Journal of Environmental Chemical Engineering 2 (2014) 1287–1293

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

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

Sulfate bioreduction and elemental sulfur formation in a packed bed reactor

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ARTICLE INFO

Article history:
Received 20 February 2014
Accepted 21 May 2014

Keywords:
SO_4^{2-}
Desulfovibrio species
Packed bed reactor
PBR
Elemental sulfur
COD/SO_4^{2-} ratio

ABSTRACT

A laboratory scale packed bed reactor (PBR) was fabricated and operated to study its feasibility on SO_4^{2-} reduction. The reactor was packed with polyurethane foam cubes and operated under different feeding and operating conditions of HRT, COD/SO_4^{2-} ratio and SO_4^{2-} concentration. Microbial community characterization showed the presence of sulfate reducing bacteria (SRB) *Desulfovibrio species* in the bioreactor. The reactor performance was found to be highly efficient than those previously reported at COD/SO_4^{2-} ratio below 0.7 and HRT of 24 h. A maximum SO_4^{2-} reduction of 97% was achieved in the PBR when operated at an HRT of 24 h with SO_4^{2-} concentration of 1500 mg/L and COD/SO_4^{2-} ratio of 0.67. Formation of elemental sulfur was noticed even in absence of any dissolved oxygen which might probably be due to oxidation of sulfides by the intermediate sulfite.

Introduction

High SO_4^{2-} containing wastewaters are generated from various industrial activities posing various environmental hazards and impacts upon its discharge into the natural environment. Biological sulfate reduction is considered as one of the most promising alternatives for the treatment of sulfate-rich effluents as compared to the conventional precipitation process with lime or calcium carbonate [1]. Biological SO_4^{2-} reduction occurs in dissolved oxygen deficient environment mediated by sulfate reducing bacteria (SRB) resulting in the conversion of SO_4^{2-} into hydrogen sulfide as the end product. The SRB are anaerobic microorganisms using SO_4^{2-} as terminal electron acceptor utilizing the carbon and energy source for their respiration [2]. SRB can utilize a broad range of electron donors, including hydrogen, formate, methanol, ethanol, molasse, lactate, acetate, propionate and butyrate, sugar, hydrocarbons and organic waste [3]. *Desulfomonas, Desulfococcus*, *Desulfobacter*, *Desulfosarcina*, *Desulfotomaculum*, *Desulfobulbus* and *Desulfovibrio* species are some of the SRB which have been widely reported. Once within the bacterial cytoplasm, the sulfate is reduced to sulfide in a series of reactions mediated by three enzymes which occur within the cell cytoplasm [4] and the pathway [5] is shown in Fig. 1. Sulfites (HSO_3^-) are the major intermediate formed within the cell cytoplasm during SO_4^{2-} reduction [5]. However, the sulfite intermediate in some cases is released into the medium to some extent and such a leakage could make sulfite available to other bacteria [6]. In absence of dissolved oxygen, the sulfides, which are the most reduced forms of sulfur, can get oxidized by the other intermediates to form elemental sulfur (S^0).

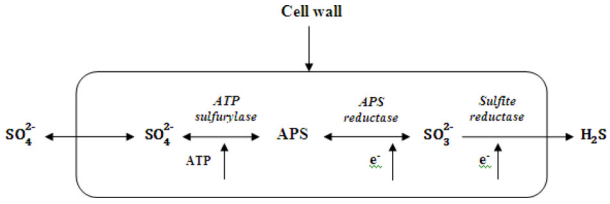

Fig. 1. Pathway of biological sulfate reduction [5].

Packed bed reactors have been widely employed for SO_4^{2-} reduction using packing materials such as glass beads, sand, polyurethane foam and other biomass support particles [7,8]. The packing material provides a large surface area for biofilm formation [9]. The biofilm formed in bioreactors with immobilized cell such as PBR provide more resistance to extreme conditions such as low pH, and high metal concentrations [10]. In such bioreactors, the biomass residence time becomes uncoupled from the hydraulic residence time; therefore it is possible to operate the reactor at high flow rate without cell washout. On the contrary, continuous reactor with freely suspended cells has to be operated at low flow rate and high residence time so as to prevent net/complete cell washout [7].

In the present study, performance of a PBR at various feeding and operating conditions such as HRT, COD/SO_4^{2-} ratio and SO_4^{2-} concentration was studied and the possibility of formation of elemental sulfur was investigated as a result of the reaction between the intermediates of SO_4^{2-} reduction. In addition to isolation and identification of the SRB responsible for SO_4^{2-} reduction in the PBR was also

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http://dx.doi.org/10.1016/j.jece.2014.05.018

carried out.

Materials and methods

Chemicals and reagents

Chemicals and reagents used in the study were either of analytical reagent (AR) grade or laboratory reagent (LR) grade. The synthetic wastewater was prepared by addition of the following components (all in g/L): KH_2PO_4, 0.5; K_2HPO_4, 0.1; $NaHCO_3$, 0.5–0.8; NH_4Cl, 0.3; $MgSO_4{\cdot}7H_2O$, 1–2.2; sodium lactate (60%), 1–1.6; $CaCl_2{\cdot}2H_2O$, 0.05; $NaMo_4{\cdot}2H_2O$, 0.03; KCl, 0.05; $CoCl_2{\cdot}6H_2O$, 0.02; $MgCl_2{\cdot}6H_2O$, 0.05; $NiSO_4{\cdot}6H_2O$, 0.01; sodium ascorbate, 0.05 and sodium thioglycollate, 0.05.

Analytical methods

Samples from the reactor were collected in centrifuge vials of 15 mL capacity prefilled with nitrogen gas. Samples for COD measurement were collected in separate vials not prefilled with nitrogen gas. All the samples were centrifuged at 8000 rpm for 5 min to separate the biomass before being analyzed for residual SO_4^{2-}, sulfides and COD in the treated effluent. Microbial population was measured as mixed liquor suspended solids (MLVSS) using a hot air oven and a muffle furnace following the procedure suggested in the standard methods [11]. pH was monitored with the help of digital pH meter (Thermo Scientific, Orion 3 Star, USA). Volatile fatty acid (VFA) and alkalinity measurement were done by direct titrimetric method as suggested by Dilallo and Albertson [12]. SO_4^{2-} was determined by turbidimetric method [11] and thiosulfate was analyzed by ion chromatography (Metrohm AG, Herisau, Switzerland) equipped with column (A Supplementary 5). Before analysis for SO_4^{2-} concentration, samples were pretreated with NaOH (6 N) and zinc acetate (1 M) to fix the sulfide in the effluent [13]. COD of samples was measured by closed reflux method as recommended in Standard Methods [11]. The COD samples were acidified with a few drops of H_2SO_4 and purged with N_2 gas instead of stirring to release the sulfides present as gaseous sulfide so as to reduce the sulfide interference with the COD value [14]. The concentration of sulfide was determined using a spectrophotometric method [15]. Sulfite concentration was determined according to procedure given in Standard Methods [11]. Due to poor solubility of bacterial sulfur in solvents, the sulfur was quantified on the basis of mass balance of SO_4^{2-} added and products formed [16]. The dried and dehydrated bacterial culture was gold sputtered and then examined under FESEM Σ (Sigma), Carl Zeiss.

Bioreactor configuration

A laboratory scale packed bed reactor (PBR) was fabricated using a Perspex cylinder of overall height 90 cm and internal diameter 11.3 cm as the main reactor unit. Polyurethane foam (PUF) cubes of approximately 2.5 cm × 1.5 cm × 1.5 cm sizes were used as supporting material for microbial growth as this material has the advantage of providing large surface area and high porosity available for the formation of bio-film [17]. Schematic diagram and detailed specifications of the reactor along with its various components are presented in Fig. 2. The bioreactor was wrapped with plastic pipes to use as water jacket, through which constant temperature water was circulated from a thermostat controlled water bath (RA8, Lauda, Germany) to maintain the temperature of the reactor content at 30 ± 0.5 °C. The influent was purged with N_2 from a nitrogen cylinder to expel out dissolved oxygen (DO), if any. The DO was kept below 10 p.p.b. as measured in DO meter (Model: DO 32 A, DKK-TOA Corporation, Japan). The PBR was operated as per the schedule given in Table 1.

The PBR was inoculated with 1000 mL of mixed microbial biomass (MLVSS = 10.65 g/L) previously acclimatized with sulfate rich

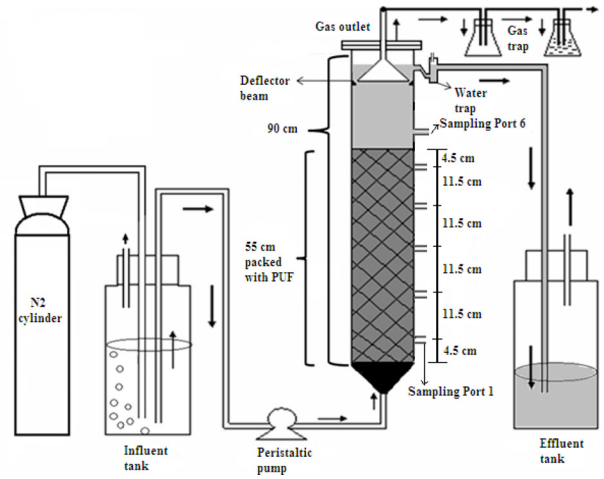

Fig. 2. Schematic diagram of the PBR system.

wastewater. During the start up phase, the PBR was fed continuously with influent having concentration of 1000 mg/L SO_4^{2-} and 1000 mg/L COD for 30 days maintaining an HRT of 48 h at an ambient temperature of 30 °C. HRT calculation was based on packed volume (5.5 L) and not the void volume of the reactor.

Feeding and operating conditions of PBR

After the start-up phase, performance of the PBR under different feeding and operating conditions namely influent COD/SO_4^{2-} ratio, HRT and influent SO_4^{2-} concentration were studied. The PBR was operated at an HRT of 30 h and constant influent COD of 1000 mg/L but with different influent SO_4^{2-} of 1000, 1200, 1400, 1600 and 1500 mg/L, thereby giving a COD/SO_4^{2-} ratio of 1, 0.8, 0.7, 0.62 and 0.67 respectively. The effect of HRT was studied at COD/SO_4^{2-} ratio of 0.67 with initial SO_4^{2-} concentration of 1500 mg/L. Steady state conditions were assumed when the effluent SO_4^{2-} and COD varied by less than 10% after a period of operation equal to a minimum of three residence times (HRT) after all the feeding and/or operating conditions were changed [18]. Once the PBR reached steady state conditions, the PBR was operated at HRTs of 30, 24, 20 and 18 h to study the effect of variation of HRT on the performance of the PBR. After optimization of COD/SO_4^{2-} ratio and HRT, the SO_4^{2-} loading rate into the reactor was increased by increasing influent SO_4^{2-} concentration to 1800 and 2200 mg/L respectively.

Partially treated samples were collected from all the six sampling ports of the PBR (Fig. 2) once the reactor reached to steady state under specific operating conditions. The schedule of profile sampling is given in Table 1. Samples were analyzed mainly for SO_4^{2-}, COD and pH. However when the influent SO_4^{2-} concentration was increased to 2200 mg/L, analysis of profile samples (day 262) were done for sulfites and thiosulfate as well.

Isolation and identification of SRB in the PBR

The microbial culture obtained from PUF particles at different reactor heights after about 100 days of the PBR operation was plated on solid Postgate B medium with 1.5 g L^{-1} agar and incubated in an anaerobic jar containing an Anaerogas pack (Hi Media) to provide anaerobic environment. Bacterial DNA was extracted with the DNA purification kit (GeneiPure™ Bacterial DNA Purification Kit, Merck) after subculturing the isolated strain named PB7 according to manufacturer's instructions. The extracted DNA was amplified for 16S rDNA gene sequence by polymerase chain reaction (PCR) (Applied