SOME QUESTIONS OF SPACE BIOENGINEERING

BY

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...for the chief malady of man is a restless curiosity about things which he cannot understand; and it is not so bad for him to be in error as to be curious to no purpose.

PASCAL (Pensees, Section 1, #18)

ABSTRACT

Zero-gravity offers selective effect on growth and metabolic activity of unicellular organisms as well as unique opportunities in purification of organic compounds. These make it possible to consider the biosynthesis and recovery of certain metabolites economically feasible in space.

Design, construction and operation of systems for the above mentioned purposes requires interdisciplinary actions within the scope of a new discipline: space bioengineering. Paper discusses the problems and perspectives of this discipline particularly in the application of bio-reactor-recovery systems in space to manufacture metabolites of high economic and scientific value. Special attention is paid to pivotal factors such as various mass transport phenomena, contamination control, automatic control of optimum environment and synchronization of the operation of the biological (biosynthesis) and the physicochemical (recovery-purification) systems. Although the space bioengineering is in its early stage of development, its role will gradually increase with the full implementation of the Space Bioprocessing Program.

INTRODUCTION

In the course of technical developments, we always witness that the human knowledge on the physical behavior of inorganic materials precedes the information on biological systems. This tradition was observed again, most recently, during the Apollo and Skylab experments which demonstrated significant changes in the physical behavior of fluids and gasses exposed to zero-gravity (1).

On the other hand, certain experiments related to the search of effects of gravitational acceleration, cosmic radiation and weightlessness on living systems revealed, among others, the possibility of culturing unicellular organisms in space. Increased specific growth rate and higher cell density obtained with S. typhimurium (2) as well as higher frequency of induction of bacteriophage in E. coli K-12 (3) indicated improved metabolic activities probably due to altered physicochemical properties of the liquids and gases under near weightless conditions.

These results initiated a study by JORDAN (4) concluding that there is a possibility to enhance the productivity of certain organic compounds if the biosynthesis takes place in space.

Most recent experiments with zero-gravity electrophoresis revealed the possibility of fine separation and (ultra) purification of biological materials (1).

These basic experimental data forecast a potential research activity and industrial application in space environment for processing biological materials. A new discipline seems to shape up which may be called as "space bioengineering".

Objective of this paper is to analyze some of the major questions related to this technology. It is emphasized, however, that the potentials and limits of this discipline have not been identified yet. All of our discussion is based on a few experimental data in space and the knowledge accumulated, mostly during the last 30 years, in biochemical engineering. It is felt, however, that this study gives some basis which can be utilized by space processing scientists during the implementation of Space Bioprocessing Program.

SPACE BIOENGINEERING

Unique properties of the space environment (particularly gravitational effects, cosmic rays) interactively influence the bioprocesses which, henceforward, requires hardware and technology substantially different from the ones employed on the Earth. Space bioengineering is concerned with the hardware design and technologies related to processing of organic compounds by means of biosynthesis and recovery-purification, at least one of which takes place in space.

As a discipline (Figure 1) space bioengineering is closely related to biochemical engineering (5) which generally deals with the theory and practice of bioconversion of materials and their recovery from a culture liquid. It has also a relationship with space microbiology (6) which deals with extra terrestrial detection of microorganisms, evaluation of behavior of terrestrial microorganisms in space and monitoring of spacecraft and astronaut microbial flora. The discipline has a close relationship with the material processing sciences specially utilizing the knowledge on unique behavior of liquids, gases and solids in zero-gravity.

Table I lists the main areas of concern related to activities in space bioengineering. At this time, we shall address ourselves only to few key questions closely related to implementation of NASA Life Sciences Program in Space, particularly the early stage of Space Shuttle and Spacelab experiments. It is anticipated, however, that gaining further practical information, the scope of discussions will broaden incorporating such questions as experimental trial of bioprocessed material in space for quality control purposes.

PROCESS DESIGN FOR SPACE EXPERIMENTS

A major objective for the initial stage of Bioprocessing Program is the demonstration of usefullness of space biosynthesis and biochemical separation techniques. Implementation of biosyntheses and recovery-purification processes in space, however, faces constraints from the viewpoint of payload, in particular regarding the requirement of relatively large quantity of water during each step of the operation. Another important constraint is the maintenance of aseptic condition during the culture and product recovery.

Usefullness of bioprocessing can be demonstrated in production of one or more organic compounds of high scientific or medical value in a quantity applicable for at least experimental purposes.

In an attempt to define the most promising materials, Table II lists various organic compounds currently produced by means of biosynthesis and biochemical recovery techniques on laboratory or industrial scale. Each process represents a type of metabolic pattern and has attractive features from experimental point of view. Accordingly,

1. Production of cell mass (SCP) or ETOH on carbohydrates can be the subject of experiments of shifting metabolic pathway in favor of one product accumulation (7),

2. Biosynthesis of gluconic acid from glucose is a classical example of combined and staged activity of various cell-bound, cell-free enzyme activities as well as nonenzymatic conversion of an intermediate into final product.

Besides, the process is well known, therefore, comparative studies can be easily made.

3. Production of oxytetracycline (OTC) has the combined characteristics of the former two processes (with the exception of nonenzymatic catalysis step). In addition, the problem of contamination is greatly reduced because of the wide spectrum of the antibiotic activity.

4. Biosynthesis of vitamin B_{12} is an example of mixed culture operation incorporating complex growth and product formation kinetics.

As it is noted on Table II with the exception of the first process, product recovery can be implemented either by chromatography or by electrophoresis.

On the other hand, the absolute (scientific, commerical) value of products #1 - #4 is low, whereas the desired quantity for application is relatively large. Even in the case of substantial improvement in biosynthesis (assuming fourfold increase in space) the needs can be fulfilled only with moving of large quantities of water.

Because of these considerations, any of the processes has short range of applicability and scientific value only in the testing stage of space biosynthesis and recovery equipment.

Product #5 has the advantage of experimental trial of eucaryote cell growth exposed to space environment as well as production of compounds of scientific and medical significance. In particular, production of growth hormones (GH), adrenocortical steroid hormones, thyrocalcitonin and parathyroid hormone may be listed here as prime candidates. In addition, electrophoresis is considered as the best means in separation of the protein and polypeptide compounds from the culture medium components (e.g. from serum). POSNER, in a short discussion (8), describes the most recent achievements (notably, direct relationship between cell mass and GH production, suspension culture of pituitary tumor cells, enhancing effect of hydrocortisone on GH production, release of hormones into the extracellular liquid). With a potential increase of cell density to 10^{12} cells per liter from 10^{9} cells per liter, gonadotropin hormone production can be substantially augmented (a cautious estimation is a fourfold increase in GH production). HIMMELFARB and his coworkers already reported 10^{8} cells per ml in a perfusion-suspension apparatus (9).

On the basis of the first experiments in space relative to fluid and gas mixing conditions, it is anticipated that the enhanced oxygen transfer will improve the cell metabolic activity leading to increased cell number, hence larger hormone production. In case of achieving

10^{12} cells per liter, a semicontinuous culture can produce about 2 G GH/L/24 hours for further recovery and purification. Also changes in normal human cells "anchorage dependency" can be anticipated in zero-G, making production of hormones by non-malignant cells possible.

EQUIPMENT DESIGN

GENERAL CONSIDERATIONS

According to experiences accumulated in biochemical engineering the following main rules must be observed in the hardware design:

1. Systems integrity. The entire system consists of three major elements: 1) Biosynthesis equipment, 2) Recovery equipment and 3) Process support subsystem. From operation point of view all those elements are considered as one unit. This principle defines the type and number of monitoring and control elements as well as the mode of operation of the system.

2. Systems flexibility. At the beginning of the experiments, particularly in the test stages at least three types of reactions can be visualized: 1) Fast, enzymatic conversion of compound A into B (where compound B is the subject of recovery), 2) Relatively fast microbiological process (doubling time = 20-60 minutes, product formation rate (dP/dt) = > 5G/L/HR) and 3) Relatively slow process (doubling time = 10-20 hrs, product formation rate < 5G/L/HR).

   Accordingly, the internal design and instrumentation of the biosynthesis subsystem must allow sufficient interchangeability which makes the implementation of enzymatic reactions (CSTR), microbial fermentations and animal cell suspension cultures possible. It is necessary to note, that in all cases, the recovery subsystem (EFO) is unchanged. This makes the application of a "buffer subsystem" necessary. This system couples or uncouples the biosynthesis and recovery subsystems depending on the differences between the kinetics of biosynthesis and the recovery.

3. Automatic operation. Because of the anticipated workload aboard the Spacelab, the system must contain extensive electronic monitoring, process analysis and control equipment which alleviates the necessity for scientists to manually operate the equipment. This question, considered to be im-

portant, both from design and process implementation points of view, will be discussed in a subsequent chapter.

SPACE BIOPROCESSING SYSTEM

Figure 2 contains a basic concept of "convertible" equipment useable as CSTR for enzymatic reactions, as well as for culturing cells in suspension. In a typical process, culture medium in Culture Vessel, 1 is seeded from a Seed Chamber. Environmental conditions are maintained according to the physiological status of the culture. Upon a condition (maximum product concentration, nutrient depletion) the culture liquid (or reaction mixture) is transferred to a dialysis system, 2 (10) where the product (among other organic compounds of same cut-off molecular weight) is removed and stored in a reservoir, 3. The non-dialyzed part of the culture is either recycled or discarded depending on the type and physiological stage of the process. Also, fresh nutrients and/or inducers can be added assuring (semi) continuous operation. The reservoir serves as a "buffer tank" if capacity problems take place in the recovery system, which is an electrophoresis apparatus.

Specific emphasize is given to the on-line measurement of process variables. With the exception of wet chemical analysis all sensed variables have computer compatible output for further data analysis.

Wet chemical analysis is performed from time to time, however, with the accumulated knowledge on the process correlations can be found between wet chemical analytical data and direct sensorial analysis. In a more advanced form, pivotal process variable(s) (11) will govern the process. This requires computer analysis and control of the entire operation.

In particular, the system is sterilized by ethyleneoxide/CO_2 gas-mixture prior to use. This is a definite deviation from the "classical" fermentation practice where steam is the primary sterilization agent. The contamination control is twofold, namely:

a) excluding foreign microflora penetration into the system,

b) controlling the spread of the culture content in the working area.

In view of the most recent findings on fluid mechanics and fluid gas interface phenomena in zero-gravity conditions, the most problematic area is the assurance of proper liquid flow and mixing conditions. This question is yet to be further analyzed.

PROCESS CONTROL

Depending on the systems configuration and the type of the bio-processing, the control of the entire process is implemented on two levels:

1) Process kinetics control,

2) Systems operation control.

The question here is the proper definition of the pivotal process variables around which the process control can be built. This requires extensive on-line, real-time analysis of the process resulting in definition of the physiological stages and the overall process kinetics.

PROCESS KINETICS ANALYSIS AND CONTROL

Analysis of the process condition can be performed introducing the signals of the on-line operating sensors into a computer which further processes the data by multivariation of the individual process variables. Figure 3 presents the logic of such an operation. According to our experiences with a highly instrumented, computer coupled pilot-plant fermentor, information related to the gas exchange conditions was found useful in detection of the physiological conditions of the culture (12).

As an example, Figures 4 and 5 show an on-line, real-time follow-up of a C. utilis culture's gas exchange condition. In this case, among other process indicators, RQ was computed which has direct correlation with the cells' physiological conditions. During the operation, samples were taken and analyzed by wet chemical analytical techniques.

Chemical analysis of the culture revealed significant correlations between RQ and some biochemical events including: 1) nucleic acid, 2) protein, and 3) ethylalcohol synthesis (Figure 5).

The drop in RQ value during the elapsed time period of 1-3.0 hours coincided with the increase in specific nucleic acid content of the culture, while the minimum RQ (EFT = 3-4 hrs.) coincided with the start of increased specific protein concentration. The increase in RQ value in the fourth hour coincided with the start of ethylalcohol formation (shaded area). Correlation obtained between the culture's metabolic activity detected by wet chemical methods and the respiratory quotient obtained via computer operation demonstrate that the latter, after the definition of correlations, can be used to determine certain transition conditions in eucaryotic cell cultures.

As a consequence, process status indicator such as RQ can be used to control the optimum environmental condtions during the culture. Figure 6 shows a double control loop concept to implement interactive control of individual process variables (T, N, Q, P, etc.: inner control loop) the composite of which creates the environmental conditions. Alteration of setpoints on the individual process controllers is based on the status of the process obtained through an analysis of the available information on culture rheology, physiology and metabolic activity (outer control loop).

In the above mentioned particular case, the culture's RQ served as process status indicator and $Q0_2$, $QC0_2$ were used to define the process kinetics. On this basis carbon and nitrogen compounds were fed in an optimum proportion resulting in suppression of ethylalcohol and increase in protein biosynthesis. By this means, environmental conditions were optimized and a fourfold increase in growth rate was obtained (7).

SYSTEMS OPERATION CONTROL

According to the principle of systems integrity the operation of biosynthesis, recovery and support subsystems will be coordinated. Figure 7 shows a concept of the systems operation control. This assumes analysis of status both for the biosynthesis and for the recovery part of the process. As it was shown, the biosynthesis stages and the kinetics can be analyzed and controlled on-line, real-time. This part of the process is considered, however, to be the most complex one exposed to unexpected disturbances. In the case of product recovery (dialysis and EFO) liquid flow rates and material concentration are considered to be the pivotal variables.

Information on status of both processes is fed into the controllers of process support system. This assures the proper rates of gas and liquid removal and purification as well as defines the availability and supply of utilities. It is well known that the microbiological processes are water extensive. Therefore, particular care must be exercised to assure the proper water recycling schedules.

The task of construction of a system with such a complexity and the payload constraints require application of microprocessors for process control purposes. These are programmed according to findings while using minicomputers in process analysis and controls of biosynthesis and recovery systems during the test stages.

CONCLUSIONS

The above mentioned examples and design considerations indicate that implementation of a Space Bioprocessing Program is technically feasible.

In addition, there are three general comments worth emphasizing with regard to this project.

First, the Program has to demonstrate the economic and/or scientific value of bioprocessing under zero-gravity conditions. This is the question of well defined process(es) and well designed equipment. Presently, there are guesses in this area and only the experimentation will give us proper answers. We have to recognize, however, that because of the unique properties of fluid-gas mixing, new process and equipment designs are an absolute necessity.

Second, we all know that the majority of the presently existing fermentation plants are constructed with more respect toward material selection, motors, pipes than regarding the importance of cells' physiology in the biosynthesis. Whatever the outcome of the Spacelab experiments will be, information obtained on the cellular metabolism can be used as source for construction of more bioprocess oriented fermentation plants on the Earth.

Third, in case of success, the bioprocessing equipment constructed for Space application, will serve as a prototype of an ultramodern fermentation system which can open new areas for the industrial microbiology.

I believe that with proper interdisciplinary efforts, we shall achieve those goals which will be beneficial both for science and the industry.

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate,
CSTR	Continuously stirred reactor,
D_i	Diameter of impeller,
DEDT	Rate of ethylalcohol (C_2H_5OH) biosynthesis,
DO	Dissolved oxygen concentration,
DPRDT	Protein biosynthesis rate in the culture liquid,
EGA	Exit gas analysis,
k_La (KLA)	Oxygen mass transfer coefficient,
N	Agitation speed,
NA	Nucleic acid content of the culture liquid,
NADH, NADH + H^+	Pyridine nucleotide content of cells (oxidized, reduced forms),
N_{Re} (NRE)	Reynolds number,
ORP	Oxidation-reduction potential (eH),
OXUP	Oxygen uptake rate of the culture,
P	Vessel head pressure,
P·	Product formation rate (dP/dt),
Q_n	Air (gas) flow rates,
$QC0_2$	Specific CO_2 release rate,
$Q0_2$	Specific O_2 uptake rate,
RQ	Respiratory Quotient,
S, S_1	Substrate, C_6 sugar,

LIST OF ABBREVIATIONS (CONTINUED)

S_2, S_n	Nutrients,
T	Culture liquid temperature,
T_d	Doubling time,
V	Culture liquid volume,
W	Energy uptake for fluid mixing,
WCA	Wet chemical analysis,
X	Cell mass,
X	Growth rate,

GREEK LETTERS

μ	Specific growth rate
ρ	Liquid density

TABLE 1

MAIN AREAS OF CONCERN IN SPACE BIOTECHNOLOGY

PROCESS DESIGN

- UNIT PROCESSES
- UNIT OPERATION
- CONCERTED OPERATION OF THE SYSTEM ELEMENTS

EQUIPMENT DESIGN

- CULTURE VESSELS
- PRODUCT RECOVERY AND PURIFICATION SYSTEMS
- INSTRUMENTATION
- SUPPORT SYSTEMS

ASEPTIC OPERATION

- MEDIA AND GAS SUPPLY STERILIZATION
- BIOHAZARD CONDITIONS

PROCESS CONTROL

- KINETICS
- CONTROL OF ENVIRONMENTAL CONDITIONS
- SYSTEMS OPERATION CONTROL

PROCESS MANAGEMENT

- ECONOMICS OF OPERATION
- EARTH-SPACE COOPERATION
- SCALE-UP IN SPACE

TABLE 2

ANALYSIS OF PROCESSES FOR SPACE BIOTECHNOLOGY

	PRODUCT	PROCESS	MAIN PRODUCTS PRIMARY (CELLS)	MAIN PRODUCTS SECONDARY	TYPE OF RECOVERY #1	TYPE OF RECOVERY #2	VALUE(A) #1	VALUE(A) $	VALUE(A) #2	ANNUAL REQUIREMENT(B) (ESTD)
1.	SCP/ETOH	C.UTILIS SUBMERGED	25 G/L	ETOH:80 G/L	FILTR.	DIST.	1.9×10^{-3}		2×10^{-2}	10^6T
2.	GLUCONATE	P.OVALIS SUBMERGED	3 G/L	GA:40 G/L	FILTR. DIAL.	CHROM. EFO	NIL		1.9×10^{-2}	10^4T
3.	OXYTETRA-CYCLINE	S.RIMOSUS SUBMERGED	8 G/L	OTC:30 G/L	FILTR. DIAL.	CHROM. EFO	NIL		2.5	10^3T
4.	VITAMINE B_{12}	MIXED CULTURE SUBMERGED	6 G/L	B_{12}:0.3 G/L	FILTR. DIAL.	EFO	FEED		4.7	10^2T
5.	GROWTH HORMONES	THYROTROPH MAMMOTROPH CELLS	10^9/L	GH:0.5 G/L	DIAL.	EFO	?		375	0.1 T

(A) PRODUCT VALUE ESTIMATED FOR PROCESSING ONE LITER CULTURE LIQUID WITH A RECOVERY LOSS OF 25%.

(B) ESTIMATED POTENTIAL NEED IN USA.

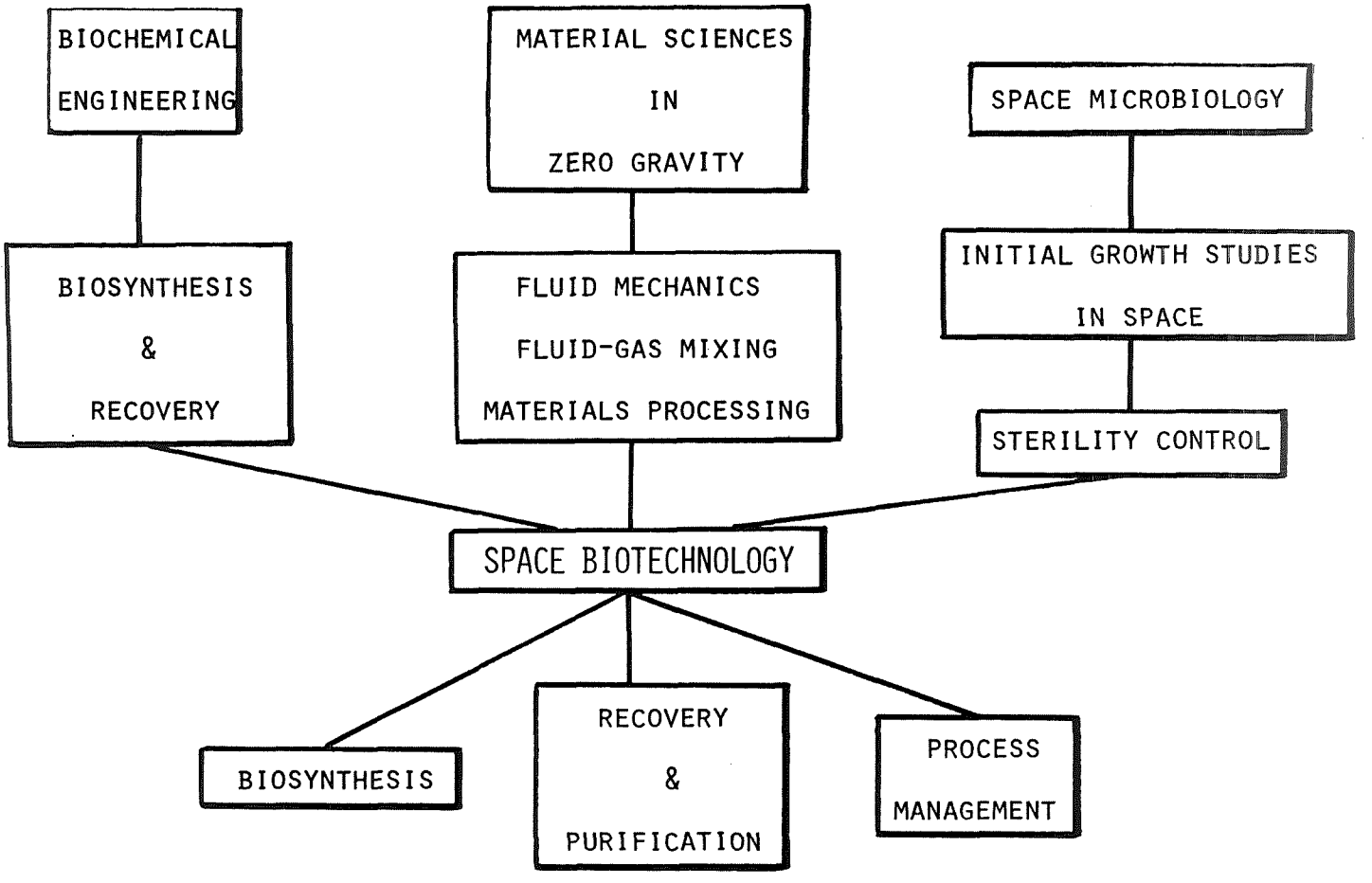

Figure 1.- Relationship between space biotechnology and other disciplines.

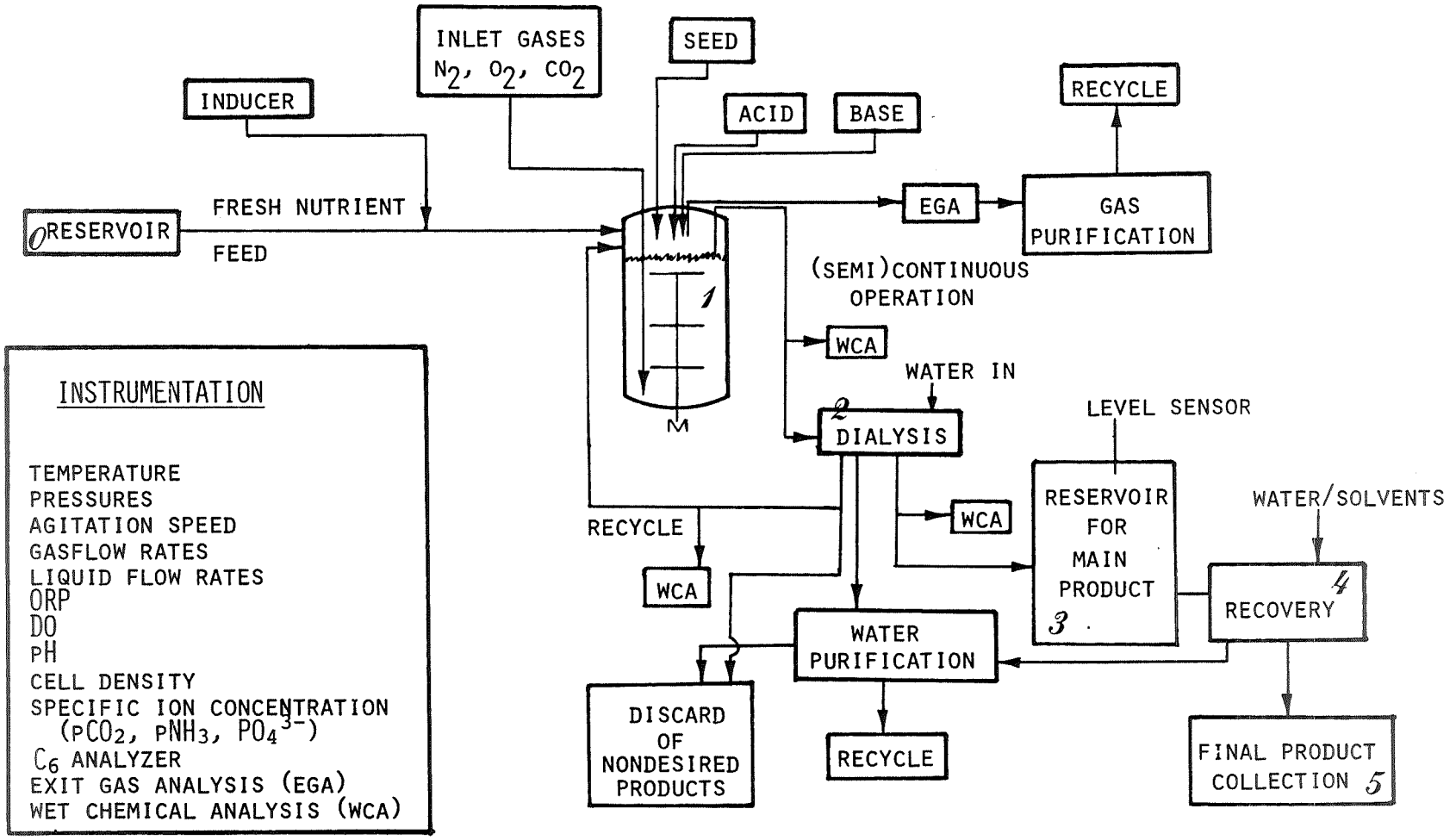

Figure 2.- Design concept of bioprocessing equipment (suspension culture).

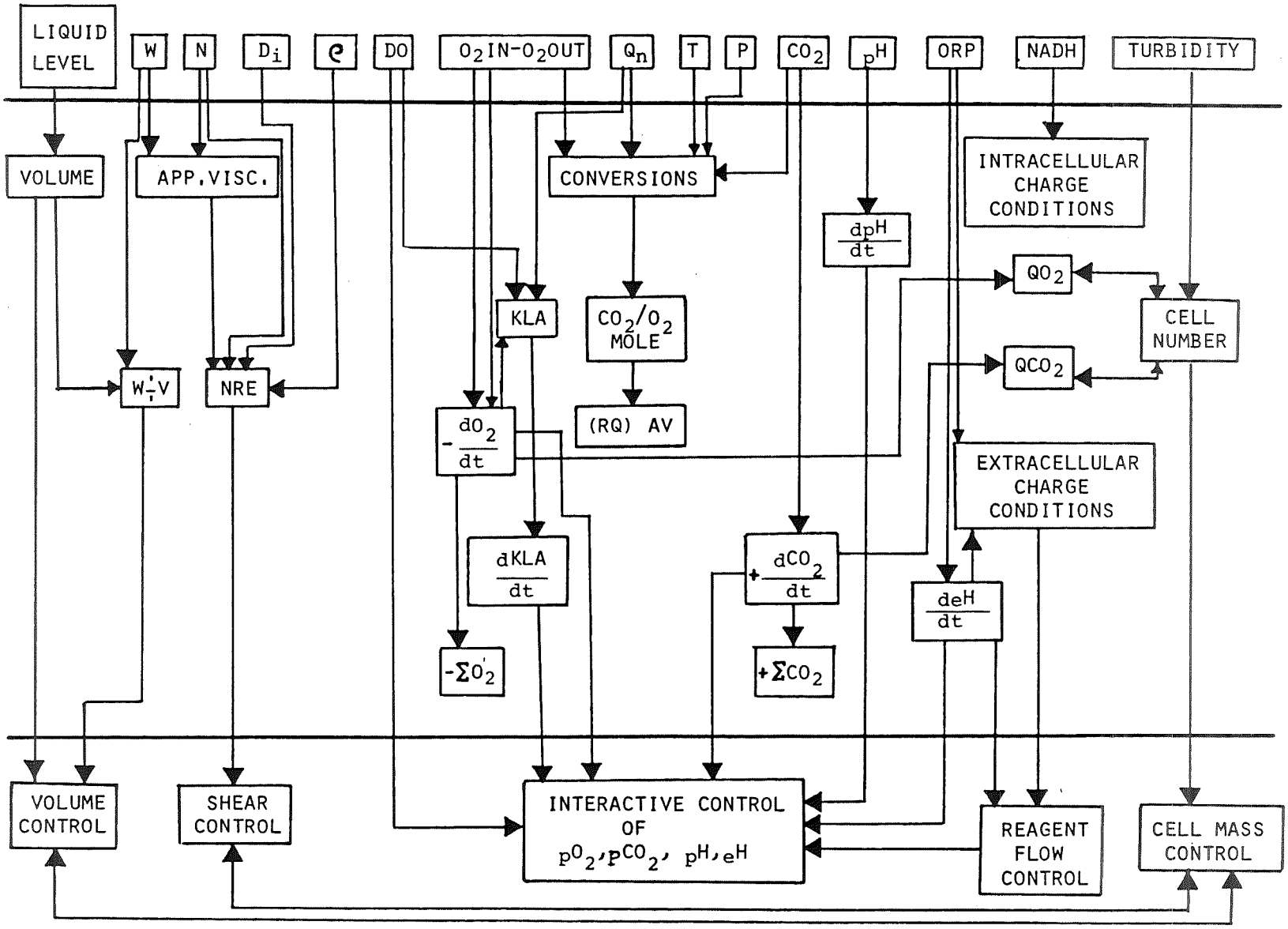

Figure 3.- Flow chart of electronic process analysis of biosynthesis processes (symbols explained in list of abbreviations).

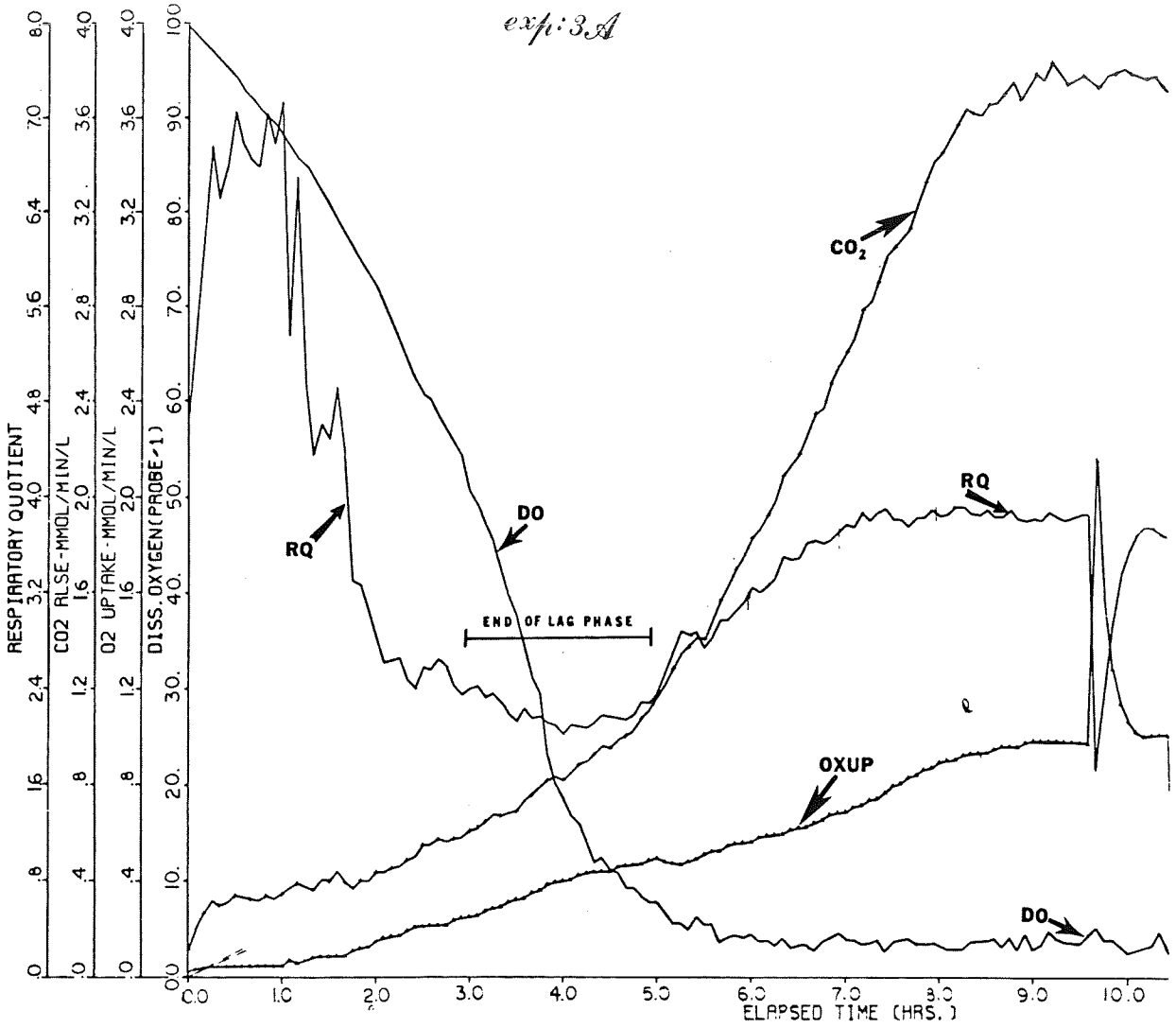

Figure 4.- On-line real-time determination of the physiological condition and process kinetics of an eucaryote cell culture.

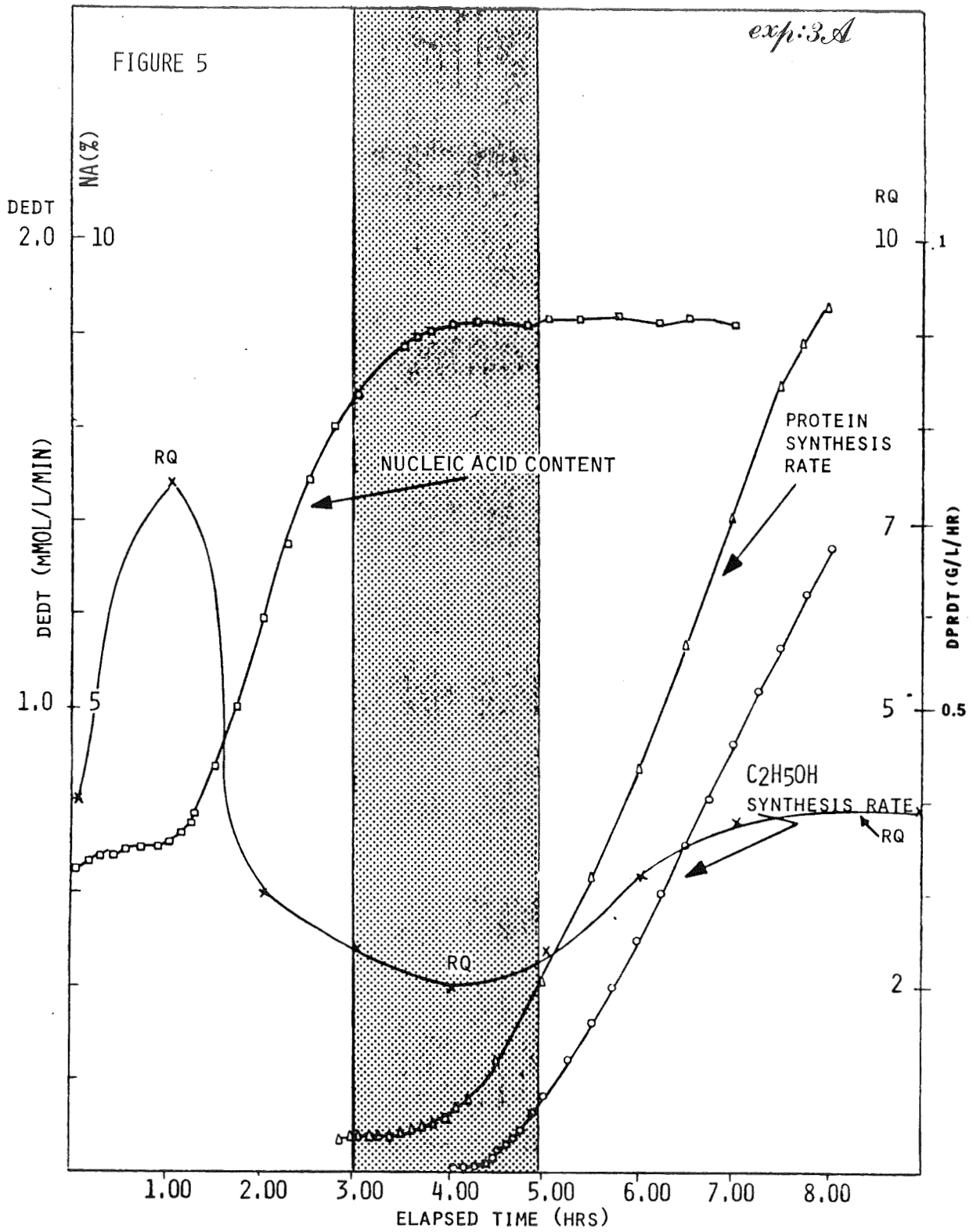

Figure 5.- Metabolic activity of C. utilis in lag-log transition.

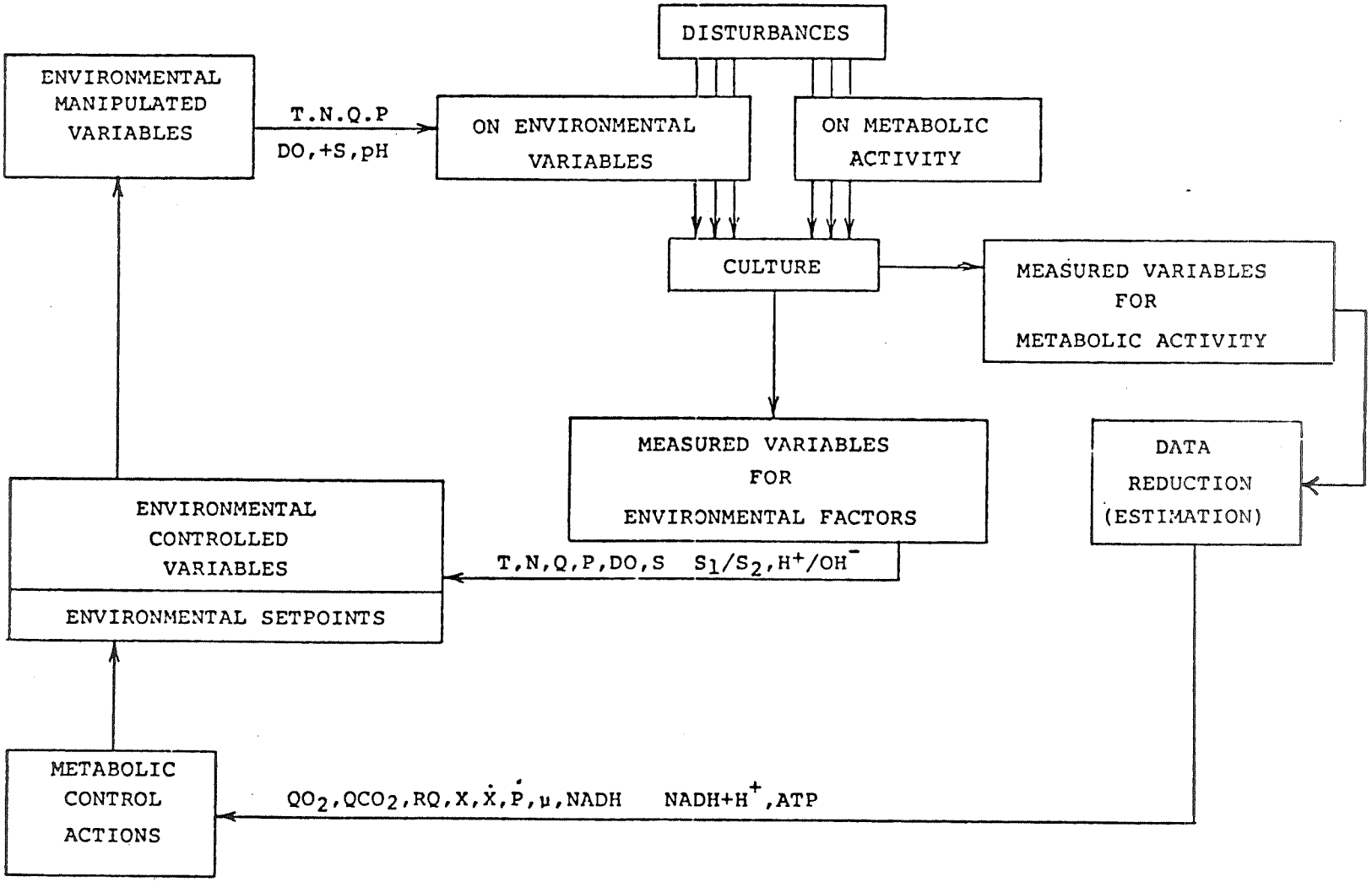

Figure 6.- Basic concept of biological interactive control.

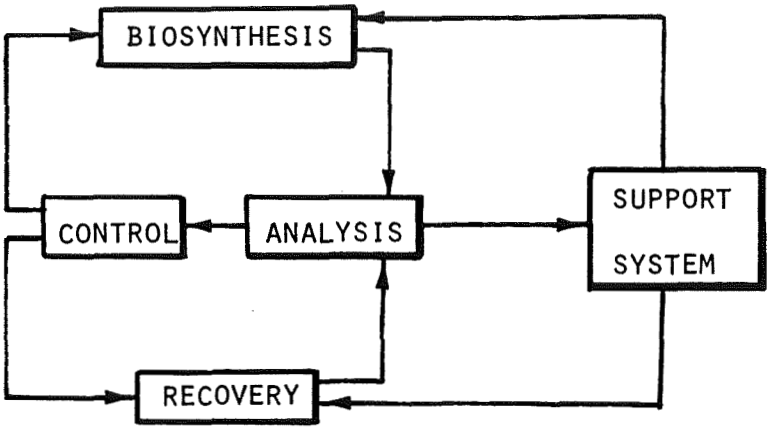

Figure 7.- Concept of systems operations control.