



Fast start-up and stable performance coupled to sulfate reduction in the nitrobenzene bio-reduction system and its microbial community

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ABSTRACT

Three laboratory-scale sequence batch reactors were set up in this study. It was found that a successfully established sulfate-reducing system — operating at COD/SO₄²⁻ ratio of 5.0 — could speed up the start-up process, leading to a high performance. It took about 100 and 60 days, respectively, for a non-sulfate-reducing system and a sulfate-reducing control system to achieve stable and high removal of NB. However, it maintained a complete NB removal in sulfate-reducing system throughout the study. Rapid and stable NB reduction was coupled to the on-going sulfate reducing process. Denaturing gradient gel electrophoresis profile demonstrated that the predominant bacterial groups in the non-sulfate-reducing system and the sulfate-reducing control system were affiliated to *Deltaproteobacterium* and *Acinetobacter*, while in the sulfate-reducing system were sulfate-reducing and sulfur-oxidizing bacteria.

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1. Introduction

Nitrobenzene (NB) is of considerable industrial importance due to its wide usage in the production of various products and intermediates such as dyes, plastics, explosives and pesticides (Mu et al., 2009). As one of the widely-used simple nitro-aromatic compounds (NAC), NB is however relatively toxic and persistent in the environment (Kuşçu and Sponza, 2009). To minimize this adverse impact, NB in the environment is strictly regulated at levels of 17 µg L⁻¹ in the US and 20 µg L⁻¹ in China, respectively (Wang et al., 2011).

A variety of possible physicochemical treatment technologies — such as adsorption, advanced oxidation processes, and electrochemical reduction — have been adopted to remove aqueous NB from wastewaters. Since these physicochemical technologies are costly and may result in a secondary pollution, attempts have been made to develop biological methods as alternative options for the removal of NB. Attentions have been therefore focused on certain NB-degrading bacterial strains isolated from activated sludge and identified as *Rhodotorula mucilaginosa* and *Micrococcus luteus* (Zheng et al., 2008, 2009). These strains were able to utilize NB as a sole source of carbon, nitrogen and energy under aerobic conditions. Due to the strong electron affinity of the nitro group and the biological toxicity of NB, however, it would be advantageous to reduce the nitro group to the corresponding amine group firstly

in anaerobic conditions, followed by oxidization under aerobic conditions. In such treatment process, the reduction of NB to aniline (AN) represents the rate-limiting step for the overall process of NB degradation (Mu et al., 2009).

The long start-up and acclimation period as well as poor performance of NB bio-reduction systems were obstacles for the application of biological method. Majumder and Gupta (2003) investigated the performance of a hybrid reactor, comprising an anaerobic trickling filter and an aeration tank, for treating NB-containing wastewater. This hybrid system took about 100 days for acclimation and start-up, achieving the maximum NB removal efficiency of 98%. Kuşçu and Sponza (2009) used sequential anaerobic baffled reactor (ABR) to reduce NB under the hydraulic retention time (HRT) of 10.38 days and the sludge concentration of 40 g L⁻¹. The acclimation period for the ABR reactor was 63 days. These studies implied the importance of developing a shorter start-up period in order to attain high and stable performances in NB bio-reduction systems for the treatment of NB-containing wastewaters.

Sulfate is widespread in natural environment as well as in wastewater treatment systems. Sulfate reduction, producing sulfide, has been widely reported to be coupled to denitrification (Wong and Lee, 2011) and precipitation of metal cations (Sahinkaya and Gungor, 2010). On the other hand, many bio-refractory organics can also be degraded by sulfate reducing bacteria (SRB) with readily-biodegradable organics being used as co-substrates. These principal bio-refractory organics of concern are benzene toluene ethylbenzene and xylene (BTEX) (Dou et al., 2008), polycyclic aromatic hydrocarbons (PAHs) such as fluorene and phenanthrene (Tsai et al., 2009), phenolic compounds like phenol (Ahn et al., 2009), and

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