



CULTIVATION OF BACTERIA WITH ECOLOGICAL CAPSULES IN SPACE

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ABSTRACT

A hermetically materially-closed aquatic microcosm containing bacteria, algae, and invertebrates was developed as a tool for determining the changes of ecological systems in space. The species composition was maintained for more than 365 days. The microcosm could be readily replicated. The results obtained from the simulation models indicated that there is a self-regulation homeostasis in coupling of production and consumption, which make the microcosm remarkably stable, and that the transfer of metabolites by diffusion is one of the important factors determining the behavior of the system. The microcosms were continuously irradiated using a ⁶⁰Co source. After 80 days, no elimination of organisms was found at any of the three irradiation levels (0.015, 0.55 and 3.0 mGy/day). The number of radio-resistance bacteria mutants was not increased in the microcosm at three irradiation levels. We proposed to research whether this microcosm is self-sustainable in space.

When an aquatic ecosystem comes under stress due to the micro-gravity and enhanced radiation environment in space, whether the ecosystem is self-sustainable is not known. An aquatic ecosystem shows what happens as a result of the self-organizational processes of selection and adaptation. A microcosm is a useful tool for understanding such processes (Beyers and Odum, 1993). We have proposed researching whether a microcosm is self-sustainable in space. The benefits of this project will be: (1) To acquire data for design of a Controlled Ecological Life Support System, (2) Possibility of microbial mutation in a space station.

We report that a hermetically materially-closed microcosm, which could be a useful tool for determining changes of ecological processes in space, was developed, and that the effects of micro-gravity and enhanced radiation on the hermetically materially-closed microcosm were estimated through measurements on the Earth and simulation models.

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Results and Discussion

Characteristics of the Microcosm A microcosm was prepared by self-selecting of biological communities collected from natural ponds (Kurihara, 1978). Two green algae (*Chlorella* and *Scenedesmus*) and a blue-green alga (*Schizothrix*) as primary producers, a protozoa (*Cyclidium*), two rotifers (*Philodina* and *Lepadella*) and an oligochaetes (*Aeolosoma*) as consumers, and bacteria (five or

more species) as decomposer were contained in the microcosm. Two hundred milliliters of half-strength Taub's solution (Taub and Dolla, 1964) containing polypeptone (0.01%) was placed in a cotton-plugged 300 ml polycarbonate square flask and sterilized. The subculture solution (10 ml) was implanted in the flask and the flask was placed in an environmental chamber at $25 \pm 2^\circ\text{C}$ under cool-white fluorescent light for 12 h (2,400 lux) and in the dark for 12 h. The organisms reached their respective population peaks on the 8th day, and after that, constant population was maintained in all species. This status was maintained for more than 150 days (Sugiura *et al.*, 1982).

A population dynamic of organisms was simulated by using mathematical models (Sugiura, 1996). We considered the relationships of organisms as in Fig. 1, developing a differential equation for each: predation, growth, metabolite generation, metabolite inhibition, etc. The time-dependent changes in the population density and the dissolved oxygen concentration were calculated. These changes almost agreed with the changes in the experimental values. The results of calculation also showed that the turnover rate of organisms is greater at an initial stage of culture, starts declining as the number of days of culture increase, and reaches a steady state after the 12th day.

The simulation results indicated that there is a self-regulation homeostasis in coupling of production and consumption, which makes the microcosm remarkably stable.

Synthesis of a Hermetically Materially-Closed Microcosm

The microcosm was open to the air, then oxygen and carbon dioxide in the water could exchange with the almost constant concentrations of these gases in the air. When the gaseous volume of the microcosm was reduced at the beginning of the culture, the population densities were not maintained. The simulation results showed that gaseous exchange of carbon dioxide and oxygen with the air is increased at the early stage, because the succession of the microcosm starts with excess organic compounds. Thus, the increase of carbon-dioxide levels and the decrease of oxygen levels in the system at the early stage might cause this phenomena (Sugiura *et al.*, 1994). Therefore, the culture flask was plugged with a screw-stopper at the 12th day after the beginning of the culture, and

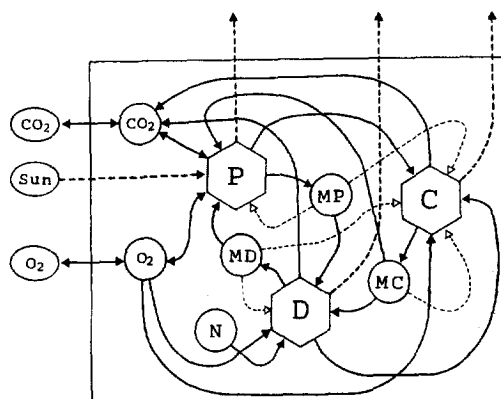


Fig. 1. Schematic diagram for the interactions in the microcosm. P, Producers; C, Consumers; D, Decomposers; N, Organic substances; MP, Metabolites from producers; MC, Metabolites from consumers; MD, Metabolites from decomposers; —, Material transfer; ---, Inhibition by materials; ·····, Electromagnetic wave.

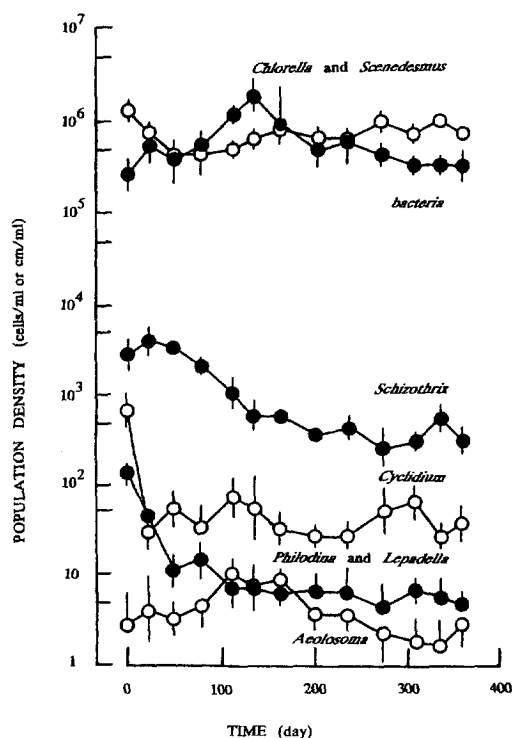


Fig. 2. Changes of population density in the hermetically materially-closed microcosm. Vertical lines indicate \pm S.D. ($n=5$).

the population densities were determined. Fig. 2 shows that the species composition is maintained for more than 365 days. This hermetically materially-closed microcosm could be more readily replicated (Sugiura, 1997). Thus, this microcosm is useful for research studies and testing.

Effect of the Material Transfer Rate in the Medium on the Activity of the Organisms The material hierarchy governs this microcosm on the Earth. For example, algae and detritus sank to the bottom. Microscopic observations showed that the colonies consisting of *Chlorella* and bacteria formed at the bottom of the flask at the mature stage (Fig. 3). Kurihara (1978) reported that the formation of colonies consisting of producers, consumers and decomposers is essential for the stability of a microcosm at its mature stage.

A non-uniform distribution of organisms was simulated using a cellular automata method (Wolfam, 1986). The ecosystem was assumed to contain only three populations, i.e., a population of bacteria, of *Chlorella*, and of rotifer. A two-dimensional region where organisms lived was divided into square cells and the population density in each cell was regarded as a discrete value. The organisms initially had a random distribution. All cells synchronously updated their densities in discrete time steps according to the deterministic rule: (1) The interaction indicated in Fig. 1 existed among organisms. (2) Rotifer wandered at random. (3) The metabolites were transferred by diffusion. A value of $0.8 \times 10^{-5} \text{ cm}^2/\text{sec}$ for the diffusion coefficient was assumed (Ishikawa *et al.*, 1996). After transient of about 2,000 steps (7 days), the random spatial distribution was attained (Fig. 4). Bacteria were separated into several groups. *Chlorella* were overlapping on most groups of bacteria. Certain groups were gradually expanding to the region, other groups were shrinking and often vanished, but the mean population density for organisms was almost constant. Fig. 4 shows the metabolites also have a non-uniform distribution. Small diffusion coefficients increased the mortality of the organisms. Large diffusion coefficients increased the population density of the rotifer, and then the system lost the balance of production and consumption. Therefore, small or large diffusion coefficients shortened the life of the system. These results suggested that the transfer of the metabolites by diffusion is one of the important factors of determining the behavior of the microcosm.

In space, there is no material flow due to convection. This condition may profoundly affect the transfer of the metabolites in the microcosm.

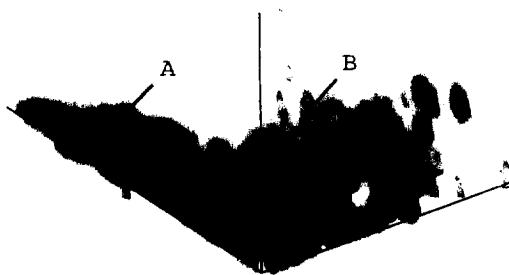


Fig. 3. A part of the colony formed at the bottom of the flask during the last stage of succession. The image is taken with fluorescent microscope method (Kawasaki *et al.*, 1995). Black areas are cells of *Chlorella* (A), grey areas are cells of bacteria (B).

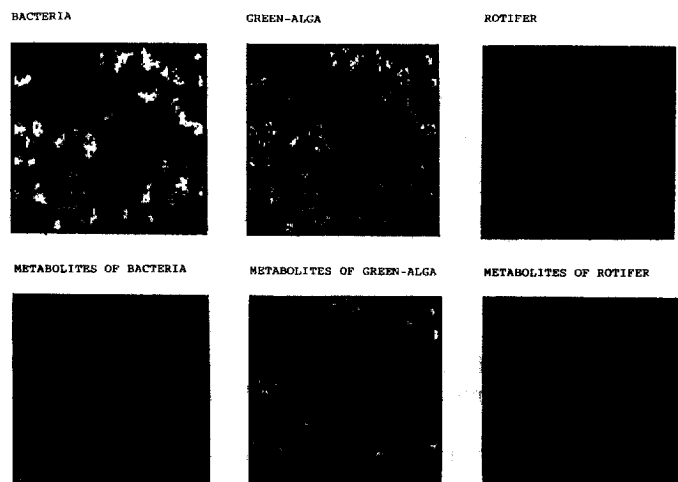


Fig. 4. Spatial distribution of the organisms and their metabolites for the mature stage (simulation results). White areas are the mounds of organism and metabolite. Light indicates density of them.

Effect of Ionizing Radiation on Species Composition of Organisms

The effects of ionizing radiation on the hermetically materially-closed microcosm were investigated. They were continuously irradiated using a ^{60}Co source. The dose levels were 0.015, 0.55 and 3.0 mGy/day, respectively. The middle dose level was equivalent to the dose level on MIR space station. After 80 days, no elimination of organisms was found in the microcosm. The lethal effects of ionizing radiation on the bacteria communities were measured and the results are shown in Fig. 5. This figure shows that there is no difference in the survival curve of bacteria among all these. The results indicated that the number of radio-resistance bacteria mutants was not increased in the microcosm at any of the three irradiation levels.

In contrast, Saito *et al.* (1995) isolated the bacteria that had received high radio-resistance on MIR space station (Fig. 6).

We have been proposing to test whether this hermetically materially-closed microcosm is self-sustainable in space. This proposal has still not been tried.

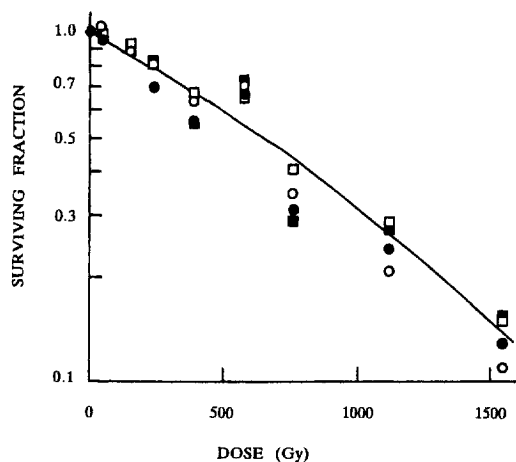


Fig. 5. Comparison of the lethal effect of ionizing radiation on the bacteria communities of the hermetically materially-closed microcosms that were treated with various radiation levels during 80 days. \square , 0.015 mGy/day; \blacksquare , 0.55 mGy/day; \circ , 3.0 mGy/day; \bullet , control.

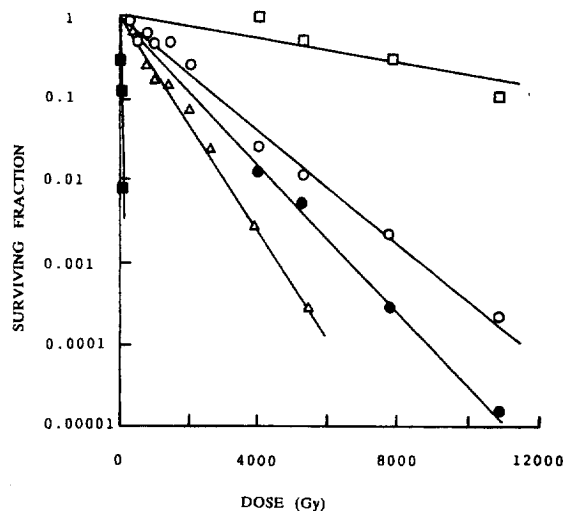


Fig. 6. The lethal effect of ionizing radiation on the bacteria collected from MIR space station. \circ , bacteria (1) collected from MIR space station; \bullet , bacteria (2) collected from MIR space station; \square , *D. Rad.*; \blacksquare , *E. Coli*; \triangle , *B. Subtilis* (spore).

REFERENCES

- Beyers, R.J., and H.T. Odum, *Ecological Microcosm*, Springer-Verlag, New York (1993).
- Ishikawa, Y., N. Shouji, and K. Sugiura, A-Life Simulation of Colony Formation in Microcosm, *CELSS Journal*, **9**, 5 (1996).
- Kawasaki, Y., T. Saito, S. Tanaka, and T. Tsuji, Selection of Fluorescent Probes Suitable for the Visualization of Cell Responses to External Stimulant, *Bioimages*, **3**, 56 (1995).
- Kurihara, Y., Studies of the Interaction in a Microcosm, *Sci. Rep. Tohoku Univ. Ser. IV*, **37**, 161 (1978).
- Saito, T., J. Koike, Y. Kawasaki, K. Kobayashi, T. Oshima, M. Sasada *et al.*, Microbiological Environment in MIR Station: Resistance to Proton Radiation, *Space Utilization Res.*, **12**, 119 (1995).
- Sugiura, K., M. Goto, and Y. Kurihara, Effects of Cu^{2+} Stress on an Aquatic Microcosm: A Holistic Study, *Environ. Res.*, **27**, 307 (1982).
- Sugiura, K., Y. Kurihara, and E. Wada, Characteristics of a Materially Closed Microbial Ecosystem, *Space Utilization Res.*, **11**, 25 (1994).
- Sugiura, K., The Use of an Aquatic Microcosm for Pollution Effects Assessment, *Water Res.*, **30**, 1801 (1996).
- Sugiura, K., Changes in Constituent Biological Species in a Materially-Closed Microbial Ecosystem, *J. Sagami Womens Univ.*, **61**, 125 (1997).
- Taub, F.B., and A.M. Dolla, A *Chlorella*-Daphnia Food Chain Study: The Design of Compatible Chemically Defined Culture Medium, *Limnol. Oceanogr.*, **9**, 61 (1964).
- Wolfram, S., *Theory and Applications of Cellular Automata*, World Scientific Publishing, Singapore (1986).