Selected organisms for systems of life-support in closed bio-ecosystems

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For the past five years, I have been conducting this session to discuss the systems of life-support in closed bio-ecosystems. Living organisms on the earth have evolved considerably since the planets origin. These organisms have adapted several functions by which they coexist, and have thus become mutually dependent upon each other. Knowledge of these functions and the ways in which they interact with the environment is essential for designing a closed-ecosystem, with a limited number of living species in harsh environments, such as extraterrestrial, deep sea or desert. The important elements related to the closed bio-ecosystem have to be discussed among the researchers who have individual specialized fields. Here, we will discuss the details of several species of organisms selected for closed bio-ecosystems. At this time, speakers of various ages will present their researches related to organisms in closed bio-ecosystems.

Keywords: closed bio-ecosystems

Use of cyanobacteria in a closed ecosystem in space

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Cyanobacteria are the first organisms that acquired the ability to evolve O_2 by photosynthesis. Thus evolved O_2 has made the environment of the Earth oxygenic. Cyanobacteria has been surviving through serious changes in natural environment, and now they are distributing all over the world. *Spirulina*, a kind of cyanobacteria, is commercially available as a food supplement. Cyanobacteria are very useful organisms when we human beings are going to expand to space. The International Space Station (ISS) is one of the closed ecosystem in space. It is composed of astronauts and microorganisms that are brought into space ship by astronauts. Incidentally, no cyanobacteria have been detected in ISS. It is necessary to keep the life of astronauts, inside of the machine-full space ship, safe and comfortable. We are trying to grow plants or microalgae in the space ship, because their photosynthetic O_2 evolution and CO_2 fixation activity would make the intra space ship environment clean and, in addition, their green color would surely make astronauts feel easy. However, the effects of space environment, such as micro gravity and cosmic ray, on the fundamental photosynthetic mechanisms have not yet been determined. Now, we are going to grow cyanobacteria in the satellite which will be launched in the Indian-Japan Space Corroboration Experiments and to estimate their photosynthetic activity in space. We have selected two filamentous cyanobacteria, *Spirulina (Arthrospira) platensis* NIES-39 and *Nostoc sp.* as the test materials. The *Spirulina* is edible and its full genome sequence has been determined. We have made full automatic on board culture chamber. The size of the chamber is 20cm depth x 20cm width x 10cm height covered with an aluminium box. The *Spirulina* cells grown under laboratory conditions were washed by centrifugation and then re-suspended in a sterile culture medium containing 5 atom % of $H_2{}^{18}O$ and 4atom % of $NaH^{13}CO_3$. Each 10 mL of cell suspension was inoculated into 6 transparent plastic bags that were placed between LED panels. The light intensity was adjusted at 20 μmoles m^{-2} sec^{-1} at the surface of a bag and bags were continually illuminated. After appropriate time intervals, each 10mL of pure ethanol was introduced to the bags by a diaphragm pump to stop the reaction. After 2 weeks experiment, the volume of gas phase of each bag was measured and then concentrations of O_2 and CO_2 were measured by newly developed GC/MS system (Shimadzu GCMS-QP2010 Plus) equipped with micro volume gas sampler. O_2 was evolved constantly under the experimental conditions though the values are fluctuated by sampling error. The isotope ratio of the evolved gas was increased as the incubation prolonged and reached at the value which is calculated from 5 atom % $H_2{}^{18}O$. Thus, effectiveness to use a stable isotope in measuring O_2 evolution was established. No CO_2 was detected under the illumination. Incorporation of ^{13}C into the cells was increased linearly with time and its value was well correlated to that of O_2 evolution. In another experiment, a terrestrial cyanobacterium, *Nostoc* sp. harvested from the field, was once dried and then put into a plastic bag (6 x 5 cm). The cells were wetted by a small amount of water and then illuminated by LED (660nm) light. After appropriate time intervals, O_2 and CO_2 concentration in the bag were measured using the GC/MS system. In the dark, O_2 was consumed and CO_2 was evolved, conversely in the light, CO_2 was consumed and O_2 was evolved. It is concluded that O_2 evolution and CO_2 fixation were precisely measured by this experimental system.

Keywords: Cyanobacteria, Space experiment, Photosynthesis

Utilization of the terrestrial cyanobacterial sheet

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The terrestrial nitrogen-fixing cyanobacterium, *Nostoc commune*, is living ranging from polar to desert. *N. commune* makes visible colonies composed extracellular polymeric substances. *N. commune* has expected to utilize for agriculture, food and terraforming cause of its extracellular polysaccharide, desiccation tolerance and nitrogen fixation. To exhibit the potential abilities, the *N. commune* sheet is made to use convenient and evaluated by plant growth and radioactive accumulation. We will discuss utilization of terrestrial cyanobacteria under closed environment.

Keywords: desiccation, cyanobacteria, bioremediation, agriculture, space, terrestrial

Species selection and making a model of a closed bio-ecosystem

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There are many differences in metabolic activity, environmental response and biological interactions between individual species. Species selection is important to make life support systems in closed bio-ecosystems, for stability of systems and a low percentage of error. For example, terrestrial cyanobacteria are useful because of CO_2 fixation ability, O_2 supply ability, and usefulness as food. Additionally, trees are useful because they can be used as herbal medicine and building material. Here, we will discuss the importance of species selection. And, we will introduce the activities of students in the Society of Eco-Engineering, who are making a model of a closed bio-ecosystem using a combination of different species.

Keywords: Closed bio-ecosystem, Life support system, Species selection, Terrestrial cyanobacteria, Tree

Study on ECLSS and Micro Ecosystem Aimed at The Optimal Design of Closed Ecological Life Support Systems

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We must produce the environment we can survive in order to live in space. Can be it possible is ECLSS (Environmental Control and Life Support System). ECLSS remove carbon dioxide and the hazardous substances generated from the human body and equipment and control oxygen concentration and humidity etc. In ground 400km ISS (International Space Station), ECLSS has created an environment that enables the activities of about six people. In the current ISS, assumes the supply of goods from the earth and they are reusing a portion of the material. Now, the system that can reuse full materials has not been developed. In recent years the manned space activity on Moon or Mars is expected, finally the development of high rate material regeneration ECLSS is desired. Aimed at the study of the complete materials circulation, Biosphere 2 and CEEF (Closed Ecology Experiment Facilities) as artificial closed ecosystem, were built. Both required a vast land and processing equipment for living of several people. Although the part of regeneration equipment of ECLSS on ISS is relatively compact, just then considering full regeneration of materials, the system becomes extremely complex and difficult.

If we can look with a macro view, the Earth is the largest closed ecosystem operated with the energy from the sun. Materials within the Earth is circulated and innumerable life exist together. They withstand to so many kinds of disturbance, but there are also species going extinct, and they has been co-exist in a much longer period than our life span. Moreover, change of number of individuals caused by disturbance goes to converge or vibration as if something control. Natural ecosystems are very stable in many aspects. The variety of factors of the self-standing stability has been studied. However, the method of incorporating it to the specific system is not clear. It is one of the dream for ECLSS developers to put a self-standing stability on ECLSS.

We carry out the two directions of approach of analysis of realistic ECLSS analysis and ecosystem function in order to propose what factor makes the ECLSS with completely material circulation (CELSS: Closed Ecological Life Support Systems). In ECLSS analysis, we simulate ECLSS with current technologies and investigate what technology can be a bottleneck and high sensitivity factors in the system by multi evaluation items. At the same time we study similarities between the actual trouble in ECLSS and disturbance in real ecosystem. In the ecosystem analysis, modeling a minimal ecosystem "microcosm", analyze mechanisms of a restoring force to a disturbance in the system. Then, we consider how self-standing stability of natural ecosystems can be applied to a specific artificial system. The final goal is to apply the self-standing stabilizing function found out from the ecosystem analysis to CELSS. At the same time we propose a system having a high stability and current technical problems for the system. Progress of our present study, we have reached only the analysis of each approach. In this presentation, we make their introductions.

Keywords: Life support system, Closed system, Ecosystems, Microcosm

The culturing of sea grape *Caulerpa lentillifera* by using waste water of kelp grouper and estimating the ability of absorbing elements contained in waste water.

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Recirculating aquaculture system has a problem in the dealing waste water. Recently, it is aimed to combine the treatment of waste water with cultivation of marine algae to prevent environmental pollutions. In this study, kelp grouper and sea grape were used for experimental fish and alga and sea grape were cultivated for 28 days by using kelp grouper’s waste water or synthetic medium (PES) in the tanks that had 50 L water volumes. Water temperature and pH were maintained at 28℃ and up to 8.0, respectively. In addition, salinities were set at 32 psu and 36 psu to confirm the tolerance of salinity, and photoperiod was controlled at 12 h light: 12 h dark in the all treatments. Sea grape cultured in 32 psu with waste water showed the highest specific growth rate (SGR) that is 7.16, similar to it of sea grape cultured with synthetic medium in 32 psu. While, the sea grape cultured in 36 psu with PES showed the lowest SGR that is 5.27. In addition, elements contained waste water, solid waste, foam waste and sea grapes were analyzed by using inductively coupled plasma atomic emission spectroscopy and total nitrogen analyzer. These results showed that the elements contained in the kelp grouper waste (i.e., waste water, solid waste and foam waste) are enough to grow up sea grape except manganese, copper and boron. Thus, these elements are needed to add to culturing water and their quantities are 189.8 μg, 99.4 μg and 157.5 μg respectively on 1 L basis.

Keywords: Kelp grouper, Sea grape, Recirculating fish culture system, Utilization of waste water, Culturing waste, Water quality

Growth of a terrestrial cyanobacterium, *Nostoc* sp. HK-01, in the poor nutrient mediums similar to the environment on Mars

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A terrestrial cyanobacterium, *Nostoc* sp. HK-01, has several abilities; photosynthesis, nitrogen fixation, usefulness as food, and space environmental tolerances. HK-01 may be utilized for bio-chemical circulation in closed bio-ecosystems, including Mars. When we introduce HK-01 to the environment on Mars, we need to define the growth of HK-01 in an environment which lacks nutrition. In this study, we tested whether akinetes (dormant cells) of HK-01 grow using components of their dead cells or/and Martian regolith simulant. We will discuss the possibility that colonies of HK-01 can be grown in an environment such as Mars.

Keywords: closed bio-ecosystems, Martian regolith simulant, terrestrial cyanobacterium

Searching for water stress proteins in terrestrial cyanobacteria, *Nostoc* sp. HK-01

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Nostoc sp. HK-01 is one of terrestrial cyanobacterium having a tolerance to desiccation stress and it has several ability, photosynthesis, nitrogen fixation and usefulness as a food, it is thought that it can be used for bio-chemical circulation in a closed ecosystem, including space.
In this study, we searched for the genes that would play an important role in the desiccation stress response. Initially, to investigate expression changes of the proteins in Nostoc sp. HK-01 cells, proteins of the desiccated cells were analyzed by SDS-polyacrylamide gel electrophoresis. The cells were dried in a desiccator. In the course of desiccation of the cells, the expression level of some proteins was increased.

Keywords: cyanobacteria, desiccation tolerance, stress protein

Evaluation of food functions of a terrestrial cyanobacterium, *Nostoc* sp. HK-01 in closed bio-ecosystems

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We study life-support in closed bio-ecosystems to provide food and oxygen for habitation in severe environments. We propose several species of organisms as candidate species. A terrestrial cyanobacterium, *Nostoc* sp. HK-01 has several unique abilities, such as photosynthesis, nitrogen fixation and tolerance to an extraterrestrial environment. Here, we propose to utilize *Nostoc* sp. HK-01 as a food resource in extraterrestrial environments such as Mars. We indicate that *Nostoc* sp. HK-01 has food functions as primary(nutritional), secondary(sensory), tertiary(physiological). We will discuss the utilization as a food resource of *Nostoc* sp. HK-01 in closed bio-ecosystems. Our results may contribute to the supply of food resources under severe conditions for life-support in closed bio-ecosystems.

Keywords: closed bio-ecosystems, cyanobacteria, food resource, Nostoc sp.HK-01, food functions

