Bioresource Technology 101 (2010) 8109–8118

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Evaluation of autotrophic and heterotrophic processes in biofilm reactors used for removal of sulphide, nitrate and COD

Kimberley Tang [a], Shijie An [b], Mehdi Nemati [a,*]

[a] Department of Chemical Engineering, University of Saskatchewan, 57 Campus Drive, Saskatoon, Canada
[b] Division of Environmental Engineering, University of Saskatchewan, 57 Campus Drive, Saskatoon, Canada

ARTICLE INFO

Article history:
Received 25 March 2010
Received in revised form 23 May 2010
Accepted 4 June 2010
Available online 26 June 2010

Keywords:
Biodesulphurization
Denitrification
COD removal
Biofilm
Loading rate

ABSTRACT

Microbial cultures originated from an oil reservoir were used in three biofilm reactors and effects of sulphide and nitrate loading rates and molar loading ratio on the removal of sulphide, nitrate and acetate, and composition of end products were investigated. Application of biofilms improved sulphide and nitrate removal rates significantly when compared with freely suspended cells. Maximum sulphide and nitrate removal rates under autotrophic conditions were 30.0 and 24.4 mM h^{-1}, respectively (residence time: 0.5 h). Oxidation of acetate occurred only at nitrate to sulphide molar loading ratios around 0.7 or higher when nitrate was present at levels higher than that required for oxidation of sulphide to sulphur. Conversion of sulphide to sulphate increased from 0% to 66% as nitrate to sulphide molar loading ratio was increased from 0.34 to 3.98. The highest nitrate and acetate removal rates in the bioreactor operated under heterotrophic conditions were 183.2 and 88.0 mM h^{-1}, respectively (residence time: 0.8 h).

1. Introduction

Water flooding is a common method for secondary oil production which is usually accompanied by reservoir souring and contamination of oil, gas and co-produced water with sulphide (Tang et al., 2009). Sulphide contaminated streams are produced also in the pulp and paper industry, petrochemical plants, tanneries, viscous rayon factories (Jing et al., 2009), and during the anaerobic digestion of sludge and agricultural wastes (Tang et al., 2009). Treatment of contaminated streams is necessary due to the toxic and corrosive nature of H_2S, prevention of SO_2 emission and acidic precipitation upon combustion of fuels, and sustainable use of water. Compared with physicochemical treatment processes, biotreatment is advantageous due to operation at low pressures and temperatures and absence of expensive catalysts. Both liquid and gaseous streams regardless of their volume and sulphide contents can be treated effectively through bioprocesses, while well established physicochemical methods such as Claus, Lo-Cat, Alkanolamine and Holmes-Stretford are generally suitable for large volume of gaseous streams containing high levels of sulphide (Tang et al., 2009).

Biotreatment of sulphide containing streams relies on chemolithotrophic sulphide-oxidizing bacteria which use either oxygen (aerobic species) or nitrate (anaerobic species) as electron acceptor, and oxidize sulphide to elemental sulphur or sulphate. Biooxidation of sulphide under aerobic conditions has attracted much attention and has been studied extensively (Alcantara et al., 2004; Ng et al., 2004; Duan et al., 2005; Lee et al., 2006; Datta et al., 2007; van der Zee et al., 2007; Cirne et al., 2008; Dumont et al., 2008; Kim et al., 2008; Li et al., 2008; Rattanapan et al., 2009; Lohwacharin and Annachhatre, 2010). However, recent trend in the literature indicates a renewed interest in anaerobic biooxidation of sulphide in the presence of nitrate (Reyes-Avila et al., 2004; Vaiopoulou et al., 2005; Cardoso et al., 2006; Ggadekar et al., 2006; Mahmood et al., 2007; Chen et al., 2008; Jing et al., 2008; Chen et al., 2009; De Gusseme et al., 2009; Jing et al., 2009; Shen et al., 2009). Positive attributes for anaerobic biotreatment include elimination of the aeration cost and safety concerns associated with treatment of gaseous streams under rich oxygen environments, and possibility of simultaneous removal of sulphide and nitrate. The latter is important as nitrate is a major water contaminants causing severe environmental problems such as eutrophication, emission of greenhouse gases and acidic deposition, as well as health problems in human and animals (Beristain-Cardoso et al., 2008; Jing et al., 2009).

Biooxidation of sulphide under denitrifying conditions has been studied in our earlier study using freely suspended cells of a microbial culture enriched from the produced water of the Coleville oil field in batch and continuous bioreactors (An et al., 2010). Effects of sulphide concentration and the molar loading ratio of sulphide to nitrate on the reaction kinetics and composition of end products were investigated. In the present study, Coleville enrichment was

* Corresponding author. Tel.: +1 306 966 4769; fax: +1 306 966 4777.
E-mail address: Mehdi.Nemati@usask.ca (M. Nemati).

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doi:10.1016/j.biortech.2010.06.037

used in three immobilized cell (biofilm) reactors and processes of biodesulphurization and denitrification (both autotrophic and heterotrophic) were studied. Effects of sulphide and nitrate loading rates and their molar loading ratio on the removal of sulphide, nitrate and acetate (COD), and composition of sulphide oxidation end products were investigated. Results have been compared with those obtained in the system with freely suspended cells and other systems with cell retention or biofilm as reported in the literature.

2. Methods

2.1. Microbial cultures and medium

Biodesulphurization and heterotrophic denitrification were studied using microbial cultures enriched from the produce water of the Coleville oil field, located in Saskatchewan, Canada. Coleville Synthetic Brine (CSB) containing per litre: 7.0 g NaCl, 0.68 g $MgSO_4{\cdot}7H_2O$, 0.24 g $CaCl_2{\cdot}2H_2O$, 0.02 g NH_4Cl, 0.027 KH_2PO_4, 0.68 $NaCH_3COO{\cdot}3H_2O$, 1.0 g KNO_3, 1.9 g $NaHCO_3$, and 0.5 mL trace element solution was used as growth medium. The trace element solution contained per litre: 0.5 mL concentrated H_2SO_4, 2.28 g $MnSO_4{\cdot}H_2O$, 0.5 g $ZnSO_4{\cdot}7H_2O$, 0.5 g H_3BO_3, 0.025 g $Na_2MoO_4{\cdot}2H_2O$, 0.045 g $CoCl_2{\cdot}6H_2O$, and 0.58 g $FeCl_3$. The medium was buffered using 6.06 g L^{-1} Tris base ($C_4H_{11}NO_3$). pH of the medium was adjusted to 7.0–7.5 using 2 M HCl.

The enrichment and maintenance of sulphide-oxidizing, nitrate-reducing culture (NR-SOB) has been described elsewhere (An et al., 2010). The main microbial component of NR-SOB enrichment, *Thiomicrospira* sp. CVO, is capable of both autotrophic and heterotrophic growth with CO_2 or acetate as carbon source, and sulphide or acetate as electron donor (energy source), respectively (Gevertz et al., 2000; Nemati et al., 2001; Tang et al., 2009). In the present study, NR-SOB enrichment was used as an inoculum to develop the heterotrophic denitrifying culture (h-NRB). For this purpose CSB medium with 10 mM of each nitrate and acetate and no sulphide was inoculated with NR-SOB enrichment (10% v/v). The resulting culture was then used as inoculum in subsequent subculturing. All the procedures and conditions used for the maintenance of the h-NRB culture were similar to those for the NR-SOB culture (An et al., 2010). In both cases cultures maintained at room temperature (23–25 °C) and subculturing was carried out on a biweekly basis.

2.2. Media for bioreactors

CSB medium containing sulphide, nitrate and acetate, or nitrate and acetate was used to study biodesulphurization and heterotrophic denitrification, respectively. CSB medium containing nitrate and acetate was autoclaved for 30 min at 121 °C. After cooling to room temperature, medium was purged with filter sterilized nitrogen gas for 30 min per litre of medium to remove the dissolved oxygen. Medium was then transferred into a sterilized collapsible medium bag (Cole-Parmer Canada, Montreal, Canada) by introducing pressurized sterilized nitrogen gas into the flask. For biodesulphurization experiments, prior to transfer to a collapsible bag, 1 M Na_2S stock solution was added to the sterilized medium (final sulphide concentration around 16 mM) and pH was readjusted to 7.0–7.5.

2.3. Specification of the bioreactors and experimental set-up

Three identical experimental systems were used in this study. Two experimental systems were used to study the biodesulphurization and the third system was devoted to heterotrophic denitrification. Each experimental set-up consisted of an up-flow bioreactor made of a glass column with a diameter of 4 cm and a height of 36 cm. Three sampling ports with rubber septum sealing were devised at 12.5 cm intervals along the length of each column. The carrier matrix used for the establishment of the biofilm was quartz sand with a mesh size of −50 to +70 (average diameter: 225 μm), and a surface area of 0.321 m^2/g. Polyvinyl chloride (PVC) tubing and a multispeed peristaltic pump were used to transfer the medium from the collapsible medium bag into the bioreactor and from the bioreactor to the effluent container through an overflow tubing. Use of collapsible bags allowed proper operation of the pumps and maintenance of the anaerobic conditions.

2.4. Experimental procedures

CSB medium containing 16.6 ± 1.6 mM sulphide (the highest level of sulphide tolerated by Coleville enrichment; An et al., 2010) and 13.5 ± 0.8 mM nitrate (average concentration ± one standard deviation) with a pH of 7.0–7.5 was used to study biodesulphurization under denitrification conditions (autotrophic denitrification). Two pore volumes of CSB medium was pumped into each bioreactor (1 and 2) using a multispeed peristaltic pump. Flow of the medium was then stopped, and each bioreactor was inoculated by injecting 20 mL of a 3 day old Coleville enrichment grown on sulphide and nitrate (NR-SOB) into each sampling port. Sulphide concentration was monitored on a daily basis until it decreased to zero in all sampling ports. The system was then switched to continuous mode by pumping medium into the bioreactor at a low flow rate (0.5 mL h^{-1}) for 3–5 days. This allowed passive cell immobilization and formation of the biofilm. Flow rate was then increased stepwise, allowing the bioreactor to reach the steady state conditions at each flow rate before increasing the flow rate to the next level. Steady state conditions were assumed when complete removal of sulphide was obtained in all sampling ports, or when sulphide concentration changed less than 5–10% over a period equal to at least two to three residence times. Both bioreactors were operated at room temperature (23–25 °C). Bioreactor 1 was used to study the effects of sulphide and nitrate loading rates on the removal rate of these compounds through increases in feed flow rate, while maintaining feed sulphide and nitrate concentrations at 16.6 ± 1.6 and 13.5 ± 0.8 mM, respectively. The effects of nitrate to sulphide molar loading ratio on the removal of sulphide, acetate and nitrate, as well as end products composition were investigated in the second bioreactor. The initial stages of operation and conditions including feed composition were similar to that applied in the first bioreactor. However, with second bioreactor the increase in feed flow rate was continued only up to 10 mL h^{-1}. Flow rate of the feed was then kept constant and molar loading ratio of nitrate to sulphide was changed in the range 0.3–4 by maintaining feed sulphide concentration at 15.4 ± 0.4 mM and varying nitrate concentration in the range 5–60 mM.

Effects of nitrate concentration and its volumetric loading rate on heterotrophic denitrification were investigated in the third bioreactor. The bioreactor was charged with CSB medium containing 32.5 ± 0.8 mM nitrate and 29.6 ± 1.2 mM acetate but no sulphide, and inoculated with Coleville enrichment grown on acetate–nitrate (h-NRB). The bioreactor was operated batchwise until nitrate concentration in all three ports dropped to zero. CSB medium was then pumped into the bioreactor at a low flow rate (1.5 mL h^{-1}) to allow establishment of the biofilm. Flow rate of the feed was then increased stepwise to a maximum value of 200 mL h^{-1}. At each flow rate sufficient time was given for establishment of steady state conditions. Following the completion of this part and in order to assess the effect of higher concentrations of nitrate, feed flow rate was decreased to 50 mL h^{-1} (48.8 ± 1.6 mL h^{-1}) and maintained at this level. Media containing higher concentrations of nitrate and acetate (approximately 50, 75, 100 or 150 mM of each