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Research article

Optimization of the operation of packed bed bioreactor to improve the sulfate and metal removal from acid mine drainage

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ABSTRACT

The present study discusses the potentiality of using anaerobic Packed Bed Bioreactor (PBR) for the treatment of acid mine drainage (AMD). The multiple process parameters such as pH, hydraulic retention time (HRT), concentration of marine waste extract (MWE), total organic carbon (TOC) and sulfate were optimized together using Taguchi design. The order of influence of the parameters towards biological sulfate reduction was found to be pH > MWE > sulfate > HRT > TOC. At optimized conditions (pH - 7, 20% (v/v) MWE, 1500 mg/L sulfate, 48 h HRT and 2300 mg/L TOC), 98.3% and 95% sulfate at a rate of 769.7 mg/L/d. and 732.1 mg/L/d. was removed from the AMD collected from coal and metal mine, respectively. Efficiency of metal removal (Fe, Cu, Zn, Mg and Ni) was in the range of 94–98%. The levels of contaminants in the treated effluent were below the minimum permissible limits of industrial discharge as proposed by Bureau of Indian Standards (IS 2490:1981). The present study establishes the optimized conditions for PBR operation to completely remove sulfate and metal removal from AMD at high rate. The study also creates the future scope to develop an efficient treatment process for sulfate and metal-rich mine wastewater in a large scale.

1. Introduction

Sulfate and metal-rich acidic wastewater generated from the mining industries is known as acid mine drainage (AMD). It possesses a serious environmental threat to the mining area because of its acidity and dissolved metal contents (Hulshoff Pol et al., 1998; Chang and Kim, 2007). The microbial treatment of AMD by sulfate reducing bacteria (SRB) is a feasible and cost effective alternative for chemical treatment. As a part of its energy metabolism, SRB uses sulfate as an electron acceptor and reduces it to sulfide that precipitates the dissolved metal as a metal sulfide. Growth of SRB also generates bicarbonate alkalinity resulting in the neutralization of the acidity present in the wastewater (Johnson and Hallberg, 2005).

The nitrogen is considered as an important constituent of cellular molecules like purine, pyrimidine, amino acids and enzyme co-factors (Prescott et al., 2005). Amino acids constitute peptidoglycan, different cellular proteins and enzymes. Purine and pyrimidine are the major components of nucleic acids, and enzyme co-factors control the different metabolic pathways. The nitrogen is reported to constitute almost 10% dry weight of bacteria (Karl et al., 2002). Therefore, the successful biological treatment of AMD requires suitable nitrogen source for SRB, which constitutes the major operational cost (Aspmo et al., 2005; Dev et al., 2015a). Different nitrogenous substrates such as tryptone (Das et al., 2013; Fortin et al., 2000), ammonium chloride (Chockalingam and Subramanian, 2006; Das et al., 2013), urea (Cocos et al., 2002; Zagury et al., 2006; Neculita and Zagury, 2008) and chitin (Daubert and Brennan, 2007; Newcombe and Brennan, 2010; Robinson-Lora and Brennan, 2010) are reported to be used to grow SRB during the treatment of AMD. Most of these substrates are costly and reported as inefficient nitrogen source. In the recent studies, cost effective nitrogen source, marine waste extract (MWE) has been developed from organic marine wastes (Dev and Bhattacharya, 2014; Dev et al., 2015b). Several million tons of organic wastes are generated in the coastal areas due to regular fishing activities. Such wastes are composed of fish scrapes, jelly

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fish, dead mollusks, shrimps, crabs and may cause major environmental threat when discarded in the seashore area. To prepare the MWE, the wastes were collected from the eastern seashore of Digha (West Bengal, India). The wastes was initially dried and pulverized. The pulverized waste was subjected to alkaline digestion at pH 12, 90 °C temperature for three hours with constant stirring. The digested product was centrifuged and filtered to separate the liquid MWE (Dev and Bhattacharya, 2014). The nutritional characteristic of MWE was reported by Dev and Bhattacharya (2014). The MWE was rich in nitrogen (13.9 mg/L) and contained other essential nutrients such as organic carbon, P, K, Na, Ca, P and trace elements (Fe, Mg, Cu, Ni, Co, Mn and Zn). The MWE has been established as an efficient and alternative nitrogen supplement for SRB growth medium (Dev and Bhattacharya, 2014).

Moreover, the role of pH, hydraulic retention time (HRT), concentration of sulfate, total organic carbon (TOC) and nitrogen in the feed have advocated the importance of process parameters towards the growth of SRB (El Bayoumy et al., 1999; Kaksonen and Puhakka, 2007). However, most of the studies related to SRB growth deals with single parameter optimization. Whereas, very few studies are available related to understanding the intricate relationship between multiple parameters involved in SRB growth (Dev et al., 2015b). Various statistical tools such as response surface model, central composite design, factorial design etc. have been explored for optimization of process conditions for SRB growth (Singh et al., 2014; Villa-Gomez et al., 2015). Use of Taguchi orthogonal design for identification of influential parameters have been extensively studied for various biological production processes viz. biohydrogen, biodiesel etc. (Roy et al., 2013). The Taguchi design uses analysis of variance (ANOVA) and orthogonal array as the tool for analysis. The orthogonal array allows the experimental design with minimum replication while ANOVA estimates the effect of parameters on the process performance. The variability is indicated by signal to noise ratio (S/N). The signal and noise presents the desired and undesired values for process performance. As the variability is inversely proportional to S/N ratio, the experimental condition having maximum S/N ratio is considered optimum (Mousavi et al., 2007). Use of Taguchi design could be used as an efficient tool for identification of influential parameters involved in the growth of SRB.

The objective of present study is to investigate the prospect of using packed bed bioreactor (PBR) for treatment of AMD collected from different sources. The process parameters governing such complex biochemical reaction includes pH, HRT, feed concentration of sulfate, TOC and MWE. The optimization and understanding the degree of influence of these parameters was done using Taguchi design. Under optimized process parameters, treatment of AMD collected from the coal and metal mine was executed using PBR for 150 d. To the best of our knowledge, the present study is a novel approach towards optimization of bioreactor operation by simultaneously considering multiple parameters to improve the metal and sulfate removal from AMD.

2. Materials and methods

2.1. Characterization of AMD

The AMD was individually collected from coal and copper mine in India. The underground coal and open cast copper mine are located in different locations in India, such as Chhattisgarh (23.33° N, 82.49° E) and Madhya Pradesh (22.02° N, 80.43° E), respectively. It was reported that both the mines produced AMD rich in sulfate and various dissolved metals (Jamal et al., 1991; Pandey et al., 2007). After collection from the main sump of both the mines, the AMD was characterized for pH, acidity, oxidation reduction potential (ORP), dissolved oxygen (DO), total dissolved solid (TDS), chemical oxygen demand (COD), conductivity, sulfate and metal contents such as Fe, Cu, Zn, Ni, Mg and Mn.

2.2. Experimental set up

In the present study, a laboratory scale PBR was used to treat the wastewater collected from the mining industries. The PBR was operated towards upflow direction and contained the following components; an influent reservoir tank, a peristaltic pump (Miclins India, PP20EX), a fixed bed column and an effluent tank (Fig. 1). The influent reservoir tank was equipped with a pH control system to maintain the pH during operation. The column was constructed of an acrylic sheet with overall height, 74.5 cm, internal diameter, 3.9 cm and working volume (V_w), 889.5 mL. To obtain better flow dispersion, the both ends of the column was encapsulated with steel adaptors and mesh (1 mm pore diameter). To reduce the oxygen flux, all the joints of the reactor were sealed with teflon tape and the butyl rubber tubing was used over the mesh. The inlet and outlet sampling port located at the bottom and top of the reactor column, respectively. Butyl rubber stopper was used to cover the ports. The bioreactor column was connected to influent reservoir and effluent tank using silicon tubes having stopper. Polyurethane beads having (3.3 ± 0.5) mm diameter were used as packing material. The packed reactor bed showed the porosity and void volume of 0.32 and 220 mL, respectively.

2.3. Inoculum

The inoculum was collected from the mixed bacterial culture that was growing in the MSRB medium and contained 96% SRB population (Dev and Bhattacharya, 2014). The inoculum was maintained in MSRB medium having the following compositions (g/L): Sodium lactate, 3.5; $MgSO_4$, $7H_2O$, 2; $CaSO_4$, 1; KH_2PO_4, 0.5 and MWE, 10% (v/v). Before inoculation into the PBR, the column was filled with reducing agent supplement (0.7% sodium thioglycolate + 0.7% ascorbic acid) and continuously sparged with nitrogen gas for 24 h to create an anaerobic condition inside. The column was covered with black paper to prevent the entry of the light inside the reactor. Subsequently, MSRB medium was added to the column and inoculated, followed by maintenance in a batch mode for 15 d. for biofilm formation. On day 16 onwards the reactor was operated in the continuous mode.

2.4. Residence time distribution study (RTD)

When the PBR reached a steady state condition in terms of sulfate reduction, the residence time distribution (RTD) study was performed to find out the flow characteristics of the column packed with polyurethane beads. The study was carried out using 2 mg Li^+/L as a tracer which was dosed in a pulse mode through the inlet sampling port. The flow rate of the tracer study was maintained to 21 mL/min which resulted in 57.23 min theoretical HRT. Each sample was collected in 2 min intervals for a period of 110 min. Subsequently, the samples were measured for Li^+ concentration. The study was carried out in triplicate and tracer response curve (C curve) was generated. The curve was analyzed for mean dispersion, variance and dispersion number (Metcalf and Eddy, 2003).

2.5. Optimization of process parameter

The optimization of the process parameters were performed using Taguchi method. This method is applied to determine the outcome of an analytical approach consisting of variable factors. Significant variance in the factor level is determined and thus it