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THE CONVERSION OF LIGNOCELLULOSICS TO FERMENTABLE SUGARS: A SURVEY OF CURRENT RESEARCH AND APPLICATION TO CELLS

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ABSTRACT

One of the anticipated uses of a bioreactor in CELSS will be to process the waste streams from plant growth chambers that are rich in non-digestible lignocellulosics. This report provides an overview of the options for converting lignocellulosics into fermentable sugars as applied to CELSS. A requirement for pretreatment is shown as well as the many available options. The available physical-chemical and enzymatic hydrolysis processes for producing fermentable sugars (saccharification) are discussed. At present physical/chemical methods are the simplest and best characterized options but enzymatic processes will likely be the method of choice in the future. The use of pentose sugars by microorganisms to produce edibles at levels comparable to conventional plants is shown. The possible use of mycelial food production on pretreated but not hydrolyzed lignocellulosics is also presented. Simple trade off analyses among some of the many possible biological pathways to regeneration of waste lignocellulosics is undertaken. Comparisons with complete oxidation processes are made. It is suggested that the NASA Life Sciences CELSS program maintain relationships with other government agencies involved in lignocellulosic conversions and utilize their experitise when the actual need for such conversion technology arises rather than develop this exerptise within NASA.

I. INTRODUCTION

The conversion of cellulosics to sugars which are usable directly as food or as chemical reagents in a Controlled Ecological Life Support System (CELSS) is a subject that has been discussed for several years. This concept provides an alternative to the oxidation of all non-edible, fixed carbon from food production processes to carbon dioxide and water. Neither option has clearly been developed relative to the tradeoffs between energy consumption, mass balances and CELSS integration issues. Certainly one of the major reasons that the conversion alternative has not been addressed in any systematic fashion is that the possibilities and challenges associated with this approach have not been examined in a CELSS context. It is the objective of this report to provide a survey of the current research into the conversion of lignocellulosics to usable sugars and to provide an assessment of the application of these technologies in a CELSS. For the purposes of this report, usable sugars are defined as fermentable sugars or sugar process streams containing monosaccharides.

It is not possible to model an actual conversion process since the exact make-up of the waste material is not yet well defined. In this report, we used the conversion of the residue from wheat as a general model for the entire process. If data is not available for this source of biomass, other sources such as municipal solid wastes, cornstover, etc. were used.

The major rationale for examining the alternative of converting lignocellulosics rather than burning them results from the need to "make every photon count" in the confines of a bioregenerative life support system such as CELSS. The overall process is the conversion of CO_2 to biomass, fixation into edibles by biological sources and the eventual oxidation of fixed carbon back into CO_2 and water. The mass flow can be diverted (essentially held up) by converting polymeric material into usable, high-energy sugars. Thus the questions that need to be examined to evaluate these kinds of alternative pathways in CELSS include the following. What can microbes and/or enzymes do in a CELSS? What can be done with the microbial product? What percentage of the available lignocellulosics can be made available to food processing streams? What are the trade-offs between biological conversions and physico-chemical processes? This survey will provide data and information about these questions and provide some conclusions and recommendations for future CELSS research.

A general, alternative pathway to processing lignocellulosics is depicted in Figure 1. Each area in the flow diagram of Figure 1 will be discussed in this report. Progress and options in these areas will be presented and their applicability to CELSS discussed. The major research effort in the U.S. for the conversion of lignocellulosics to monomeric components suitable for fermentation has been provided by the Department of Energy at research institutions, national laboratories and universities. Information was obtained from the literature, private conversations with researchers in the field and from attendance at appropriate meetings and the abstracts from these meetings.

One of the forums for research in this area has been an annual symposium on the biotechnology for fuels and chemicals sponsored by Oak Ridge National Lab and the DOE Energy Conversion and Utilization Technologies Program. At the tenth annual meeting held in May of 1988, an open discussion of the progress in the field of conversion of cellulosics to fuels and chemicals was held. Figure 2 outlines some of the highlights of that discussion. Relative to CELSS, it is obvious that many of the concerns of this body of research are applicable to CELSS waste treatment processing. It will be useful for the NASA CELSS program to keep abreast of developments in this field of research since advances here will be applicable to CELSS problems.

II. COMPOSITION OF CELSS BIOMASS AND ASSOCIATED CHALLENGES

The fixation of carbon dioxide into edible biomass will most likely be performed by typical agronomical species. A set of candidate crops has been defined and includes wheat, potatoes, lettuce, soybeans, and others (1). In every case, the dry weight mass ratio of edible to non-edible material is about 0.5 (harvest index). Even in the highly controlled and confined growth chambers, harvest indices are on the order of 0.5. The composition of these inedible portions is comprised of cellulose, hemi-cellulose, lignin, crude protein and ash. An example of these compositions is provided in Table 1. The data shows values from wheat cultivars used in actual intensive growth chambers to analyze possible CELSS candidate food crops and on typical wheat straw purchased at a local feed store. The values are very similar except for the lignin content. This value is often variable because of the various methods used to analyze the data and most methods underestimate the actual lignin content because often relatively severe methods are required to isolate the lignin which also degrades this material. More comprehensive explanations of common and accurate analytical techniques can be found in the literature (2). One of the better methods for use in comparing lignin content is the so-called Klason lignin value. This is derived by dissolving the biomass in 72% sulfuric acid. Any undissolved residue is considered lignin. Values can vary widely as is shown in Table 1 and are a function of method. Note that in the Van Soest data (3), the values are lower than that given by Klason or spectrophotometric data. Other data give values for lignin in straws that more closely approximates the Klason and spectrophotometric data given in the literature. It is known that annual plants do not usually have high lignin contents because they have no need to provide long-term resistance to biodegradation (4). The candidate species chosen for CELSS are all annuals and thus their lignin content will likely be low.

The availability of fermentable sugars from the inedible biomass is on the order of 50-68% of the inedible dry weight. This represents a significant fraction of usable material for food and/or chemicals production. The lignin is the intractable material that must be fractionated from the biomass in order to process the remainder of the polymeric material. Its use as a chemical feedstock is of interest to bioconversion schemes (5) but is less likely to serve any good purpose in CELSS and should probably be considered as a feedstock for process heat, i.e. conversion to CO_2 and water especially since lignin possesses the highest specific heat content of biomass (12,700 BTU/lb). The remaining mineral content of the plant will eventually enter process streams and be recycled. It will be shown below that the process heat and mineral content of plants could be used to provide energy and chemicals for the conversion of inedibles to fermentable sugars.

Although there are more extensive descriptions of the structure of a lignocellulosics such as wood and straw, the following is a brief but useful summary of such compositions. "Cellulose, hemicellulose, and lignin are found within the wood cell wall... In general, cellulose fibrils are surrounded by hemicellulose, which is embedded in a lignin matrix. Crystalline cellulose is viewed as the reinforcing framework, sort of the steel rods, of the cell walls. The hemicelluloses and lignins support the fibrils." A more detailed description of these components helps to clarify the complexity of plant cells.

"Cellulose is a single polysaccharide. It's a polymer of...glucose. A single, unbranced cellulose molecule, about 3-5 μm in length, comprised of about 7,000 to 12,000 glucose units. Hydrogen bonding occurs begween hydroxy groups within the sugar sequence, and also among adjacent cellulose molecules that band together along their long axes to form bundles called elementry fibrils. Glucose molecules in fibrils are regularly aligned; about 70% of the structures are crystalline. Cellulose is the chief structural polysaccharide of plant cells...

"The next major constituent...are the hemicelluloses... Hemicelluloses, comprised mainly of D-glucose, D-mannose, L-arabinose, andD-xylose, have molecular chains that are often branched. There are about 100 to 200 sugar residues per molecule. Hemicelluloses have higher water solubilities than cellulose, are more readily hydrolyzed, and have less ordered structures.

"Lignins are amorphous, cross-linked phenolic polymers that occur uniquely in vascular plants and comprise 20-30% of most wood.' Molecular weights range from the thousands to the hundreds of thousands. Lignins are made from three cinnamyl alcohols, p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. They differ from each other in the number of methoxyl groups substituted on the benzene ring" (6).

Studies have shown that the lignin in plant material presents a real physical barrier to the effective hydrolysis of cellulosics into monomeric substrates (4, 7, 8). Considerable effort has been made to produce methods and means to accomplish the clean separation of lignin from polysaccharides without degradation of the polysaccharide material. The need for two general stages of pretreatment has been suggested by research results. One stage usually involves the mechanical pretreatment of the biomass material by sizing and milling but may also include some chemical or physical processes such as acid/base and heat (usually in the form of steam) or even radiation. The next stage involves some form of fractionation of the lignin from the polysaccharides. It has repeatedly been shown that both steps are necessary for the effective extraction of monomeric sugars from the biomass material. It is possible to circumvent the second step through the direct growth of fungal biomass on biomass ground into small particles and this option will be discussed below. However, the more typical processes involve both steps. The delineation between the stage one and stage two pretreatments is not always distinct and more often these two stages are combined. In this report they are discussed under two separate headings but note that many second stage treatments are really combinations of both stage one and two pretreatments and that first stage pretreatments often employ some type of hydrolysis.

III. FIRST STAGE PRETREATMENT

The process of rendering raw biomass suitable for further pretreatment and enzymatic or microbial hydrolysis usually involves mechanically sizing or thermally disrupting and fractionating the raw material. Chemical treatments are often coupled with these processes or used as the major source of pretreatment. In many cases, these chemicals are added to assist in simultaneously fractionating and hydrolyzing the lignocellulosics. Figures 3 and 4 show simple flow diagrams for pretreatment and hydrolysis for lignocellulosics.

A. Mechanical

Methods have been proposed and studied for the preparation of wheat straw for enzymatic hydrolysis (9). Mechanical grinding permits positive temperature control, wet and dry processing, no chemical cleanup or recyle and is relatively simple to operate. This comminution technique has only recently been applied to pretreatment of lignocellulosics and may include the use of shredding, knife mills, hammer mills, disk refining and vibratory rod milling. Another similar treatment is agitation bead milling. These methods are commonly used in the food and pharmaceutical industries to obtain fine grinds and dispersions. Results from published work (7, 10) showed that only a two fold increase in enzymatic hydrolysis resulted from such pretreatment methods and that fermentability was a function of particle size. Others observed increased ethanol production using such pretreated material indicating increased enzyme susceptability (11). These methods all require relatively high power consumption and replacement of mechanical parts both of which are drawbacks in a CELSS context.

B. Thermochemical pulping

The use of steam at moderate temperatures (>160^{o} C and 100 psig) solubilizes cellulose and delignifies lignocellulosics. Combined with the use of caustic this method is widely employed to treat lignocellulosics by the pulp and paper industry. These methods work although they can be energy intensive. Efforts to employ variations of thermochemical methods for pretreatment of lignocellulosics for ethanol production have been conducted and are known as autohydrolysis, and steam explosion. Some studies found thermochemical processes the most workable in pretreating municipal solid waste (MSW) (11). In all cases the chemistry is simple and hydrolysis occurs due to mild, acid-catalyzed reactions which cleave glycosidic bonds and acetate groups in hemi-celluloses; cleave, to varying degrees, the glycosidic linkages in cellulose; and hydrolyze the α-ether linkages in lignin. Autohydrolysis and rapid steam hydrolysis are similar processes. Here temperatures are in the 220 to 275 °C range but residence times are short. The process fractionates the lignocellulosics while steam explosion does not. Some reports of 95% glucose recovery (of available glucose) and 70% pentose recovery (of available pentoses) using an 8% slurry, 100 sec residence times and 240°C have been given (12). At 2% substrate levels, the yields were quantitative. Others have reported similar results (13, 14) and generally claim that this type of process improves enzymatic hydrolysis capability of the thermally treated material and depolymerizes cellulose and hemi-cellulose. Lignin is not affected to any great degree. An example of a variation in this method employed steam heating of lignocellulosics at 200 °C for 15 min followed by acid hydrolysis at pH 1.0 for 4 hours at 100 °C. The result was very fermentable xylose fractions. The hydrolysates were only suitable for fermentation when the xylose concentration was <30 g/L probably due to the several inhibitors of fermentation that were also produced including acetic acid, lignin monomers, furfural and other volativle fatty acids (15). Additionally, in actual operation, acid hydrolysis processes can produce metal ions and often produce increases in concentration of sugars but not in yield (16). Steam Explosion - In this process, lignocellulosics are superheated and extruded which causes tremendous disruption of the fibrous material when the pressure changes. The cellulose is rendered more enzymatically susceptible to hydrolysis by this procedure although about 10% is lost in the process (17). The process tends to disrupt the amorphous regions of the cellulose and increase surface area although the crystallinity of the material is not greatly disrupted. Substrate concentrations on the order of 6% solids are generally used. Considerable work has been conducted to examine the best methods to enzymatically treat steam exploded wood and recover the enzyme. This will be discussed further below.

The energy intensive requirements of thermochemical approaches are not conducive to CELSS, but overall these processes are simple, workable, and some are relatively mature technologies. The possible applications in a CELSS should probably be more closely examined with emphasis on trade-off analyses for energy and weight compared to the weight of cellulosic conversion systems.

C. Chemical pretreatment

Agents such as sodium hydroxide, ammonia, chorite, sulfur dioxide, amines and acid (both dilute and concentrated) have been proposed and used. In the forest products industry, the use of alkaline pretreatments prevails. Other solvating agents known to solubilize cellulose include iron/tartrate, cadmium or copper and ethylene diamine and others (7, 11). Chemical pretreatments were not considered good candidates for treating MSW largely due to the unknown effect of chemicals and solvating agents on the diverse composition of MSW (11).

An example of a chemical pretreatment process using high acid concentrations illustrates the difficulties in using such processes. Acid (20% HCL or 60% H_2SO_4) treatment of lignocellulosics produces a very good product that is free from many of the side products resulting from thermochemical or mild acid hydrolysis processes. Electrodialysis has been proposed as a method to recover the sulfuric acid (18). An initial calculation showed that it would require 1.1 faraday to move one mole of H_2SO_4 across a membrane and that current efficiencies of 0.26 are required to make the process feasible. Another major expense is the cost of membranes. Commercially, the cost of membranes is roughly equal to the entire capital cost of the rest of the system. These membranes must be able to withstand 20% HCl and 60% H_2SO_4.

In biomass conversion technologies the arguements for acid treatments always must address the main drawback to this approach: the high cost of recycle necessary to make this pretreatment cost effective. There are additional problems which are of minor economic importance in the commercial arena but which may be major factors in a CELSS. These include equipment maintenance, storage of residual acid, and the production of fermentation inhibitors (which presumably would be unsuitable for both fermentation and inclusion into food items). The use of these agents presents another problem - the toxicity of agents (containing elements such as cadmium) that are used to process biomass that eventually becomes food. Technically for chemical pretreatments, the major problems for CELSS would be the recovery and recycle of acids, bases and other chemicals, the durability and replacement of operating and storage vessels, and the environmental containment of reagents. If chemical pretreatment processes were deemed useful or possible in CELSS, it is at least clear where the developmental work is required. Such definition is not as obvious for the less well understood enzymatic and other pretreatment processes.

D. Electron Irradiation

This method has been used to render cellulose more susceptible to hydrolysis. Emert and co-workers treated MSW with 10-100 Mrad of a 100 kW electron beam accelerator (11). Alcohol production decreased with increased irradiation. The complex effects of radiation on a diverse material leading to unknown products and reactions were considered to be the cause of this poor result. A major drawback for CELSS applications would be the power requirement. With increased understanding of the effects of space radiation on the more homogeneous waste streams in CELSS this method may be useful. However, considerable research would be required since even the effect of radiation on simple tissues is incompletely understood and lignocellulosic waste streams are much more complex.

IV. DIRECT USE OF FIRST STAGE PRETREATED LIGNOCELLULOSICS AS SUBSTRATES FOR MICROBIAL GROWTH

The direct use of only moderately pretreated biomass as substrates for fungal growth has actually been employed in commercial processes. Many organisms possess hemi-cellulases and efforts have been made to isolate such organisms and use them to directly degrade ligno-cellulosics (19). Fungal species are usually able to attack the cellulose complex while simpler eucaryotes and procaryotes require solubilized materials. Two examples, both from Canadian sources, illustrate the use of the fungal process.

A. Mushroom production

An integrated process using the fungus, Pleurotus sajor-caju growing on corn stover in submerged cultural conditions has been proposed (20). Some moderate pretreatment is required and involves base hydrolysis of the corn stover to solubilize hemi-celluloses and some lignin. The species is also known to fix nitrogen although initial experiments did not exhibit this property. The product produces 40-45% protein of moderate quality (i.e., low in sulfur amino acids). Additional research is being carried out to move this process towards commercialization and including fuel production as an added bonus.

B. Waterloo SCP I & II Processes

Early work by Canadian researchers demonstrated that from ground, milled and thermally/chemically pretreated (hot water and/or alkali) biomass such as cornstover, straw, or bagasse a semi-solid substrate could be provided for cellulolytic fungi (21). The fungi could be grown to high density under relatively simple conditions. Original efforts concentrated on the fungus Chaetomium cellulolyticum but have progressed to a type II process using Neurospora sitophila (22). This was done because work with the former strain showed that unless growth conditions were carefully controlled, mycotoxins were formed (as was also the case with another fungal food, Fusarium graminearum often suggested as a source of SCP - single cell protein).

These candidate processes are attractive to CELSS food production largely because of their inherent simplicity. Few pretreatment requirements, simple growth requirements and a safe, "mushroomy" type product that is more acceptable as food provide advantages in a CELSS system. The negatives include high energy requirements for mixing and harvesting mycelial mats, low growth rates and mycotoxin production if the wrong strains are used. However, the growth rates are still higher than plants. These processes also claim to produce BOD (biological oxygen demand) free water which would be an advantage in CELSS.

Twelve years ago, a survey of the existing SCP production facilities world wide was made. This was at the height of the interest in SCP. There were several processes either under development or in production phase that employed the following types of biomass substrates: whey, sulfite liquor, coconut, cellulose, and wood pulp (23). Few of these remain today because of the poor economics however their use suggests that direct utilization of CELSS waste streams is possible.

V. SECOND STAGE PRETREATMENT

Pretreatments that are specifically designed to disrupt lignocellulosics, fractionate components and carry out simutaneously the hydrolysis of the cellulosics comprise what can be called second-stage pretreatment. Figure 4 depicts the two generally accepted methods for hydrolyzing lignocellulosics. Since most of the effort in this field has been carried out by laboratories intrerested in fuels and chemicals, it must be remembered that most efforts are evaluated relative to the fermentability of the resultant "pretreated biomass." Although fuels and chemicals are not our primary objective, the fact that the polymeric sugars are rendered fermentable meets the CELSS criterion for usable sugars. The main thrust of current efforts in this field of research involve acid and/or enzymatic hydrolysis. Research and development into base hydrolysis and solvent assisted base or acid catalysis has also been conducted. As noted above, the caustic treatment of wood is an old technology for the paper and pulp industry and was the basis for the advanced studies being carried out today. There is a significant body of literature and experience that describes these issues and this report will make no attempt to comphrensively examine them. This report will highlight the main features of the processes and the reader may consult the references for further details.

A. Mechanical/Acid Catalyzed

Acid hydrolysis of cellulosics is an old technology that is relatively well understood. Integration of mechanically pretreated biomass into reactors and the efficient recovery of the product are major areas of research (5). Although in DOE Biochemical conversion program and its research teams and contractors do not consider all aspects of lignocellulosic conversion research they certainly address most of the major issues in these bioconversion processes. The following are short summaries of some of the major findings in this DOE sponsored program.

- o The size of the pretreated material is crucial in yielding efficient acid hydrolysis conversion.
- o Incorporation of xylose fermentation improves overall

process kinetics.

- Concentrated acid processes produce high yields (up to 100%) but acid consumption and recovery are expensive.
- Dilute acid hydrolysis processes exhibit sugar degradation problems and hence lower yields. Research into understanding this phenomenon is underway as well as developing efficient reactor designs.
- Removal of by-products inhibitory to fermentation may be necessary to overall process economics (The economics may not be of concern to CELSS but the presence of undesirable by-products will be).
- Methods to improve process economics include use of resins, solvent extraction and adsorption to isolate the product from the process streams.

1. <u>Example of one process</u>

The following is a more detailed description of one kind of acid hydrolysis process and illustrates some of its features. A correlation between mechanical pretreatment and subsequent hydrolysis of cellulose was examined. Coarse shredding of wheat straw was accomplished with hammer-type shredders followed by further comminution with rotary knife mills, hammer mills or laboratory knife mills. The water soaked material (3%) was then fed into a disk refiner and this output was examined for response to acid and enzyme hydrolysis. Breakage of the lignin-hemicellulose-cellulose barrier and subsequent accessability of the cellulose to enzyme and acid hydrolysis was a primary concern. The data revealed that mechanical treatments could increase digestability only about two fold even under extended treatments but that chemical pretreatments produced dramatic effects even at rather mild conditions (95^oC). They showed that the lignin-hemicellulose-cellulose complex could be completely broken and that the removal of residual lignin was required for successful enzyme hydrolysis. It did appear that removal of hemi-cellulose was required and necessary for good digestability. Acid-detergent pretreatments were not sufficiently better to warrant further use (10, 24).

Additional work has led to a better description of the kinetics of hemicellulose degradation (24, 25). Here it was found that at low temperatures, 95^oC, complete xylan degradation could occur if long incubation times, 1-2 days, were used. Further results were obtained which strengthened the relationship between hemi-cellulose removal and cellulose digestability. In addition, the use of other more vigorous pretreatment methods such as explosive decompression appear to increase surface area of the biomass but are not prerequisites for high enzyme digestability. Dilute acid pretreatments could also be carried out successfully in slurries of 20-40 wt % solids (25). (Flow diagrams and equipment requirements are found in all of these published studies). The yield of solubilized xylose was only 70-80%. For CELSS, such yields might border on satisfactory.

Economics will not be the main driver for a CELSS waste processing subsystem and thus concentrated acid hydrolysis might be more favorable for inclusion in CELSS than dilute acid hydrolysis since the production of by-products is negligible. However, the recycling of acid may prove to be an insurmountable problem not from an economic or technical point of view, but from an environmental perspective. The other advantages and drawbacks to this process are discussed above (III.C.).

B. Mechanical/enzymatic hydrolysis

Whereas acid hydrolysis involves two basic steps to obtain fermentables - pretreatment and hydrolysis, the process of enzymatic hydrolysis must also consider enzyme production and recovery (recovery in this instance is analogous to recovery of the acid). This process is well known but not as well understood and significant amounts of research have been conducted to understand the basic chemical/biochemical reactions (5). The following list also summarizes the research efforts for DOE laboratories and is typical of the research into this topic worldwide.

- The key to increasing digestibility of lignocellulosics appears to be directly related to increasing the cellulose surface area available to the enzymes.
- Prehydrolysis to a) open surface pore sizes and permit more enzymatic attachments and b) remove xylans is important.
- Steam explosion usually is not employed as a pretreatment method since it degrades hemi-cellulosics into furan compounds.
- Staged prehydrolysis steps using acid followed by base permit the removal of hemi-cellulose and lignin which then enhances enzyme digestibility.
- Understanding the role of feed-back inhibition by glucose and cellobiose are important aspects of improving process flow and economics.
- Simultaneous saccharification and fermentation (SSF) of pretreated biomass is a possible route to direct utilization of lignocellulosics. For fuels production, the low rates of enzyme production make this process expensive.

However for CELSS, this would not be as much of a consideration (other considerations are discussed below).

- Research into improving the production of cellulolytic enzymes has yielded good results and currently a few commercial companies are marketing low cost cellulolytic enzyme preparations at reasonable costs (the market here is interestingly enough the food processing industry).
- Methods to reduce power consumption in mixing highly viscous suspensions of cellulose, lignin and yeast have shown early promise.
- New organisms which operate at higher temperatures or that are genetically modified to improve some aspect of the process are being examined.

This area of research has recently (10 years) had a heavy investment of time and effort. Only now have some of the basic questions regarding the biochemistry of the cellulolytic hydrolysis process begun to be answered. Again the major problem in this type of hydrolysis is the recovery of the enzyme. Work in Sweden has shown that it may be possible to recycle the enzymes and efficiently obtain glucose in reasonable quantities. By washing and recirculating the undigested cellulose enzyme recovery factors of near 90% have been obtained (17). This is the minimum level of recovery at which the overall process becomes economically feasible. The actual machinery and equipment is relatively simple but would likely require a considerable amount of water and power.

C. <u>Mechanical/base catalzyzed hydrolysis</u>

This is the method long used by the pulp and paper industry. It involves the milling of lignocellulosics and treatmenting them with alkali (5%) at 130 to 180°C. Complete delignification occurs but the hemicellulosic fraction is rendered non-fermentable. Milder conditions, 25°C and long treatment times, result in partial delignification but no degradation of pentosans (26). This process does, however, require high chemical consumption (estimated at 5-20% w/w oven dry biomass) (10, 27). This is a low technology approach using mild conditions that does produce the desired results. This approach does not lend itself to production type processes which are favored by industry. However, it may be applicable to CELSS if sufficient quantities of caustic can be produced from the ash of oxidized lignin and unhydrolyzed biomass (i.e., from fractionated lignin used for process heat). Neutralization is another factor that must be considered for CELSS and the source of acid in a CELSS is not obvious.

D. <u>Mechanical/base catalyzed hydrolysis with solvents</u>

The solvent pretreatment or organosolv processes have been employed to ameliorate the negative effects of direct base catalyzed delignification of biomass material. To avoid large losses of fermentable hemicellulosic degradation products and to reduce the costs involved with large consumption of base, alcohols have been employed along with base catalysis. The hemicellulose is less soluble in alcoholic solutions and thus is not degraded.

In initial experiments, it was demonstrated that 70% of the lignin could be removed using mild alcohol-alkali-water mixtures [50% (v/v), 25°C for 72 hrs] with only 5% loss of pentosans. Base requirements were found to be 0.1g NaOH consumed per gram of cornstover for adequate lignin removal. The enzymatic hydrolysis of the treated cornstover was increased by four-fold over the untreated cornstover. The extent of utilization of the treated material by bacterial fermentations was 87% (27).

Since these initial studies, the organic solvent method has been expanded to examine the kinetics and wider applications of this process including the use of mild acid catalyzed processes. In the case where no acid is added, the relatively high temperatures and eventual degradation of the lignocellulosics, results in production of organic acids such as acetic acid, which then catalyze the further hydrolysis of hemi-celluloses and promote delignification. In the cases where acid is added, researchers have found that the use of acid assists in rendering the cellulose more digestible by enzymes. In one study, H_3PO_4 acid at 0.02 to 0.008 M and 130 oC was found to make the cellulose nearly 100% digestible by enzymes (28).

VI. QUALITY OF OUTPUT FOR CELSS APPLICATIONS

The definition of usable sugars as fermentable sugars is easily demonstrated by those wishing to produce fuels or chemicals by microbes. Usable sugars in a "food" context requires a further definition. Many of the monosaccharides can be directly used as a food source or processed into edible components. These are largely the hexoses. The pentose sugars are not of themselves a good source of carbohydrate and must be futher processed in some fashion. A typical, commercial method is to use these sugars as substrates for microbial food production. An example of such a process is yeast grown on sulfite waste liquors in paper processing plants which liquors are largely comprised of pentose sugars (23). Additionally, reports have been published which describe the actual food value from two or three yeast strains that might be candidates for CELSS unconventional food production (29). The conversion factor or harvest index for converting monosaccharide to edible components by these candidate yeast strains was on the order of 0.5. This edible portion is comprised of about 6-20% protein and the balance in carbohydrate, usually in the form of trehalose and glycogen with some small fraction of lipid. The overall microbial "harvest index" value can be increased if only protein is desired. These results coupled with the conversion of lignocellulosics to monosaccharides show that microbial food production can serve as a useful food production and waste processing subsystem for CELSS. The mycelial or fungal food produced as described in Section IV above is very similar to that produced in mushroom production. This microbial food is easily processed into food products suitable for man (30). Its quality is similar to other microbial food with a likely deficiency in the sulfur amino acids (29).

VII. APPLICATIONS TO CELSS

The major purpose of this survey was to examine the field of biomass conversion and determine areas that might be applicable to the CELSS system. There were two aspects of this survey: 1) identifying the lignocellulosic conversion systems that are available and 2) making some evaluation as to the relevance of these conversion systems to CELSS food production and waste processing systems. The material presented above largely addressed item 1 and some of item 2. What follows are additional comments on that survey and on the applicability of the conversion alternative to CELSS.

1. The list of candidate food production species for CELSS

food production schemes is largely made up of annuals and/or plants with low lignin content. This is an advantage since this will lessen the pretreatment requirements and ease isolation of polysaccharides for hydrolysis into usable sugars. The use of low lignin plants will be advantageous from a waste processing point of view.

2. It does not appear possible to avoid any pretreatment of the biomass prior to hydrolysis. Some sort of mechanical and other (thermochemical, chemical, etc.) pretreatment processes will be necessary to render the biomass suitable for hydrolysis to the monosaccharides. This will require equipment and varying amounts of water, depending upon the process used and the possible regeneration of some chemicals such as caustic. Some of these processes are quite simple but most are usually energy intensive. Some will require additional research before they can be considered as possible candidates (electron radiation). While some processes may be very effective (e.g. concentrated acid pretreatments), they also increase the complexity of processing since environmental problems of acids, base for neutralization and possible toxic by-products such as metal ions must be considered. Trade-off analysis are clearly necessary but will not be useful until candidate systems are chosen and designs are implemented. From an engineering point of view in systems where pressurized vessels are employed, the cost of building such systems rises geometrically with the increase in pressure. It should be noted that in the Soviet Union a large industry is in place that utilizes acid-hydrolyzed biomass for animal feed, although not for CELSS type applications (Grohmann, personal communication).

3. The hydrolysis of lignocellulosic fractions can occur with any one of the following methods acid, base, enzymatic and organosolvents after the lignin has been fractionated out of the lignocellulose. This fractionation step is a prerequisite to full recovery of usable sugars from the lignocellulosic materials. The hemi-cellulosic fractions are generally quickly solubilized followed by the slower hydrolysis of the cellulosic fractions. To avoid the attendant problems of using acids and acid leachate products and the environmenal problems of solvents, the base-catalyzed hydrolysis or enzymatic hydrolysis procedures appear to be best suited for CELSS.

Some other aspects of these hydrolysis processes are important to consider in evaluating the best choice for CELSS. The base catalyzed or enzymatic processes require lots of water and power for mixing. If water becomes limiting, then acid hydrolysis is actually less water intensive. Acid hydrolysis using 20-30% slurries and acid hydrolysis giving 10% sugar solutions have been reported (25). In all of these processes, cellobiose is produced and acts as an inhibitor and its conversion to glucose must be considered. In the area of enzymatic hydrolysis, it is not possible to immobilize these enzymes since they are acting on a solid substrate, i.e. solid-solid reactions don't readily occur. Some suggestions have been made to employ simultaneous saccharification and fermentation (SSF processes). Commercially, this is one area of interest in the alcohol fuels area for it proposes to eliminate some problems in multi-step processing. In many ways the Waterloo Process is analogous to this approach. However, in the production of usable sugars no consideration has been given to finding a strain of microbial species that is both cellulolytic and edible. Thus, a strain would have to be found that meets the following initial criteria: it produces cellulases and hemi-cellulases and no toxins. In addition, it will probably have to meet some minimum production requirements such as tolerance to high concentrations of sugars and alcohols, etc. Again, trade-off analyses will be required when the CELSS system is better defined.

The use of base catalyzed hydrolysis is low technology but is effective and caustic may be easier to deal with than acid from an environmental perspective. Enzymatic processes are environmentally suited for hydrolyzing cellulosic fractions. Enzymatic processes have not reached their full potential largely due to the lack of understanding of the basic chemistry and biochemistry of these systems. As this research area matures, it is expected that it will become more suitable for CELSS applications.

4. The use of fungal decomposition, fractionation hydrolysis and production of single-cell food is a low technology process that is very attractive. Some pretreatment is necessary, but grinding, hot water and some caustic can usually render the biomass suitable for fungal growth. This presumes that the end product-fungal food-is the desired output rather than glucose which could then be processed into food items. There are some problems with power (for mixing) and some water requirements but they would be small compared to other processes. The main consideration to be made here is the trade-off on the output of the waste processing stream. Is it to be glucose or a microbial food product?

5. With DOE supporting this work, it would appear that the

CELSS program will be the beneficiary of results supported by another government agency. This will reduce the cost to NASA. However, it would be necessary to keep abreast of the developments by DOE researchers and determine how new advancements might aid CELSS researchers. When design scenarios for CELSS begin to be implemented, it would be prudent for NASA to involve DOE R&D people rather than try and develop all the expertise for waste processing of lignocellulosics themselves.

VIII. TRADE-OFF ANALYSES AND CONCLUSIONS

The accompanying analysis is a compendium of "napkin" calculations trying to relate the quantity of energy necessary to make food verses that which is necessary to decompose it. As a necessity there are many assumptions that one needs to rely on in order to carry out such an analysis. Even though the results obtained here are only approximations, they do offer some insight into the complex trade-offs that will be necessary in developing a CELSS model system. Most of the values cited here come from two NASA publications: NASA Conference Publication 2480 (31) and NASA Contractor Report 177422 (32) and a published report (33).

The principles in this analysis are the food source wheat and the inedible waste lignin. Given the best of all worlds 100 g (426 Kcal) of wheat (all values are for dried material) could give, if properly processed, 84 g (314 Kcal) of edible material and 16 g (112 Kcal) of inedible lignin. From this it can be calculated that the lignin comprises about 16% of the weight and contains 26% of the original energy (31). Unfortunately it is difficult to interpret the magnitude of these values within the CELSS system unless these can be related to some other parameter. This leads use to the second stage of analysis.

In order to start, we'll assume that the lignin produced by the plant needs to be recycled. To do so, we use the process of wet oxidation. Analysis of this process (32) shows that the process requires 1.3 Kw of power (303 w electricity + 275 btu/h heat) to process a waste mixture of 10% feces/90% urine at 330 cc/h. If 10% of the slurry is feces then in one hour we would process 33 g giving a final energy cost of 0.039 Kw/g/h. If we assume the composition, by mass, of feces and lignin to be the same then the 16g of lignin waste would require 0.624 Kw/h or 536 Kcal. Remember the original lignin has trapped only 112 Kcal of energy. Thus you need almost 5 times the amount of energy to get rid of lignin by wet oxidation than what it originally contained.

Another way to look at the problem is to see if one can relate the amount of energy involved in both making and disposing of lignin in some other terms (32). What we chose was surface area necessary to grow the food source in the first place. In order to supply the daily energy requirement for an astronaut (2800 Kcal/d), one would need 680 g of seed/d of wheat seed. A typical cultivator gives 15 g of seed/m^2/d. In order to produce the 680 g/d, 45 m^2 of surface area would be required. If light for photosynthesis is generated by electricity, then 27 Kw of electricity would be required per day (0.6 Kw/m^2 x 45 m^2). Now let's transgress a little. If a harvest index of 0.45 is chosen for wheat, then the 680 g/d of seed comes from 1511 g of wheat. Sixteen percent or 242 g of the wheat is lignin which amounts to approximately 1694 Kcal or 26% of the wheat energy content. 26% of the wheat energy is in lignin or 7 Kw or 11.7 m^2 of surface area (7 Kw x 0.6 Kw/m^2). It takes by wet oxidation about 5 times the energy to break lignin down than it takes to make it, therefore wet oxidation would take 35 Kw of photosynthetic energy or 58 m^2 of growing surface area. The total area for lignin both to make and break would be about 70 m^2 or about 1.5 times the original area for cultivation.

In conclusion, the above mentioned analysis suggests that the amount of energy necessary for growth and disposal of lignin is significant. By using this type of analysis, it might be possible to get a better understanding of the trade-offs that will be necessary in order to make CELSS successful. It is also possible that this type of analysis might be helpful in describing areas of future work and thus help direct the overall effort.

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Table 1 Composition of Wheat cultivars (straw, roots, stems, leaves, etc.) and other Selected Plant Materials

Source (reference)	Crude Protein	Hemi-Cellulose	Cellulose	Lignin	
				Klason or Spectrop.	Van Hoest
All values are shown as a % of the source material only					
Yecora Rojo (3)					
Overall	–	–	–	15.7	7.7
Stems	6.6	22.9	32.6	–	5.7
Leaves	11.8	27.2	25.8	–	3.7
Roots	–	43.7	25.2	–	4.3
Veery 10 (3)					
Stems	3.7	22.8	35.0	–	7.3
Leaves	9.0	24.0	26.9	–	3.6
Wheat Straw (10,24)	–	24.8	42.7	18	–
Wheat Straw (2)	–	–	–	13.9	–
Oat Straw (2)	–	–	–	14-22	–
Rice Hulls (2)	–	–	–	40	–

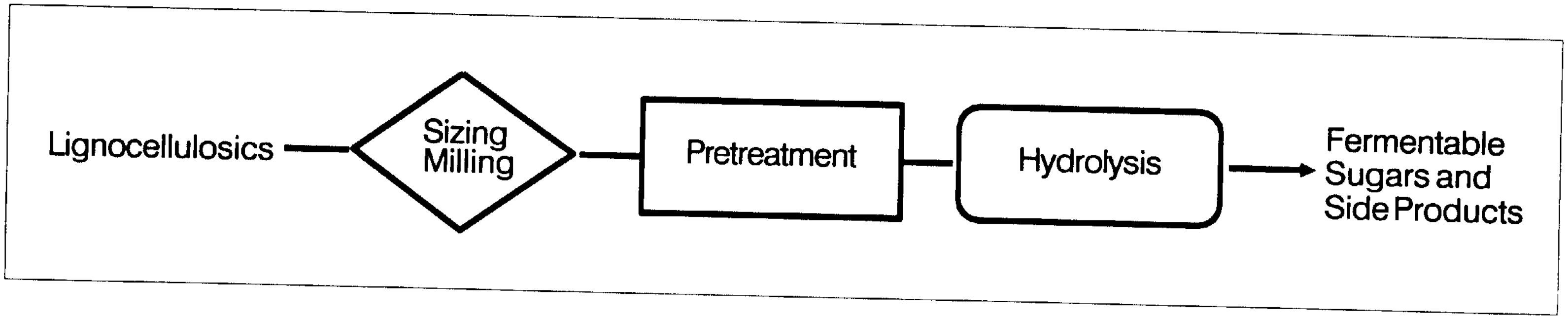

Figure 1. Methods for the conversion of lignocellulosics to usable sugars all involve the same basic steps

The most important processes that are under investigation are as follows:

1. Pretreatment of raw materials
2. Making cellulolytic and other enzymes
3. Hydrolyzing cellulose to glucose
4. Fermentation of xylose to alcohols

Separating hydrolysis and fermentation is favored because it gives more control in the mixing and matching of enzymes

Most research in the study of enzymatic processes has been done on the \ enzyme from the fungus, Trichoderma

Most fermentation studies have involved yeasts because of their consistent ability to ferment sugars at high efficiencies.

Areas needing more study:

- ○ Better biological catalysts and better biological reactors (design and operational concerns)
- ○ Developing a standardized measure of digestability

Figure 2. Conversion of Cellulosics to Sugars: Overview of Current Research and Challenges

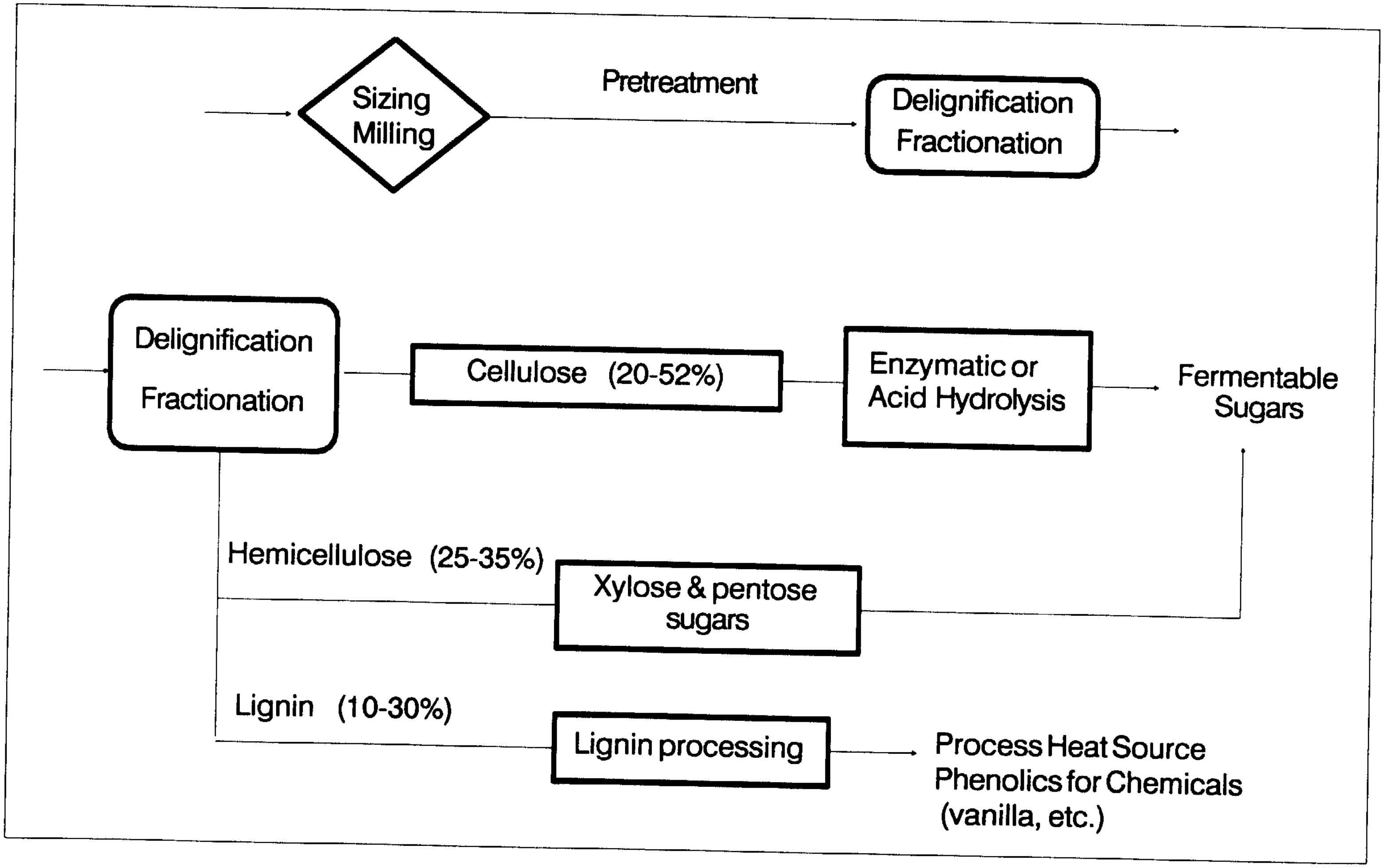

Figure 3: Pretreatment and pretreatment with fractionation

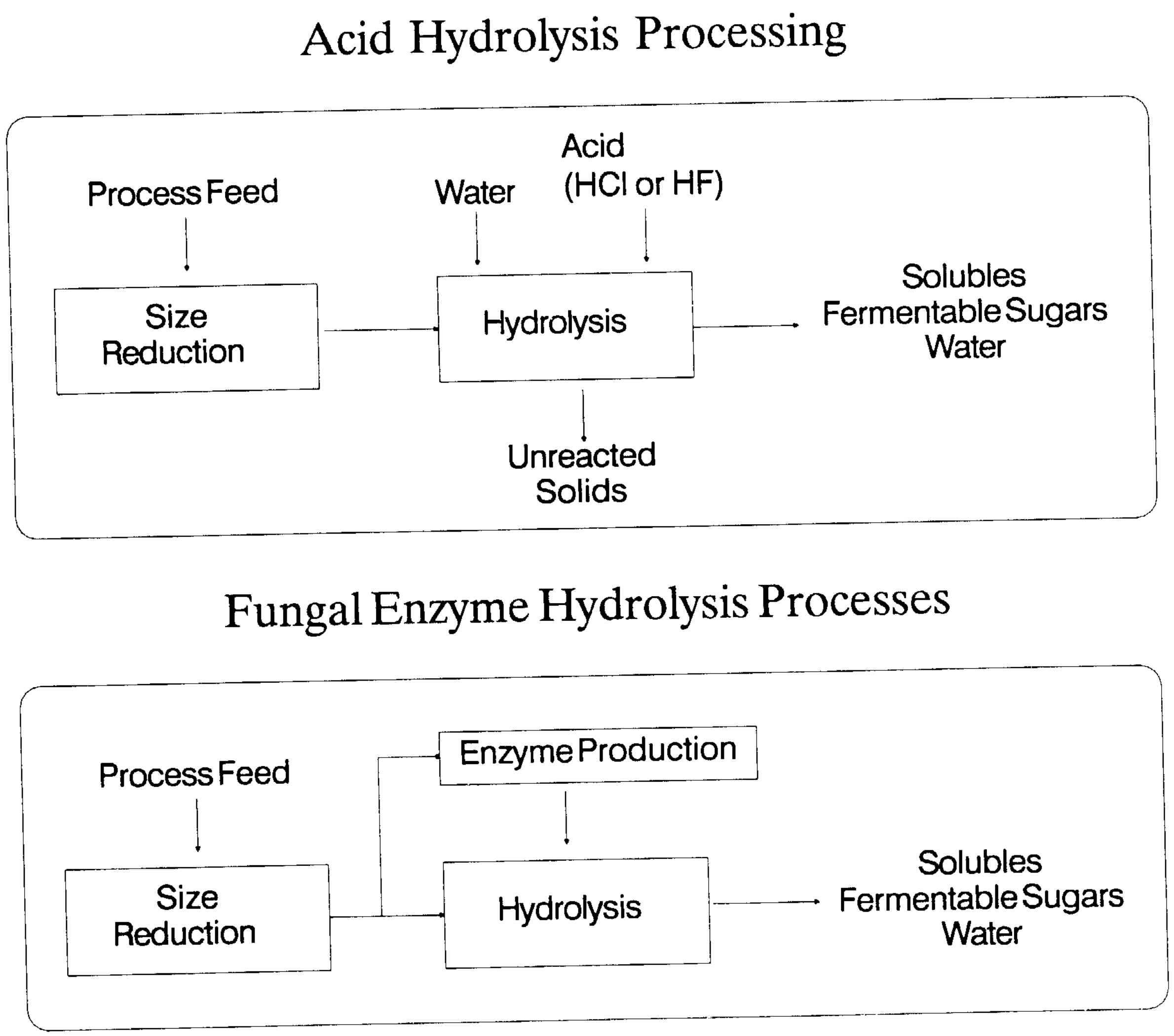

Figure 4. Hydrolysis of Sized, Milled and Pretreated Lignocellulosics