

Civil Engineering Research



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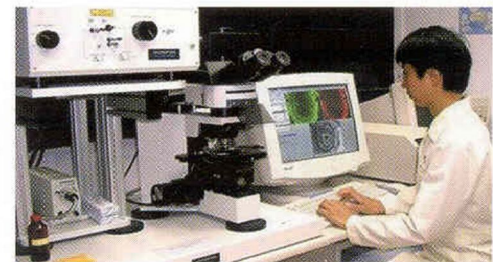
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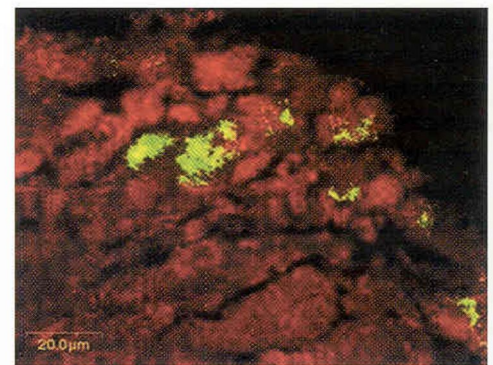
Achieving Excellence in Civil Engineering Research

Civil engineers have traditionally played an important role in developing infrastructures which shape city skylines and enable the exploitation and harnessing of the earth's natural resources that underpin the economic growth engine. Human activities create stresses on the environment and affect the natural cycles in soil, atmosphere and water bodies. These stresses have led to the degradation of natural habitats and adverse global scale environmental impacts such as ozone layer depletion and global warming. As civil engineers address problems associated with aging urban infrastructures, growth in human population and economic activities continues to exert ever increasing demand for new infrastructures. Amid signs of intensifying natural disasters such as floods, forest fires, typhoons and earth quakes, civil engineers also have to face emerging needs to protect the population against manmade threats such as biological and chemical terrorism and structural blast damages. There is also an increasing awareness of the need to build resource-efficient structures to preserve the environment, and maintain a sustainable relationship with the earth's natural resources. Civil engineers are being challenged to deliver products and services of higher quality and greater efficiency. To remain efficient and relevant, civil engineers will need to continue to advance the knowledge in such diverse disciplines as solid mechanics, environmental fluid dynamics, structural dynamics, smart materials technology, and molecular biotechnology.

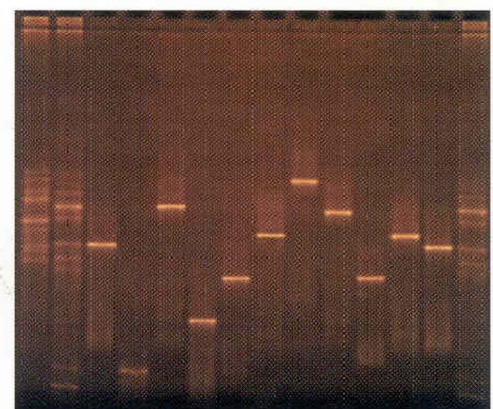
The School, besides performing its primary role of providing education, also places equal emphasis on research and development of innovative engineering solutions to problems relevant to the natural and built environment. The R & D efforts of the School have gained recognition from the industry as reflected by its winning of two **Singapore Institution of Engineers Prestigious Engineering Achievement Awards** in 2004. The first award was for outstanding research achievements in **Biogranulation**, a powerful and versatile system for high-performance biological wastewater treatment developed by a research team led by **Prof Tay Joo Hwa**. In this system, compact microbial aggregates or "granules" are cultivated which can be



Using a confocal laser scanning microscope to investigate granular biofilms



Confocal laser scanning microscope image of a granule section hybridized with molecular probes



DNA fingerprint techniques are routinely used to investigate the microbial ecology of the granular biofilms

Application of Iron-Reducing Bacteria for Phosphate Removal from Returned Liquor of Municipal Wastewater Treatment Plant

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Introduction

Discharge of phosphorus into surface water caused its eutrophication to appear in excessive growth of phototrophes, dissolved oxygen depletion, and decline of water quality. The environmental standards of different states recommend that total phosphorus concentrations in streams discharging into reservoirs should be less than 0.05 - 0.10 mg l⁻¹. Meanwhile, the concentration of phosphate in municipal wastewater is usually from 1 to 20 mg l⁻¹. Significant portion of phosphate, from 10% to 50%, is supplied to aerobic tanks of wastewater treatment plant (WWTP) with returned liquor, which is liquid fraction after anaerobic digestion of activated sludge and anaerobic sludge dewatering. Phosphate concentration in returned liquor is from 50 to 200 mg l⁻¹. Flow rate of returned liquor is approximately 4% of sewage flow rate. Therefore, removal of phosphate from returned liquor can significantly decrease supply of phosphate to aerobic tank of WWTP. A new method for phosphate removal or recovery from wastewater is an application of iron-reducing bacteria (IRB) for reduction of Fe(III) in cheap iron hydroxides, for example, iron ore, wetland iron ore, or iron-containing clay [1]. IRB produce Fe(II) precipitating phosphate [2,3]. Applications of microbial reduction of iron in the treatment of fat-containing wastewater [1], removal of phosphate during anaerobic digestion of activated sludge [3], and the combination of microbial reduction and microbial oxidation of iron for the treatment of food-processing wastewater [4] have been reported.

The aim of this research was to examine efficiency of enrichment culture and isolated strains of IRB for the removal of phosphate from returned liquor of municipal WWTP using ferric hydroxide as a source of Fe(III).

Materials and methods

Suspension of anaerobic sludge with the content of total solids (TS) 5.5% (w/v) was collected from the local municipal wastewater treatment plant. Returned liquor was obtained by centrifugation of suspended anaerobic sludge at 3000 rpm for 15 min. Suspension of ferric hydroxide was prepared by slow neutralization of 250 mmol l⁻¹ FeCl₃ solution by 2 mol l⁻¹ NaOH. Selection of enrichment cultures was made in an anaerobic chamber Bacton Anaerobic/Environmental Chamber (Sheldon Manufacturing, Inc., Cornelius, OR, USA), which was filled in by the following mixture of gases, v/v: N₂, 90%; H₂, 2.5%; CO₂, 2.5%. Pure culture of iron-reducing bacteria (strain BK) was isolated on the solid agar medium with returned liquor. Phylogenetic identification of IRB pure cultures was conducted using PCR of 16S rRNA gene and its sequencing. The interaction

of IRB cells with the particles of Fe(OH)₃ was observed with epifluorescent microscope BX-FLA-3 (Olympus, Japan) and confocal laser scanning microscope (CLSM) Fluoview300 (Olympus, Japan). 120 ml bottles were filled with 50 ml of mixture of return liquor, distilled water, suspension of ferric hydroxide and inoculum of iron-reducing bacteria. The mass ratio between Fe(III) added to initial phosphate concentration was 2. Negative control was a process without additions of Fe(III) and inoculum. Abiotic control was performed by the addition of 15% (v/v) of ethanol. The concentration of phosphate was measured every three days by Vanadomolybdophosphoric Acid Colorimetric Method. The Phenanthroline Method was used to determine the concentration of Fe(II). The concentration of volatile fatty acids (VFA) was determined using HPLC (Perkin Elmer, Norwalk, CT, USA).

Results and discussion

Anaerobic sludge from municipal wastewater reclamation plant served as an inoculum for enrichment cultures of iron-reducing bacteria. The characteristics of anaerobic sludge were as follows: a moisture content, 94.5±1.4%; volatile solids content, 64.7±0.2% of TS; total iron concentration, 24.6±0.9 mg g⁻¹ of TS; pH, 8.3±0.1. The composition of returned liquor was as follow, mg/l: total organic carbon, 679; total Fe(II), 65; soluble Fe(II), 58; PO₄³⁻, 128; and volatile fatty acid (VFA), 1097. Concentrations of VFAs in returned liquor were as follow, mg l⁻¹: acetic acid, CH₃COOH, 10; isobutyric acid, (CH₃)₂CHCOOH, 531; valeric acid, CH₃(CH₂)₃COOH, 159; isocaproic acid, (CH₃)₂CH(CH₂)₂COOH, 397.

High content of iron in anaerobic sludge ensured the presence of iron-reducing bacteria in its microbial community. The final concentration of Fe(II) in enrichment culture was 345 mg l⁻¹. Efficiency of biotransformation of Fe(III) into Fe(II) was 88%. The IRB concentration in EC increased during 10 days of cultivation from 10³ colony-forming units (CFU) ml⁻¹ at the beginning of the process to 10⁷ CFU ml⁻¹ and 10⁸ CFU ml⁻¹ after seven and 10 days of cultivation, respectively. According to CLSM, almost all bacterial cells were alive; percentage of viable cells was 93%, and share of dead cells was 7% (Figure 1).

The cells of iron-reducing bacteria were embedded into slime and attached to small (5 to 20 μm) particles of iron hydroxide. There were no cells attached to big (100 to 500 μm) particles of Fe(OH)₃ without slime matrix (Figure 2). These data confirm the suggestion that direct contact between iron-reducing bacteria and Fe(III) sources is required for Fe(III) reduction.

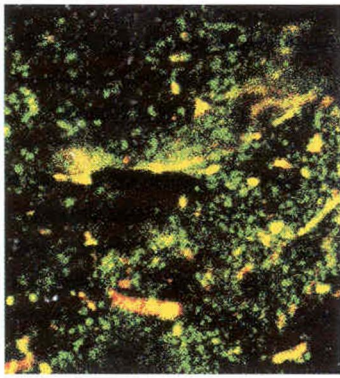


Figure 1. Stained cells of IRB on the surface of iron hydroxide particles. Viable cells are green and dead cells are yellow ones.

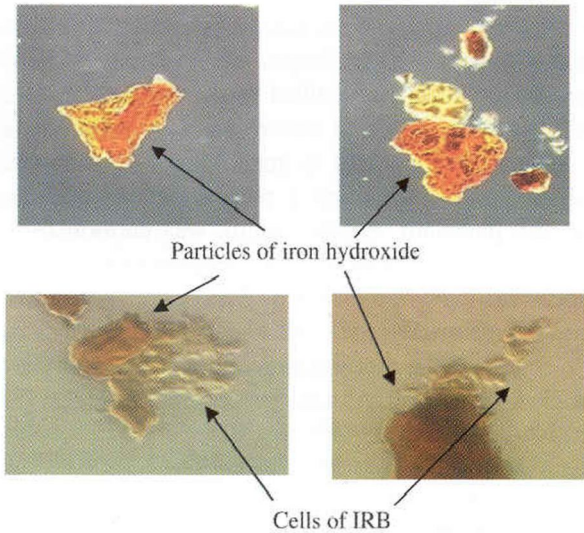


Figure 2. Interaction of IRB cells with the particles of iron hydroxide.

Pure culture of iron-reducing bacteria (strain BK) was isolated on the solid agar medium with returned liquor. It was Gram-negative, rod-shaped facultative aerobe. The determination of the nearest phylogenetic neighbor sequences for 16S rRNA gene sequences of the strain BK by BLAST search program showed that it was the representative of species *Stenotrophomonas maltophilia* close to strain *S. maltophilia* SB5 (identity = 99%). The full sequence for *S. maltophilia* strain BK has been deposited in the GenBank database under accession number AY641540. The iron reduction and phosphate removal efficiencies of strain BK and enrichment culture were studied (Figure 3).

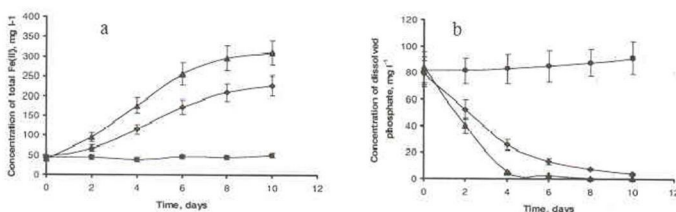


Figure 3. Changes of total ferrous concentration (a) and phosphate concentration (b) during the experiments with the different inocula of iron-reducing bacteria: no inoculum (●), inoculum of pure culture of *S. maltophilia* strain BK (◆), inoculum of enrichment culture (▲).

The initial contents of VSS were 0.37 mg ml⁻¹ and 0.63 mg ml⁻¹ in the experiments with strain BK and enrichment culture, respectively. The maximum rates of Fe(II) production were 2.58 mg g⁻¹ VSS h⁻¹ and 2.96 mg g⁻¹ VSS h⁻¹ for enrichment and

pure cultures, respectively. There were no significant changes in the content of Fe(II) neither in control (without addition of Fe(III) and inoculum), nor in abiotic control (Fig. 3). The concentration of phosphate decreased from 82 to 7 mg l⁻¹ after 6 days of incubation and to 5 mg l⁻¹ after 4 days of incubation when strain BK or enrichment culture was applied, respectively. The rates of phosphate removal were 33 mg g⁻¹ VSS d⁻¹ for the strain BK and 30 mg g⁻¹ VSS d⁻¹ for enrichment culture. The concentration of phosphate increased from 81 mg l⁻¹ to 91 mg l⁻¹ in control on day 10. It was stable in abiotic control, 83 mg l⁻¹.

Organic matter of returned liquor from anaerobic digester of activated sludge consisted mainly of branched fatty acids. The majority was represented by isobutyric and isocaproic acid. Decomposition rate of fatty acids (C₂ - C₆) with a branched chain (iso form) is smaller than that of acids with a straight chain. Maybe, the presence of branched fatty acids in returned liquor caused slow growth rate of IRB as well as rates of Fe(III) reduction and phosphate removal. Notwithstanding initial high concentration of Fe(II) (around 50 mg l⁻¹) in returned liquor, phosphate was not precipitated in control. It probably caused the chelation of Fe(II) with organic acids and inability of chelated Fe(II) to react with phosphate and precipitate it. Therefore, the important function of IRB was not only the reduction of Fe(II), but also biodegradation of branched fatty acids preventing precipitation of phosphate by iron chelation. The rate of iron reduction of *S. maltophilia* strain BK was 2.96 Fe(III)g⁻¹ VSS h⁻¹ more than that of the enrichment culture. The rate of phosphate removal was 33 mg g⁻¹ VSS d⁻¹ for the strain BK. Thus, environmental engineering conclusion is that it would be possible to remove or recover phosphate from returned liquor with initial phosphate concentration of 70 mg l⁻¹ for 0.5 h of hydraulic retention time if the biomass of iron-reducing bacteria in bioreactor for the treatment of returned liquor is increased to 99 g l⁻¹.

Conclusion

Bacterial reduction of ferric hydroxide enhanced phosphate removal from returned liquor. The obtained data could be used for the design of a new biotechnology of anaerobic removal or recovery of phosphate from returned liquor of municipal WWTP.

References

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