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Phenol and sulfide oxidation in a denitrifying biofilm reactor and its microbial community analysis

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ABSTRACT

A microbial consortium attached onto a polyethylene support was used to evaluate the simultaneous oxidation of sulfide and phenol by denitrification. The phenol, sulfide and nitrate loading rates applied to an inverse fluidized bed reactor were up to 168 mg phenol–C/(l d), 37 mg S^{2-}/(l d) and 168 mg NO_3^-–N/(l d), respectively. Under steady state operation the consumption efficiencies of phenol, sulfide and nitrate were 100%. The N_2 yield (g N_2/g NO_3^-–N) was 0.89. The phenol was mineralized resulting in a yield of 0.82 g bicarbonate–C/g phenol–C and sulfide was completely oxidized to sulfate with a yield of 0.99 g SO_4^{2-}–S/g S^{2-}. 16S rRNA gene-based microbial community analysis of the denitrifying biofilm showed the presence of *Thauera aromatica*, *Thiobacillus denitrificans*, *Thiobacillus sajanensis* and *Thiobacillus* sp. This is the first work reporting the simultaneous oxidation of sulfide and phenol in a denitrifying biofilm reactor.

1. Introduction

Nowadays there is a growing interest to develop technologies for the removal of complex mixtures of organic and inorganic compounds from industrial wastewaters such as the chemical and petrochemical industry. These wastewaters have an important environmental impact due to their high concentration of sulfide, ammonium and aromatic compounds [1]. Hydrogen sulfide is corrosive and a malodorous compound that exerts an oxygen demand. Additionally, it is extremely toxic to living plants and organisms, and at a level of 0–5 mg/l in the air, it can be detected easily [2]. Levels greater than 10 mg/l, can affect human health, while levels of 500–1000 mg/l can cause death [3]. There are several studies which show that sulfide is inhibitory for many microorganisms. This inhibitory effect is presumed to be caused by unionized hydrogen sulfide, because only neutral molecules permeate well the cell membrane [4,5]. Another possibility is that sulfide may combine with the iron of ferredoxin and cytochrome in the cell, stopping the electron transport system [6].

Phenolic compounds are toxic, carcinogenic, mutagenic and teratogenic at high concentrations [7]. They have an important effect on bacterial membrane. However, there are reports of bacteria that resist high phenols concentration. One such resistance mechanism is the isomerization of cis-unsaturated fatty acids to the trans-configuration, as it was seen by phenol-degrading *Pseudomonas putida* P8 [8]. The chains of trans fatty acids molecules can align closer together in a biological membrane than those in the cis-configuration, then a more rigid membrane is formed [9].

On the other hand, wastewater streams containing nitrogen compounds may cause serious environmental problems if these compounds are not properly removed before discharge into the receiving water bodies. A high nitrogen concentration in the receiving waters can lead to eutrophication, i.e., algal blooms and/or fish death in rivers, lakes, and coastal areas [10,11].

Sulfide, phenol and nitrogen compounds can be eliminated by physicochemical or biological methods. The physicochemical methods are expensive because of the energy consumption and other chemical substances, generating undesirable and toxic

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residual products that need further treatment [12]. Anaerobic biological processes have been considered as alternatives to remove aromatic compounds [13]. Phenol biodegradation has been reported under sulfate-reducing [14], methanogenic [15], humus reducing [16] and denitrifying [17] conditions. The stoichiometric reaction for phenol oxidation under denitrifying conditions is shown in Reaction 1, indicating that the C/N stoichiometric ratio is 0.92:

$$C_6H_6O^- + 0.2H_2O + 5.6NO_3^- \rightarrow 6HCO_3^- + 0.4H^+ + 2.8N_2$$
$$\Delta G^{\circ\prime} = -3071\ \text{kJ/reaction} \quad (1)$$

Some denitrifying bacteria are chemolithoautotrophic and use reduced sulfur compounds such as elemental sulfur (S^0), sulfide (S^{2-}), thiosulfate ($S_2O_3^{2-}$), or sulfite (SO_3^{2-}) as electron donors [18–20]. The stoichiometry of the complete sulfide oxidation under denitrifying conditions is shown in Reaction 2, where the stoichiometric S/N ratio is 1.43:

$$S^{2-} + 1.6NO_3^- + 1.6H^+ \rightarrow SO_4^{2-} + 0.8N_2 + 0.8H_2O \quad \Delta G^{\circ\prime}$$
$$= -743.9\ \text{kJ/reaction} \quad (2)$$

Comparing the $\Delta G^{\circ\prime}$ values (Reactions 1 and 2) show that organotrophic denitrification is more spontaneous than lithotrophic denitrification. However, Reyes-Ávila et al. [21] using acetate as electron donor indicated that the lithoautotrophic process is faster.

Sulfide can be removed via anaerobic processes by chemolithoautotrophic microorganisms such as *Thiobacillus denitrificans*, which has been extensively studied under anoxic or aerobic conditions [22–24]. Sulfide in presence of an organic molecule such as acetate can be removed via denitrification process either in continuous or batch mode [21,25]. The simultaneous removal of sulfide and phenol using a denitrifying biofilm has not been reported. Nonetheless, it has been demonstrated that biofilm structures allow attached microorganism to tolerate high concentrations of toxicants (i.e., sulfide) without any apparent toxic effect [26,27]. The objective of this study was to evaluate the simultaneous removal of sulfide and phenol under well-defined stoichiometric denitrifying conditions using a biofilm reactor. The microbial community analysis of the biofilm is also presented.

2. Materials and methods

2.1. Inverse fluidized bed reactor (IFBR) and culture medium composition

An inverse fluidized bed reactor [28], also known as down-flow fluidized bed reactor, was used in this study. The IFBR consisted of a glass column of nominal volume of 2 l (1.14 m of height and 4.8 cm of internal diameter) and a working volume of 1.7 l. The experimental set up of the IFBR is shown in Fig. 1. The biofilm carrier was low density polyethylene (267 kg/m^3) of 0.4 mm mean diameter, occupying 20% of the working volume. The mineral medium (pH 7.0) was prepared as follows (g/l): K_2HPO_4 (0.80); KH_2PO_4 (0.3); NH_4Cl (0.15); $MgCl_2$ (0.02); $NaHCO_3$ (0.16), and trace elements solution supplied at 2 ml/l. The trace element solution contained (g/l): EDTA (0.05), $CuSO_4{\cdot}5H_2O$ (0.015), $CaCl_2{\cdot}2H_2O$ (0.07), $MnCl_2$ (0.03), $(NH4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.015), $FeCl_3$ (0.015). The sulfide and phenol were the electron donors, while nitrate was the electron acceptor. The IFBR was equipped with a double wall, through which water, heated in a thermostatic waterbath, was recirculated to maintain the reactor temperature at 30 ± 1 °C. The down-flow liquid velocity was 4 m/h and the bed expansion was between 50 and 60% of the working volume. The gas produced was measured with a calibrated column by liquid (400 g NaCl/l) displacement and its molecular nitrogen and carbon dioxide content was determined by gas chromatography from daily gas samples.

2.2. Inoculum, biomass immobilization and continuous operation

The IFBR was operated in batch mode for 5 d to allow the microbial colonization of the plastic carrier using phenol as carbon and energy source and nitrate as electron acceptor. The nitrate and phenol concentrations were 490 and 180 mg/l, respectively. The IFBR was inoculated with a denitrifying sludge obtained from a lab scale continuous stirred tank reactor fed with nitrate and acetate; under steady state conditions the consumption efficiencies of nitrate and acetate were 100% and the denitrifying yield (g N_2/g NO_3^-–N) and bicarbonate yield (g HCO_3^-–C/g acetate–C) were close to 1. The initial concentration of volatile suspend solids (VSS) in the IFBR was of 0.9 g/l. The liquid of the reactor was replaced with freshly prepared medium every 24 h (for five cycles). The immobilized volatile solids (IVS) at the end of the batch operation were of 1.2 kg IVS/m^3 of dry carrier.

After the polyethylene particles were colonized, the IFBR was operated in a continuous mode under organotrophic conditions fed with phenol and nitrate. The IFBR was operated with two hydraulic retention times (HRT): 1.9 and 0.9 d. The experimental C/N ratio was 1.05, with a 13% carbon excess according to Eq. (1). Once the IFBR reached a steady state under organotrophic conditions at HRT of 0.9 d, it was fed with sulfide as a second electron donor, at a constant loading rate of 37 mg S^{2-}/(l d). The NO_3^-–N fed was enough to completely oxidize the sulfide to sulfate and also to oxidize approximately 87% of phenol to CO_2. The denitrification was evaluated through the consumption efficiencies of substrate and the product yields: denitrifying yield (g N_2/g NO_3^-–N), bicarbonate yield (g HCO_3^-–C/g phenol–C) and sulfate yield (g SO_4^{2-}–S/g S^{2-}).

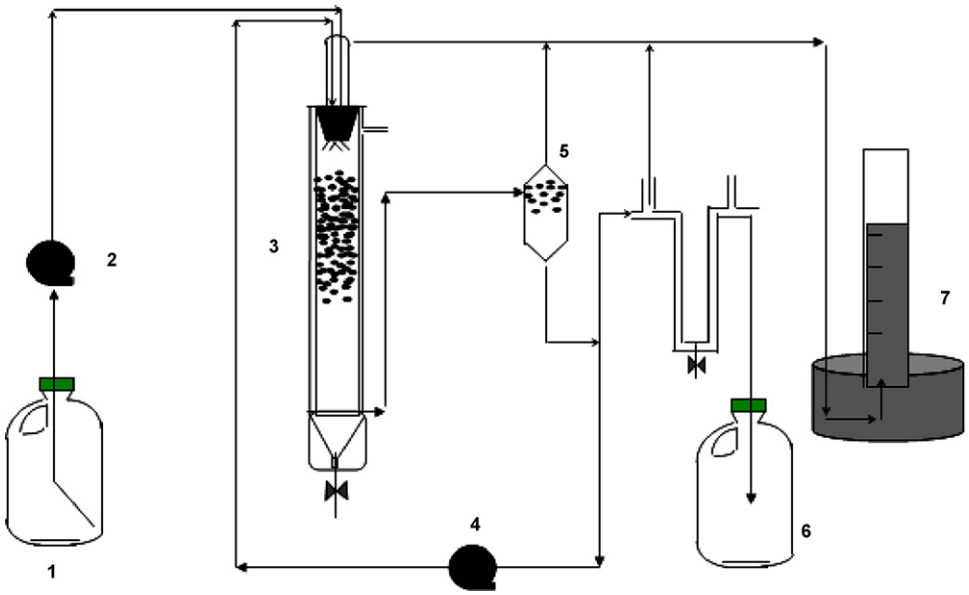

Fig. 1. Experimental set up of the inverse fluidized bed reactor (IFBR): (1) influent; (2) influent pump; (3) bioreactor; (4) liquid recirculation pump for bed expansion; (5) sludge trap; (6) effluent; (7) gas collection.