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 SO₄²⁻ concentration, such as 1500, 3500, 5500 and 7500 mg L⁻¹. With increase in SO₄²⁻ concentration, SO₄²⁻ reduction rate, its efficiency, SRB specific growth rate, HS⁻ generation rate were deteriorated. In the presence of 1500 mg L⁻¹ SO₄²⁻ concentration, rate and efficiency of SO₄²⁻ 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 SO₄²⁻ concentration was considered optimum to support highest SO₄²⁻ reduction and SRB activity in MSRB medium.

Keywords sulfate reducing bacteria, COD/SO₄²⁻ 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 SO₄²⁻ and metal rich effluents is through biological sulfate reduction. In this approach SO₄²⁻ 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 wastewatrer 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, NH₄Cl, NH₄HCO₃, (NH₄)₂PO₄ 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 $\mu$, where $x$ indicates bacterial cell concentration. Similarly sulfate reduction can be measured as $r_{s\text{O}_4}$, 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$^{-1}$. 
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, to obtain 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.

Acknowledgement

Financial support provided by Indian Institute of Technology Kharagpur, India is gratefully acknowledged.

References