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Research article

Process integration for biological sulfate reduction in a carbon monoxide fed packed bed reactor

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ABSTRACT

This study examined immobilized anaerobic biomass for sulfate reduction using carbon monoxide (CO) as the sole carbon source under batch and continuous fed conditions. The immobilized bacteria with beads made of 10% polyvinyl alcohol (PVA) showed best results in terms of sulfate reduction (84 ± 3.52%) and CO utilization (98 ± 1.67%). The effect of hydraulic retention time (HRT), sulfate loading rate and CO loading rate on sulfate and CO removal was investigated employing a 1L packed bed bioreactor containing the immobilized biomass. At 48, 24 and 12 h HRT, the sulfate removal was 94.42 ± 0.15%, 89.75 ± 0.47% and 61.08 ± 0.34%, respectively, along with a CO utilization of more than 90%. The analysis of variance (ANOVA) of the results obtained showed that only the initial CO concentration significantly affected the sulfate reduction process. The reactor effluent sulfate concentrations were 27.41 ± 0.44, 59.16 ± 1.08, 315.83 ± 7.33 mg/L for 250, 500 and 1000 mg/L of influent sulfate concentrations respectively, under the optimum operating conditions. The sulfate reduction rates matched well with low inlet sulfate loading rates, indicating stable performance of the bioreactor system. Overall, this study yielded very high sulfate reduction efficiency by the immobilized anaerobic biomass under high CO loading condition using the packed bed reactor system.

1. Introduction

Sulfate is one of the most common anionic species found in the environment second to only bicarbonates. The sources of sulfate in water bodies are both natural and anthropogenic, e.g. water passing through gypsum salt containing soil, atmospheric deposition, municipal and industrial discharges, etc. (Hulshoff et al., 2001; Mokone et al., 2012). Effluent from industries such as those involved in edible oil production (Wu et al., 2010), molasses fermentation (Li et al., 2011), tannery operations (Calheiros et al., 2012), food production (Chitapornpan et al., 2013), coal burning power plants (Xu, 2011) and pulp and paper processing (Kamali and Khodaparast, 2015), majorly contributes towards sulfate pollution in the environment (Sinharoy et al., 2015; Kiran et al., 2017; Goswami et al., 2017a).

Despite the large amount of sulfate entering into the environment, less attention is paid towards its mitigation as the environmental risk associated with sulfate is low compared with the other organic or inorganic pollutants (Papirio et al., 2013). Sulfate pollution is, however, a concern as it leads to numerous secondary environmental problems. Excessive quantities of sulfate released into the environment can affect drinking water supplies, thereby causing toxicity to various life forms (Kuo and Shu, 2004). Sulfate acts as an electron acceptor and gets converted to sulfide (H_2S) in an environment lacking oxygen or nitrate. This produced H_2S causes stench and corrosion problems (Sawyer et al., 2003). The discharge limits for sulfate in wastewater are 500 and 400 mg/L as per the United States Environment Protection Agency (USEPA) and Central Pollution Control Board of India standards, respectively (USEPA, 2002; Indiawaterportal.org), whereas, according to the World Health Organization (WHO) regulation only 250 mg/L of sulfate is permissible in drinking water (WHO, 1996). Therefore, it is highly necessary to treat sulfate rich wastewater before releasing into the environment.

Both physicochemical and biological methods are used for treating sulfate rich wastewater. Even though physicochemical methods are found suitable, drawbacks such as the need for solid-liquid separation, sludge disposal problem, high operation and energy cost are some major limitations of the same (Sarti and Zaiat, 2011). To overcome such limitations of physicochemical methods,

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research direction is changed towards biological sulfate removal using sulfate reducing bacteria (SRB). The major advantages of using SRB in sulfate rich wastewater treatment include minimal sludge production, reduction in the number of potential pathogens i.e. microorganisms, and removal of other co-pollutants, e.g. heavy metals (Dev et al., 2017; Van Den Brand et al., 2015). Compared with biological treatment, chemical treatment such as lime addition is not feasible for high sulfate (>1500 mg/L) containing wastewater, as the dissolution and precipitation of gypsum are at equilibrium below this value (De Godoi et al., 2017; Kiran et al., 2017; Geldenhuys, 2003). However, the process requires an external carbon and energy source, which seriously limits its large-scale applications (Dries et al., 1998). Among the different electron donors used in this biological process, including alcohols and short chain volatile fatty acids; hydrogen (H_2) is considered more striking because of the low free energy of the reaction involved, which strongly favors sulfate reduction more than any other anaerobic process (Weijma et al., 2002).

On the other hand, sulfate reduction using pure H_2 is not economically feasible, thus requiring alternative and cheaply available source for H_2. In this context, *in situ* produced H_2 from biological carbon monoxide (CO) conversion can be considered a better substitute to pure H_2 (Parshina et al., 2010). Only a few studies have reported the capability of SRB to utilize CO as the sole source of carbon and energy for sulfate reduction (Sipma et al., 2007; Sinharoy et al., 2015). However, the CO is known to be toxic to most live forms, and also the low amount of H_2 produced seems to limit the process efficiency. Hence, it is necessary to find an appropriate hydrogenogenic CO utilizing bacteria capable of sulfate reduction, which can detoxify CO as well as reduce sulfate present in wastewater utilizing the *in situ* produced H_2. Moreover, for achieving high process efficiency, suitable bioreactor system need to be evaluated that can improve the gas liquid mass transfer for both CO and H_2. Packed bed bioreactor with counter current flow is well-known for its high mass transfer efficiency. Also, it is well established that immobilization aids in overcoming substrate toxicity by avoiding direct exposure of microorganism to CO and it further avoids biomass washout from the reactor even at high pollutant loading rates (Kuo and Shu, 2004). A number of studies have reported the use of CO, syngas (CO/H_2) or pure H_2 for biological sulfate reduction (Hao et al., 2014). However, the effect of different process parameters, such as initial Pco, sulfate concentration, etc. on sulfate reduction using CO as the sole carbon and energy source is not explored in detail. Further the use of immobilized anaerobic biomass for simultaneous CO conversion and sulfate reduction has not been reported so far in the literature. Hence, this study examined the potential of anaerobic microbial consortium immobilized in the form of beads for sulfate reduction by utilizing CO as the sole carbon source, both under batch and continuous CO fed conditions using a packed bed bioreactor. The specific objectives of this study were as follows: (a) preparation and characterization of cell immobilized beads, (b) optimization of process parameters such as CO, sulfate concentration and bead amount on sulfate removal and CO utilization and (c) performance evaluation of packed bed bioreactor with cell immobilized biomass on sulfate reduction using CO as the sole carbon and energy source.

2. Materials and methods

2.1. Biomass source and its activation

Anaerobic biomass containing mixed consortia was obtained from a large scale upflow anaerobic sludge blanket (UASB) reactor (located at Kavoor, Mangalore, Karnataka, India) treating common effluent, i.e. combination of domestic sewage and industrial wastewater generated by small/medium scale industries located in that area. Detailed characterization of the biomass was reported previously by Sinharoy et al. (2015). For initial activation of the sludge biomass, 10% (*v/v*) of the anaerobic biomass was added to 200 mL serum bottle containing mineral salt media (MSM). After nitrogen purging, the bottles were incubated in orbital shaker set at 30 °C and 150 rpm for 2 days. Such freshly grown biomass was immobilized using alginate (calcium/PVA) for carrying out the sulfate reduction experiments, as detailed in Section 2.2.

The composition of the MSM (g/L) is as follows: sodium chloride (0.3), ammonium chloride (0.2), calcium chloride dihydrate (0.11), magnesium chloride hexahydrate (0.1), potassium dihydrogen phosphate (0.1), ferric chloride (0.945), copper chloride (0.013), zinc chloride (0.07), cobalt chloride (0.065), sodium molybdate (0.021), manganese chloride (0.63), nickel chloride (0.13), and yeast extract (0.5). The pH of the media was adjusted to 7.0 with the help of 1N NaOH.

2.2. Preparation, characterization and reuse potential of immobilized anaerobic biomass

For biomass immobilization, two types of beads were prepared: the first bead type was prepared with only sodium alginate and the other with polyvinyl alcohol (PVA) and sodium alginate as per the procedure described by Covarrubias et al. (2012). Briefly, 40 mL of the anaerobic sludge biomass was collected by centrifugation and washed twice with phosphate buffer saline (PBS). The biomass was then mixed with 20 mL alginate solution of required concentration (1–3.5%, *w/v*) and stirred for 10 min. The mixture was added drop wise into a chilled 10% $CaCl_2$ solution to form the beads, which were cured further for 1 h in the same solution with proper mixing and finally washed with saline. For preparing PVA-alginate beads, the same procedure was followed; however, 8–12% (*w/v*) PVA and 1% (*w/v*) sodium alginate were taken along with the biomass for preparing the suspension. Besides, 3% boric acid was added to the 10% $CaCl_2$ solution for crosslinking of beads. Table 1 presents the composition of the different beads tested in this study.

The prepared beads of different compositions were examined for their biological activity and mechanical strength. The biological activity was measured by the capability of the immobilized biomass to reduce sulfate by utilizing CO as the sole source of carbon and energy. Experiments were performed using 120 mL serum bottles sealed with polytetrafluoroethylene (PTFE) septum. The serum bottles were filled with 50 mL mineral salt media (MSM) of pH 7.0 along with 2 g (wet weight basis) immobilized beads (calcium alginate or PVA-alginate) as the inoculum. The bottles were purged with nitrogen gas prior to the experiments and 250 mg/L of sulfate was added into the media. The initial Pco in the bottles was 90 kPa. The bottles were incubated at 30 °C and 150 rpm on a rotary orbital incubator shaker. All the experiments were performed in triplicate. Bottles containing blank beads without any immobilized biomass served as the negative control in these batch experiments.

Table 1
Composition of calcium alginate and PVA-alginate beads used for biomass immobilization.

Calcium alginate beads		PVA-alginate beads	
S. No.	Sodium alginate (% *w/v*)	PVA (% *w/v*)	Sodium alginate (% *w/v*)
1	1	8	1
2	1.5	10	1
3	2	12	1
4	2.5		
5	3		
6	3.5		