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Impacts of produced water origin on bacterial community structures of activated sludge

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ABSTRACT

The purpose of this study was to reveal how activated sludge communities respond to influent quality and indigenous communities by treating two produced waters from different origins in a batch reactor in succession. The community shift and compositions were investigated using Polymerase Chain Reaction–denaturing gradient gel electrophoresis (PCR–DGGE) and further 16S ribosomal DNA (rDNA) clone library analysis. The abundance of targeted genes for polycyclic aromatic hydrocarbon (PAH) degradation, *nahAc/phnAc* and *C12O/C23O*, was tracked to define the metabolic ability of the *in situ* microbial community by Most Probable Number (MPN) PCR. The biosystem performed almost the same for treatment of both produced waters in terms of removals of chemical oxygen demand (COD) and PAHs. Sludge communities were closely associated with the respective influent bacterial communities (similarity > 60%), while one sludge clone library was dominated by the *Betaproteobacteria* (38%) and *Bacteriodetes* (30%) and the other was dominated by *Gammaproteobacteria* (52%). This suggested that different influent and water quality have an effect on sludge community compositions. In addition, the existence of catabolic genes in sludge was consistent with the potential for degradation of PAHs in the treatment of both produced waters.
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Introduction

During gas and oil extraction, a waste stream called produced water is generated, which contains relatively high concentrations of hydrocarbons including alkanes and polycyclic aromatic hydrocarbons (PAHs), and is in general treated with a biological process. Owing to their persistence and potential deleterious effects on the environment and human health, PAHs have long been an important group of pollutants of environmental concern (Chowdhury et al., 2009).

Since the first PAH-degrading bacterial isolate was acquired from crude oil deposits 80 years ago, extensive studies have focused on the biodegradation of PAHs (Cerniglia, 1992). Bacterial groups belonging to *Pseudomonas* spp., *Sphingomonas* spp., *Burkholderia* spp., *etc.*, have been found to be mainly responsible for the degradation of PAHs (Peng et al., 2008).

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With the development of culture independent molecular techniques, more candidate PAH degraders have been found in different environments contaminated with PAHs. At the same time, the genes encoding dioxygenase and catechol cleavage dioxygenase, such as the *phnAc*/*nahAc* and *C12O*/*C23O* genes, have been confirmed to be the key functional genes during PAH mineralization. The functional gene abundance has been used as an indicator of PAH degradation ability at the community level (Tuomi et al., 2004).

Previous studies have shown that low molecular weight PAHs in produced waters were effectively removed through biological treatment (Wang et al., 2007). In such a system, high community similarity between the raw produced water and wastewater treating biomass was observed, suggesting the potential role of the indigenous bacterial community from the oil reservoir in the biodegradation of the hydrocarbons, including PAHs. It is known that the environmental conditions for different oil reservoirs, such as the temperature, oil compositions, salinity, *etc*., could be very diverse, which may lead to significant differences in indigenous bacterial communities. For example, in the high-temperature oil reservoirs in the North Sea oil-field, the microbial community was dominated by *Firmicutes* (about 55%) with 20% *Bacteroidetes* (Hakon et al., 2008), while *Proteobacteria* and *Firmicutes* were dominant in the produced water from mesophilic oil reservoirs (Pham et al., 2009), and a single phylotype of *Arcobacter* was found in an oil reservoir with low temperature (Grabowski et al., 2005). Since such a substantial difference in the indigenous bacterial communities existed in different oil reservoirs, it is interesting to know how the different indigenous bacterial communities will impact the sludge bacterial communities as well as the correlations among community composition, function and wastewater treatment performance of a biological system.

In this study, two different produced waters, one from HeBei oilfield (HBPW) and another from XinJiang oilfield (XJPW), with different pollutant compositions, salinity and community composition, were used to evaluate the impacts of water quality and indigenous community on activated sludge using a batch reactor. The reactor was used to treat HBPW stably for two months before switching to treat XJPW. The community shift and compositions were revealed using PCR–DGGE (denature gradient gel electrophoresis) combined with 16S rDNA clone library analysis. At the same time, the sets of catabolic genes were monitored by Most Probable Number–Polymerase Chain Reaction (MPN–PCR) (Tuomi et al., 2004; Zhang et al., 2008) using primer sets for general detection of the genes encoding *nahAc*, *phnAc*, *C23O*, and *C12O*. The result of this study will allow a better understanding of the response of activated sludge communities to produced water quality and indigenous communities. The co-variation of the bacterial community and functional performance suggest a pathway to more efficient design and operation of produced water treatment processes.

1. Materials and methods

1.1. Simulated system and sample collection

A conventional aerobic sludge system was set up to evaluate the performance and microbial community composition and succession of activated sludge. The system was operated in a sequential batch reactor at 45 ± 1°C and natural pH (7.2–7.4), the same as that of the existing full scale system. The reactor was mixed by aeration with HRT at 2–2.5 days and the organic loading rate ranged from 125–150 mg chemical oxygen demand (COD)/(L · day). The reactor was started up with the initial biomass concentration of 4 g/L. Sludge volume was tracked as the indicator of biomass and sludge volume index (SVI) was 100 mL/g, along with stable effluent COD. Both the activated sludge and HBPW were taken from an existing oilfield bio-treatment system in eastern China. Both effluent and activated sludge were sampled every three days for COD, PAH and molecular biology analyses. After 8 weeks, with stable performance in terms of effluent COD, only the influent was substituted by another produced water XJPW with the same activated sludge and operating parameters as above. The effluent and activated sludge was also sampled every three days for PAH and molecular biology analysis for another 8 weeks until the effluent COD was stable. The last sludge samples for produced water HB as influent (HBPW clone library) and produced water XJ as influent (XJPW clone library) were chosen for clone analysis. Biomass was evaluated during the running process without discharge.

1.2. Quantification of PAHs and water quality analysis

Both influent and effluent water samples (250 mL) from HBPW and XJPW were taken for PAH analysis according to the protocol of the US Environmental Protection Agency (US EPA) and were processed immediately (Chen et al., 2005). The concentrations of PAHs in the extracts were determined by an Agilent 6890GC equipped with a 5973 mass selective detector using an HP-5 fused silica capillary column (60 m × 0.25 mm × 0.25 μm film thickness) under the selected ion monitoring mode. The recoveries for surrogate standards fell within a fairly narrow range and, for individual PAHs, were between 73.2% ± 5.6% and 90.3% ± 6.2%.

The COD, total organic carbon (TOC), redox potential (ORP), and chlorate were determined, respectively, according to the standard methods (Andrew et al., 1998).

1.3. Detection and enumeration of catabolic genes

DNA templates from quantitative sludge samples (0.1 g) and influent water samples (10 mL) were independently extracted in triplicate and serially diluted 10-fold, and three samples of each dilution step were subjected to PCR. The MPN number was determined as described previously (Zhang et al., 2008), based on the cut-off probability theory of Kohno and Fukunaga (1998). The programs of touchdown PCR and primers of *phnAc*/*nahAc*, *C12O*/*C23O* and eubacteria 16S rRNA gene (*rrs*) were described in Wang et al. (2007).

1.4. Cloning of PCR-amplified 16S rRNA genes and sequencing

After primary screening of PCR–DGGE (Zhang et al., 2008; Wang et al., 2007), an activated sludge sample taken just before switching influent and the last one for XJPW treatment were subjected to clone analysis. Undigested PCR products amplified by primers 27 F (5′AGAGTTTGATCMTGGCTCAG3′) and 1492R

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