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Sulfate Reduction and Growth Kinetics of Sulfate Reducing Bacteria While Using Marine Waste Extract as Nitrogen Source

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Abstract Sulfate reduction and SRB growth kinetics were studied in growth media for SRB (Dev et al., 2014), MSRB containing marine waste extract as nitrogen source with increasing SO_4^{2-} concentration, such as 1500, 3500, 5500 and 7500 mg L^{-1}. With increase in SO_4^{2-} concentration, SO_4^{2-} reduction rate, its efficiency, SRB specific growth rate, HS^- generation rate were deteriorated. In the presence of 1500 mg L^{-1} SO_4^{2-} concentration, rate and efficiency of SO_4^{2-} reduction, SRB specific growth rate and HS^- generation rate were found highest, 99.56%, 94.58 mg/L·h, 0.099 d^{-1} and 13.03 mg/L·h, respectively. Therefore, 1500 mg L^{-1} of SO_4^{2-} concentration was considered optimum to support highest SO_4^{2-} reduction and SRB activity in MSRB medium.
Keywords sulfate reducing bacteria, COD/SO_4^{2-} ratio, sulfate concentration, rate of sulfate reduction, bacterial specific growth rate.

Introduction

Increasing population and industrial activities have led to the generation of high amount of wastewater. Effluents from industries like pulp and paper, fertilizer, metallurgical, chemical and mining are rich in heavy metals and sulfate (Lens et al. 1998). When discharged untreated, such effluents are highly dangerous to the fresh water bodies, environment and human activities. Sulfate is considered to be a major pollutant which increases the salinity of receiving water bodies and thus reduces the availability of potable and usable water. The heavy metals are highly toxic and may cause severe health defects if consumed (Gray 1997).

One of the major ways of treating such SO_4^{2-} and metal rich effluents is through biological sulfate reduction. In this approach SO_4^{2-} is reduced to HS^- which precipitates the dissolved metal as metal sulfide form. The reaction also generates bicarbonate alkalinity which increases pH of wastewater. Use of biological sulfate reduction in the treatment of sulfate rich wastetwater is advantageous to other available treatment technologies. Application of different sulfidogenic bioreactors in the treatment of sulfate rich wastewater has been studied extensively, from laboratory to field application (Johnson and Hallberg 2005).

One of the major causes that cannot support effective SRB activity and results in poor sulfate reduction performance is mainly the growth the substrates which lack nutrients especially nitrogen (Robinson-Lora and Brennan 2009). SRB growth media generally contains nitrogen sources like bactopeptone, bactotryptone, NH_4Cl, NH_4HCO_3, $(NH_4)_2PO_4$ etc. It is impractical to use commercial nitrogen source for the growth of SRB in the large scale treatment of sulfate rich wastewater because of high cost. Protein rich fraction extracted from organic marine waste which was termed as marine waste extract (MWE) has been used as alternative and cost effective nitrogen source for SRB (Dev et al. 2014).

In this current work kinetic study on SRB growth and sulfate reduction was performed in a batch reactor using MWE as nitrogen source in the presence of different sulfate concentrations.

Bacterial specific growth rate can be measured using the formula μ, where x indicates bacterial cell concentration. Similarly sulfate reduction can be measured as r_{so4} , where s indicates SO_4^{2-}concentration.

Kinetic data in the presence of a broad spectrum of sulfate concentration works as a base to design an efficient biological sulfate reduction process. This paper describes the effect of different sulfate concentrations and COD/SO_4 ratios on the kinetics of SRB growth and biological sulfate reduction in SRB growth media containing MWE as nitrogen source.

Materials and Methods

SRB inoculum

SRB mixed culture grown in MSRB medium was used as inoculum in this kinetics study. The inoculum contained 96% of SRB population.

Study of the Sulfate reduction and SRB growth kinetics

Sulfate reduction and SRB growth kinetics was studied in MSRB medium in the presence of varying concentration of SO_4^{2-}, such as 1500, 3500, 5500 and 7500 mg/L. In MSRB medium marine waste extract is used as nitrogen source. The entire study was performed in batch mode. Incubation time was maintained to 240 h. Samples were collected at the beginning and subsequently after the completion of the incubation time. Immediately after collection, measurement of ORP HS^-, SO_4^{2-} and bacterial cell concentration were performed. Chemical oxygen demand (COD) was measured for samples collected at the beginning of the batch study. From SO_4^{2-} and bacterial cell concentrations data sulfate reduction efficiency, rate of sulfate reduction and bacterial growth were measured.

Analysis

ORP was analyzed using ORP electrode (9778BNWP, Thermo scientific). The ORP values were corrected with hydrogen electrode. HS^- was measured using the method 4500 E (APHA 2005). SO_4^{2-} was measured according to the method described by Roy et al. (2011). The bacterial cell concentration was obtained by counting the total bacterial cell stained with 4, 6-diamidino-2-phenylindole (DAPI) (Das et al. 2013). The cells were observed under epifluroscence microscope (Carl Zeiss, Germany, Model- AXIO Scope. A1) equipped with filter set 49 (excitation wavelength 365 nm).

Result and Discussion

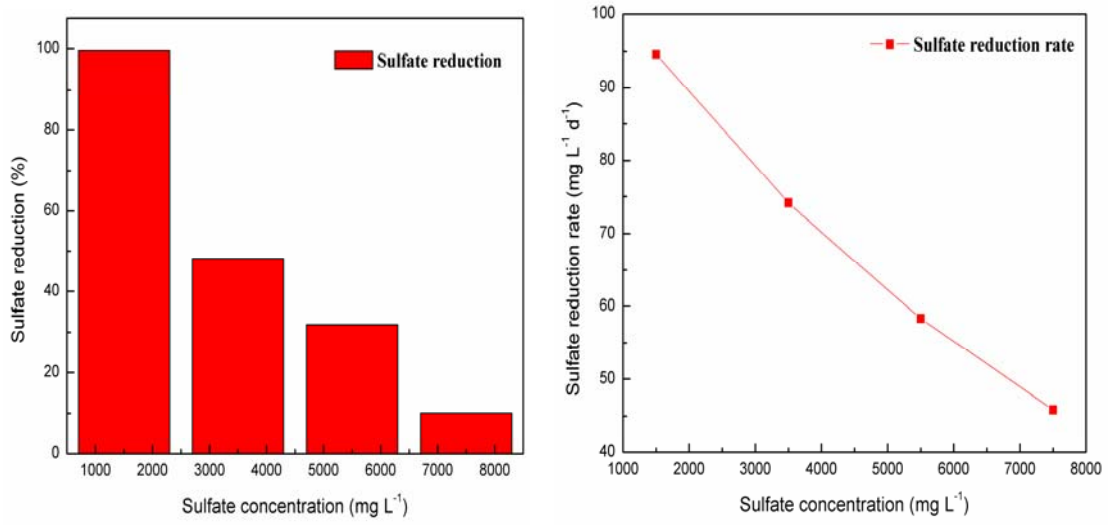
Efficiency and rate of sulfate reduction

The ability of MSRB medium to support SO_4^{2-} reduction decreased upon increasing the SO_4^{2-} concentration (fig. 1A). Medium containing 1500, 3500, 5500 and 7500 mg/L of SO_4^{2-} could able to support 99.56, 48.10, 31.89 and 10.09 % SO_4^{2-} reduction, respectively.

Similarly the rate of sulfate reduction also decreased upon increasing the SO_4^{2-} in growth media. SO_4^{2-} reduction rates of 94.58, 74.16, 58.33 and 45.83 mg L^{-1} d^{-1} were observed in growth media containing 1500, 3500, 5500 and 7500 mg L^{-1} of SO_4^{2-}, respectively (fig. 1B).

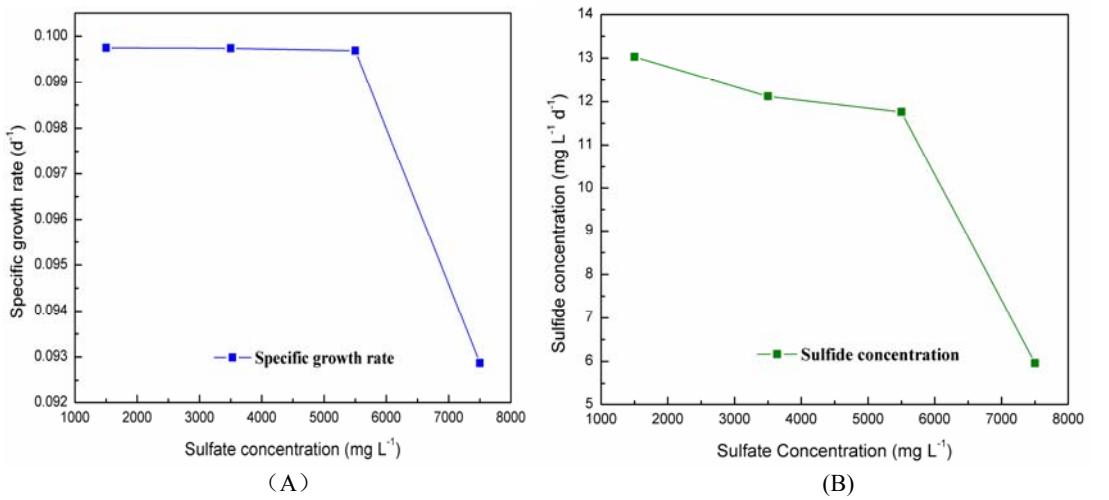
Specific growth rate

Specific growth rate of SRB varied little in the MSRB media containing 1500, 3500, 5500 mg L^{-1} of SO_4^{2-} concentration and the value was near to 0.1 d^{-1} (fig. 2). But upon increasing the SO_4^{2-} to 7500 mg L^{-1} the specific growth rate of SRB decreased to 0.09287 d^{-1}.



(A) (B)

Fig. 1 *Efficiency of SO_4^{2-} reduction (A) and rate of SO_4^{2-} reduction (B) with increasing sulfate concentration in media.*



（A） (B)

Fig. 2 *Specific growth rate of SRB (A) and sulfide generation rate (B) with increasing concentration of sulfate in growth media.*

At the end of the batch study all the growth media exhibited ORP value in the range of -257.4 to -287.4 mV. As the SO_4^{2-} concentration changed, the COD/SO_4^{2-} ratio of the media also varied. MSRB media containing 1500, 3500, 5500 and 7500 mg/L of SO_4^{2-} exhibited COD/SO_4^{2-} ratio of 8.94, 3.76, 2.07 and 1.27. The optimum SO_4^{2-} concentration and COD/SO_4^{2-} ratio which could support optimum SO_4^{2-} reduction efficiency, rate, specific growth rate and sulfide generation rate were 1500 mg/L and 8.94, respectively. Higher sulfate concentration could have inhibitory effect on the SRB activity. Therefore, upon increasing SO_4^{2-} concentration, decline in SO_4^{2-} reduction efficiency and rate were found. Similar results which exhibit the inhibition of SRB growth by sulfate concentration above 2500 mg/L has been reported by Al-Zuhair et al. (2008). Similarly, with increasing initial sulfate concentration, decrease in the sulfate reduction ability is reported by Oyekola et al. (2010). In our study it was found that the bacterial population decreased upon increasing the SO_4^{2-} concentration. Generation of sulfide serves as a suitable indicator of SRB activity (Bayoumy et al. 1999). In our study generation of sulfide decreased upon increasing the sulfate

concentration. Therefore, it can be clearly assumed that high SO_4^{2-} concentration plays a significant role in inhibiting the growth of SRB.

The result clearly indicates that 1500 mg/L SO_4^{2-} concentration and COD/SO_4^{2-} ratio of 8.94 were optimum to support the highest SO_4^{2-} reduction and SRB activity in a medium containing marine waste extract as nitrogen source. Therefore, toobtain highest rate and efficiency of SO_4^{2-} reduction in large scale treatment of SO_4^{2-} rich wastewater, the SO_4^{2-} concentration and COD/SO_4^{2-} of the wastewater should be maintained to the respective values as mentioned earlier. Either the modification of marine waste extract dosing or the dilution of wastewater containing higher SO_4^{2-} concentration may be performed to reach that operational condition.

Conclusions

Both SO_4^{2-} concentration and COD/ SO_4^{2-} ratio have significant impact on SRB growth. In this study SO_4^{2-} concentration above 1000 mg/L and COD/SO_4^{2-} ratio below 8.49 exhibit negative impact on SRB growth and SO_4^{2-} reduction. Therefore, it can be concluded that SO_4^{2-} of 1500 mg/L and COD/SO_4^{2-} ratio of 8.49 is optimum for SRB mixed culture in the presence of marine waste extract as nitrogen source.

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