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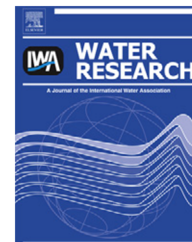

Aerobic degradation of sulfanilic acid using activated sludge

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ABSTRACT

This paper evaluates the aerobic degradation of sulfanilic acid (SA) by an acclimatized activated sludge. The sludge was enriched for over three months with SA (>500 mg/L) as the sole carbon and energy source and dissolved oxygen (DO, >5 mg/L) as the primary electron acceptor. Effects of aeration rate (0–1.74 L/min), DO concentration (0–7 mg/L) and initial SA concentration (104–1085 mg/L) on SA biodegradation were quantified. A modified Haldane substrate inhibition model was used to obtain kinetic parameters of SA biodegradation and oxygen uptake rate (OUR). Positive linear correlations were obtained between OUR and SA degradation rate ($R^2 \geq 0.91$). Over time, the culture consumed more oxygen per SA degraded, signifying a gradual improvement in SA mineralization (mass ratio of O_2: SA at day 30, 60 and 120 were 0.44, 0.51 and 0.78, respectively). The concomitant release of near stoichiometric quantity of sulphate (3.2 mmol SO_4^{2-} released from 3.3 mmol SA) and the high chemical oxygen demand (COD) removal efficacy (97.1%) indicated that the enriched microbial consortia could drive the overall SA oxidation close to a complete mineralization. In contrast to other pure-culture systems, the ammonium released from the SA oxidation was predominately converted into nitrate, revealing the presence of ammonium-oxidizing bacteria (AOB) in the mixed culture. No apparent inhibitory effect of SA on the nitrification was noted. This work also indicates that aerobic SA biodegradation could be monitored by real-time DO measurement.

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1. Introduction

Wastewaters generated from textile factories often contain sulfonated aromatic amines, which are primarily originated from the reductive cleavage of sulfonated azo dyes (10–50% of the applied dyes remain in the wastewater) (O'Neill et al., 1999). Sulfanilic acid (4-aminobenzenesulfonic acid, SA) is one of the most representative sulfonated aromatic amines (Perei et al., 2001). Due to environmental and health concerns, SA-contaminated wastewaters need to be treated prior to its discharge into the environment (Chung and Cerniglia, 1992; Oh et al., 1997; Topac et al., 2009). Currently, aerobic biodegradation is considered as the most effective and environmentally benign approach to treat SA-contaminated wastewaters (Tan et al., 2005). However, the negatively charged sulfonyl group of SA molecule is known to suppress biodegradation by most heterotrophic microbial communities due to the low permeability of SA through bacterial membranes (Hwang et al., 1989). An acclimatization period is often required to enrich an efficient SA-degrading microbial community in the treatment process (Tan and Field, 2005; Tan et al., 2005).

Earlier research has investigated the aerobic degradation of SA by using microbial enrichments. For example, the pioneering works by Feigel and Knackmuss (1988, 1993) have shown that a defined co-culture of *Hydrogenophaga palleroni* S1

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and *Agrobacterium radiobacter* S2 could effectively degrade SA under aerobic conditions. Some other SA-degrading pure strains such as *Pseudomonas paucimobilis* (Perei et al., 2001), *Agrobacterium* sp. PNS-1 (Singh et al., 2004), *Pannonibacter* sp.W1 (Wang et al., 2009), *Ralstonia* sp. PBA and *Hydrogenophaga* sp. PBC (Gan et al., 2011) have also been reported. Arguably, these studies have only limited practical implication as they mainly focused on the use of either pure or co-cultures. To practically treat SA-contaminated wastewaters, mixed microbial cultures such as activated sludge could be of significance.

Activated sludge obtained from municipal wastewater treatment plants has been demonstrated as an effective mixed culture inoculum for starting up SA-contaminated wastewater treatment processes. For instances, Tan et al. (2005) reported that SA could be aerobically degraded using activated sludge previously contaminated with a mixture of sulfonated aromatic compounds. In a separate study, efficient acclimatization of a SA-degrading mixed culture was achieved even using activated sludge that has not been previously exposed to SA (Carvalho et al., 2008). Nonetheless, the relationship between oxygen consumption and SA degradation in these aerobic mixed culture processes has not been properly defined.

Since oxygen is the primary electron acceptor for these microbial processes, understanding the relationship between the kinetics of SA degradation and oxygen consumption would facilitate process optimization of aerobic SA wastewater treatment. Therefore, the objectives of this study were (i) to enrich an aerobic SA-degrading mixed culture using activated sludge that has not been previously exposed to SA as microbial inoculum, and (ii) to evaluate the kinetics of SA degradation and oxygen consumption of the enriched culture. The relationship between oxygen consumption and SA degradation was elucidated, and the feasibility of using real-time dissolved oxygen (DO) measurement as a strategy to obtain SA degradation kinetics during the treatment process was explored. To our knowledge, these issues have not been previously addressed particularly in a mixed culture environment. This work would shed light on our fundamental understanding of biological removal of sulfonated aromatic amines in wastewaters.

2. Materials and methods

2.1. Chemicals

Sulfanilic acid (SA) (4-aminobenzenesulfonic acid, CAS number 121-57-3) was purchased from Sigma–Aldrich (Australia). Some selected physical–chemical properties of SA are: density 1.485 g/mL (25 °C), water solubility in 10 g/L (20 °C) and Henry's constant 8.89×10^{-13} m^3/mol (25 °C). A working stock solution of SA (5 g/L) was prepared by dissolving the SA in deionized water. All chemicals used in this study were of analytical grade.

2.2. Bacterial inoculum and SA containing synthetic wastewater

Activated sludge was obtained from a domestic municipal wastewater treatment plant in Perth, Australia, and was stored at 4 °C prior to use. Unless specified otherwise, the basal medium used in this work had a composition of (mg/L): NH_4Cl 125, $NaHCO_3$ 125, $MgSO_4 \cdot 7H_2O$ 51, $CaCl_2 \cdot 2H_2O$ 300, $FeSO_4 \cdot 7H_2O$ 6.25, and 1.25 mL L^{-1} of trace element solution, which contained (g/L): ethylenediamine tetraacetic acid (EDTA) 15, $ZnSO_4 \cdot 7H_2O$ 0.43, $CoCl_2 \cdot 6H_2O$ 0.24, $MnCl_2 \cdot 4H_2O$ 0.99, $CuSO_4 \cdot 5H_2O$ 0.25, $NaMoSO_4 \cdot 2H_2O$ 0.22, $NiCl_2 \cdot 6H_2O$ 0.19, $NaSeO_4 \cdot 10H_2O$ 0.21, H_3BO_4 0.014, and $NaWO_4 \cdot 2H_2O$ 0.05 (Cheng et al., 2010). In some experiments where the effects of the background ammonium and sulphate were tested, NH_4Cl and $FeSO_4 \cdot 7H_2O$ were omitted in the medium (i.e. Fig. 6). Defined SA concentration in the synthetic wastewater was prepared by adding a known volume of the SA stock solution to the basal medium. pH was adjusted to 7.0 by using phosphate buffer (KH_2PO_4 and K_2HPO_4, 15–30 mM).

2.3. Reactor configuration and general operation

A 2-L glass continuously-stirred tank bioreactor was used in this study to aerobically acclimatize the SA-degrading culture. The working volume of the culture medium was 1.5 L. An adjustable aeration pump was used to supply oxygen to the suspended culture medium at an air flow rate that was varied from 0 to 1.74 L/min. The suspension liquor was continuously stirred by using an overhanging turbine impeller stirrer to maximize mass transfer. A DO sensor and process monitor (TPS Pty. Ltd., Australia) was used to measure the DO concentration in the suspension liquor. The DO data was periodically recorded into an excel spreadsheet using a LabVIEW computer program. The reactor was operated in batch mode at room temperature (25 ± 2 °C).

2.4. Experimental procedures

2.4.1. Reactor start-up

The enrichment process was initiated by mixing the activated sludge (10%, v/v) with the medium to obtain an initial mixed liquor suspended solids (MLSS) concentration of 2000 mg/L. The initial SA concentration in the medium was 500 mg/L during the first two weeks of operation (weeks 1 and 2). During this period, medium renewal was performed every 2 days. For medium renewal, the aeration pump and the overhanging mixer were switched off to allow a complete sludge settlement (ca. 30 min) and only the supernatant was decanted. From week 2 to 8 (i.e. two months after the initial start-up), the initial SA concentration was gradually increased to about 1000 mg/L and medium renewal was performed daily. No sludge wastage was performed during the initial two months to prevent washout of microorganisms relevant to the process. After the initial two months until the end of the acclimatization (i.e. 5 months), 75 mL of the mixed liquor was wasted daily to maintain a steady range of a MLSS of 5000–6000 mg/L (sludge age = 20 days). During the entire acclimatization process, DO concentration in the reactor was maintained at over 5 mg O_2/L to ensure sufficient supply of DO.

2.4.2. SA degradation of the acclimatized culture at different initial SA concentrations and different aeration rates

SA degradation kinetics of the acclimatized sludge was quantified after the initial 2 months of acclimatization. SA

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