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主　論　文　の　要　旨

論文題目　Promotion of Biological Nitrogen Fixation and Biological Hydrogen Production using Humin as Extracellular Electron Mediator
(細胞外電子伝達物質の固体腐植ヒューミンを用いた生物学的窒素固定および生物学的水素生産の促進)

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論　文　内　容　の　要　旨

This study was conducted for the promotion of two important microbial reactions, biological nitrogen fixation (BNF) and biological hydrogen (Bio-H_2) production, by using extracellular electron mediating function of solid-phase humin under anaerobic conditions. Both reactions are crucially important not only for maintaining global biogeochemical cycle but also have great impact on socio-economic and global environmental health. However, slow kinetic of natural BNF and low product yield of Bio-H_2 owing to metabolic constrains of Bio-H_2 producers leads to the supreme dependency on energy and carbon intensive artificial nitrogen fixation and H_2

production, in last few decades. This study achieved significant promotion in both BNF and Bio-H_2 production using humin.

Promotion of BNF was observed for both anaerobic microbial consortia enriched under nitrogen deficient condition as well as different nitrogen fixing type strains (known as diazotrophs). Promotion of Bio-H_2 was obtained in dark fermentative hydrogen production (DFHP) by anaerobic microbial consortia enriched under nitrogen deficient condition. Humin extracted from Kamajina paddy field soil was used in this study as model humin

The first outcome of the study was the identification of the role of humin as redox mediator to promote BNF of the microbial consortium, in which electrochemically reduced humin worked as sole electron donor to activate the BNF of washed and starved microbial consortium under anaerobic condition without presence of any other external organic carbon as electron source. In the presence of organic carbon, humin was reduced by humin reducers in the microbial consortium, and the reduced humin further donated electrons to the consortium for promoting BNF activity of the consortium to the levels of 5.7-11.8 times under nitrogen-deficient conditions, showing the potential of humin as extracellular electron mediator (EEM) to promote BNF. Promotion of anaerobic BNF activity was also observed using various humin from

different origins although the degree of promotion differed, which suggested the ubiquitous phenomenon of humin to promote BNF. The next generation sequencing (NGS) of partial 16S rRNA genes showed the predominance of Clostridiales (Firmicutes) in the consortia. These findings suggest the effectiveness of humin as a solid-phase extracellular electron mediator for the promotion of anaerobic BNF activity.

The second outcome of the study showed that humin has unique potential to promote DFHP by the N-fixing anaerobic consortium through the supply of extracellular electrons to both nitrogenase and hydrogenase of the microbial consortium. Presence of humin promoted the Bio-H_2 production beyond the capacity of the consortium, which had reached saturation with the optimum concentrations of the two different organic carbon, glucose, and mannitol, as substrate. This consequently achieved the higher product yields of Bio-H_2 under nitrogen-deficient conditions with glucose as substrate and humin as the extracellular electron mediator: 3.74–4.12 mol-H_2/mol-glucose. These findings also showed a new approach of DFHP through the simultaneous action of nitrogenase and hydrogenase of the microbial consortium. *Clostridium* and *Ruminococcus* were suggested as major hydrogen producers in the consortia

The final part of the study was carried out to investigate the taxonomic spectrum of different diazotrophs promoted by humin, level of BNF promotion and mechanism of

BNF promotion by humin. The findings obtained here showed that humin possesses the potential to promote BNF activity of a broad spectrum of diazotrophs, including alpha-, beta-, gamma-, and delta-Proteobacteria, Firmicutes, Actinobacteria, and Archaea, which harbor nitrogenases classified into clusters I, II, and III, regardless of whether molybdenum or vanadium nitrogenase, by extracellular electron transfer to intact microbial cell. The level of promotion reached in the range from 194 % *of Azotobacter vinelandii* (the lowest) and 916 % (the highest) of *Ensifer fredii* in the cultures compared to the level achieved by the organic carbon source in absence of humin (100 %). This level of BNF promotion was achieved by the reduced humin, causing ATP synthesis in the cells of diazotroph in absence of any other external electron source as well as direct electron donation to the Mo-Fe protein of the nitrogenase in the cells without relying on the biological electron transfer system. Therefore. it can be suggested that these mechanisms could result in BNF promotion in wide spectrum of non-modified (wild type) diazotrophs beyond the level of their biochemical capacity.

In conclusion, the findings of this study show promising function of humin to promote BNF and Bio-H_2 production as EEM. Therefore, considering the promotion of BNF activity and Bio-H_2 production under nitrogen-deficient conditions and the ubiquitous distribution of humin in the environment, the outcomes of this study would contribute

for the basis to the development of a sustainable technology toward greener nitrogen fixation and Bio-H_2 production.