C_Biodegradation and Bioremediation

C-1

Semi-Rational Engineering of a Cold-Active Acetyl Xylan Esterase for Substrate Selectivity

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Cold-active acetyl xylan esterase catalyzes the hydrolysis of glucose penta-acetate and xylan acetate, reversibly producing acetyl xylan from xylan. It showed relatively broad substrate specificity toward several acetylated compounds and cephalosporin antibiotics. The acetylation activity of this enzyme may be effective for the semi-synthesis of new antibiotics. To make this enzyme to accommodate broader substrates for the modification of cephalosporin antibiotics, we used covalent docking using Autodock vina and HotSpot3D webserver. We found the 7 amino acid residues critical for substrate binding. Theses residues were substituted by site saturation mutagenesis and the substitutes showing better activity against linalyl acetate and terpinyl acetate were screened and identified by sequencing. The affinity of more active mutants were evaluated using Autodock.

Keywords : Semi-rational approach, cold active enzyme, promiscuity

C-2

Isolation and Characterization of *Pseudomonas* sp. JPS-CO_2 using Carbon Monoxide as a Sole Carbon Source

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Carbon monoxide is a representative harmful gas among air pollutants, and various attempts have been made to remove carbon monoxide from the atmosphere. The research team is developing a new bio-based air purification system using microorganisms to remove carbon monoxide from the atmosphere. To this end, I first tried to isolate a new bacterial strain that can grow by using carbon monoxide in the atmosphere as the sole carbon source. First, carbon monoxide was supplied to a specific bioelectrochemical reactor (3V, 10-s pulse) to promote microbial metabolism, and mixed strains obtained from the environment were inoculated into the reactor and cultured further for 4 weeks. As a result of microbial enrichment cultivation, a new strain that can grow dominantly was finally obtained, and as a result of 16-rRNA analysis of this strain, it was named as *Pseudomonas* sp. JPS-CO_2. As a result of investigating the availability of carbon monoxide on the isolated microbial strain, it was found that 0.5% of carbon monoxide supplied to the reactor was effectively removed after 10 days of cultivation. This result is expected to be developed as a new concept of bio-air purification system to remove carbon monoxide from the atmosphere in the future.

Keywords : Air pollutants, carbon monoxide, *Pseudomonas*

C-3

Biodegradation of Plastics in Anaerobic Condition by *Enterobacter* spp. Isolated from Gut of *Tenebrio molitor*

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Polystyrene, also widely known as Styrofoam™, is a polymer of styrene and one of a major plastic that pollutes the aquatic environments. Large quantities of polystyrene have been introduced into the environment, resulting in the hazardous accumulation of the material in ecosystems. To solve this problem, it is utmost importance on how to effectively treat the polystyrene wastes. In this study, 105 strains were isolated from the gut of mealworm (*Tenebrio molitor*) larvae, which is known to chew and digest polystyrene. Ten bacterial strains successfully grew on selective-media containing polystyrene as a sole carbon source. Subsequent incubation of the strains on polystyrene-media produced clear zones around the bacterial colonies, indicating that they would be functionally involved in polystyrene degradation. 16S amplicon sequencing results suggested that the isolated strains are closely related to *Enterobacter* spp. Especially, Strain of SLAM LG3 formed a clade which was distinct from all other *Enterobacter* species, suggesting that SLAM LG3 is a promising candidate as a novel polystyrene-degrading bacterium. In combination with the functional studies, our study provides potentials of novel polystyrene-degrading microorganisms to be applied for petroleum-based plastic degradation.

Keywords : *Tenebrio molitor*, Polystyrene (PS), degradation

C-4

Metagenomic Analysis of Eco-Friendly Treatment Process of Textile Dye Wastewater Bioaugmented with the Novel Microbial Consortium CES-1 at a Full-scale System

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The presence of very small amounts of dyes in water (less than 1 ppm for some dyes) is highly visible and affects the aesthetic merit, water transparency and gas solubility in waterbodies. The hypothesis is that the azo-dye could be degraded via pathways including azo-dye reduction, aromatic compound degradation, amino acid metabolism, nitrogen and phosphorus cycle and TCA cycle. The activities of dye wastewater treatment plant were evaluated before and after the addition of CES-1. Total genomic DNA samples were extracted from different treatments with different time variables. The effect of CES-1 bioaugmentation on the microbial community structures and functional genes of each treatment step was explored by high-throughput sequencing and metagenomic analysis. Through the addition of CES-1, the sludge reduction rate over 20 months after the bioaugmentation was 26% (reduction from 6.18 to 5.00 in sludge per ton of influent COD). The removal rate of COD increased up to 97.8% in 50 days and the removal rate of other parameters increased as well. *Massilia timonae* and *Verminephrobacter* sp. were dominant in the bioaugmented samples. The metagenomic analysis revealed the key enzymes involved in azo dye and aromatic compound were well annotated including azo-reductase (acpD), catechol 2,3-dioxygenase (dmpB), protocatechuate 4,5-dioxygenase, alpha chain and beta chain (ligA and ligB). These enzymes were dominant in CES-1 and three separate groups of sample such as primary aeration (PA), secondary aeration (SA) and sludge digestion of control (PA_0303_17 and SA_0303_17), 50 days (PA_0629_17, SA_0629_17 and SD_0303_17) and 531 days' (PA_1026_18, SA_1026_18 and SD_1026_18). TCA cycle provides abundant NADH and FADH2 to the electron transfer chain, NADH and FADH2 eventually transfer electrons to the azo dyes through cytochrome. Among them, NdoR and nahA is combined with the complex structure to transmit electrons to azo dyes. This proves the bioaugmentation of CES-1 in the treatment made an impact on enhancing the metabolic pathways in dye degradation. fadE, ENO and *Glucanobacter _unclassified* were clustered with PA_1026_18 and commonly present in all the bioaugmented samples after 531 days. The high consistency of the analyses mentioned above indicated that the network analysis is a reasonable and powerful tool to provide us new insights and eligible biomarker in complex environmental examples. The intrinsic relationships between the microbial community structures and functions of enzymes for the dye metabolism were analyzed using sophisticated algorithms. This understanding of metabolic pathways in the dye wastewater treatment system will undoubtedly contribute to an optimized and efficient operation of the treatment system and any other wastewater treatment systems.

Keywords : Bioaugmentation, biodegradation, metagenomics

C-5

Biosorption of As(III) using *Saccharomyces cerevisiae* SRCM 501804 Isolated from Korean Turbid Rice Wine: Isotherm and Kinetic Studies

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In this study, *Saccharomyces cerevisiae* SRCM 501804 (Genbank accession No. MZ068240) was isolated from Korean turbid rice wine (*Makgeolli*). Dried biomass of *S. cerevisiae* SRCM 501804 showed superior biosorption capacity of trivalent arsenic (As(III)) in aqueous solution. The characterizations of the *S. cerevisiae* SRCM 501804 were performed by phylogenetic analysis, Fourier transform infrared spectroscopy (FT-IR), MINTEQ, and point of zero charge (pH_{pzc}). Additionally, the influence of pH (2.0-10.2), biomass dosages (0.01-0.09 g), contact times (0-120 min) and initial concentration (51.2-281.5 mg/L) on the biosorption of As(III) were evaluated. Dried biomass of *S. cerevisiae* SRCM 501804 removed 90.1% of the As(III) from a 40.8 mg/L within 1 h. The biosorption isotherm and kinetic results showed the Langmuir isotherm and Pseudo-second order models well fitted. The biosorption experiments showed that *S. cerevisiae* SRCM 501804 could be used as effective biosorbent for the As(III) biosorption in aqueous solution.

Keywords : *Saccharomyces cerevisiae,* trivalent arsenic, biosorption

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C-6

Characterization of DDT-Degrading Soil Bacteria Isolated from a DDT-Contaminated Soil

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Dichlorodiphenyltrichloroethane (DDT) is one of organochlorine insecticides, which was prohibited for its toxicity in most of the world in the 1970s and 1980s. However, it has been frequently detected in various environments for its non-biodegradability and can have adverse effects on organisms over a long time. We isolated six DDT-degrading bacterial strains from a DDT-contaminated soil in Korea. They were affiliated with the genera *Flexivirga*, *Rhodopseudomonas*, *Paralcaligenes*, *Nitrobacter*, *Mesorhizobium*, and *Oleiharenicola*, respectively. They degraded 14~77% of 1.5 mg/L of 4,4'-DDT within 34 days in minimum media, among which, Paralcaligenes sp. KSB-10 showed the highest degradation rate (77%). Interestingly, DDE and DDD, the known metabolites of DDT were not detected in the HPLC chromatograms at day 34 for all the isolates. When strain KSB-10 was inoculated into minimum media containing various concentrations (2.3~9.3 mg/L) of technical grade of DDT (4,4'-DDT:2,4'-DDT=1:0.4) and incubated for 19 days the degradation rate decreased from 54% to 19% as the DDT concentration increased. In addition, the degradation rates of 2,4'-DDT were generally lower than those of 4,4'-DDT, indicating that the former is more difficult to degrade than the latter or the degradation rate decreases at lower concentrations.

Keywords : DDT, biodegradation, bacteria

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C-7

Biological Upgrading of Ethanol-Assisting Depolymerized Lignin: from Waste to Value-Added Product

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Waste lignin is dramatic increasing from pulp, paper, and biofuel production. More than 100 million tons of waste lignin is being generated annually. Although many attempts have been made to establish sustainable lignin valorization, but it remains challenging. In this work, we present a new approach for lignin valorization by biological co-upgrading lignin and its organic solvent - ethanol from depolymerization process into protocatechuic acid and polyhydroxyalkanoic acid. *Pseudomonas putida* KT2440 with high efficient ethanol utilization was used for biological upgrading of ethanol-assisting depolymerized lignin containing various lignin monomers at a concentration of 77 mg/mL. To produce value-added products, *P. putida* KT2440 was engineered by knocking out the protocatechuate 3,4-dioxygenase, construction of formaldehyde utilization pathway, and reconstruction acetaldehyde direct conversion to acetyl-coenzyme A using aldehyde dehydrogenase from *Dickeya zeae*. Additionally, we demonstrate the promoting of the utilization of formaldehyde on the growth and production of value-added products. Finally, the engineered strains are able to produce 6.73 ± 0.26 mg/L of PCA with a 17.5% (w/w) yield of total lignin monomers, and 303.66 ± 26.75 mg/L of PHA with 21.26% (w/w) of dry cell weight from 0.5 mL of ethanol-assisting depolymerized lignin. This study represents a sustainable approach for lignin valorization, which employs ethanol-solvent of depolymerization of lignin as the co-substrate without separation process.

Keywords : Lignin valorization, polyhydroxyalkanoic acid, co-upgrading

C-8

Are there Any Fungi that Can Decompose of Plastic in Soil Fungal Communities?

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In agriculture, plastics play an important role in increasing production, such as controlling weeds, maintaining soil temperature, and preventing water loss. But, Plastic (agricultural waste) was known to accumulate in the soil as one of the main factors of environmental pollution and affect the soil ecosystem. To solve the soil pollution caused by plastics, we conducted a study to find fungi capable of degrading plastic in the fungal community of agricultural plastic waste. To find fungi capable of degrading plastic, collected agricultural waste plastic from agricultural land, fallow land, and dumpsite. And then, each sample's DNA was extracted and through Next-Generation sequencing analysis. As a result, identified the fungi of 522 strain and each sample's dominant species. These results suggest that there may be fungi that can degrade agricultural plastic in the fungi community of agricultural plastic waste. Further study, we will analyze the characteristics of bio-degradable fungi of plastic by using fungi isolated from agricultural plastic waste. This data will provide a new experimental field of perspective for bio-remediation.

Keywords : Biodegradation, fungi, plastic

C-9

Cesium Removal Using *R. erythropolis*

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Nuclear power generation is eco-friendly and economical because it can obtain a lot of energy with a small amount of fuel compared to fossil fuels. It is being developed as a sustainable energy source to secure a stable power supply and carbon dioxide reduction. However, due to the wastewater from nuclear power plants and radioactive materials released from Fukushima, Chernobyl nuclear power plant accidents, the need for environmental restoration research is increasing. Cesium is a representative radioactive material. Adsorption, coagulation, precipitation, reverse osmosis, and ion exchange are used to remove cesium, but there are limits to practicality due to the complexity of the treatment method with high cost. To overcome the problems of the existing processes, an economical, easy-to-treat, and eco-friendly bioremediation method is newly attracting attention in radioactive waste treatment. In this study, the aerobic bacteria *R. erythroplis* was used to remove cesium dissolved in aqueous solution. As a result of conducting the experiment under various conditions, its cesium removal efficiency is up to 86% after 24 hours of incubation. During bacterial growth, the maximum removal efficiency of cesium was showed when the stationary phase is start. As a result of analyzing the bacteria using FE-TEM, cesium was intensively accumulated in some specific points inside of the bacteria. In addition, we can check that the bacteria which removed cesium grew 3-4 times longer than control bacteria. Removal of heavy metals and radioactive materials using bacteria is eco-friendly and has high economical, which shows the possibility of solving environmental pollution caused by nuclear power plant wastewater or accidents.

Keywords : Bacteria, bioremediation, Cesium

C-10

Analysis of Microbial Communities in Waste Plastic Films and Soils Collected from a Landfill Site in Gochang-gun, Korea and Their Potential to Degrade Polyethylene Films

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Non-degradability of plasticware has been recognized as serious environmental problem. Biodegradation of plastics by microorganisms has a potential to alleviate the rapid accumulation of plastic wastes. To identify and isolate the microbes to degrade plastics, we carried out the metagenomic study for waste plastic films and nearby soils collected from a landfill site located in Gochang-gun, Korea, in which plastic wastes have been buried for more than 20 years. The analysis of bacterial and fungal communities has been in progress using DNA and RNA extracted from the waste films and soils. Physical and chemical parameters of landfill soils were measured and observed as high pH values. Fourier-transform infrared (FT-IR) spectroscopy to the films showed peaks related to −OH functional group implied as erosion of the films. When pure low-density polyethylene (LDPE) films have been cultured with landfill soil and waste film samples for 2 months, functional groups containing oxygen such as −OH group appeared in the FT-IR spectroscopy analysis, suggesting partial oxidation of LDPE films. Bacteria and fungi have been isolated from the oxidized LDPE films for further study. These results suggest that the microbiome enriched in the plastic films has potential to degrade LDPE films.

Keywords : Polyethylene, biodegradation, metagenome

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C-11

A Study on the Selection of Water Purification Species Using Microalgae Biofilm

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Contamination of livestock wastewater, fertilizers, pesticides, etc., which appear as nonpoint sources of pollution, cannot be predicted and prepared, so it is necessary to rely on the purification of rivers themselves. Microalgae have been reported to remove nitrogen and phosphorus from the water and to remove many of the pollutants in the water as a primary producer that grows through photosynthesis. It has been confirmed that many kinds of microalgae grow wild in Korea, and in particular, the efficiency of the treatment of nutrients is known according to the type of microalgae. Therefore, microalgae growing in domestic rivers were selected and compared with their ability to remove toxic heavy metals. In order to effectively carry out these studies, microalgae clusters that form colonies in rivers were collected. In addition, the ability of the colony to remove contaminants was first confirmed. After that, the effects of individual microalgae constituting the colony were compared. Microalgae colonies were mainly found in puddles of water or in calm spots in rivers, and had viscous or filamentous forms. Microalgae such as *Coccomyxa* sp., *Cladophora* sp., *Acutodesmus* sp., And *Gloeocystis* sp. Showed different treatment efficiencies for nitrogen, phosphorus and heavy metals. *Chlococcum* sp. and *Gloeocystis* sp. Showed high removal efficiency of nitrogen and phosphorus. While *Acutodesmus* sp. showed high removal efficiency of heavy metals such as Cu, Pb and Zn. From these results, the removal of toxic heavy metals through microalgae is effective. If the microalgae treatment characteristics of the selected microalgae are appliedto the streams in the event of pollution, it is expected to increase the efficiency of the river's self-cleaning by increasing the biological purification capacity.

Keywords : Microalgae, biofilm, purification

C-12

One-Pot Chemical and Biological Depolymerization of PET Using a Novel Biocompatible Catalyst, Betaine

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Due to its unique physical characteristics, poly(ethylene terephthalate) (PET) has been broadly usedin numerous industries. However, PET poses significant environmental problems worldwide due to itslow decomposition and recycling rate. Since replacing PET with other substances is almostimpossible, an efficient procedure for PET recycling is needed in a circular economy. Here, wedesigned an integrated process consisting of depolymerizing PET and converting PET monomers intohigh value-added products with a one-pot process for the paradigm shift to a method for resourcerecovery of PET components. The center of our approach is the use of a biocompatible and greencatalyst, betaine, in the glycolysis process that allows the entire PET glycolysis slurry to be used as asubstrate that can directly be applied to additional bioprocesses without any separation steps. Basedon the density functional theory (DFT) analysis, by not only synergistic effects between the anion andcation groups of betaine but also strong hydrogen interactions between PET, EG, and betaine,betaine effectively catalyzed PET depolymerization. Through the PET glycolysis with betaine and theoptimized enzyme hydrolysis for the PET glycolysis slurry, PET was decomposed to terephthalate(TPA, 31.0 g/L, 62.8% mol/mol) and ethylene glycol (EG, 11.7 g/L, 63.3% mol/mol) at high yields andhigh titers. This process has been applied to the conversion of the PET hydrolysate (TPA and EG) toprotocatechuic acid (PCA) and glycolic acid (GLA), respectively. We suggest that this one-pot chemo-bioprocess Integrating chemical glycolysis, enzymatic hydrolysis, and bioconversion for PETdepolymerization and recycling, is highly promising for the upcycling of waste PETs.

Keywords : PET recycling, betaine, depolymerization

C-13

Direct Removal of Harmful Cyanobacterial Species Using an Adsorption Process and the Potential Applications as Lipid Source

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In the present study, we applied an adsorption process to remove and recover the harmful pollutant *Microcystis aeruginosa* using polyethyleneimine (PEI)-modified chitosan-waste biomass composite fiber sorbent (PEI-CBF). The enhancement of the positive binding sites on the sorbent after PEI modification is important factor to ensure the removal potential of the adsorption processing for *M. aeruginosa* cell removal from an aqueous solution. The PEI-CBF could remove 90.5% of *M. aeruginosa* cells without requiring additional processes, while CBF removed 22.7% under the same experimental conditions. In the cell removal process using PEI-CBF, the *M. aeruginosa* cells were bound to the PEI-CBF without cell lysis and damage. From the cell-loaded PEI-CBF, 95.3% of adsorbed cyanobacterial cells were recovered via a desorption process in an alkalic solution and ultrasonication. In addition, the total lipid content of the recovered *M. aeruginosa* cells was similar as that of non-adsorbed *M. aeruginosa* cells. Furthermore, the cell removal performance of regenerated sorbents was almost entirely maintained. Our adsorption process can be applied as a breakthrough technology to allow the conversion of the environmental pollutant *M. aeruginosa* to energy resources by recovering and controlling the cells without substrate loss by cell lysis.

Keywords : *Microcystis aeruginosa,* adsorption, waste to resource

C-14

Dynamics of Microbial CH4-Oxidation Potential in Rhizosphere during Rhizoremediation of Diesel-Contaminated Soil

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Methane is a representative non-CO_2 greenhouse gas. Diesel-contaminated soil is one of the potential methane gas sources. For soil remediation, it is necessary to develop the technology that can satisfy not only pollutant degradation but also CH_4 mitigation. In this study, microbial CH_4-oxidation potential in rhizosphere was evaluated during rhizoremediation of diesel-contaminated soil planted with maize and tall fescue. . The CH_4 oxidation rates in the maize rhizosphere were constantly increased from 1.1 μmol·g-dry $soil^{-1}·h^{-1}$ at 0 d to 1.5 μmol·g-dry $soil^{-1}·h^{-1}$ at 83 d. The CH_4 oxidation rates in the tall fescue remained almost constant at 1.1 μmol·g-dry $soil^{-1}·h^{-1}$ for 83 d. The dynamics of CH_4 oxidation functional gene, *pmoA*, in the maize and tall fescue rhizosphere showed the similar pattern to that of CH_4 oxidation rate in each rhizosphere. In the maize rhizosphere, the abundance of methanotrophs ranged 0.61~2.67%, and its abundance in the tall fescue rhizosphere was 0.79~2.99%. The dominant methanotrophs were almost the same in the maize and tall fescue rhizosphere. *Sphingopyxis*, *Methylocapsa*, *Methylosarcina*, *Methylococcus*, *Methylocystis* were dominant methanotrophs. The use of these methanotrophs in rhizoremediation process is expected to mitigate CH_4 emission during the remediation of contaminated soil.

Keywords : Rhizoremediation, methane oxidation rate, methanotrophs

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C-15

Evaluation of N_2O-Reducing Activity in Rhizosphere during Rhizoremediation of Diesel-Contaminated Soil

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Diesel-contaminated soil is considered a major anthropogenic N_2O source. Various physico-chemical and environmental factors in soil can affect nitrogen cycle including N_2O-reduction. However, there are few researches on N_2O-reduction activity in diesel-contaminated soil. In this study, N_2O-reduction performance in rhizosphere was characterized during the rhizoremediation of diesel-contaminated soil. Three planting treatments (no planting, maize and tall fescue planting) of out door pot experiment were conducted for 110 days. In all experimental groups, the N_2O-reduction activity gradually decreased as the remediation time elapsed. That is, the N_2O-reduction activity decreased with decreasing residual diesel concentration in soil. The N_2O-reduction rates in the soil without planting were higher than those in the soil planting with maize or tall fescue. However, the *nosZ*I, functional gene for N_2O reductase, abundances were not correlated to the N_2O reduction activities in all experimental groups. The *nosZ*I abundances increased after 83 day in the no planting soil and tall fescue planting soil (150.15 - 291.48 gene copy number • g-dry $soil^{-1}$). The *nosZ*I abundances in the maize planting soil increased after 19 day (170.58 - 273.74 gene copy number • g-dry $soil^{-1}$). Based on bacterial community structures, the abundances of major N_2O-reducing bacteria such as *Hyphomicrobium, Sphingomonas, Pseudomonas* increased during the initial period (0 - 19 d). Their abundances at the day 19 were 14, 15 and 24 % in the no planting soil, tall fescue planting soil, and maize planting soil, respectively. The abundances in all experimental groups gradually decreased to 7 - 8 % until the day 40, and they maintained during 40 - 110 day. These results can be used to establish strategy for the mitigation of N_2O emission during rhizoremediation of contaminated soil.

Keywords : Rhizoremediation, nitrous oxide reduction, diesel-contaminated soil

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C-16

Effect of Compost Amendment on Bioremediation Performance of Diesel-Contaminated Soil

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Bioremediation using microbial activity is well-known for the remediation technology of contaminated soil. Compost amendment can increase microbial activity by supplying available nutrients, resulting in the enhancement of bioremediation efficiency. Compost addition can also affect the CH_4 and N_2O emission and the soil microbial community structure. In this study, to evaluate the compost amendment effect on bioremediation performance, low nutrient barren soils mixed with different ratios of compost (0, 5, 10 and 20%, w/w) were contaminated with diesel of 10,000 mg-TPH·kg-dry $soil^{-1}$. The diesel removal efficiency was improved by increasing the compost addition amount. After 103d, the diesel removal efficiency was 54% in the soil without compost, and it was 85% in the soil with 20% compost. The CH_4 oxidation potential in all treatments increased during diesel bioremediation. The CH_4 oxidation potential rate in the soil with 20% compost was 1.79 times higher than that in the soil without compost. However, the N_2O reduction potential decreased over time, and there was no significant effect of compost amendment. The compost amendment affected bacterial community structures, and the abundances of *Immundisolibacter, Acidibacter* and *Terrimonas* were increased by compost amendment. The information obtained in this study can be utilized as a meaningful reference for the innovation of bioremediation technology.

Keywords : Bioremediation, compost amendment, diesel contaminated soil

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C-17

Degradation of Bisphenol A by *Sphingobium* sp. A3 Isolated from Contaminated Soil

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Bisphenol-A (BPA) is an endocrine-disrupting compound (EDC) that mimics the function of estrogen causing damage to reproductive organs. Bacterial BPA degradation has been consider as a cost effective and eco-friendly method compared with physical and chemical methods. In this study, we found BPA-degrading bacteria from soil and characterized its BPA-degradation efficiency. The soil sample was collected at a waste dump site and a BPA-degrading enrichment culture was conducted aerobically using mineral salts medium with BPA as sole carbon source. We isolated the strain A3 from the BPA-degrading enrichment culture, and the evaluation of its BPA degradation ratio showed that the strain A3 degraded more than 70% of 200 ppm of BPA in the medium for 24 hours. The bacterial identification and phylogenetic analysis based on 16S rRNA gene sequence showed that the strain A3 was closely related with *Sphingobium yanoikuyae* ATCC 51230^T, and when tested on R2A medium, the growth of strain A3 was observed at a temperature range of 15 to 35℃ and pH range of 5.0-9.5.

Keywords : Biodegradation, bisphenol A, *Sphingobium* sp. A3

C-18

Effect of *Rouxiella* sp. S1S-2 on *in vitro* Assay for Odor Reduction in Livestock Eenvironment

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Odor generation is one of the most common concern in livestock farm. In this study, among various odorous compounds, we focused on microbial odor control against ammonia (NH3) and amine. For this, we performed *in vitro* assay to screen bacterial strains harboring odor reduction potential. The evaluation of NH3 and amines removal efficiencies using gas detector tubes, from control to bacterial-treated pig manure slurry in odor emission chamber, showed that strain S1S-2 showed a high removal efficiency against NH3 and amine odor. Especially, the treatment of 5% (w/v) freeze-dried powder showed removal efficiency of > 85% for NH3 and amines. The bacterial identification using 16S rRNA gene sequencing showed that the strain S1S-2 belong to a strain of the genus Rouxiella. In conclusion, we screened the strain *Rouxiella* sp. S1S-2 harboring high potential of NH3 and amines removal and this strain could be a candidate in the future field experiment studies for odor management in livestock.

Keywords : *Rouxiella* sp. S1S-2, odor reduction, NH3 and amine

C-19

Isolation of Plant Growth-Promoting Rhizobacteria Having Heavy Metal Tolerance and Diesel Degradation Capacity

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Soil contamination with hazardous pollutants such as heavy metals and oil causes the disturbance of the soil biota due to decreasing the microbial activity and diversity. For the remediation of contaminated soils, rhizoremediation is attracting attention as a promising technology. The introduction of promising microbial agents into contaminated soil is one of the strategies to enhance rhizoremediation performance. In this study, heavy metals (Cu, Cd, and Pb) tolerant-rhizobacteria were isolated from the rhizosphere of *Zea mays* and *Festuca arundinacea*, which were cultivated in heavy metals/diesel-contaminated soil. Additionally, plant growth promoting (PGP) traits and diesel removability of the isolates were evaluated. Among the 44 isolates, three strains with outstanding PGP traits (IAA, siderophore, and ACC deaminase productivity), heavy metal tolerance, and diesel removability were selected and identified. *Novosphingobium* sp. CuT1 showed a high resistance of heavy metals at 10 mM of Cu and Pb, this bacterium had a diesel removability of 40.5%. *Sphingomonas* sp. PbM2 and *Bacillus* sp. PbT3 could grow at 1 mM of heavy metals, and the strain PbT3 exhibited a diesel removability of 77.7%. These results suggest that the isolated rhizobacteria have potential as the candidates for enhancing the rhizoremediation performance soil contaminated with heavy metals and diesel.

Keywords : Heavy metal tolerance, plant growth-promoting rhizobacteria, rhizoremediation of contaminated soil

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C-20

Effect of Petroleum Hydrocarbons Concentration on Rhizoremediation and CH_4 Emission

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Rhizoremediation is a sustainable approach for petroleum hydrocarbons (PHs)-contaminated soils using the synergism between plants and microorganisms in rhizosphere. This study investigated the effects of initial PHs concentrations on rhizoremediation performance, CH_4 emission, and bacterial community structures in soil planted with tall fescue (*Festuca arundinacea*). The soils were artificially contaminated with diesel at initial concentrations of 5,000 (T5), 10,000 (T10), 30,000 (T30), and 50,000 (T50) mg-TPHs·kg-soil^{-1}. Five kg of soil was put in a pot, and 10-12 tall fescues seedlings per a pot were planted. The pot experiments were conducted for 85 days. PHs concentrations did not decrease up to day 22, but significantly declined on day 37. On day 85, the PHs removability was 49.8% (T5), 67.2% (T10), 43.6% (T30), and 36.5% (T50). CH_4 emission was affected by initial PHs concentration, in which CH_4 emission was approximately 4 times higher in the highest initial PHs concentration (T50) than that of the lowest one (T5). PHs removability exhibited a positive relationship with *Rhizobium*, *Halothiobacillus*, and *Geobacter*, while CH_4 emission was positively associated with *Stenotrophomonas*, *Acidicapsa*, *Gemmatirosa*. The results in this study provided a comprehensive information for both rhizoremediation and CH_4 emission in PHs-contaminated soils.

Keywords : Rhizoremediation, CH4 emission, petroleum hydrocarbon

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C-21

Degradation of Polyethylene Terephthalate (PET) through Extracellular PETase in *Corynebacterium glutamicum* and Surface - Displayed PETase in *Pichia pastoris*

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Poly (ethylene terephthalate) (PET) is the rigidly structured polyester that is used in fabrics and storage materials. PET causes several natural problems because chemical degradation of polyester plastic requires high-temperature, high-pressure. This process makes large amounts of toxic substances that could destroy the environment. Therefore, it is necessary to find ways to decompose PET in an eco-friendly way. Meanwhile, PET hydrolase (PETase) expressed from Ideonella sakaiensis has been found to have a very high effect on PET decomposition, and this is thought to be the starting point for solving environmental problems caused by plastic. In this study, we designed recombinant PETase from *Corynebacterium glutamicum* and *Pichia pastoris*, respectively. We used secretion signal to make PETase from *C. glutamicum* to move out of the cell wall in *C. glutamicum*, and the enzymatic activity of PETase was verified. Also, we designed a whole-cell biocatalyst by displaying PETase on the surface of *Pichia pastoris* SMD1168. PETase was fused to a *TIP1* DNA(TIP630), encoding the glycosylphosphatidylinositol (GPI)-anchored protein of *Saccharomyces cerevisiae*. The localization of the surface-expressed PETase was confirmed by flow cytometric analysis and immunofluorescence confocal microscopy using FITC-labeled secondary antibody. The degradation of PET to MHET, BHET, and TPA using both recombinant strains was analyzed by HPLC. We report the results here.

Keywords : PETase, secretion signal, glycosylphosphatidylinositol (GPI)-anchored protein

C-22

Optimization of Cultural Conditions for *Rhodococcus* sp. 3-2 for Biodegradation of Fungicide Benomyl and Carbendazim

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The development and increased utilization of chemical fertilizers for agricultural purposes are recognized all over the world. Despite their usefulness, to protect crops and livestock, they have potential risks to food safety, ecosystem and all the living things. Likewise, benomyl and carbendazim that are considered, as a carcinogen is a major pollutant detectable in the environment. In this regard, *Rhodococcus* sp. was reported to reduce the harmful effects of benomyl and carbendazim. Therefore, in this work, the growth conditions of microorganisms such as *Rhodococcus* species that have an efficiency of biodegrading of benzimidazole fungicides was evaluated. Initially, optimization of growth parameters such as carbon source, nitrogen source, mineral source, temperature, pH and agitation speed for *Rhodococcus* sp. were determined in lab scale. In addition, for the mass production and field application of *Rhodococcus* species for agriculture purposes, cost efficient production with higher efficacy using the economical carbon and nitrogen sources are warranted. Therefore, for enhanced growth of *Rhodococcus* sp., we have developed a defined media and optimization were performed for their growth types. Furthermore, the storage stability of *Rhodococcus* sp. in, liquid (L) and new types of formulation in powder form(P) was developed and optimized for their effect on fungicide at different temperature for at least 6 months. Based on the results, L and P were found to maintain their viable cell count at > 1.0 x 10^8 CFU/g at low-temperature conditions. Firstly, growth optimization of *Rhodococcus* sp. in 5L jar-fermenter with the optimized defined medium were carried out and OD at 600nm, pH, viable cell count was determined. Secondly, industrial scale culturing with the optimized defined media (1.5 ton) were carried out, and viable cell counting the liquid samples and powder types. Further, the degradation of benomyl and carbendazim by liquid or powder form of *Rhodococcus* sp. in the field will be studied to increase the crop yield without causing harm to ecosystem and approaching the sustainable agricultural system.

Keywords : Residual pesticide, biodegradation, microorganisms

C-23

Biological Upgrading of 3,6-anhydro-L-galactose from Red Seaweeds to a New Platform Chemical, 3,6-anhydro-L-galactitol for Producing Bioplastics

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Producing petrochemicals from renewable biomass instead of fossil fuels has received much attention in the manner of sustainable development. Recently, marine algae are gaining importance owing to their advantages over lignocellulosic feedstock. Particularly, red microalgae have higher carbohydrate contents and simpler composition among marine algae. In red macroalgal carbohydrates, 3,6-anhydro-L-galactose (AHG) is the main monomeric sugar composing agarose along with D-galactose. However, AHG is not a common sugar and is chemically unstable. Thus, not only monomeric AHG but also red macroalgal biomass itself cannot be efficiently converted or utilized. To break through this problem, we biologically upgraded AHG to its sugar alcohol, 3,6-anhydro-L-galactitol (AHGol), an anhydrohexitol, a new platform chemical that much stable than AHG. For the upgrade of AHG to AHGol, agarose passes a chemical hydrolysis process, producing agarobiose (AB) and a biological process, subsequently, converting AB to AHGol using metabolically engineered *Saccharomyces cerevisiae* for effective production of AHGol with high titers and yields. The produced AHGol was further converted to another platform chemical for plastics, isosorbide. To our knowledge, this is the first that demonstration of upgrading a red macroalgal biomass component to a platform chemical *via* a new biological route, by using an engineered microorganism.

Keywords : Red-microalgae, chemo-bio process, 3,6-anhydro-L-galactitol (AHGol)