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Isolation and Characterization of Denitrifying Monoterpene Degraders

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Enrichment cultures capable of various monoterpenes degradation under O_2-free denitrifying conditions were established with ditch samples from forest, and the transformations of monoterpenes were observed in the enrichment cultures. Geraniol(4mM) were totally degraded after an incubation period of 6 weeks and linalool(4mM), nerol(4mM) after 4weeks incubation. PCR cloning and sequencing of environmental 16s rDNA genes were used for the characterization of microbial communities of enrichment samples. The enrichments showing positive activity for the degradation of geraniol, linalool and nerol contained 22, 3 and 5 bacterial isolates, respectively.

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Bacterial Community Analysis of Good and Poor Denitrifying Activated Sludges from Continuous Reactors

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The bacterial community structures of good (90%) and poor (24%) denitrifying activated sludges were analyzed by culturable methods and non-culturable methods. The sludge samples were obtained from two continuous reactors (CRs) for nitrate removal. In culturable methods, eight denitrifying bacteria (DB) and two nitrate-reducing bacteria (NRB) were isolated from activated sludge in the good denitrifying continuous reactors (CR-Ⅰ), and four DB and three NRB were isolated in the poor denitrifying continuous reactor (CR-Ⅱ). *Pseudomonas* sp. (especially, rRNA group Ⅰ *pseudomonas*) was isolated as a predominant part of the DB in both CRs. Most-probable number (MPN) of NRB and NIOB in CR-Ⅰ were 2.82×10^7 and 6.31×10^4 CFU/ml, respectively and MPN of NRB and NIOB in CR-Ⅱ 2.69×10^4 and 9.33×10^3 CFU/ml, respectively. In non-culturable methods, total cells were 3.71×10^{12} in CR-Ⅰ and 5×10^9 in CR-Ⅱ cells per ml of activated sludge by 4,6-diamidino-2-phenylindole (DAPI) staining method. The proteobacteria specific probe EUB 388 visualized approximately 76% in CR-Ⅰ and 52% in CR-Ⅱ of DAPI-stained cells by fluorescence in situ hybridization (FISH), and beta-proteobacteria were dominant and formed the characteristic flora in both CRs. In 16S rDNA clone libraries, we obtained dominant seven recombinant 16S rDNAs in CR-Ⅰ and five recombinant 16S rDNAs in CR-Ⅱ by restriction enzyme analysis (REA). REA of 16S rDNA clone libraries showed that the genera of NRB (at least 30.8% of the total 104 clones) in CR-Ⅰ were *Zoogloea ramigera* PDS1, *Alcaligenes defragrans* PDS3, *Rodobacter capsulatus* PDS4, Denitrifying-Fe-oxidizing bacterium PDS5, and *Azoarcus* sp. PDS6, and the genera of nitrifying bacteria (26.9% of the total 104 clone libraries) in CR-Ⅱ are *Nitrospira* sp. PNS1 and *Nitrosomonas* sp. PNS4. These results indicated that the activity of denitrification in was directly proportional to the number of DB and NRB from activated sludges in continuous reactor, but many nitrifying bacteria was declined activity of denitrification in continuous reactor.