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FOOD PRODUCTION and GAS EXCHANGE SYSTEM using BLUE-GREEN ALGA (SPIRULINA) for CELSS

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#### ABSTRACT

In order to reduce the cultivation area required for the prowth of higher plants in space adoption of algae, which have a higher photosynthetic ability, seems very suitable for obtaining oxygen and food as a useful source of high quality protein.

The preliminary cultivation experiment for determining optimum cultivation conditions and for obtaining the critical design parameters of the cultivator itself has been conducted.

Spirulina was cultivated in the 6-liter medium containing a sodium hydrogen carbonate solution and a cultivation temperature controlled using a thermostat. Generated oxygen gas was separated using a polypropyrene porous hollow fiber membrane module. Through this experiment, oxygen gas (at a concentration of more than 46%) at a rate of  $100 \sim 150$  ml per minute could be obtained.

#### INTRODUCTION

To support the long duration stay of human beings in space, technology to recover  $O_2$  gas from  $CO_2$  gas exhaled by human beings and a continuous food production system must be established. Environmental control system hardware for supporting life science experiments plans to use solid amine for removing excessive  $O_2$  gas and some kind of salcomine for recovering excessive  $O_2$  gas produced by higher plants and algae. Otherwise, Sabatier or Bosch method is also planned to be used for reduction from  $CO_2$  gas to  $O_2$  gas. Though these physico-chemical methods are very effective for gas exchanges, they have no ability to producing foods.

no ability to producing foods. The utilization of biological methods in space is advantageous for producing food and also for conducting gas exchange simultaneously. However, there are various problems with this method. If we plan to conduct the gas exchanges using only higher plants a very large cultivation area is required. The required quantity of  $O_2$ gas per day for supporting a crew is about 0.9 kg. Harvesting of 1.0 kg dry weight of leaf vegetables such as lettuce through about 3 month cultivation time requires about 1.6 kg of  $O_2$  gas and produces 1.2 kg of  $O_2$  gas. To get 0.9 kg of  $O_2$  gas, for example, cultivation area where produces 70 kg of lettuce is required. Therefore, another bio-species such as algae having higher photosynthetic ability is profitable to be adopted in Controlled Ecolgical Life Suppord Systems (CELSS).

#### UTILIZATION OF ALGAE AS FOOD RESOURCE

To use algae as food, it is desirable to establish the following conditions; (1) Mass production ability. (2) High quality protein and mineral biomass. (3) Lower (2) Righ quality protein and mineral biomass. (3) Lo production costs. (4) No toxicity. (5) Acceptable taste.

Chlorella, a green alga, has been studied for about 30 years and could be used as the protein source, but utilization of Chlorella as food in CELSS seems very difficult for following reasons; (I) A high speed centrifugal separator which needs very high energy consumption must be used for harvesting because of its very small size (nearly 5  $\mu$ m). (2) Since the cell wall consists of crude fiber, it is indigestible by the human body without pulverization. (3) Contamination by a virus or a bacillus occurs easily because the nutrient solution is weakly acidic. weakly acidic.

Then, Spirulina, a kind of blue-green alga, has been proposed because of the following distinctive features; (i) Harvesting is very easy because the size is about 100 times that of Chlorella. (2) Cell is made of viscous

polysaccharide and is very easily digested. ③ Contamination seldom occures because the medium is heavily alkaline.

These characteristics cover most of the weak points of These characteristics cover most of the weak points of Chlorella and furthermore, the protein contained in Spirulina is high quality. About 30 kinds of Spirulina have been found, but only several species among these have good properties with high protein, no toxicity, high propagating ability and big size for harvesting. Especially, <u>Spirulina platensis</u> and <u>Spirulina maxima</u> are the well-known species produced in industrial scale. Since the range of the suitable cultivation temperature for Spirulina is from 30 to 40 °C, the waste heat from other featilities in CEISS can be used

facilities in CELSS can be used.

facilities in CELSS can be used. As shown in Fig. 1b the alkaline medium, range of its pH is from 9 to 12, has a very high utilization efficiency of  $CO_2/1/$ . Spirulina utilizes sodium hydrogen carbonate (NaHCO<sub>3</sub>) as the source of CO<sub>2</sub> necessary for photosynthetic reactions. NaHCO<sub>3</sub> is dissociated into HCO<sub>3</sub> or  $CO_3^{2-}$  in the solution as shown in Fig. 1. When the photosynthetic reaction of Spirulina proceeds, the concentration of  $CO_3^{2-}$  increases relatively. The rise of pH value as shown in Fig. 1a results from the progress of this reaction/2/. To maintain continuous cultivation, pH value must be held within some range (i.e. 9~10). If pH value goes over the limit value, CO<sub>2</sub> must be provided in the culture solution to reduce pH value. These operations are shown as the discontinuous broken lines of pH in Fig. 1a. On the other hand, since the medium for Chlorella is weakly acidic, solubility of CO<sub>2</sub> are not so high, therefore, it is very difficult to increase the concentration of CO<sub>2</sub> in the culture solution. To use Spirulina in CELSS seems more suitable than Chlorella from comparing these characteristics of Chlorella and Spirulina.

#### NUTRITIVE VALUES AND PRACTICAL USE OF SPIRULINA IN CELSS

The nutritive values and components of Spirulina are very important factors to use it as food. The organic components of Spirulina have the following distinctive features : features; (1) Abundant protein content. (2) Carbohydrate consists of glycogen (so called animal starch). (3) Containing linoleic acid and linolenic acid (unsaturated fatty acids). (4) Containing an abundant vitamin complex, in comparison with another algae, especially vitamin B<sub>12</sub> and vitamin E. (5) Containing pigments such as chlorophyll and carotenoid ( $\beta$ -Carotene) which changes into vitamin A. (6) Containing many mineral components.

As to protein, not only quantity but also quality is very important factors to keep animal's lives. The quantity of essential amino acids of Spirulina nearly satisfies the values recommended by the FAO (Food and Agriculture Organization) except for lysine and cystine (3). Furthermore, the human body needs digestible and absorbable proteins. Fig. 2 shows the comparison of digestibility by pepsin/2). It is clear from the data shown in Fig. 2 that Spirulina has better digestion properties in comparison to Chlorella.

When human and animals stay in the micro gravity environment for a long time, the phenomena such as calcium decrease in bones and the deterioration of muscular function are reported. Fig. 3 shows the clinical examples for the changes of values of calcium and potassium in blood electrolyte when Spirulina was given every day on the ground /5/. Since these data show the increasing tendencies of calcium and potassium in blood, it is expected some effects to prevent the occurence of these harmful phenomena. From the data described





above, Spirulina seems a good candidate for food in CELSS.

## OXYGEN GAS RECOVERING EXPERIMENT

To cultivate algae in the micro gravity environment, many technical problems must be solved. One of those problems is to establish an effective method to gather and recover  $O_2$  gas which is generated by algae and remains in the medium. To separate oxygen gas from the medium, it is not suitable to use a centrifugal separator which would disturb other experimental equipment. Therefore, a membrane separation technique which works without mechanical moving components seems more suitable. As for the membranes, various properties regarding permeability of gas are affected by shapes or material composition of membranes.

material composition of membranes. Table 1 shows the properties of representative membranes /5/. Plain membrane has a good property for gas permeability, but it has a demerit in mechanical strength if the membrane thickness is reduced in order to get higher permeability. In the result, some compromising between both characteristics is required. Usually O<sub>2</sub> gas generated by algae remains in the form of bubble that is gas itself, in the medium solution. Then, utilization of the porous membrane enabling to pass gas only seems to be better than the plain membrane. Table 1 shows that the membrane made from silicone has a superior property for gas permeation, but, Table 2 /5/ shows that porous type membranes have much better gas permeation characteristics than plain silicone membranes. For these reasons, we carried on the O<sub>2</sub> gas recovering

characteristics than plain suitcone memoranes. For these reasons, we carried on the  $O_2$  gas recovering experiment using a porous hollow fiber membrane module, manufactured by Mitsubishi Rayon Co., Ltd., as the gas separator. The characteristics of this module are shown in Table 3 and the pictures of scanning electron micrographs of hollow fiber are shown in Fig. 4/6/. One of the hollow fibers is small in inside diameter, and wall thickness. Then, the module which is composed of bundles of hollow fiber is designed very compact. The dimension of this module is 215 mm (L) × 40 mm (D). A very large membrane area can be obtained by using hollow fiber membranes. Fig. 5 shows the block diagram of the  $O_2$  gas recovering experiment equipment now being used in our laboratory.  $O_2$  gas blown into the cultivation tank is carried to the membrane module along the water flow and a part of the  $O_2$  gas permeates the membrane, therefore, for clearing the permeation property of the membrane, the quantity and concentration of permented  $O_2$  gas are measured by the oxygen gas analyzer.

The cultivation tank is made from acrylic resin and has a round shaped top in order to collect oxygen gas concentratively and also has an inner volume of about 0.009 m<sup>3</sup>. For the permeation ability estimation of the membrane, pure water was used and its temperature was set at 30 °C (same condition as Spirulina cultivation) and also 99.99% high purity  $O_2$  gas was used insted of the  $O_2$  gas generated by Spirulina.







Fig. 3. Variation of Blood Electrolyte

One of the experimental data for  $O_2$  concentration variation of recovered gas is shown in Fig. 6. For determing the accurate dissolved  $O_2$  gas quantify, the dissolved  $O_2$  concentration in pure water was reduced to about 1.7 - 1.8 ppm before the experiment. The flow rate of pure  $O_2$  gas from  $O_2$  bottle was 50 cc/min. and the flow time was set for 10 minutes.  $O_2$  gas which permeated the membrane was continuously collected by the suction pump and its concentration was measured by the suction pump and its concentration of  $O_2$  gas did not change, but after that it rapidly increased. The flow rate of  $O_2$  gas at the membrane area, such as 0.3, 0.5, and 1.0 m<sup>2</sup> were tested, but there appeared only small differences between them. As shown in Fig. 6, the maximum value of  $O_2$ 

As shown in Fig. 6, the maximum value of  $O_2$ concentration is about 73%. It took about four hours until the dissolved  $O_2$  concentration went down to the value of the start time. From these results, it is clarified that the membrane module works effectively to recover not only the bubbled but also the disolved  $O_2$  gas.

TABLE 1 O2 and CO2 Permeability on Various Polymer

Polymer	O <sub>2</sub> Permeability	CO <sub>2</sub> Permeability
Polyvinyliden Chloride	16	13
Polyester	176	304
Cellulose Acetate	1,705	8,680
	r <del>-</del> 3,500	15,965
Polyethylene	3,200	5,390
	L−8,800	27,000
Polypropylene	2,900	9,905
Polystyrene	4,850	23,790
Polytetrafluoroethylene	17,600	48,000
Ethyl Cellulose	25,600	104,000
Silicone Elastmer	1,568,000	8,040,000

Membrane thickness 25µ conversion, 25°C, cm3/m2.atom.24hr

TABLE 2 Property of Membrane for Artificial Lung

Polymer	Structure	Total thickness $(\mu)$ /	Gas permeability	
	on den de	Thickness of membrane $(\mu)$	02	CO <sub>2</sub>
Silicone Elastmer	polyester reinforcement	190/160	140	770
Polysiloxanepolycarbonate	uniformalized	50/50	170	730
Polyalkylsulfon	applied porous polypropylene menbrane	25/2.5	1100	4600
Ethylcelluloseperfluorobutylete	applied polyolefin cloth	175/2.5	880	4700
Polypropylene membrane	porous	25/25	very	very
Teflon membrane	porous	500/500	yery good	good very good

#### GAS CLOSED EXPERIMENT

It was clear that O<sub>2</sub> gas has been obtained from the solution by using the porous hollow fiber membrane module. Then, we have carried out gas closed experiments using the Spirulina cultivator and a fish breeding tank. O<sub>2</sub> gas generated from Spirulina is provided to Tilapia (a kind of tropical fresh water fish) and CO<sub>2</sub> gas exhausted from Tilapia is supplied to Spirulina, through the membrane gas separator and the artificial lung that is the hollow fiber membrane module.

We have shown the block diagram of this experiment system in Fig. 7. Algae cultivator is made from acrylic resin with 180 mm diameter and 300 mm length. The species used in these experiments are <u>Spirulina</u> oscillatoria and <u>Spirulina platensis</u>. The medium solution contained mainly NaHCO<sub>3</sub> and its temperature was controlled at 30 °C by thermostat. Light energy was supplied through optical fibers or fluorescent lamp into the cultivator. The time to reach saturation in culture of Spirulina was 7 ~ 10 days in several experiments.

The airtight fish breeding tank is 280 mm diameter  $\times$  500 mm length and three Tilapias (length of 15 cm and weight of 160 g) have been bred in it. We plan to use Tilapia as protein supply resource in CELSS and have researched on developing a breeding technology and equipment which works well in micro gravity environment. O<sub>2</sub> gas for Tilapia was supplied through an artificial lung which is the same as the gas seperator and the variation of DO<sub>2</sub> was monitored for this gas closed experiment. The differences of DO<sub>2</sub> values supplied by room air and O<sub>2</sub> from Spirulina cultivator are shown in Fig. 8. The experiment started when the value of DO<sub>2</sub> went down to 0.1 ppm from about 4 ppm. Water circulation rate was 2.0 liter/min. and O<sub>2</sub> gas supply rate was 100 cc/min. Fig. 8 shows that Spirulina has a good O<sub>2</sub> supply ability. When using Spirulina, the maximum value of O<sub>2</sub> gas concentration reached 46.0 ~ 50.2 %. The airtight fish breeding tank is 280 mm diameter  $\times$  500

permeability : cm3/min.m2 atom

TABLE 3 Characteristics of Porous Hollow Fiber Module

Material	Polypropylene	
Inner diameter	200 µm	
Wall thickness	22 µm	
Porosity	45 %	
Pore Size	0.036 μm	
Gas flux	7×104 1/m <sup>2</sup> hr 0.5atm	
Bubble point	12.5 kg/cm <sup>2</sup>	
Effective area	0.3, 0.5, 1.0 m <sup>2</sup>	

#### CONCLUSIONS

A long term experiment to cultivate Spirulina for more than one month was successfully conducted. Successively, in order to examine the quantity of  $O_2$  gas generated by Spirulina, the closed gas circulation experiment combined the algae cultivator and the breeding tank with three adult Tilapias. The experiment term reached 32 days and it was clean that the continuous experiment operation time depended on the life time of the gas separator. The ability of the separator was decreased by algae which covered the surface of the membranes. To conduct long term experiments, this problem must be solved, for example, to wash out adhesive Spirulina on the surface of fibers. fibers.

Further work is required to establish the technologies to harvest algae automatically, to develop long life gas separators and artificial lungs and to conduct an experiment including not only gas circulation but also food supply to fishes or animals in the complete closed system.

#### REFERENCES

- H. Tamiya, et al, Algal Culture Method, 1965.
  T. Ogawa, J. Ferment. Technol., 1974.
  N. Fujii, Mystery of Spirulina.
  K. Aroishita, Spirulina for Health and Beauty, 1981.
  K. Atsumi, et al, High Polymer for Medical Supplies, p. 25 ~ 32, 1978.
  Catalogue of Hollow Fiber Membranes, MITSUBISHI RAYON CO., LTD.



Fig. 4. Picture of Hollow Fiber by Scanning Electron Micrographs







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