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Research article

Understanding the performance of sulfate reducing bacteria based packed bed reactor by growth kinetics study and microbial profiling

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ABSTRACT

A novel marine waste extract (MWE) as alternative nitrogen source was explored for the growth of sulfate reducing bacteria (SRB). Variation of sulfate and nitrogen (MWE) showed that SRB growth follows an uncompetitive inhibition model. The maximum specific growth rates (μ_{max}) of 0.085 and 0.124 h^{-1} and inhibition constants (K_i) of 56 and 4.6 g/L were observed under optimized sulfate and MWE concentrations, respectively. The kinetic data shows that MWE improves the microbial growth by 27%. The packed bed bioreactor (PBR) under optimized sulfate and MWE regime showed sulfate removal efficiency of 62–66% and metals removal efficiency of 66–75% on using mine wastewater. The microbial community analysis using DGGE showed dominance of SRB (87–89%). The study indicated the optimum dosing of sulfate and cheap organic nitrogen to promote the growth of SRB over other bacteria.

1. Introduction

Sulfate reducing bacteria (SRB) are known to utilize sulfate as an electron acceptor and reduce it to sulfide. SRB mainly belongs to five major bacterial groups such as *Deltaproteobacteria, Clostridia, Thermodesulfobacteria, Thermodesulfobiaceae, Nitrospirae, Euryarchaeota,* and *Crenarchaeota*. The SRB are obligate anaerobes and thrive in wide range of habitats such as anoxic sediments, hydrothermal vents, hypersaline aqueous environments, hydrocarbon seeps, mud volcanoes, and bioreactors treating acid mine drainage (AMD) (Muyzer and Stams, 2008).

Because of its ability to reduce sulfate, the SRB is usually used to treat sulfate-rich wastewater generated from several industries such as mining, chemical, electroplating, tannery and metal processing (Lens et al., 2002). Treatment of sulfate-rich wastewater was reported using different bioreactor configurations such as continuous stirred tank reactor (CSTR), fluidized bed reactor (FBR), up-flow anaerobic sludge blanket bioreactor (UASB), membrane bioreactor (MBR), anaerobic hybrid reactor (AHR), packed bed reactor (PBR) and gas-lift reactor (Kaksonen and Puhakkaa, 2007). The efficiency of the treatment process can be improved by attaching the slow growing anaerobic SRB on the carrier materials kept inside the bioreactor. Most of the recent studies reported the use of PBR because of its operational robustness and reduced maintenance requirement during the treatment (McMahon and Daugulis, 2008; Bernardez et al., 2012; Brahmacharimayum and Ghosh, 2014).

Improvement of the treatment process is dependent on the activity of SRB. Therefore, it is important to study the growth kinetics of a specific SRB population to predict and control their biochemical activity. While sulfate is used as electron acceptor for SRB, the nitrogen serves as an important macro-nutrient. Influence on growth by varying sulfate and nitrogen concentration has been widely reported in SRB (El Bayoumy et al., 1999; Moosa et al., 2002; Al Zuhair et al., 2008; Oyekola et al., 2010; Dev et al., 2015). Though, very few reports are available related to the role of nitrogen on the growth kinetics of SRB. Most these growth kinetic studies were performed for suspended cells, whereas little focus was given on the microbial culture immobilized on a solid surface.

There is a difference in the growth kinetics between the suspended and immobilized bacterial community (Pallud and Van Cappellen, 2006). SRB is also dependent on the fermentative bacteria for successful utilization of the growth substrate (Oren, 2010).

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List of abbreviations

Abbreviation/symbols, Full name	
SRB	Sulfate reducing bacteria
CSTR	Continuous stirred tank reactor
FBR	Fluidized bed reactor
UASB	Up-flow anaerobic sludge blanket reactor
MBR	Membrane bioreactor
AHR	Anaerobic hybrid reactor
PBR	Packed bed reactor
MWE	Marine waste extract
FISH	Fluorescence *in-situ* Hybridization
FITC	Fluorescein isothiocyanate
DAPI	4, 6-diamidino-2- phenylindole
DGGE	Denaturing gradient gel electrophoresis
PCR	Polymerase chain reaction
BLAST	Basic local alignment search tool
NCBI	National center for biotechnology information
COD	Chemical oxygen demand
TDS	Total dissolved solid
TKN	Total Kjeldahl nitrogen
μ	Specific growth rate (h^{-1})
μ_{max}	Maximum specific growth rate (h^{-1})
K_s	Half saturation constant (mg/L)
K_i	Growth inhibition constant (mg/L)
S	Substrate concentration (mg/L)
X	Biomass concentration (mg/L)

Therefore, the growth kinetics of SRB enriched mixed culture is related to its microbial community structure. It is also important to study the microbial community at the substrates concentrations.

Most of the previous studies reported only sulfate reduction kinetics in PBR giving less focus to the SRB growth (Baskaran and Nemati, 2006; Bernardez et al., 2012). The present study reports the growth kinetics of SRB enriched mixed culture that was studied using PBR at a wide range of sulfate and nitrogen source concentrations. Subsequently, the microbial community structure was analyzed at the respective concentration of sulfate and nitrogen that could provide highest growth rate. Further, the same concentration of sulfate and nitrogen was used to study their ability to support sulfate and metal removal from the wastewater. To best of our knowledge, no such reports are available where the study of growth kinetics of SRB in PBR using cheap organic nitrogen source has been done.

2. Materials and methods

2.1. Inoculum and growth media

The SRB mixed culture was collected from the sulfidogenic bioreactor that was used in the study of Das et al. (2013). Subsequently, the mixed culture was repeatedly subcultured in Postgate B medium (Postgate B 1984). To create the anaerobic condition, the growth medium was prepared with deionized water which was boiled and subsequently cooled under the continuous flow of nitrogen. The pH was maintained at 7.2 by the addition of 0.1 N NaOH. Subsequently, the medium was autoclaved, cooled and inoculated with the mixed culture. Finally the medium was supplemented with 10% (v/v) reducing agent (0.7% sodium thioglycolate+0.7% ascorbic acid). The mixed culture developed after the final subculture was used as inoculum in the subsequent study.

2.2. Comparison of the nitrogen sources

A batch study was conducted to compare the effectiveness of MWE with that of commercial nitrogen sources for their ability to support sulfate reduction. The composition of MWE was reported by Dev and Bhattacharya (2014). The MWE contained an average of 13,951 mg/L of Total Kjeldahl Nitrogen (TKN) in terms of ammonia. On the basis of their frequent use in standard growth media, the commercial nitrogen sources selected in the study included tryptone, corn steep liquor, yeast extract, NH_4HCO_3, and NH_4Cl. In the batch study, the growth media was prepared in different batches corresponding to the different nitrogen source selected in the study. The nitrogen sources were respectively supplemented into the SRB growth medium Postgate B. During the supplementation, the nitrogen compounds present in the Postgate B (NH_4Cl and yeast extract) were replaced with the same amount of the nitrogen sources selected in the study. Similarly, about 10% v/v MWE having the nitrogen content equivalent to what was present in conventional Postgate B was added by replacing the NH_4Cl and yeast extract. The batch study was conducted for 168 h and samples were collected at each 24 h intervals to measure the concentration of sulfate, dissolved sulfide, total cell count and SRB population.

2.3. Continuous study

The continuous study was performed in a laboratory scale upflow PBR. After the inoculation and formation of biofilm, bacterial growth kinetics was studied at varying concentration of sulfate and nitrogen.

2.3.1. Upflow PBR

Two identical upflow PBR setups were constructed and termed as PBR1 and PBR2, respectively. The PBR system 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 column was constructed of an acrylic sheet with overall height, internal diameter and working volume (V_w) of 74.5 cm, 3.9 cm and 889.5 mL, respectively. It was encapsulated at both ends with steel adaptors and mesh (1 mm pore diameter) to obtain improved flow dispersion. Butyl rubber tubing was used over the mesh, and all the joints of the reactor were sealed with teflon tape to minimize the oxygen flux. The reactor was equipped with an inlet and outlet sampling port located at the bottom and top of the column, respectively. The ports were capped with butyl rubber stopper. Silicon tubes with an attached stopper were used to connect the bioreactor with the influent reservoir and effluent tank. The column was packed with 500 g of polyurethane beads which had (3.3 ± 0.5) mm diameter. The void volume and porosity of the reactor bed were 220 mL and 0.32, respectively. Before packing the column, the beads were rinsed with water, followed with soaking in 10% HNO_3 for 48 h, and repeated washing by distilled water and finally, rinsed with deionized water.

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. The reactor column was filled with the MSRB medium (Dev and