

# Study of archaea community structure during the biodegradation process of nitrobenzene wastewater in an anaerobic baffled reactor



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## ABSTRACT

Nitrobenzene (NB) is an important industrial raw material in organic synthesis. However, successful biological treatment is challenging since NB wastewater is biologically toxic. During the experiment, the performance was examined during the acclimation process of NB in an anaerobic baffled reactor (ABR). The removal efficiencies of NB and chemical oxygen demand were 98% and 90%, respectively. Furthermore, by applying polymerase chain reaction-denaturing gradient gel electrophoresis (DGGE) technology of 16SrDNA, this paper analyzes the structural change of the archaea community in the ABR before and after NB acclimation and identifies the dominant community. The sequence structure analysis of archaea 16S rDNA in DGGE profiles shows that after NB biodegradation, the archaea-dominant community primarily consists of *Methanobacterium beijingense* 8-2, uncultured Archaeon TA04, and uncultured *Methanobacterium* sp. isolated from environmental samples, which may be the important functional archaea in an ABR for NB biodegradation. The study of the population structure distribution and the dominant archaea community is helpful for elucidating the mechanisms of the anaerobic biodegradation mechanism of NB.

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## 1. Introduction

Nitrobenzene ( $C_6H_5NO_2$ ; NB) is an organic compound that has been designated as a “priority pollutant” by the United States Environmental Protection Agency on the basis of its known or suspected carcinogenicity, mutagenicity, teratogenicity, or high acute toxicity (Majumder and Gupta, 2003). NB has been so widely used in the creation of industrial chemicals such as aniline, aniline dyes, drugs, explosives, paint, pesticides, and shoe, floor and metal polishes that NB wastewater caused by improper handling might contaminate surface and groundwater and negatively impact human health. Among various physical, chemical and biological methods for the removal of NB wastewater, the biological treatment option is the most cost effective. The stability of NB makes it very resistant to biological and chemical degradation (Li et al., 2010).

The anaerobic baffled reactor (ABR) is a new third-generation anaerobic reactor installation that integrates upflow anaerobic sludge beds (UASB) with a staged multi-phase anaerobic reactor (Zhu et al., 2008). The ABR has been used to treat high sulfate wastewaters (Saritpongteeraka and Chairapat, 2008), soybean protein processing wastewaters (Zhu et al., 2008), low strength complex wastewaters (Krishna et al., 2008), domestic wastewaters with bamboo carriers (Feng et al., 2008), heavy oil-produced wastewaters with low strength soluble wastewaters (Krishna et al., 2009) and high concentrations of salt and nutrients (Kennedy et al., 2006; Ji et al., 2009). ABR has longer biomass retention times, which favors the growth and multiplication of bacteria with long generation periods. As a result, ABR has potential advantages in the treatment of toxic wastewater. Constructed wetlands that are eco-friendly, low maintenance and low cost have been used for the treatment of municipal wastewater, industrial wastewater and agricultural runoff (Liang et al., 2009; Nyquist and Nyquist, 2009; Ong et al., 2009). During the ABR process, NB is presumed to be converted to aniline, which is more readily biodegradable in the subsequent aerobic process.

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The development of molecular biology tools has contributed to the detection, quantification and identification of the microbial communities involved in wastewater treatment (Parales et al., 2000; Delbes et al., 2001a,b; Huang et al., 2005). Cloning and sequencing of 16S rDNA gene fragments provide information about microorganism phylogeny (Garcia-de-Lomas et al., 2007; Fernández et al., 2008; Nadais et al., 2011). However, studies on the molecular identification of the microbial communities in ABR processes based on cloning and analysis of the nucleotide sequences of genes encoding the 16S ribosomal rDNA are quite limited.

In this study, NB removal efficiency was investigated during the acclimation process. Our research aimed to understand the archaea community of the activated sludge in the five compartments (1–5) of the ABR using denaturing gradient gel electrophoresis (DGGE) fingerprint analysis. We also used cluster analysis of the DGGE data to assess the structure and composition of the archaea population before and after NB acclimation.

## 2. Materials and methods

### 2.1. Experimental equipment

Fig. 1 shows a flow chart of ABR and the constructed wetland in this study. The operation was continuous throughout the experiment. The volume of ABR reactor and constructed wetland were 12.8 L ( $415 \times 100 \times 308$ ) and  $6 \text{ m}^3$  ( $4 \times 1 \times 1.5$ ). Temperature control was accomplished using a temperature controller. The study was conducted at mesophilic conditions of  $30 \pm 0.1^\circ\text{C}$ . The seed sludge was inoculated by a full-scale UASB reactor treating corn wastewater. NaOH was added to make an influent pH value of 6.5–7.5. Each compartment of the ABR was filled with elastic packing made of polyethylene plastic. This kind of packing made the gas and water distribution uniform, enabled the biofilm to grow easily and had high mass transfer efficiency. The ABR system has a hydrological retention time of 24 h. The ABR reactor contained five compartments (1–5). Each compartment consisted of an up-flow zone and a down-flow zone. The use of reed beds with developed roots in the constructed wetland had a vertical subsurface flow as well as good filtration and adsorption for the suspended solids. Fig. 1 shows a schematic diagram of the ABR as well as the constructed wetland treating NB wastewater.

### 2.2. Sample collection and analysis

NB was measured by gas chromatography-mass spectrometry (6890GC/5973MSD; Agilent). Samples were withdrawn from the liquid media at the beginning and end of each treatment period. Clear supernatants were analyzed for chemical oxygen demand (COD). The samples were analyzed in triplicate and the average values were reported. Each pH measurement was performed using the relevant probes and analyzer.

The mixed sludge samples were randomly collected from the five compartments of the ABR. The samples were bead-beaten for 15 min with glass beads in TENP medium (50 mM Tris, 20 mM EDTA, 100 mM NaCl, 0.01 g/mL PVP; pH 10) using a Mini BeadBeater (HMS-350) to allow for the total desorption of the immobilized microorganisms. The TENP medium (with the microorganisms in suspension) was centrifuged at 10,000 g for 5 min. The liquid was then removed and the pellets of activated sludge were washed three times by centrifugation using phosphate-buffered saline (PBS) medium (8 g/L NaCl, 0.2 g/L KCl, 1.44 g/L  $\text{Na}_2\text{HPO}_4$ , 0.24 g/L  $\text{KH}_2\text{PO}_4$ ; pH 7.4) three times at 15,000g. The resulting pellet was resuspended in PBS buffer and frozen at  $-20^\circ\text{C}$  for total DNA extraction.

### 2.3. DNA extraction

The DNA was extracted from the samples of the five different compartments (1–5) using NB in the ABR. The activated sludge samples from compartments 1–5 of ABR were washed with TENP buffer. The depositions were washed three times by centrifugation using PBS buffer for 5 min at 10,000g at  $4^\circ\text{C}$ . The 20mg bacterial pellet was re-suspended in 200  $\mu\text{L}$  of extraction buffer containing 10 mg/mL of lysozyme followed by incubation for 1 h at  $37^\circ\text{C}$ . After lysis, 10  $\mu\text{L}$  of freshly prepared proteinase K (10 mg/mL) and 200  $\mu\text{L}$  of 10% sodium dodecyl sulfate were added to the lysates and incubated at  $55^\circ\text{C}$  for 1 h. After centrifugation for 3 min at 13,000g, the supernatants were deproteinized by extraction in saturated phenol and once in chloroform-isoamyl alcohol (24:1). The samples were then centrifuged at 13,000g for 2 min at  $4^\circ\text{C}$  and mixed with 1 M sodium acetate and ethanol on ice for 1 h at  $-20^\circ\text{C}$ . The precipitated DNA was subsequently recovered by centrifugation for 10 min, washed with 70% (v/v) ethanol and centrifuged at 13,000g for 10 min, dried and then dissolved in 50  $\mu\text{L}$  of TE. DNA quality was

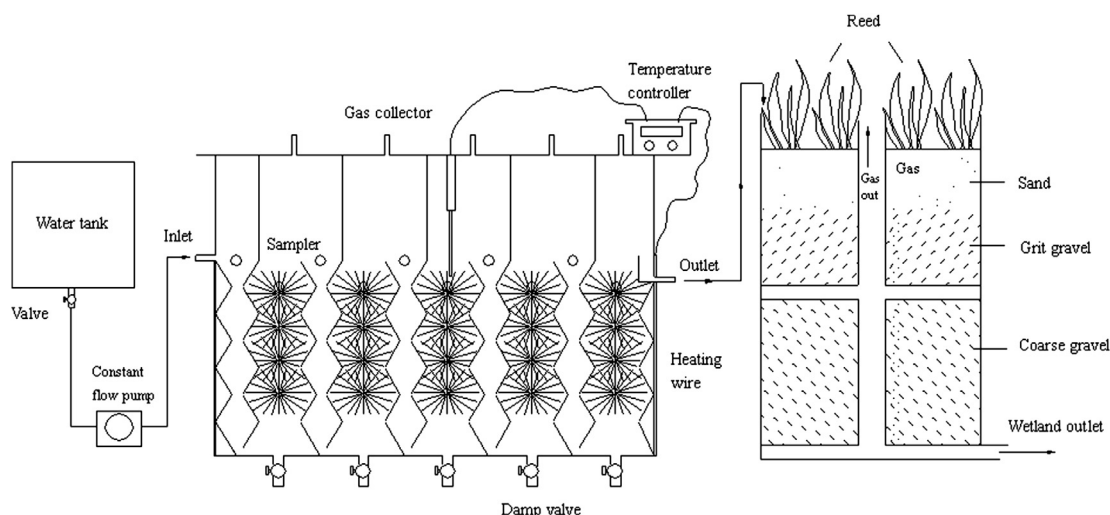


Fig. 1. Picture of reaction equipment.

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