DESIGN AND OPERATION OF AN ALGAL PHOTOBIOREACTOR SYSTEM

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ABSTRACT

A photobioreactor system has been designed, constructed, and implemented to achieve efficient oxygen production for a closed ecological life support system (CELSS). The special features of this system are the optical transmission system, uniform light distribution, continuous cycling of cells, gravity-independent gas exchange, and an ultrafiltration unit. The fiber optic based optical transmission system illuminates the reactor internally and includes a light source which is external to the reactor, preventing heat generation problems. Uniform light distribution is achieved throughout the reactor without interfering with the turbulent regime inside. The ultrafiltration unit exchanges spent with fresh media and its use results in very high cell densities, up to 10^9 cells/ml for *Chlorella vulgaris*. The prototype photobioreactor system was operated in a batch and continuous mode for over two months. The oxygen production rate measured at 4-6 mmoles per liter of the culture per hour under continuous operation, is consistent with the expected performance of the unit for the provided light intensity.

1 INTRODUCTION

One of NASA's more important challenges in achieving manned flight in space for prolonged periods of time is to have an on-line workable and efficient CELSS which provides oxygen, food, and water for humans and recycles the wastes. Many life support systems have been designed that use algal cell cultures to produce oxygen /3, 5, 9/. Algal cultures are primary candidates for inclusion in a bioregenerative system because they typically grow rapidly, have metabolism that can be controlled, produce a high ratio of edible to nonedible biomass, and have gas-exchange characteristics compatible with human requirements /9/. Successful utilization of microalgae in CELSS requires an energy efficient and compact photobioreactor.

The issues to be addressed in an efficient design include optimal lighting techniques and configurations with an emphasis on lighting efficiency, gravity independent gas-liquid separation, minimal heat transfer, and minimal cell adherence to the surface /1/. Some of these problems such as optimal lighting techniques and selection of appropriate wavelengths have already been addressed by Mori /6/. In the present work we have designed, constructed, and implemented a prototype photobioreactor which satisfies some of the critical design parameters.

2 ORDER OF MAGNITUDE CALCULATIONS

The primary design of the bioreactor system was based on order of magnitude calculations of light and specific surface area required for the reactor to support one person's oxygen requirements. The volumetric oxygen production rate depends on three primary factors:

$$\text{Volumetric oxygen production rate} = \text{(Specific oxygen production rate)} \tag{1}$$
$$= \text{x (Chlorophyll content)} \tag{2}$$
$$= \text{x (Cell density)} \tag{3}$$

Numerical values for these three quantities can be estimated.

1. The specific oxygen production rate is an intrinsic property of algal biochemistry which is directly proportional to the chlorophyll content of the cells. Representative values for this quantity are 50-400 moles oxygen produced per hour per mole of chlorophyll /7/. This rate may vary with physical conditions but for practical purposes must be considered as an inherent biological constraint.

2. The chlorophyll content is a function of cell type and operating conditions such as temperature and light intensity. It could therefore be varied by strain selection and genetic engineering. The chlorophyll content is on the order of 0.5-1.0 femto moles chlorophyll per cell /4/.

3. The cell density is primarily a function of bioreactor design, with the illuminated surface area to volume ratio and the rate of oxygen removal being the most important design variables. Assuming a cell volume on the order of 30 femto-liters/cell (typical for *Chlorella vulgaris*), the packing density of cells will be about 3×10^{10} cells per milliliter. Therefore a density of about 10^9 cells/ml or 3% (vol/vol) may be considered as a reasonable design goal.

Using these numerical values for the three parameters, the volumetric oxygen production rate can then be estimated to be 10^{-4} moles oxygen generated per milliliter of culture per hour. One human being requires approximately one mole of oxygen per hour /5/, so a 10 liter unit should satisfy this need if the estimated volumetric oxygen production rate could be attained. The photobioreactor must sustain cells at these high concentrations, and maintain high growth and oxygen production rates.

Production of each oxygen molecule through the photosynthetic pathway requires 8 photons of light in the blue and red region of the spectrum /2/. Thus, the minimum light requirement is about 800 μEinsteins/ml/hr, or about 40 mW/ml of light at the required wavelengths. This amount of light corresponds to about 0.4 kW of light energy per person. One of the main factors that must be considered in photobioreactor design is the light penetration distance at the required cell density. The light intensity is described in Beer's law as an exponentially decaying function of distance and cell density. For *Chlorella pyrenoidosa*, it has been shown that the penetration distance is about 1 cm at cell concentrations of about 10^8 cells/ml /8/. Thus at 10^9 cells/ml the penetration distance is about 1 mm. These calculations indicate that the required specific area for the reactor is about 5-10 cm^2/cm^3, and the desired light intensity will range between 4-8 mW/cm^2 at the proper wavelengths. These calculations form a basis for the theoretically achievable oxygen yield.

3 PHOTOBIOREACTOR DESIGN

Our prototype photobioreactor system is shown in Figure 1. It has a volume of 600 ml and a specific illuminated area of 3.2 cm^2/cm^3. The light source is a Xenon lamp that provides 3.2 W of light in the visible portion of the spectrum into the chamber. The light intensity at the illuminated surfaces is about 1 mW/cm^2. About 60% of this light falls into the blue and red region of the spectrum which can be utilized by the cells, resulting in about 0.6 mW/cm^2 of usable light. This intensity is about an order of magnitude below the desired intensity.
The gas exchange device is external to the reactor, and a closed loop system is used to circulate the culture between the reactor and the gas exchange device. The circulation rate is calibrated so that the incoming stream to the photobioreactor is low in oxygen and the existing stream is close to saturation. The gas exchange process of the culture is carried out in hollow fiber cartridges, which can be used as a single unit, or in serial or parallel arrangements. In the serial arrangement oxygen can be stripped off the culture in one unit under low pressure, and carbon dioxide can be dissolved in the culture in another unit under high pressure to increase the solubility. The gas supplied to the cartridge is a mixture of nitrogen, oxygen, and carbon dioxide, whose compositions can be controlled. The effluent gases from the hollow fiber cartridge flow through a condenser, trapping the water vapor prior to analysis of the gas composition. The pH, dissolved oxygen, and dissolved carbon dioxide concentrations in the inlet and outlet streams are acquired every 5 minutes by a Macintosh computer. The on-line data acquisition system provides a direct measurement of the carbon dioxide fixation and oxygen production rates. An on-line ultrafiltration unit is used to dialyze the culture medium at a relatively high flow rate. This unit will allow us to selectively separate the waste and/or secreted products and exchange them with fresh media.

4 MATERIALS AND METHODS

Chlorella vulgaris Emerson strain, from Carolina Biological Supply, was cultured in the bioreactor system in N-8 medium at a pH of 5.6. The medium consisted of (mg/lit): $Na_2HPO_4.2H_2O$, 260; KH_2PO_4, 740; $CaCl_2$, 10; Fe EDTA, 10; $MgSO_4.7H_2O$, 50; KNO_3, 1000; and trace elements such as $Al_2(SO_4)_3$, $MnCl_2$, $CuSO_4$, and $ZnSO_4$.

Algal cell concentration was measured with a Coulter Counter Model ZM. This system also includes a Coulter Channelizer which can measure particle size distributions. The culture was cultivated in the system with a circulation flow rate of ≈ 2 liters per minute. The gas composition to the cartridge was controlled by multitube flowmeters. The input gas composition to the hollow fiber was kept at 15% O_2, 15% CO_2, and 70% N_2 with a total flow rate of 300 ml/min. The rate of ultrafiltration was about 8 ml/min, and the molecular cut-off of the membrane was 100 kD. The light intensity inside the reactor was measured by a LI-COR light meter model LI-185 from LAMBDA Instruments Corporation, which indicated an intensity of 0.6 mW/cm^2 of useable light inside the reactor, and the light energy provided to the reactor was about 3.2 mW.

5 RESULT

Chlorella vulgaris, inoculated at 10^7 cells/ml in the reactor, was grown up to 10^9 cells/ml in the photobioreactor system in a batch mode (Figure 2). The ultrafiltration unit is critical in achieving cell densities greater than 10^8 cells/ml. The specific oxygen production rate increased following medium dialysis. Thus prior to dialysis, the culture is either nutrient limited or there are secreted growth inhibitory factors that are accumulated in the culture. The nutrient limitation factor has been ruled out by dialyzing the culture against medium with high concentrations of nitrate (Figure 2). The pH drops to about 5.6, which is the pH of fresh medium, and rises after dialysis is stopped. The optimization and control of the pH is of significant importance in keeping the culture in a favorable environment for growth and this is an easy parameter to control once the optimum pH is identified. The system was switched to continuous mode on day 54, with a dilution rate of 0.15 per day. The oxygen production rate under these conditions was in the range between 4-6 millimoles per liter of culture per hour (Figure 3). This amount of oxygen corresponds to a steady state concentration of 4×10^8 cells/ml, and a dilution rate of 0.15/day in the system, which in turn will
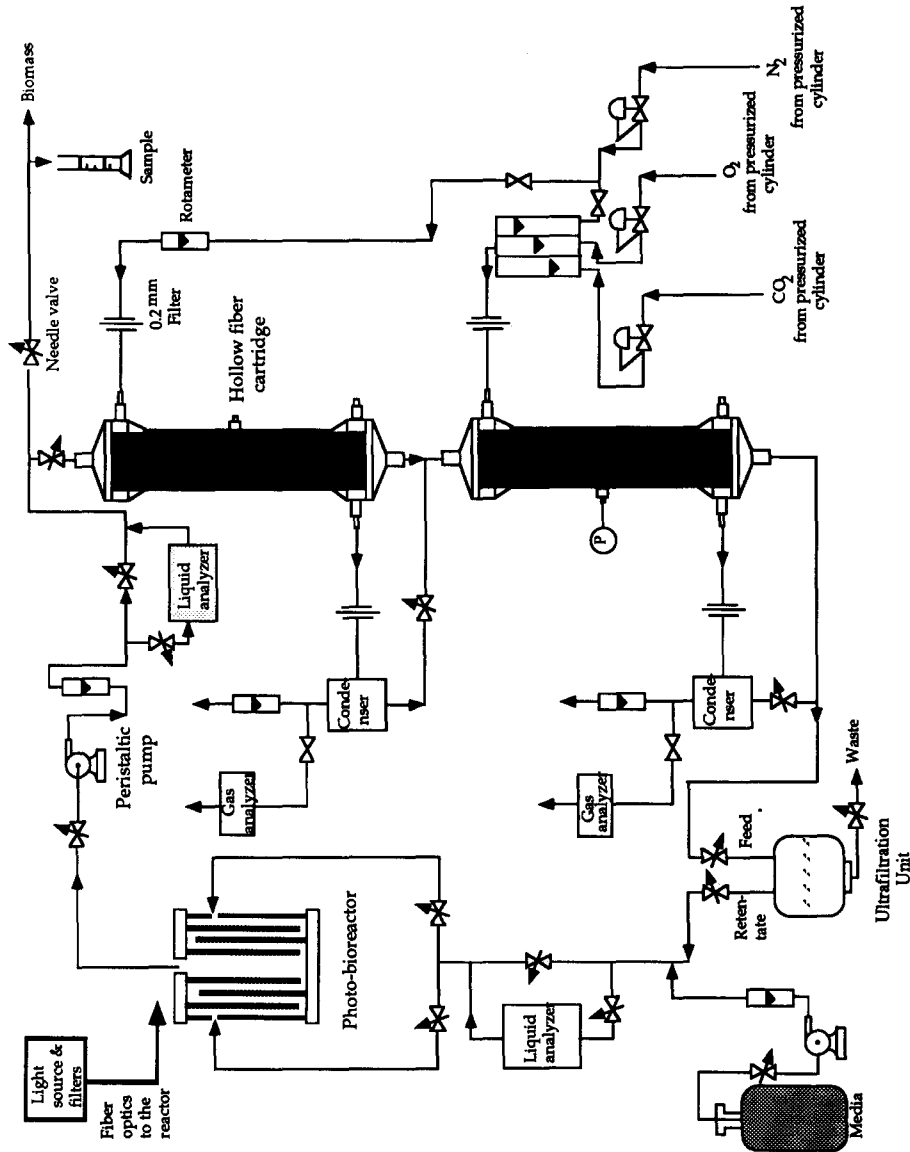
Fig. 1. The photobioreactor system.

correspond to a 200 liter unit required to support one human being. The production rate of oxygen is close to the order of magnitude calculations based on the provided light energy (3.2 W) and specific area of the reactor (3.2 cm^2/cm^3).
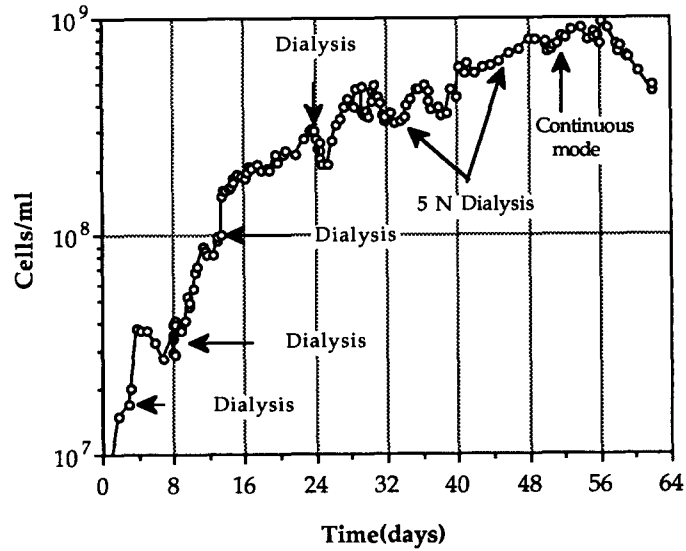
Fig. 2. The growth curve of *Chlorella vulgaris* in the photobioreactor system.
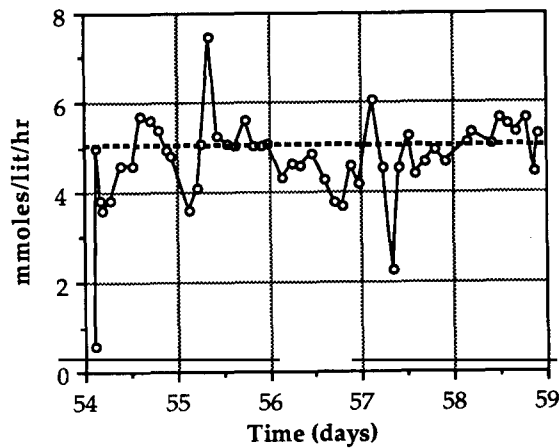

Fig. 3. The oxygen production rate after the system was switched to continuous mode.

6 CONCLUSIONS

A photobioreactor primarily for the use of CELSS has been designed, constructed, and implemented to meet the following requirements:

1. Uniform distribution of light throughout the reactor.
2. High illuminated surface-to-volume ratio (a key parameter in reactor design).
3. High efficiency gravity independent gas-liquid exchange.
4. Elimination of detrimental effects of UV light and heating effects of IR light through selection of appropriate light wavelengths prior to illumination in the reactor.

Based on the provided light and nutrients to the photobioreactor, the performance of the system meets the estimation of the achievable growth rate and oxygen production rate. This performance can be improved by about one order of magnitude by increasing the light intensity at the illuminating surfaces inside the reactor, in which case a 20 liter unit would be able to support a human being. One of the most significant features of this unit is its satisfactory performance over a two month period, which is a good measure of its reliability.

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