UPM-SHIZUOKA UNIVERSITY INTERNATIONAL COLLOQUIUM *2014*

March 3rd 2014
Agriculture Hall, Faculty of Agriculture,
Universiti Putra Malaysia

ABSTRACT

IMPACTS OF HYDROPHILIC POLYSACCHARIDES ON BACTERIAL ATTACHMENT AND MEMBRANE BIOFOULING

Yosuke Tashiro[1], Keitaro Yoshida[2], Thithiwat May[2] and Satoshi Okabe[2]
Affiliation [1]Shizuoka University, Hamamatsu, Shizuoka 432-8561 Japan,
[2]Hokkaido University, Sapporo, Hokkaido 060-8628 Japan
Email: tytasir@ipc.shizuoka.ac.jp (Y. Tashiro)

Membrane bioreactors (MBRs) become important options for wastewater treatment and reclamation. However, membrane fouling hampers the widespread application of MBRs due to severe flux decline and high-energy consumption, leading to operational and maintenance cost increase and frequent chemical washes. Membrane fouling is complex and occurs as a result of accumulation of bacterial cells, extracellular polymeric substances (EPS) and other soluble microbial products on membrane surfaces and membrane pore clogging. The impacts of specific EPS components required for biofilm formation on initial bacterial attachment to MF membranes and subsequent membrane fouling are not well understood. Therefore, further studies are obviously required to better understand the interactions between EPS and membranes.

In this study, we focused on a hydrophilic polysaccharide, which is one of EPS produced by several γ-proteobacteria and has unique characteristics of repressing bacterial attachment at the beginning stage of biofilm formation and developing voluminous three-dimensional biofilms at the later stage. Here we investigated the effects of the hydrophilic polysaccharide on biouling on several properties of membrane materials and obtained new insights into the association between bacterial EPS production and biofouling. We established a novel screening system to detect the bacterial attachment on several kinds of membrane used for the wastewater treatment and found that the hydrophilic polysaccharide inhibits the attachment on hydrophobic membranes. Moreover, we built a bench-scale cross-flow filtration system and investigated the effect of the hydrophilic polysaccheride on the transmembrane pressure, the accumulated biomass on membrane and the filtration resistance. The results showed that the hydrophilic polysaccharide causes severe membrane clogging regardless of low biomass on the membrane. Thus, roles of polysaccharides in biofouling process are complex, and biomass on membrane and biofouling are not coincidental.

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ABSTRACT

POPULATION DYNAMICS AND CELL-TO-CELL INTERACTION IN A MODEL MICROBIAL ECOSYSTEM

Kenshi Suzuki[1], Fatma Azwani[2], Yuma Inuzuka[3], Yosuke Tashiro[1], and Hiroyuki Futamata[1]
[1,3] Shizuoka University, Hamamatsu, 432-8561 Japan,
[2]Universiti Putra Malaysia, 43400 UPM Serdang, Selangor
f0330226@ipc.shizuoka.ac.jp, f0012019@ipc.shizuoka.ac.jp, tytasir@ipc.shizuoka.ac.jp,
achkawa@ipc.shizuoka.ac.jp, thfutam@ipc.shizuoka.ac.jp

Abstract. Cell-to-cell interactions have a crucial role in microbial ecosystems. Since natural environments have a huge diversity of microorganisms interacting each other, it is too complex to analyze all things in microbial ecosystems. In this study, mixed chemostat cultures were constructed to analyze the dynamics and succession of microbial ecosystem by using three different strains, *Pseudomonas* sp. C8, *Ralstonia* sp. P10, and *Comamonas testosteroni* R2. Real-time PCR was used to monitor the population densities of three strains. In a mixed culture containing three strains, they showed equilibrium population densities at early stage. However, C8 and R2 became dominants and P10 was about two orders of magnitude lower than those of C8 and R2 in the transition to carbon starved condition. The other hand, R2 only dominated in coculture with C8. Furthermore, P10 and R2 showed equilibrium population densities in theirs coculture. These results suggested that these strains shared the role in this model ecosystem and some interactions play important roles. To understand bacterial interaction, we characterized the substance to affect the growth. A supernatant from a strain used in this study was filtrated by 0.2 μm membrane and was added (10% [v/v]) in the other culture. These analyses revealed that the supernatant from C8 (C8-supernatant) repressed significantly the growth of other strains. Interestingly, the growth repressed with C8-supernatant was almost completely recovered by addition of $FeCl_3$. Although the growth of R2 was strongly repressed by C8, R2 became dominant in the model ecosystem without addition of $FeCl_3$. This result suggested that R2 has another recovering mechanism from the growth repression with C8-supernantant. To obtain a clue for revealing this interesting subject metabolomic analysis is currently under investigation.

UPM-SHIZUOKA UNIVERSITY INTERNATIONAL COLLOQUIUM *2014*

March 3rd 2014
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ABSTRACT

ANALYSIS OF THE GROWTH REPRESSING COMPOUNDS SECRETED FROM *PSEUDOMONAS* SP.C8

Yuma Inuzuka[1], Kenshi Suzuki[2], Fatma Azwani[3],Yosuke Tashiro[2], Hajime Kobori[4], Wei Tao Qui[4], Hirokazu Kawagisi[4], and,Hiroyuki Futamata[2]
Affiliation: [1, 2]Shizuoka University, Hamamatsu 432-8561 Japan, [3]Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, [4]Shizuoka University, Shizuoka, 422-8529, Japan
Email: f0012019@ipc.shizuoka.ac.jp, f0330226@ipc.shizuoka.ac.jp
tytasir@ipc.shizuoka.ac.jp, thfutam@ipc.shizuoka.ac.jp

Microbes have been contributed to wide-ranging industries. Although most microbial industrials are based on mixed microbial communities, it remains poorly understood how microbes interact there. To maintain efficient microbial activities in microbial industries, it is important to understand cell-cell interactions in complex microbial ecosystems. In the previous study, the interaction of phenol-degrading bacteria including *Pseudomonas* sp. C8, *Ralstonia* sp. P-10 and *Comamonas testosteroni* R2 was analyzed and we found that the supernatant of C8 (C8SP) strongly represses the growth of P-10 and R2. The aim of this study is to understand how they affect complex microbial ecosystems. To analyze the effect of C8SP on environmental microorganisms, microbes derived from the paddy soil was inoculated to an inorganic medium with 0.2 mM phenol in the presence and absence of 10%(v/v) C8SP. When phenol was completely consumed in the culture, 10%(v/v) culture was transferred to a fresh medium with or without C8SP respectively, and this step was repeated 15 times. In the analysis of microbial community dynamics, bacterial community structures were finally stable at different equilibrium, suggesting that C8SP is useful for controlling microorganism community structures. Of C8SP fractionated with different molecular weight, the fraction less than 5000 Da exhibited the growth repression. C8SP was partitioned with *n*-hexane-, ethyl acetate-, *n*-butanol-, and H_2O-soluble components. A growth test using R2 showed that the growth repressing compound(s) was in the H_2O-soluble components. Interestingly, of the H_2O-soluble components fractionated with different molecular weight, some fractions exhibited the growth repression of strain R2 but not strain P-10. Identifying growth-controlling compounds and resolving the mechanism how C8SP affects the growth of microorganisms would establish a new strategy to control complex microbial communities.

UPM-SHIZUOKA UNIVERSITY INTERNATIONAL COLLOQUIUM *2014*

March 3rd 2014
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ABSTRACT

ELECTRONIC FLOW FOR EFFICIENT REDUCTIVE DECHLORINATION OF TRICHLOROETHENE IN A ENRICHMENT CULTURE

Kenji Suzuki[1], Hiroyuki Morioka Naruaki Sato, Teruaki Hanji, Yosuke Tashiro, and Hiroyuki FUTAMATA[2]
Affiliation [1 & 2]Shizuoka University, Hamamatsu 432-8561 Japan
Email: f0912072@ipc.shizuoka.ac.jp, tytasir@ipc.shizuoka.ac.jp, thfutam@ipc.shizuoka.ac.jp

Abstract. Chlorinated ethenes, such as trichloroethene (TCE), are persistent groundwater pollutants. Bioremediation strategies for these contaminanted sites are based on reductive dechlorination. To develop efficient reductive dechlorination, it requires an understanding of the electron flow in microbial ecosystem. A dechlorinating microbial enrichment culture (LS) was constructed with citrate as electron donor, which converted TCE to ethene via *cis*-1,2-dichloroethene and vinyl chloride (VC). The 5th transfer culture LS completely dechlorinated TCE to ethene in about 3 weeks. Methane production rate increased after completion of dechlorination. It took over 3 months to dechlorinate TCE completely in the 6th LS, mainly because of slow VC-dechlorinating rate. Methane was produced more in the dechlorination process. Accumulation and decrease of acetate corresponded to methane production in the 5th and 6th LS. Archaeal communities in 5th LS and 6th LS were compared to clear how methane was produced. Clone library analyses targeting archaeal 16S rRNA gene revealed that hydrogentrophic methanogen *Methanocorpusculm* spp. were dominant archaea in the 5th LS, and aceticlastic methanogen *Methanosaeta* spp. were dominant in the 6th LS. Real-time PCR analysis showed similar population densities of *Dehalococcoides* in both LSs. These results indicated that the interspecies hydrogen transfer between anaerobic acetate oxidizers and *Dehalococcoides* might be important for efficient dechlorination. Furthermore it was suggested that there is need to obviate the competition between anaerobic acetate oxidizers and aceticlastic methanogens. The effect of lactate on dechlorination property of 9th LS was investigated, and the result showed that the addition of lactate promotes dechlorination of vinyl chloride (VC), suggesting that lactate was powerful tools for controlling electronic flow, resulted in efficient TCE-dechlorination.

UPM-SHIZUOKA UNIVERSITY INTERNATIONAL COLLOQUIUM *2014*

March 3rd 2014
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ABSTRACT

EFFECT OF EXTERNAL RESISTANCE ON THE ELECTRICITY GENERATION AND MICROBIAL COMMUNITY STRUCTURE IN MICROBIAL FUEL CELLS

Hiroko Kubota, Yutaka Kato, Kei Suzuki, and Hiroyuki Futamata
Shizuoka University, Hamamatsu, Japan, 432-8561
f0012046@ipc.shizuoka.ac.jp, f5345018@ipc.shizuoka.ac.jp, and thfutam@ipc.shizuoka.ac.jp

Abstract Format. Effects of external resistance on microbial ecosystem and electricity-producing properties in microbial fuel cells (MFCs) were investigated. Two kinds of MFCs were constructed with lake sediment as inoculum, lactate as electron donor and different external resistances, 10 Ω (LR-MFC) and 1000 Ω (HR-MFC). Polarization curve analyses revealed the electricity producing potential of these MFCs. By comparing with the potential of LR-MFC, HR-MFC enabled to decrease and maintain the internal resistance lower, resulted in the production of higher power density. Cyclic voltammetry analyses revealed that the electricity producing property of LR-MFC changed from indirect extracellular electron transfer (EET) to direct EET, while direct EET had occurred in HR-MFC. These results indicated that exoelectrogens-biofilm had developed on the surface of anodes in both MFCs. Multidimensional scaling (MDS) analyses based on PCR-DGGE profiles revealed that bacterial community structures of LR- and HR-MFCs were almost similar until day 38, after which these bacterial community structures were significantly different from each other and were stable at different dynamic equilibrium with fluctuation. The dynamic equilibrium of HR-MFC fluctuated less than that of LR-MFC, suggested that selective bias for bacterial enrichment on the anode of HR-MFC was stronger than that of LR-MFC. Clonal analyses targeting 16S rRNA gene showed that *Geobacter* spp. dominated approximate 20% and 32% of biofilm communities on the anode of LR- and HR-MFCs at day 197, respectively. However, the proportion of *Geobacter* spp to analyzed clone number decreased 5% and 0% on the anode of LR- and HR-MFCs at day 427, respectively. On the other hand, *Firmicutes* dominated 40 to 60% of biofilm communities on the anode of LR- and HR-MFCs at day 427. These results suggested that higher external resistance is useful for controlling the exoelectrogen ecosystem, in which *Firmicutes* instead of *Geobacter* was selectively enriched on the surface of anode.

UPM-SHIZUOKA UNIVERSITY INTERNATIONAL COLLOQUIUM *2014*

March 3rd 2014
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ABSTRACT

PRODUCING MECHANISM OF RECHARGEABLE BIOMINERAL

Yusuke Chiba, Kei Suzuki, Hiroki Mochihara, Yusuke Tashiro and Hiroyuki Futamata
Shizuoka University, Hamamatsu, Japan, 432-8561
yusuke070809@yahoo.co.jp, f5345018@ipc.shizuoka.ac.jp, h.mochihara@yahoo.co.jp,
tytasir@ipc.shizuoka.ac.jp and thfutam@ipc.shizuoka.ac.jp

Abstract. Microorganisms that were obtained from the biofilm on the surface of anode in an MFC produced precipitate under anaerobic conditions with lactate as electron donor and ferric citrate as electron acceptor. Interestingly, the current density was increased approximate 80-folds by addition of the precipitate into anode chamber in MFC. Furthermore, electrochemical analyses demonstrated that the precipitate was one of rechargeable biomineral. Scanning electron microscope (SEM) and energy dispersive X-ray spectrometry (EDX) analyses revealed that the shape was flat and polygon and that the biomineral consisted of phosphorus, iron, calcium, titanium, and oxygen derived from a medium. When the biomineral was produced, the sulfate was consumed completely. In parallel, the concentration of ferric ion in a supernatant decreased, and the concentration of ferrous ion in the biomineral increased concomitantly. The biomineral was not produced under the condition of pH 4.7 and the sulfur in the supernatant was not consumed. The biomineral was produced under the absence of sulfate but the iron in the biomineral was almost ferric. These results indicated that the reduced sulfate (sulfide) was required for reduction of ferric. Denaturing gradient gel electrophoreses analysis was performed to investigate what kinds of microorganisms play major role in biomineralization. The results showed that *Pelosinus* sp. and *Desulfovibri*o sp. dominated in the compound-producing culture. These results suggested that sulfate-reducers and iron-reducers played major role in the biomineralization including chemical reaction. However, it is unknown that how bacteria produce the biomineral, how the biomineral is charged and what kinds of chemical reaction occur. These interesting subjects are currently under investigation.

UPM-SHIZUOKA UNIVERSITY INTERNATIONAL COLLOQUIUM *2014*

March 3rd 2014
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Universiti Putra Malaysia

ABSTRACT

DEVELOPMENT OF THE EFFECTIVE WASTEWATER TREATMENT PROCESS BY CONTROLLING ELECTRONIC FLOW

Takuya Hosokawa[1], Yosuke Tashiro[2], Hiroyuki Futamata[2]
[1,2] Shizuoka University, Hamamatsu, 432-8561 Japan
f0012116@ipc.shizuoka.ac.jp, tytasir@ipc.shizuoka.ac.jp, thfutam@ipc.shizuoka.ac.jp

Activated sludge process under aerobic conditions is used in wastewater treatment. However, there are some problems, including cost and greenhouse gas generation. Anaerobic wastewater treatment would be a way to solve these problems by inhibiting excess generation of activated sludge. But anaerobic wastewater treatment needs higher treatment efficiency under low concentration of chemical oxygen demand (COD) towards the practical use. To exploit the efficient anaerobic wastewater treatment, microbial fuel cells (MFCs) were constructed to control electronic flow under anaerobic condition. We prepared 6 reactors containing microbes derived from paddy soil, and investigated the effect of biominerals (BM), which is formed by microbes under anaerobic conditions, on electricity generating capacity. COD removal efficiency indicates the performance of wastewater treatment, and is important factor for continuously treatment. In the MFC without BM (Reactor IV), COD remained approximate 1500 mg/L and propionic acid was accumulated. On the other hands, the MFC with BM (Reactor V) showed that 76% of COD was removed for 10 days. The amount of coulomb obtained from Reactor V was about 800-folds higher than Reactor IV. In theory, Reactor V was capable of repressing 36% of the generation of excess sludge compared with aerobic condition. These results suggested that the electronic flow in the system changed by adding BM, suggested the potential for effective wastewater treatment under anaerobic condition.

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March 3rd 2014
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ABSTRACT

INTERSPECIFIC CELL-CELL COMMUNICATION WITH MEMBRANE VESICLES SECRETED BY BACTERIA

Yusuke Hasegawa[1], Hiroyuki Futamata[2] and Yosuke Tashiro[2]
Affiliation [1]Department of Physical Engineering, Faculty of Engineering, Shizuoka University, Department of Applied Chemistry and Biochemical Engineering, Graduate School of Engineering, Shizuoka University
Email: f0012105@ipc.shizuoka.ac.jp, thfutam@ipc.shizuoka.ac.jp and tytasir@ipc.shizuoka.ac.jp

Abstract: Many bacteria secrete membrane vesicles (MVs), which are bacterial extracellular particles in the size of 50-250 nm-diameter and have liposome structures. It has been known that MVs have a role in the transfer of several signals for the cell-cell communication. However, it remains unknown how they behave among interspecies bacteria. This study aims to understand what type of the behavior MVs show in heterogeneous communities and to find out a mechanism of MV fusion to the cell. Here we screened high MV-producing bacterial strains with a high-throughput MV quantitative method and characterized their MVs with the transmitted electron microscopic (TEM) imaging analysis. *Buttiauxella agrestis* JCM 1090^T, which was the highest MV-producing bacterium tested in our experiments, was used as a model MV donor and the attachment of MVs on bacterial cells was investigated by flow cytometry and TEM imaging analysis. The result showed that MVs derived from *B. agrestis* JCM 1090^T easily attach to own cells as compared to other bacterial cells, suggesting that *B. agrestis* JCM 1090^T MVs are used as an intraspecies communication tool rather than interspecies. Here we also established a method of analyzing the interaction between MVs and cells. It will hold the promise of building a system, in which MVs convey useful materials to target cell. The final goal of this study is to control specific cells in heterogeneous communities with bacterial MVs.