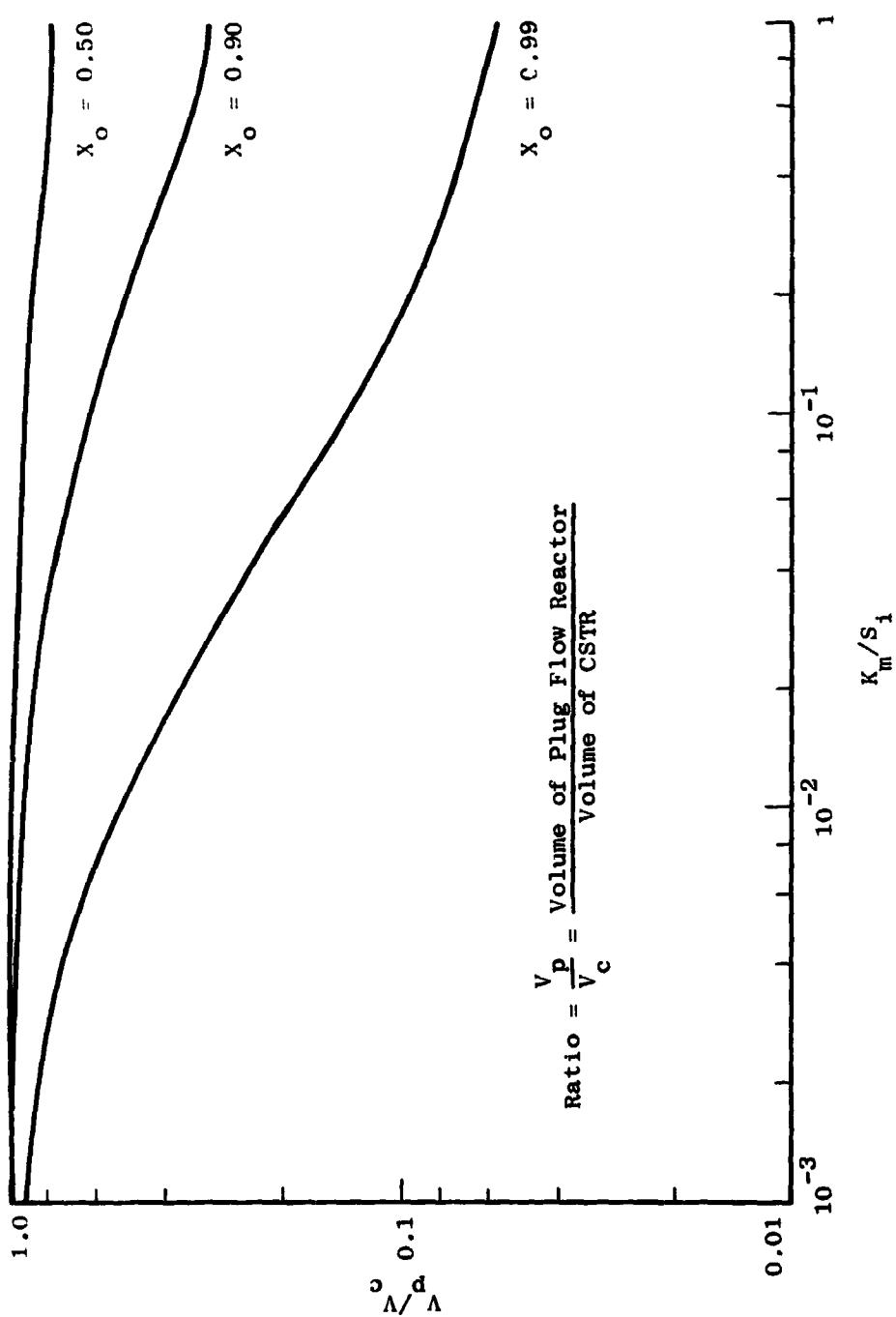
Fig. 7. RATIO OF VOLUMES.

plant requires flows of about 100,000 gpm. When these flows are passed through columns containing beds with small particle sizes (necessary to achieve high surface areas and high enzyme loadings), the pressure drops and resultant horsepowers are unacceptable to economic design.

Using the Ergun equation for pressure drop through a packed bed;

$$\frac{\Delta p}{L} g_c = 150 \frac{(1 - G)^2}{\epsilon^3} \frac{\mu U_o}{(\emptyset_5 dp)^2} + 1.75 \frac{(1 - \epsilon)}{\epsilon^3} \frac{\rho S_o^2}{\emptyset_s dp}$$

where

U_o = superficial tower velocity, cm/sec

dp = particle diameter, cm

μ = fluid viscosity, gm/cm-sec

g_c = 980 gmf/gmn cm/sec^2

ρ_s = fluid density, gm/cm^3 $\emptyset_s$ = sphericity

L = bed depth, cm   = 1, for spheres

Δp = gm/cm^2 pressure drop

and using spherical particles in water, we have $\mu = 0.01$, $\rho = 1$, $\emptyset_s = 1$, $\epsilon \cong 0.4$, we get

$$\frac{\Delta P}{L} \text{ (in p}_{s_i}\text{/ft)} = 3.73 \times 10^{-3} \frac{U_o}{dr^2} + 7.3 \times 10^{-3} \frac{U_o^2}{dp}$$

converting flow, F, in gallons/min to U_o using the tower diameter D_T:

$$\left(\frac{\Delta P}{L}\right) = 0.3 \frac{F}{D_r^2 dp^2} + 47.3 \frac{F^2}{D_G^4 dp}$$

for a tower 100 feet tall, this becomes

$$(\Delta P)dp^3 = 2.6 \times 10^5 \left(\frac{F}{A}\right) + 3.5 \times 10'' (F/A)^2$$

where A = surface area required for enzyme, cm^2. If C_o = molarity of output and molecular weight glucose = 180,

$$F = 92/C_o, \text{ gmp}$$

$$\Delta p \; dp^3 = 2.4 \times 10^7/C_o A + 3 \times 10^{15}/(C_o A)^2$$

Assume a reaction rate of R, μ-mole G/mgm Enzyme-HR and an enzyme load of C_E, mgm E/cm^2 surface. For a production of 100 tons glucose/day, we have 2.1×10^{10} μ-mol/HR. Thus,

$$\Delta p \; dp^3 = 1.13 \times 10^{-3} \left[\frac{RC_E}{C_o}\right] + 6.73 \times 10^{-6} \left[\frac{RC_E^2}{C_o}\right]$$

The horsepower is proportional to flow $\times$ pressure drop. Finally,

$$\text{Horsepower} = \frac{6.05 \times 10^{-5}}{dp^3 C_o} \left[\frac{RC_E}{C_o}\right] + \frac{3.6 \times 10^{-7}}{dp^3 C_c} \left[\frac{RC_E}{C_o}\right]^2$$

A foreseeable reaction rate would be of the order of

$$R = 3 \times 10^4 \; \mu\text{-mol G/mgm E - HR}$$

$$C_o = 10^{-3} \; M$$

For C_E, take a value of 100 mgm enzyme/gm glass with a particle diameter of 0.0462 cm. The volume of this particle is $\pi/6 \; dp^3$, cm^3 and the density is 2.2 gm/cm^3. If we assume no porosity so that all the enzyme is on the surface, the enzyme we can fix is about:

$$100 \; \frac{\text{mgm Enzyme}}{\text{gm-glass}} \times 2.2 \times \frac{\pi}{6} \; (0.0462)^3 \cong 1.13 \times 10^{-2} \; \text{mgm E}$$

The surface area $\pi dp^2 = 6.7 \times 10^{-3} \; cm^2$

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$$\therefore C_E = \frac{1.13 \times 10^{-2} \text{ mgm E}}{6.7 \times 10^{-3} \text{ cm}^2} = 1.69$$

The horsepower for this set of conditions (100 tons/day, 100 foot deep bed, particles 0.0462 cm, etc.) is:

$$Hp = \frac{6.05 \times 10^{-5}}{(0.0462)^3 (10^{-3})} \left[\frac{3 \times 10^4 \times 1.69}{10^{-3}} \right] + \frac{3.6 \times 10^{-7}}{(0.0462)^3 (10^{-3})} \left[\frac{3 \times 10^4 \times 1.69}{10^{-3}} \right]^2$$

$$Hp \cong 10^{16} ::$$

This is so absurd that it is difficult to see how any reasonable change in parameters could ever make packed towers acceptable. No further consideration, therefore, was given to these and only CSTR's were considered.

I. Enzyme Kinetic Equations Used for Simulation

In order to compute reactor residence times and thus the sizes of the reactor vessels needed, it is necessary not only to describe the flow field behavior within the reactor (e.g., CSTR or plug flow) but also to describe the kinetics of the reactions involved. In the case of enzyme reactors, expressions which describe such kinetics may be simple, or they may be relatively complex, depending upon the reaction to be described and associated simplifying assumptions. Such expressions divide into only a few classes of mathematical problem areas, although from the point of view of the chemistry involved, the expressions may be difficult, if not impossible, to construct.

As an example, consider a single substrate, monomolecular enzymatic chemical reaction. One widely accepted model for such a reaction is [1]:

$$\frac{dP}{dt} = V = \frac{V_{max} \cdot S}{K_m + S} = -\frac{dS}{dt} \tag{1}$$

in which:

P = product concentration at time t in moles/liter

S = substrate concentration at time t in moles/liter

V = reaction rate (velocity) in moles/liter/minute

V_{max} = maximum reaction rate in moles/liter/minute

K_m = Michaelis constant in moles/liter

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As with any mathematical model of a physical reality, assumptions are inherent in the formulation of the model; these may reflect physical reality, or may be introduced in the interests of mathematical simplicity [2].

Equation (1), known in the literature as the Michaelis-Menton equation, constitutes the simplest possible model of an enzymatic chemical reaction. The chemistry involved in the formation of product in such a reaction is clearly defined and no possible ambiguities exist with regard to the mechanism of its formation [1]. Unfortunately, such a simple model is, in many cases, either obviously not correct or hopelessly idealistic. Broadly speaking, there are three situations in which the use of Michaelis-Menton kinetic models are inappropriate:

(1)   When multisubstrate/multiproduct reactions occur

(2)   When inhibition of catalysis takes place

(3)   When a significant "back reaction" exists due to unfavorable thermodynamic equilibrium

For [3] above, consider the case of a reaction in which two substrates combine to form a single product, a not-infrequent occurrence in the processes of interest in the manufacture of synthetic food. In such a case, two models or pathways for the formation of product can be imagined [4], ordered and nonordered. The two chemical rate equations and their associated mathematical models are quite different, depending upon which pathway is used to represent the reaction.

Secondly, it is possible (and, in fact, highly probable) that reaction inhibition effects will occur in enzymatic chemistry. In such a situation, product or substrate concentration above a certain threshold level slows down catalysis and affects a reaction rate slower than that predicted by simple kinetic models which do not include such inhibition effects. Often the mechanism and/or kinetic data associated with inhibition are difficult to obtain for reasonable ranges of operating conditions.

The existence of a significant "back reaction" (conversion of desired product back into original substrate) causes few conceptual difficulties from the viewpoint of mathematics, although from a computational standpoint such a reaction may complicate the algebra involved. Back reactions are usually assumed to have the same form as their associated forward reaction, perhaps with some changes in inhibition terms and/or number of products and substrates.

Naturally, inhibitions, back reactions, and multisubstrate, multiproduct reactions are in no way mutually exclusive. Many enzymatic chemical reactions and processes exhibit all three of these complicating effects to some degree, and therefore involve extremely cumbersome, though mathematically tractable, expressions.

Most published information regarding enzymatic reactions is in the form of first-order differential equations which describe the rate of

formation of product of the reaction. An example of such a display of reaction rate information is equation (1). More generally, such equations take the form:

$$\frac{dP}{dt} = V = f(S_1, S_2, \ \ldots \ S_n, P_1, P_2, \ \ldots \ P_n, K_1, K_2, \ \ldots \ K_n, K_{I1}, K_{I2}, \ \ldots \ K_{In})$$

(2)

in which:

$$S_1, S_2, \ \ldots \ S_n = \text{substrate concentrations at time } t \text{ in moles/liter}$$

$$P_1, P_2, \ \ldots \ P_n = \text{product concentrations at time } t \text{ in moles/liter}$$

$$K_1, K_2, \ \ldots \ K_n = \text{chemical reaction rate constants in moles/liter}$$

$$K_{I1}, K_{I2}, \ \ldots \ K_{In} = \text{inhibition constants (when present) in moles/liter}$$

It is important to note that this information may not be directly usable by the designer of a chemical reactor. Continuous flow CSTR's, for example, require rearrangement of such data into a form suitable for use by such a designer (see the discussion on CSTR's). This difficulty, although somewhat subtle, is nonetheless real. The format of presentation of chemical model information cannot be divorced from the type of reactor and/or plant which is to be designed to house the reaction(s). This follows directly from the assumptions inherent in the choice of a reactor type which describe the flow field within such a reactor.

J. Reactor Sizing Calculations and Numerical Solution Techniques

As has been previously stated, analytical determination of reactor sizes is tractable only when a single (or perhaps double) reaction takes place in a reactor vessel. When it becomes desirable to place more than one reaction in a given vessel (usually to take advantage of favorable reaction energetics and thermodynamics), the complexity of the resulting mathematics dictates the use of automated, numerical solution techniques. Conceptually, the mathematical expressions themselves do not change or become more complex, but the number of coupled variables involved precludes hand computational methods. The type of numerical technique to be utilized of course depends on the form of the equations to be solved. This form, in turn, is dependent upon two factors:

(1) The form of the mathematical kinetic model representing the reaction (does it contain inhibition, does it have a back reaction, is it multisubstrate, etc.).

(2) The type of reaction vessel to be used (CSTR, plug flow, continuous, batch process, etc.).

For reactors useful for volume production of synthetic food, it is anticipated that all such reactors will be continuous flow, perhaps with certain amounts of recycle of reactor output. The reactors themselves may utilize CSTR technology, or may be plug flow reactors. These reactor types and assumptions, together with kinetic models which describe the reactions taking place within the vessels, constitute a mathematical model of coupled reaction/reactor food production. We note here that food production schemes which use single reaction reactors are also mathematically modeled; the salient characteristic of this discussion is the fact that solution of coupled reaction models is intractable by hand calculation techniques.

1. Coupled Reaction Plug Flow Reactors: Simulation Techniques

As previously described, plug flow reactors assume only longitudinal motion of reactant "plugs" down the length of the reactor. Because this motion can be expressed in terms of reactor length and, through flow velocity, in terms of reactor residence time, it is possible to design (size) a reactor for a single plug flow reaction directly from a differential rate equation describing the reaction kinetics; this differential equation is of the form of Equation (2).

When more than one reaction takes place within a plug flow reactor, several differential equations are involved, each describing a particular reaction within the reactor vessel. Assuming that the reactions are coupled,[*] that is, assuming that the product(s) of one reaction constitute the substrate(s) of a subsequent reaction, their describing differential equations will also be coupled. Thus a mathematical model for a plug flow reactor containing coupled enzymatic reactions consists of the set of first-order, nonlinear differential equations:

$$\frac{dP_i}{dt} = V_i = f(S_i, K_i, \text{ and perhaps } K_{Ii}) \qquad i = 1, 2, \ldots n \qquad (1)$$

$$n = \text{\# of reactions}$$

together with the initial conditions:

$$P_i(0) = P_{io} \qquad (2)$$

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[*] This is not really an assumption; the only reason for placing more than one reaction inside a given vessel is to take advantage of favorable coupling kinetics. It can therefore be stated a priori that any reactor containing more than one reaction will arrange those reactions so that the product(s) of one become the substrate(s) of another.

and the coupling relationships:

$$P_i = S_j \ , \qquad i \neq j \qquad\qquad (3)$$

which describe how products of, say, the ith reaction are substrates of the jth reaction, and thus serve to couple the n equations (1) to one another. Usually $P_{i_0} = 0$ for all i (no product of any of the n reactions enters the top of the reactor) although this is not the case when a recycle stream exists. The detailed mathematical form of equations (1) is determined by the actual kinetic models at hand for each of the reactions to be used.

The solution to a set of first-order, coupled, nonlinear ordinary differential equations is a well-investigated problem. Many techniques can be used to determine the time history of product formation within the reactor. Among the easiest to use and most readily available are Runge-Kutta and Euler numerical integration schemes. Since it is expected that the formation of products with time will be a relatively smoothly-behaved function, elaborate predictor-corrector techniques are not warranted. Accuracy of such a solution can readily be tested by stepsize variation within the numerical integration routine selected.

Output for such a simulation would consist of the time history of all of the products and substrates within the vessel. Based on some desired final conversion percentage of the final (nth) product, the residence time required can then be determined and thus the actual size of the reactor established.

2.   Coupled Reaction CSTR's: Simulation Techniques

A CSTR operates at the outlet concentration(s) of the reactants and substrates present within the reactor. Because of this assumption (dependent upon the assumed perfect mixing within the CSTR) the effective reaction rates are governed by substrate levels at quite a low level. If one could, for example, operate a CSTR with a 99 percent conversion of substrate to product, the reaction would then operate at only a 1 percent substrate concentration--a low level of concentration indeed. It is thus clear that, for high conversion rates with a CSTR, the reactions contained within such a vessel will not operate anywhere near substrate saturation levels. This observation allows simplification of the mathematical expressions governing the behavior of reactions within CSTR's.

Consider a CSTR operation with n reactions of the Michaelis-Menton form. In such a reactor, there will be n equations of the form of Equation (1). If the reactor operates at reasonably high conversion rate, the substrate outlet concentrations will be low. Therefore, it is possible to linearize the Michaelis-Menton equation as follows:

$$\frac{dP}{dt} = V = \frac{V_{max} \cdot S}{K_m + S} \doteq V_{max} \cdot S \quad \text{for small} \quad S \qquad (4)$$

In order for the reactor to be operating at steady-state, all of the reactions within the reactor must operate at the same reaction rate, thus there will be $(n-1)$ equations of the form:

$$V_i = V_{(i+1)} \qquad i = 1, 2, \ \ldots \ (n-1) \qquad (5)$$

The n unknowns in these $(n-1)$ equations are the substrate levels present at the steady-state operating condition. The nth relationship between the substrate levels is determined from a mass balance on the overall reactor:

$$S_{in} = S_{out_1} + S_{out_2} + \ldots + S_{out_\eta} + P_{out} \qquad (6)$$

in which the subscripts "in" and "out" refer to input and output of the reaction vessel. Substitution of Equation (6) into any of the $(n-1)$ relationships of (5) will yield a system of $(n-1)$ equations in the remaining $(n-1)$ substrate concentrations; the nth substrate concentration [the one for which Equation (6) was substituted] is determined directly from the mass balance equation (6). Because the kinetic equations (4) are linearized, this set of equations can be written in matrix form:

$$\underline{A} \ \underline{S} = \underline{C} \qquad (7)$$

in which $\underline{A}$ is an $(n-1) \times (n-1)$ matrix, $\underline{S}$ is an $(n-1)$ vector of substrate concentrations and $\underline{C}$ is an $(n-1)$ vector of known values of substrate inlet concentrations and desired product outlet concentrations (a constant vector).

Solution of Equation (7) for the $(n-1)$ unknown substrate concentrations proceeds directly from matrix theory of solution of simultaneous linear algebraic equations:

$$\underline{S} = \underline{A}^{-1} \ \underline{C} \qquad (8)$$

The computation of Equation (8), coupled with a solution for the nth substrate concentration from Equation (6) determines the rates of each reaction with a coupled-reaction CSTR directly from Equations (4). These rates, in turn, establish the size of the reactor since they require certain residence times for running reactions to completion or desired level of completion.

It is conceivable that a CSTR may be run at a relatively low level of completion of desired product due to size and/or pumping requirements associated with high completion levels of given reactions. In such a case, the linearization of Equation (4) is obviously invalid. Two possibilities are evident:

(1) The problem may be solved with (obviously invalid) linearized kinetic equations anyway; this is attractive because of the mathematical simplicity thus afforded the reactor designer.

(2) The full nonlinear kinetic expressions may be used. This would follow the above development up to the point of solution for the substrate concentrations in Equations (7) and (8). Some form of solution technique amenable to nonlinear, coupled algebraic equations would then need to be employed.

Because of the complexity of solution techniques for nonlinear algebraic equations (and even their availability), strategy (1) is the more attractive at this point. Obviously a trade-off exists between computational difficulty and accuracy of results, as in any simulation. The direction chosen would be most dependent upon the outlet concentration chosen; a very low outlet concentration (say, 10 percent product or so) would probably require nonlinear equations and solution techniques to be used in order that anything like meaningful answers result.

A final note: since not all kinetic expressions involve only one substrate and/or product, the linearization of Equation (4) must be extended to multisubstrate, multiproduct reactions. Usually in such a reaction, one or the other product or substrate will dominate. If this is not the case, matrix techniques offer no possibility for solution and again nonlinear techniques must be used.

REFERENCES

1. Sidney Bernhard, The Structure and Function of Enzymes, W. A. Benjamin and Co., New York, New York, 1968, p. 73.

2. Sidney Bernhard, Op. Cit., p. 74.

3. Sidney Bernhard, Op. Cit., p. 13.

4. Sidney Bernhard, Op. Cit., p. 93.

Appendix 5.2

SETTLER DESIGN

In an ideal rectangular gravity settling basin the paths of all settleable discrete particles will be straight lines and particles with similar settling velocities will move in parallel paths. The diagram below shows a settling pattern for a typical longitudinal section of an ideal rectangular basin. Any particle settling in a moving liquid will be displaced in a direction and with a velocity that is the vector sum of its own settling velocity, V_2, and the velocity, V_1, of the surrounding liquid. For a basin depth D_1, a basin length L, is required to remove the settling particle. At the same flow velocity V_1 and a basin depth D_2, a basin length L_2 would be required to remove the particle.
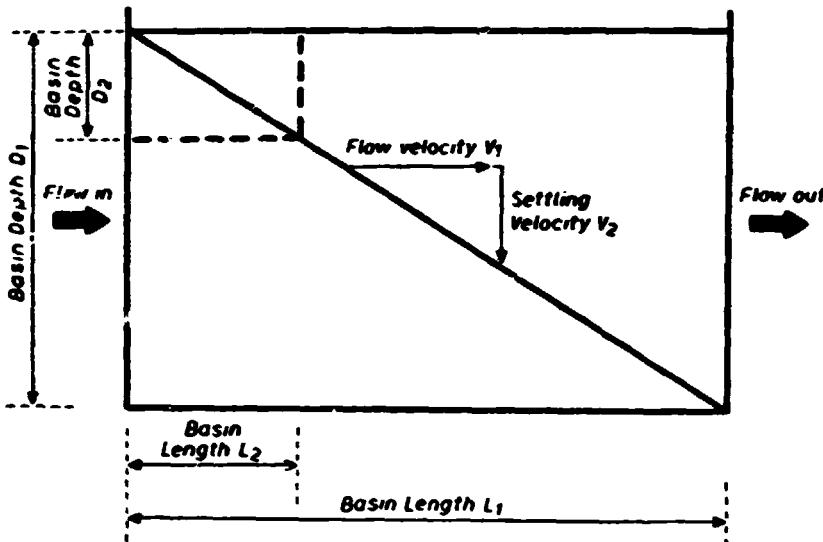
IDEALIZED SETTLING PATHS OF DISCRETE PARTICLES IN A HORIZONTAL FLOW TANK.

In such a system, as shown above, the settling time is expressed as

$$t = D/V_2 \quad (1)$$

where

D = basin depth, feet

V_2 = settling velocity, feet per minute

t = time, minutes

With a basin depth of D_1, the required basin length or horizontal length of settling path to remove these particles from the flowing liquid is a function of horizontal liquid flow velocity V, and settling time t:

$$L = V_1 t \tag{2}$$

where

    L = length of settling path or basin, feet

    V_1 = surrounding liquid velocity, feet/minute

From Equation (1) it is seen that a decrease of basin depth D will result in a decreased settling time. Equation (2) indicates that a reduction of velocity V, and/or a decrease in settling time t results in a reduced length of settling path L. This is expressed by

$$L = \frac{V_1 D}{V_2} \tag{3}$$

In considering an ideal settling basin with width W and length L, the cross-sectional area A in a plane perpendicular to the direction of settling is expressed by

$$A = LW \tag{4}$$

For continuous-flow sedimentation, the particles settle at a characteristic settling velocity and the liquid at any depth becomes clarified as soon as those particles initially at the surface pass through it. The rate of clarification q is

$$q = V_2 A \tag{5}$$

From Equation (5) it is obvious that the rate of clarification is a function of basin settling area and particle settling velocity.

The detention time required to clarify a volume of liquid is now expressed as

$$T = \frac{Q}{q} = \frac{Q}{V_2 A} \tag{6}$$

where

T = detention time, minutes

Q = volume of liquid, cubic feet

with detention time being dependent only upon settling velocity and settling area.

V_2, the particle settling velocity, is more normally expressed as

$$V_2 = \frac{Q}{TA} \tag{7}$$

with the units normally being gallons per square foot per minute. This expression of the particle settling velocity is called the overflow rate.