

# 主論文の要約

論文題目 **Study on heterotrophic denitrification systems requiring less organic carbon assisted by solid-phase humin**  
(固体ヒューミンを用いた有機炭素消費抑制型の従属栄養脱窒システムに関する研究)

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## 論文内容の要約

Nitrate contamination of groundwater is one of the most globally widespread environmental problems. The major source is nitrogen fertilizer applied in the agriculture area. Researchers have been testing ways to remove nitrate from groundwater. The traditional technologies such as ion exchange, reverse osmosis and electro-dialysis are energy consuming and costly. In contrast, heterotrophic denitrification using microorganisms to convert nitrate to nitrogen gas coupled with organic carbon oxidation to carbon dioxide was applied extensively in the groundwater nitrate remediation. However, excess of biomass growth, residues of organic carbon introduced and microbial metabolites in groundwater hampered its application. These limitations have provoked research efforts to develop energy-efficient alternatives for reducing the organic carbon usage. Thus, a study is conducted to develop microbial

denitrification systems requiring less organic carbon source and to elucidate the role of solid-phase humin in microbial denitrification reactions.

In Chapter 1, some basic concepts such as microbial denitrification, bioelectrochemical systems, and solid-phase humin are introduced as essential background. The motivation of this study has been also described.

In Chapter 2, a bioelectrochemical system (BES) assisted by humin has been developed as one of the approaches to reduce the organic carbon usage for heterotrophic microbial denitrification. A harmless, non-electrotrophic *Pseudomonas stutzeri* was selected as a bacterium for BES. The electrochemically-reduced humin enhanced the microbial denitrification reactions of *P. stutzeri* but not abiotic nitrate reduction. The enhanced current for denitrification was observed only with the presence of both *P. stutzeri* and solid-phase humin. This corresponded with the results that the cyclic voltammetry analysis showed the redox capacity only in the *P. stutzeri* BES incubated with solid-phase humin. These results suggested that the solid-phase humin functioned as a direct electron donor for the denitrification reactions of *P. stutzeri*. Nitrogen balance and the estimation of the first order rate constants of the consecutive reactions in denitrification suggested that the electrochemical enhancement appeared mainly in the reduction of nitrite to nitrous oxide, while the solid-phase humin enhanced whole reducing reactions from nitrate to nitrogen gas. The achieved nitrate removal rate was  $0.07 \text{ g NO}_3\text{-N/m}^3\cdot\text{cm}^2\cdot\text{h}$ , which was higher than the previous reports ranging from  $0.004\text{--}0.008 \text{ g NO}_3\text{-N/m}^3\cdot\text{cm}^2\cdot\text{h}$ , suggesting the humin-containing *P. stutzeri* BES poised at  $-500 \text{ mV}$  (vs. SHE) was highly competitive with other existing denitrifying BESs. It is worth noting that this high nitrate removal rate was achieved electrochemically with non-electrotrophic *P. stutzeri* by using solid-phase humin.

In Chapter 3, a synthetic microbial co-culture system has been introduced as another approach to reduce the organic carbon usage in denitrification. The co-culture system is comprised of an acetogenic bacterium *Sporomusa ovata* and a denitrifying bacterium *P. stutzeri*, with hydrogen as a sole external electron donor and CO<sub>2</sub> as the carbon source. Nitrogen balance study showed the reduction of nitrate to nitrogen gas without the accumulation of nitrite and nitrous oxide in the co-culture system. *S. ovata* did not show nitrate reduction to ammonium in the co-culture system. Addition of humin enhanced the nitrate reducing capacity when acetate was deficient in the system. The electron balance analysis indicates that significant portion of hydrogen was utilized for denitrification in co-culture systems. The effects of humin in the co-culture system were the additional electron donor and redox conditioner. The nitrogen removal rate of the humin-containing co-culture system reached to 0.19 Kg NO<sub>3</sub><sup>-</sup>-N. m<sup>-3</sup>. d<sup>-1</sup>, the mass ratio of carbon (consumed as acetate) to nitrogen (removed by nitrate reduction) was estimated to be 1.34 ± 0.16, The stable denitrification activity during 61 days of successive subculturing suggested the robustness of co-culture systems.

In Chapter 4, humins extracted from eight soils and a river sediment have been examined on the effect on microbial denitrification, and characterized polyphasically. The results demonstrated that humins extracted from Nagoya university farm soil fertilized by cattle manure, Kamajima soil, Nagano paddy soil and Nagano upland soil enhanced the microbial denitrification of *P. stutzeri*, while humin extracted from Arako river sediment showed inhibition effect. Although Fourier-transform infrared analysis showed that all the humins exhibited similar spectra with different absorbance intensity, indicating their similar chemical structure of functional groups, the elemental compositions varied among sources. Statistical analyses showed the organic part and

aromaticity of humin may be factors affecting the reducing capacity of humin, while the protein-derived organic matter contained in humin was not considered as the functional group in this case.

In Chapter 5, the achievements of this study and their environmental significance are summarized, for example, the findings that humin enabled electron transfer from electrode to non-electrotrophic bacteria has important implications for the widespread application of BES for enhancing the microbial denitrification. In addition, the remaining issues and important points for the application of the studied system have been also pointed out.

In Chapter 6, the conclusion is drawn and the possible application of humin-assisted BES and co-culture system are also prospected.

Thus, this study has established a BES and a co-culture denitrification system for heterotrophic denitrification requiring less organic carbon. Both systems were assisted by solid-phase humin and functioned efficiently for the nitrate removal without using external organic carbon source. The results also extended our understanding that how humin works in the microbial denitrification reactions. This study provides a basis to design a humin-assisted bioremediation strategy with low organic carbon usage for groundwater nitrate removal.