Research in closed bio-ecosystems

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Researchers in this session have been studying useful life-support systems in closed bio-ecosystems since 2011. The final goal of our studies is to inhabit extraterrestrial environments. There are many types of research related to closed biological and technical systems. This research should be shared among a wide range of people engaged in many different areas of research, because the results will be inherited by future generations. Here, I will introduce our session’s outline and the contents of each individual presentation.

Keywords: closed bio-ecosystems, extraterrestrial environments

Dissolution of a woody biomass (*Albizia falcataria*) with an ionic liquid, 1-H-3-methyl imidazolium chloride ([HMIM]Cl)

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[Introduction]
Trees will play important roles in space agriculture as suppliers of oxygen, wooden materials and woody biomass[1)]. Woody biomass contains cellulose, hemicellulose and lignin and wide variety of useful chemicals. Effective utilization of the woody biomass will bring benefit in the closed ecosystem. To produce various useful materials from woody biomass, kraft pulp method, acid hydrolysis, and enzymatic saccharification have been studied. However, these method require severe conditions such as high temperatures and high pressures.
An ionic liquid is a salt that a melting point below about 100˚C and has unique characteristics such as excellent solubility, low volatility, incombustibility and low viscosity[2)]. Recently, it has been reported that some ionic liquids can dissolve cellulose and lignocellulose, and the solvent can be utilized as green solvent to produce cellulose films, acetyl cellulose and carboxymethyl cellulose. In this experiment, dissolution behavior of a woody biomass (*Albizia falcataria*) to a low cost ionic liquid (1-H-3-methyl imidazolium chloride) are investigated.

[Materials and methods]
Wood chips (*Albizia falcataria*) were provided by Nankai Plywood Co., Ltd. They were ground using a willy meal, and 40-80 mesh were used for experiments. To synthesize 1-H-3-methyl imidazolium chloride ([HMIM]Cl), 1-methyl imidazole and hydrochloric acid were mixed in ice box and stirred for 24 hours, the resulting liquid was washed with ether and evaporated *in vavuo*. Synthesized [HMIM]Cl was identified by NMR. Wood meals (0.5 g) and [HMIM]Cl (15 g) were added into a flask and refluxed at 90 to 120˚C for 1 to 24 hours. The mixture were filtrated, wash with 150 mL of 1,3-dimethyl-2-imidazolidinone and water, and dried at 105˚C to calculated insoluble residues. The lignin content in the insoluble residue were determined as Klason lignin. FT-IR spectra of the insoluble residue were recorded. Used [HMIM]Cl was recovered to add ethanol, filtrated and evaporated *in vavuo.*

[Results and discussion]
In the wood meal treated with [HMIM]Cl at 90˚C, the content of insoluble residue decreased in 1 hour, then the content did not changed with increasing reaction time. FT-IR spectrum of insoluble residue at 90˚C for 1 hour showed disappearance of a peak at 1740 cm^{-1} derived from C=O of acetyl group in hemicellulose. In the wood meal treated with [HMIM]Cl at 120˚C, the content of insoluble residue decreased with increasing reaction time, and the lignin content in the residue increased with increasing reaction time. FT-IR spectrum of insoluble residue at 120˚C for 24 hour showed disappearance of the peak in hemicellulose, and a peak at 1429 cm^{-1} derived from bending vibration of CH_2 in cellulose. Peaks at 1600 cm^{-1} and 1460 cm^{-1} derived from aromatic rings in lignin were clearly found in the spectrum. From these results, lignin was concentrated in the insoluble residue by dissolution with [HMIM]Cl. Recycled [HMIM]Cl after cellulose dissolution had almost the same dissolution rate compared with unused [HMIM]Cl, however, recycled [HMIM]Cl after wood meal dissolution showed low dissolution rate compared with the unused ionic liquid. Low-molecular lignin degradation products could not completely precipitate in used [HMIM]Cl by addition of ethanol.

[References]
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Keywords: ionic liquid, biorefinery, falcata

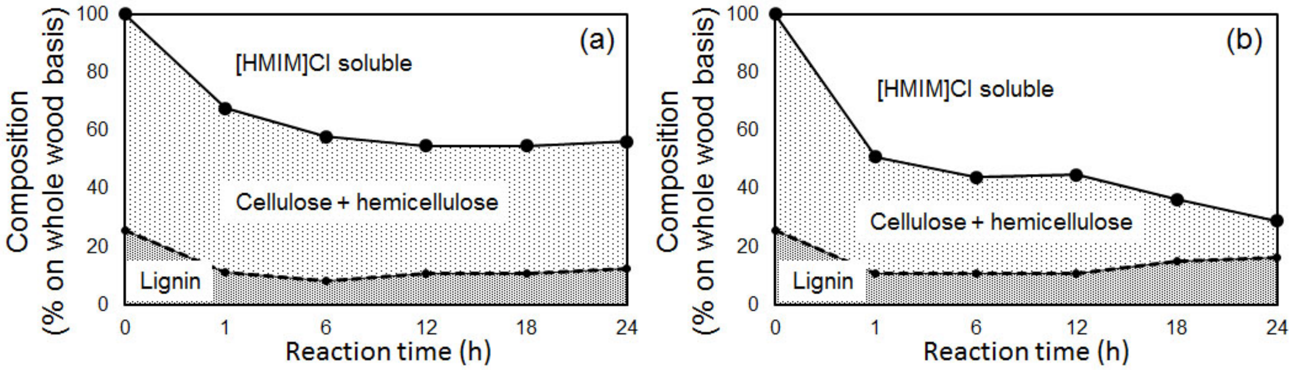

Fig. 1. Composition of wood meal treated with [HMIM]Cl at (a) 90˚C and (b) 120˚C.

Growth and tolerance of a terrestrial cyanobacterium, *Nostoc* sp. HK-01 under harsh environment

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A terrestrial cyanobacterium, *Nostoc* sp. HK-01, has several abilities; photosynthesis, nitrogen fixation, utility as food, and tolerance to extraterrestrial environments. *Nostoc* sp. HK-01 may be utilized for biochemical circulation in closed bio-ecosystems, such as Mars. Tolerance to extraterrestrial environments are important for transportation to Mars. Akinetes, which are dormant cells of *Nostoc* sp. HK-01, are tolerant against dry heat, upto 100 °C for 10h. These results indicate that some functional substances which provide tolerance against heat exist in the akinete cells. Akinetes of *Nostoc* sp. HK-01 could be transported to Mars. Before we introduce *Nostoc* sp. HK-01 to Mars’ environments, we need to understand its growth in an environment with poor nutrition. We tested whether akinetes of HK-01 can grow using components of their dead cells or/and Martian regolith simulant. We will discuss the possibility that a colony of HK-01 can be grown in an environment with poor nutrition. *Nostoc* sp. HK-01 could contribute to soil formation from Martian regolith, so that plants could grow. *Nostoc* sp. HK-01 is a good candidate for an initial organism to introduce into the Martian environment.

Keywords: Closed bio-ecosystems, Cyanobacteria, Dry heat tolerance, Martian regolith simulant, Nostoc sp. HK-01

Draft genome sequence analysis of the extreme environment grown cyanobacterium, *Nostoc* sp. HK-01 (NIES-2109)

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Cyanobacteria are photosynthetic organisms to produce oxygen on the earth for our life. Terrestrial cyanobacteria are living in extreme environment. Isikurage (*Nostoc commune*) is a nitrogen-fixing terrestrial cyanobacterium and living ranging from polar to desert. *Nostoc* sp. HK-01 (NIES-2109, hereafter HK-01) was isolated from crust of Isikurage. Previous reports suggest that HK-01 survives under low vacuum condition such as Mars and that dried HK-01 tolerates for high temperature and high gamma-ray radiation. HK-01 has expected to utilize for space environment. Recently, we determined a draft genome sequence of HK-01. Here, we are trying to analyze HK-01 genes *in silico* and will discuss material cycle and stress tolerance.

Keywords: cyanobacteria, extreme environment, genome, space

Growth of candidate materials and element cycle on Martian Regolith simulants

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The preparations for the establishment of a manned Mars mission have been starting and lots of results related to the mission have been accumulated. The candidate materials, cyanobacterium and plants, were cultured with Martian Regolith simulants. They could grow well on the Martian Regolith simulants as well as their suitable medium. Here, we will show their growth and element cycle on Martian Regolith simulants.

