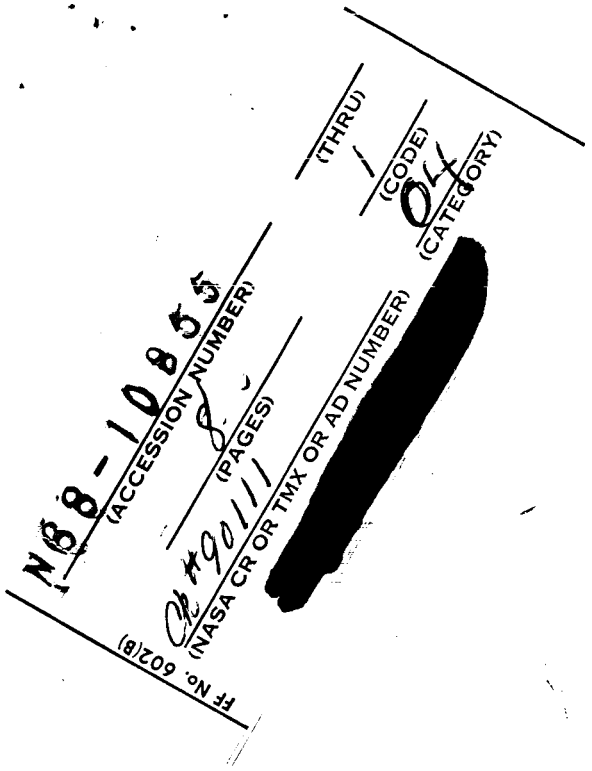

Engineering Requirements for Culturing of Hydrogenomonas Bacteria

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ABSTRACT

Experimental results obtained with a continuous culture system for the cultivation of Hydrogenomonas eutropha for waste management in a life-support system indicate that a reliable and stable system can be designed under the present state-of-the-art. The present system provides for control of hydrogen, oxygen, carbon dioxide, pH, cell density, temperature, urea, and ammonia during growth. The culture system design is adaptable to operation in a zero-gravity field, and should be adaptable to integration with proposed water electrolysis and product recovery systems for waste management in an overall life support system.

THE POTENTIAL USEFULNESS of cultures of Hydrogenomonas bacteria for waste management in a life-support system has been discussed by Bongers (1)* and Schlegel (2). Carbon dioxide and urea, major waste products from human metabolism, can be assimilated by the organism as the sole sources of the carbon and nitrogen required for growth. Hydrogen and oxygen are also assimilated during the growth process, and the major products of the culture are bacterial cells and water. These organisms derive energy for growth from the reaction

$$H_2 + 1/2\ O_2 \longrightarrow H_2O \qquad (1)$$

One concept for a complete cycle for life support in a closed environment includes waste utilization by the Hydrogenomonas culture; replenishment of atmospheric oxygen with oxygen produced by electrolysis of water; and utilization of harvested cellular material as a source of food (3, 4). Only a part of the electrolytic oxygen is required to balance human consumption. The remainder of the oxygen and all of the electrolytic hydrogen are supplied to the culture. Ideally, the inputs and outputs of materials to and from the human occupants, the bacterial culture, and the water electrolysis system can be brought into approximate balance across their respective interfaces by controlling only the growth of the culture and the electrolysis of water. Thus, conversion rates can be established in response to normal human requirements; waste carbon dioxide, urea, and hydrogen can be utilized; and the only net input to the total system is electrical energy.

The performance potential of this system was evaluated by Jenkins (5) in 1965, with "tentative best estimates" based on incomplete data. System weight and energy requirements appeared encouragingly low, but confirming data were required.

This paper describes the continuing work in this laboratory from which we are evolving a better understanding of the environmental requirements for culturing Hydrogenomonas bacteria. Some of the engineering requirements for con-

*Numbers in parentheses designate References at end of paper.

struction materials and for maintaining favorable environmental conditions are well established. Others are not so well defined, in particular the selection of an adequate control system of minimum complexity. The current status of culture control in an operating system is therefore discussed, and alternatives are compared.

SELECTION OF MICROORGANISM

An environment suitable for growth of Hydrogenomonas spp. matches the requirements imposed by the concept of a closed life-support cycle, as outlined above. All the studies in this laboratory have been made with H. eutropha; a culture of this organism was obtained initially from the laboratory of the Research Institute for Advanced Studies through the courtesy of Dr. Leonard Bongers. It was reported that H. eutropha grows more rapidly in the required environment (H_2, O_2, CO_2, urea system) than other species, and hence would be a suitable selection for the study of continuous culture.

ESSENTIAL FEATURES OF CULTURE APPARATUS

Foster and Litchfield (3) have described the essential features of the first model of the automatic Hydrogenomonas culture system, which was designed to maintain a constant environment suitable for growth of the organism. The apparatus was specifically adapted for research to determine optimum environmental conditions. Therefore it was more flexible in operation, more highly instrumented, and more precisely controlled than may be necessary in an engineering design for continuous culture apparatus to be operated only within a limited, near-optimum range for each environmental variable. Three subsequent research models of the apparatus have each evolved from the preceding one, with instrumentation and controls added or modified to study the growth environment and the response of the organism to individual variables. All models have incorporated three essential subsystems:

1. An environmentally isolated culture vessel, containing both the suspension of cells growing in the liquid medium and the associated gas-phase inventory of a mixture of carbon dioxide, oxygen, and hydrogen, which supplies dissolved gases to the liquid phase.
2. An analytical system, with appropriate sensors and readout devices for measuring the value of each significant environmental variable in the culture vessel.
3. A control system, with feedback responses to each analytical measurement, which adds or removes materials or heat to counteract any departures from selected environmental conditions.

EXPERIMENTAL STUDIES

OBJECTIVES - The experimental studies were intended to define the environmental conditions under which the culture would operate most efficiently as a part of the total life-support cycle described previously. Efficiencies can be variously defined, depending on the requirements of the specific mission in which the life-support system is to be used. In all cases weight, volume, and power requirements must be reduced as much as possible; reliability and stability must be achieved; and the quality of the cell product must be suitable for use as a food supplement after appropriate processing. However, in some cases maximum cell production rates may not occur with minimum energy input. The same is true for maximum conversion rates of carbon dioxide in a unit volume of culture, and similarly for urea conversion, and for hydrogen conversion. All the environmental variables interact in a complex manner to influence the growth of the bacteria. Therefore, the experiments were planned for the broad objective of understanding, at least qualitatively, some of the tradeoffs involved in reaching maximum rates of conversion or production.

CRITERIA FOR EVALUATION OF RESULTS - It is informative to compare the overall response of the organism to its environment in terms of its growth, which occurs with periodic cell division. The average period is constant under steady-state conditions of growth in a constant environment. This means that the rate of increase (dC/dt) in cell density C at time t is proportional to the cell density:

$$dC/dt = kC \qquad (2)$$

For convenience, we compare growth in various environments in terms of the magnitude of the growth rate constant k. Dimensional units are C, grams (dry weight) of cells per liter of culture; t, hours; k, hours^{-1}

An informative evaluation of environmental influences on the growth of the organism can be obtained from a comparison of the rates of assimilation or conversion of each of the three gases. Both the total gas consumed and the proportions of the individual gases in the system are significant. Usually the gas conversion rate is proportional to cell density in a constant environment. Often a significant change in environmental response is indicated by a difference in the proportion of carbon dioxide converted, relative to hydrogen and oxygen. In the most active growth state, gases are converted in the proportions 6:2:1 for H_2:O_2:CO_2, respectively. In essentially inactive but viable cultures which are "resting," the proportions of these gases may approach 6:3:0. We have chosen therefore to normalize the composition of the assimilated gas mixture relative to 6 parts of hydrogen, and to express gas conversion rates as follows:

$$\frac{dg}{dt} = GC\,(r_{H_2} + r_{O_2} + r_{CO_2}) \qquad (3)$$

where:

$\frac{dg}{dt}$ = Mixed gas consumption rate, liters at standard temperature and pressure per (hour) (liter of culture)

C = Cell density, grams (dry weight) per liter of culture

r_i = Conversion ratio of gas i normalized so that $r_{H_2} = 6$

G = Specific gas-consumption constant

Qualitatively, the experimentally determined values of both k (Eq. 2) and G (Eq. 3) reflect environmental influences on the organism, one in terms of cell production and the other in terms of gas assimilation.

There is as yet no equivalent equation relating the rate of urea conversion to culture variables by a proportionality constant that is characteristic of environmental effects. The conversion of urea as a part of the growth process is discussed qualitatively in the next section.

GROWTH RESPONSES TO ENVIRONMENTAL CONDITIONS - We present here a qualitative summary of growth responses to environmental variables based on the experimental results obtained from many quantitative measurements of the environment and the corresponding growth of the organism in both batch and continuous cultures in the apparatus described above. This appraisal of the growth responses serves as a guide to, and justification for, the estimates of engineering requirements for a waste management system projected in the following section.

Temperature - All experiments in this laboratory have been made at 30 C. Brief excursions up to 35 C have had no measurable effect. It has been reported from experiments in other laboratories that the optimum temperature for the culture is about 33 C, and the tolerable maximum approaches 40 C. In the range between 25-40 C, the primary effect of temperature is probably on the chemical equilibria in the liquid phase, the rates of chemical reactions, and the solubility of the gases in the liquid that are in equilibrium with the gas phase mixture. At temperatures near the lower limit, gas solubility is increased somewhat, but reaction rates are reduced slightly, including growth itself. Near the higher temperature limit and beyond, growth is likely to be inhibited by undesirable effects on the enzyme systems that catalyze conversion reactions within the cells. The controls in the experimental culture apparatus have maintained the pre-set culture temperature to plus or minus 0.2 C, but a control to maintain temperature within plus or minus 2 C may be acceptable.

Pressure - The experimental apparatus is designed to withstand internal pressures up to about 20 psi above ambient. In this range, there is no expected or measurable effect of pressure on the growth of the organism itself. There is an expected effect of pressure on the solubility of the gases in the liquid phase culture, maintained in equilibrium with the mixed gas phase by vigorous stirring. Thus, the solubility of the gases can be approximately doubled by increasing system pressure from ambient to 15 psig. An equivalent control of the proportions of the gases dissolved in the liquid phase can be achieved by varying the partial pressures of the individual gases in the gas phase. The usual practice in the experimental cultures has been to maintain the average pressure a few pounds above ambient, and to permit fluctuations in pressure of up to 5 psi above the minimum pressure set point. These fluctuations result from the intermittent additions of individual gases on demand from the control equipment, which replaces each gas at the average rate at which it is consumed to maintain constant environmental conditions.

Composition of Gas Phase - The composition of the gas phase controls the concentrations of the dissolved gases available to the organism, as pointed out above. All three individual components of the gas mixture can be varied between rather wide limits as long as each is replaced as rapidly as it is consumed. Oxygen is the critical component and must be held below about 15% maximum concentration in the gas mixture; otherwise, oxygen has inhibitory effects on growth because it tends to inactivate the enzyme hydrogenase that mediates the utilization of hydrogen as an energy source. Experimental experience indicates that oxygen can fluctuate between perhaps 7-14% without notable effects on growth. Others have estimated that about 13% is optimum. Our observations have included experimental cultures in which oxygen rose to greater than 20% because of control failure; growth rate was reduced because of this and perhaps other unbalanced environmental conditions, but, in particular, the harvested cellular product had an unpleasant odor that was not present during suitable culture conditions.

Carbon dioxide in the gas phase was maintained by the automatic controls at concentrations between about 2-6% in many of the experimental cultures. The rate of consumption of carbon dioxide during growth appears not to be affected in this range, nor indeed at much higher concentrations. In some experimental cultures, the pH was controlled by the addition of carbon dioxide to the gas phase to form bicarbonate in solution and to counteract a trend toward rising pH in the liquid phase. It was observed that there was no measurable effect of high carbon dioxide concentration in solution until the gas phase composition included more than 25% carbon dioxide.

Hydrogen makes up the remainder of the gas phase composition. Its concentration in the gas phase is thus in the range of 80-85%. It has no measurable effect over a much wider range, and others have reported that hydrogen may vary between partial pressures equivalent to about 5-90% at slightly above one atmosphere total pressure.

pH - The hydrogen ion concentration of the liquid phase, as expressed in pH units, is usually considered as a primary and significant variable which has an optimum value that favors the best growth of an organism in a bacterial culture. The control of pH may be selected as a method for maintaining optimum conditions in many cultures because it is convenient to measure precisely, and to stabilize within a narrow range by including a two-component chemical buffer in the solution of nutrient salts comprising the liquid medium.

It is certainly true that a shift in pH may signal a depar-

Table 1 - Basal Medium for Hydrogenomonas Eutropha

Major Elements	Concentration, grams/liter	Trace Elements	Micrograms/ liter
		H_3BO_3	200
KH_2PO_4	0.68	$CoCl_2 \cdot 6H_2O$	80
K_2HPO_4	0.27	$CuSO_4 \cdot 5H_2O$	8
$MgSO_4 \cdot 7H_2O$	0.1	$MnCl_2 \cdot 4H_2O$	80
NaCl	0.1	$ZnSO_4 \cdot 7H_2O$	100
$CaCl_2$	0.1	$Na_2MoO_4 \cdot 2H_2O$	50
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$*	0.01	$NiCl_2 \cdot 6H_2O$	70

pH 6.5: Demineralized double distilled water

*Sterilized separately, then added aseptically.

ture from optimum conditions in a growing culture when the composition of the solutes is relatively constant during steady-state growth. Nevertheless, pH alone is not a specific measure of nutrient composition, and the pH value may vary over a considerable range if nutrients that affect growth are present in acceptable amounts. Experience has shown that the pH range of the liquid medium may be varied from about 6.6-7.2 with acceptable growth in the culture, and that it may stabilize at any point in this range, depending upon the proportions of ammonia and bicarbonate plus phosphate buffer present in solution. We have observed extreme variations to 5.5 on the acid side and to 7.8 on the alkaline side during the stabilization process as growth proceeds after inoculation, without apparent permanent effect or injury to the organism. These variations were deliberately permitted by using only a lightly buffered medium in order to study the stabilization process. Presumably, for continuous growth under approximately optimum conditions, one could buffer the solution more heavily at a pH value of 6.8-7.0 as a matter of choice.

Composition of Liquid Medium - The basal medium used for dilution of the culture to maintain a constant cell density and for starting the culture by inoculation contains the inorganic salts as shown in Table 1. To the basal medium is added carbonate and bicarbonate as the primary source of carbon by dissolution of carbon dioxide from the gas phase as outlined above.

Urea is present in the liquid medium as the sole nitrogen source in the nutrient. In the experimental cultures, it has been added to the basal medium in concentrations that have varied between 0.5-1.5 grams/liter in general. After inoculation, and while the cell density is being allowed to increase to the level that would be efficient for continuous culture, the urea concentration usually decreases at a relatively rapid rate. Some of it may be assimilated directly, and another part may be hydrolyzed with the formation of some ammonia and a consequent rise in pH.

Additional supplies of urea have usually been added as a small volume of concentrated urea solution in water in response to one of two types of control. Either the controls may respond to a signal that the urea itself has fallen below a preset minimum concentration, or they may be programmed to respond with urea addition to a preset minimum concentration of ammonia, measured by an ammonia analysis or by a pH measurement equivalent to ammonia in an otherwise constant composition of the medium. In either case, the concentration of urea in a system is usually maintained at less than 0.1 gram/liter. When the control is set to be activated by a minimum level of ammonia concentration, the residual urea concentration in equilibrium with its hydrolysis product, ammonia, is unmeasurably low.

In the course of the experiments on the growth of Hydrogenomonas eutropha in this laboratory, it has become clear that the conversion of urea as the sole nitrogen containing nutrient available to the organism is a complex process. The results have indicated, and it is postulated, that there are two paths for urea conversion into cell substance. By one path, there may be conversion of one-half of the urea nitrogen without the intermediate release of ammonia to the medium. The conclusion that only one-half of the urea is converted directly is based on accurate nitrogen balances in the system comparing urea inputs and ammonia inventories with the corresponding incremental increase in cellular nitrogen.

By the second path, the presence of urea causes synthesis of urease enzyme by the cells and urease is released to the medium. In the presence of this enzyme, urea is rapidly

hydrolyzed with ammonia release, and the cells in turn assimilate ammonia as the primary nitrogen source.

It has appeared that the growth of the organism is considerably more rapid by the first path of urea assimilation without ammonia release. However, there is a rate controlled process involved in the conversion of urea by the first path, which tends to deplete an unidentified intermediate as the cell density increases. When the cellular demand for nitrogen cannot be satisfied by the first path because of the time lag inherent in the rate controlled conversion process, urease synthesis increases abruptly, and urea is thereby hydrolyzed very rapidly. Then the cells shift to ammonia assimilation at a slower growth rate.

König and Schlegel (6) have described experiments with Hydrogenomonas cultures that appear to us to have similar implications, although their interpretation differs from ours. They have recorded a cyclic variation of ammonia and of urease with time as growth proceeds. We agree with their interpretation that ammonia represses urease formation and serves as the primary nitrogen source when it is present. When ammonia is depleted by the growing organism, urea derepresses urease synthesis and more ammonia is formed. Thus, there are cycles in urease and in ammonia that are out of phase as the cell density increases. Their data points for urea appear also to show a cyclic variation in the slope of urea concentration curves indicating variation in the rate of urea conversion, but they have chosen to plot smooth curves between the scattered data points. Their published data are insufficient to demonstrate related variations in growth rate, if they did occur, as we have observed them in our cultures.

It would be desirable to favor the rapid growth rate postulated as characteristic of the first proposed path by controlling the composition of the liquid medium, if this could be accomplished. Procedures for minimizing or preventing the release of urease by the organism in response to its environment have not yet been found. However, a very dense inoculum consisting of washed cells, when returned to a fresh medium, has exhibited rapid growth rate to cell densities higher than are obtainable with a dilute inoculum. This implies that the high cell density itself is not a limit on growth rate if ammonia formation mediated by urease activity could be diminished.

Composition of Product - The composition of the harvested cellular material varies somewhat with the environmental conditions and the composition of the liquid medium in which the cells were grown. In particular, the concentration of urea or ammonia appears to affect the nitrogen content of the cells. Actively growing cells obtained from a medium containing 0.1-1.0 grams of urea per liter of culture have about 12.0-12.5% nitrogen. Nitrogen deficient cultures may yield slowly growing cells with nitrogen contents below 8%. Jenkins (5) has described preliminary feeding tests with very small amounts of harvested cells from this laboratory as indicating that the cellular material is a good protein source. The current effort is in production of much larger quantities of cellular material to support more comprehensive feeding studies in another laboratory, but further data are not yet available.

Growth Rates and Consumption Rates - As a basis for possible calculations of the capacity for waste conversion and for production of cellular material as a protein supplement in engineering type equipment, we present two sets of estimates of numerical values of the parameters presented in Eqs. 2 and 3. At maximum rates of growth, the growth rate constant k in many cultures has been about 0.23 hr^{-1}. The cell density may increase at this rate up to 5 or 6 grams/liter (dry weight). Thus, the rate of cell production at 5 grams/liter (dry weight) cell density would be $0.23 \times 5 = 1.15$ grams/(hour) (liter of culture).

During periods of active growth of the culture, the rate of gas consumption increases in exact proportion to the increase in cell density, so that the specific gas consumption constant remains at the fixed level of 0.36 liter/(hour)(gram dry weight). The gas proportions are in the ratios 6:2:1 for H_2:O_2:CO_2, respectively. If this rapid growth rate is maintained to a cell density of 5 grams/liter (dry weight), the rate of consumption of carbon dioxide alone would therefore be $0.36 \times 5 \times 1 = 1.8$ liters CO_2/(hour) (gram dry weight). Hydrogen and oxygen consumption rates are 6 and 2 times the carbon dioxide rate, respectively. Much experimental effort has been devoted to the study of procedures for maintaining rapid growth and high rates of cell production and gas conversion under steady-state conditions. Such a steady-state is difficult to achieve, and has not been maintained consistently for long periods.

Commonly, the gas rates pass through a sharp maximum at a particular time in the growth curve which can be read from plots of gas rates against time, with a precision of only a few minutes. This time corresponds exactly with an amount of cell nitrogen in the inventory of growing cells in the culture equal to one-half the urea nitrogen converted at that point.

Following the maximum in gas rates, gas consumption decreases somewhat and then levels off and remains approximately constant. During the same interval, the cell density continues to increase with growth at a lower rate.

Not much effort has been spent in studying continuous cultures at these lower rates. However, for purposes of calculation we estimate that continuous cultures could be maintained for long periods at cell densities of 5 grams/liter, with a growth rate constant of k = 0.10 to 0.12; a specific gas consumption constant of 0.1; and gas consumption ratios of 6:2:0.5 (H_2:O_2:CO_2). It will be seen that these estimated values of the culture parameters represent a much less efficient waste management system than would be possible with the maintenance of an active culture in a continuous steady-state at the values repeatedly observed for shorter periods of up to 8 hr. We plan to continue studies directed toward improvement of culture efficiency concurrently with, and following our present effort to produce cell material in

quantities of up to 25 lb dry weight for use in nutrition studies.

PROJECTED ENGINEERING REQUIREMENTS

An engineering design of a prototype apparatus for waste management in continuous culture of Hydrogenomonas eutropha has not yet been made. This section itemizes the probable requirements for such an apparatus, based on experience with the experimental operation of the research system.

CULTURE VESSEL - The research apparatus has a culture vessel constructed from a Pyrex cylinder with stainless steel plates at the ends held together with external tie rods and sealed with Teflon gaskets. All entries to the vessel are through the top and bottom plates. Total internal volume is about 56 liters, and the volume of the liquid phase can be maintained optionally at 10-30 liters by adjusting the height of an overflow tube inserted through a sealed gland in the bottom plate. Note that the volume of an active culture to balance the carbon dioxide waste from one man would be about 20 liters, whereas the conservative values of the parameters estimated above for a slower growing continuous steady-state culture would require about 120 liters of culture for a one-man capacity.

It has been convenient to use a vessel with Pyrex walls so that the state of the culture could be observed qualitatively during operation. Only inert materials can be used in contact with the salt containing nutrient medium to prevent corrosion and toxic effects on the organism from corrosion products. It is possible to construct an engineering model entirely of stainless steel, but a sight glass would probably be required or some other means of checking the volume of liquid present. The research design is much easier to clean and to check for accumulation of cell residues than would be a culture vessel constructed entirely of stainless steel.

The temperature of the liquid phase in the research apparatus is maintained at ± 0.5C, which is easily possible with available temperature controls. It is probable that ± 2 C would be an adequate temperature control. An electric immersion heater jacketed in stainless steel is inserted through the bottom plate and sealed; also a cooling coil is required, constructed of stainless steel tubing. Heating is necessary only to bring the liquid phase up to operating temperature, and thereafter cooling is required for temperature control to dissipate the reaction heat from growth of the culture and and the mechanical heat generated by stirring.

The stirring impeller is driven by a shaft through a sealed bearing in the bottom plate. The stirring motor mounted on the frame below the culture vessel uses a V-belt drive to a pulley on the shaft. In the present apparatus, the stirring system is probably overdesigned with a 1-1/2 hp motor, and it is possible that a 1/2 hp motor might be adequate to provide the violent agitation needed for maintaining equilibrium between the gas phase and the liquid at high gas consumption rates.

Other entries through the top and bottom plates include a gas supply tube through the top extending below liquid level; a pressure tube through the top leading to a pressure measuring diaphragm; a number of sealable entries through the bottom for manual sampling from the liquid phase, and for inserting sensors to measure dissolved oxygen and carbon dioxide. It is possible that a pH sensor could also be inserted directly into the liquid culture through the bottom plate, but the present design incorporates a sampling loop containing the pH electrode in a separate chamber to facilitate maintenance and to separate bubbles from the liquid before pH is measured. A peristaltic pump moves a small flow of liquid continuously through the loop with an intake in the bottom plate and return of the sample through the top plate.

ANALYTICAL SYSTEM - The research apparatus provides for individual measurement in the culture of pH, oxygen, carbon dioxide, urea, ammonia, total pressure and hydrogen by difference, cell density, temperature, and accurate measurements of the amounts added of oxygen, carbon dioxide, urea, hydrogen, and liquid medium as a diluent. It seems probable that the analytical system could be greatly simplified to measure the appropriate environment for a steady-state continuous culture.

The projected requirements for analyses would include frequent or continuous measurements of pressure, pH, cell density, and oxygen, supplemented by infrequent manual analyses of the carbon dioxide content of the gas phase. Oxygen measurements are presently made with a polarographic electrode. The engineering designer might be able to substitute an infrared system for gas measurement that would be applicable to both oxygen and carbon dioxide.

Cell density has been determined by spectrophotometric measurement of the optical density of the liquid phase diluted quantitatively to reduce the optical density to a measurable value at a wavelength of 650 nanometers. It has been reported to us that the RIAS Laboratory measures cell density without dilution in a specially constructed optical cell with an extremely short light path. It would probably be advisable to include a sampling loop with a flow pump in the engineering prototype design, as described above.

Bubbles must be removed from the sample stream in a bubble separator before measuring the optical density in an optical flow cell, with or without dilution, and before a stable pH measurement can be made with a glass electrode system. The zero point for optical density must be recalibrated occasionally by diverting the sample stream and passing nutrient solution containing no cells through the system. When a sampling loop is used, calibration can be accomplished without breaking any connections by a suitable valving arrangement in the flowing stream. Occasionally, a cleaning solution and a water rinse must be used in the optical cell to remove filmy cell deposits on the optical windows. The calibration system can also be used for passing a cleaning solution through the optical cell with the same bypass valves. A liquid phase oxygen sensor cannot be included in a sampling loop, because a significant part of the oxygen is consumed in the unstirred sample before it can reach the point of measurement in the loop.

The analytical system could presumably be modified for satisfactory operation at zero gravity by incorporating a centrifugal bubble separator in the loop ahead of the positive displacement peristalic pump, so that only liquid phase would be fed into the analytical section of the loop. Operation under zero-g might be an advantage in the culture vessel itself, since intimate mixing of the gas and liquid phases is required. This mixing is now produced by strong agitation with considerable power consumption. A much smaller agitator with less power would conceivably be adequate in the absence of a gravity field.

ENVIRONMENTAL CONTROL - Signals from the analytical system are used to activate subsystems of the environmental control system in order to maintain selected optimal conditions for cell growth in the culture. The three gases are stored under pressure to supply the culture system as required through solenoid operated metering valves. Oxygen is controlled by admitting a new supply when the oxygen sensor signals a deficiency. It is projected that a lightly buffered nutrient solution in the liquid culture would signal a carbon dioxide deficiency by rising pH, and that carbon dioxide would be added on the basis of a maximum pH signal generated from the pH electrode system. With both oxygen and carbon dioxide under specific control, a pressure sensor could be set to add hydrogen whenever the total pressure falls below a preset minimum. The essential features of this projected system differ from the presently used research control system only by the substitution of pH control for carbon dioxide in place of a direct measurement and control of dissolved carbon dioxide in the culture.

A supply of basal nutrient medium with added urea would be stored under ambient pressure and added to the culture vessel as a diluent in response to a signal from the cell density analysis that a preset maximum cell density had been exceeded. The nutrient would be added by a positive displacement peristaltic pump against above-ambient system pressure. At the same time, an equal volume of liquid cell suspension would be removed from the culture vessel through a parallel tube in the same pump. The outflowing cell suspension is collected in an appropriate storage vessel for further processing. The concentration of urea in the nutrient supply would be adjusted in accordance with the setting of the cell density control to balance the input of urea nitrogen against the removal of nitrogen in the harvested cells and their suspending liquid.

In the event that experimental studies of continuous steady-state cultures in a prototype engineering apparatus showed some tendency for a time-based unbalance between urea conversion and cellular nitrogen production, an alternate control system might be useful, in which a separate supply of concentrated urea solution could be added to the culture vessel by a low pH signal indicating that ammonia derived from urea hydrolysis was depleted by cell growth. In this alternate projected control system, carbon dioxide might be added in constant proportion with the hydrogen in response to the low pressure signal. The proportions of hydrogen and carbon dioxide might be adjusted manually at infrequent intervals to balance exactly the proportionate consumption of the two gases by a stable steady-state culture.

Processing - The harvested cell suspension must be centrifuged to separate the cells from their suspending nutrient liquid. This is a relatively severe requirement for the projected system, because the only process that now appears to be technically feasible is centrifuging the suspension at about 60,000 g to separate the cells. It has been our experience that the cells must be re-suspended in water and recentrifuged to wash out the associated urea and inorganic salts, and three such washes are necessary. The washed cell paste presumably would need to be further treated to be converted into an acceptable protein containing food supplement, but further processing is not properly a part of the present discussion.

SYSTEMS INTEGRATION - Ultimately, the Hydrogenomonas culture system must be integrated with a water electrolysis system and a product recovery system. The present design of the Hydrogenomonas culture system would be adaptable to operation in a zero gravity field with a diminution in power requirements for agitation. A coupling of the culture system with a rotating electrolysis cell might well be feasible but has not been investigated to date. Integration of the culture system with suitable methods for harvesting and processing of cell substances also appears feasible but needs to be investigated in the future.

SUMMARY AND CONCLUSIONS

The applicability of Hydrogenomonas culture as part of a closed life-support cycle for waste management depends both on the specific requirements of the mission and on the degree of efficiency that can ultimately be achieved in the conversion of wastes and the production of a food supplement. It is concluded that a reliable and stable continuous culture system can be designed and operated under the present state-of-the-art. At present, the steady-state obtained in continuous cultures over an extended period of days is perhaps five-fold less efficient in culture volume and specific conversion rates than is indicated for more active cultures which have been demonstrated experimentally for periods of hours. Research on possible improvement in culture procedures and conditions to obtain the projected large increase in culture efficiency is continuing as a secondary objective in the current work directed primarily toward the production of pound quantities of Hydrogenomonas cells for use in nutritional studies.

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