

Analysis of Microbial Communities Responsible for Enhanced Biological Phosphorus Removal in an Anaerobic/Aerobic SBR Reactor using Stable Isotope Probing Approaches

Shipeng Lu, Minjeong Park, Che Ok Jeon

EB-NCRC, Gyeongsang National University, Jinju, GyeongNam, 660-701, Korea

TEL: +82-55-751-6252, FAX: +82-55-759-9363

Abstract

Microbial communities responsible for enhanced biological phosphorus removal (EBPR) were analyzed using DNA-stable isotope probing (SIP)¹ and RNA-SIP² approaches in an anaerobic/aerobic sequencing batch reactor (SBR) supplied with acetate as sole carbon source. The SBR was inoculated with activated sludge from a wastewater treatment facility performing just carbon removal. SBR was operated with a cycle of fill, anaerobic, aerobic, settling, and drawing phases. Phosphate release during the anaerobic period and phosphate uptake in the aerobic period gradually increased with time. After about 2-month, steady state operation with complete phosphorus removal could be achieved. At 3-month operation, the enriched microbial community was subjected to SIP with ¹³C-labeled sodium acetate to biomark the DNA of phosphorus accumulating microorganisms. The extracted DNA and RNA were fractionated using CsCl and CsTFA ultracentrifugation, respectively. The fractionated [¹³C] DNA and RNA from the SIP experiment were separately subjected to t-RFLP and rRNA gene sequence analyses. Small subunit ribosomal DNA inserts from two 16S rRNA gene libraries were PCR amplified and digested using *Hae*III and *Hha*I to analyze restriction fragment length polymorphism (RFLP). The dominant clones (11 of 42 clones) in the [¹³C] DNA clone library was closely related to *Dechloromonas* with low sequence identity within the *betaproteobacteria*, while the dominant clones (25 of 41 clones) in the [¹³C] RNA clone library were affiliated into uncultured *betaproteobacteria* from environmental samples.

References

1. Jeon, C.O., W. Park, P. Padmanabhan, C. DeRito , J. R. Snape, and E.L. Madsen (2003) Discovery of a Previously Undescribed Bacterium with Distinctive Dioxygenase that is Responsible for *in situ* Biodegradation in Contaminated Sediment. *Proc. Nat. Acad. Sci.* **100** (23), 13591-13596.
2. Manefield M., A. S. Whiteley, R. I. Griffiths, and M. J. Bailey (2002) RNA Stable Isotope Probing, a Novel Means of Linking Microbial Community Function to Phylogeny. *Appl. Environ. Microbiol.* **68**, 5367-5373.