A STUDY ON EFFICIENCY OF FIVE DIFFERENT CARBON SOURCES ON SULFATE REDUCTION

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ABSTRACT

Normally sulfate containing wastewaters from various industrial activities such as pulp and paper industries, mining and mineral processing, production of explosives, scrubbing of flue gases, petrochemical industries, galvanic processes, battery, paint and chemical manufacturing etc. is treated using physicochemical and biological methods. Even though physicochemical methods are effective, the overlying limitations of separation and appropriate disposal of the solid phase, relatively high cost and energy consumption limit their usage. Biological sulfate reduction has been recognized as an efficient method for sulfate removal from wastewater owing to heavy metal removal, recovery of metals from sulfide sludge and low sludge generation in spite of the major problem associated with the production of sulfide. The biological sulfate reduction process is mediated by a group of microorganisms known as Sulfate Reducing Bacteria (SRB). The SRB form a specialized group of microbes that use sulfate as terminal electron acceptor for their respiration. The presence of sufficient amount of electron donors is essential as a minimum COD to sulfate mole ratio of 0.67 is required for achieving theoretically possible removal of sulfate. Hence, in COD deficient wastewaters, addition of external carbon sources is a must in order to achieve complete reduction. Thus, the availability of carbon forms a critical limiting factor for the microbial activity. Usually low-molecular-weight organic compounds are utilized by SRB as electron donors. This study was carried out to analyze the potential of the enriched mixed consortium to utilize different carbon sources such as acetate, dextrose, ethanol, formate and lactate as the sole carbon source for reduction of sulfate. Out of the five sources, lactate was found to be the most efficient carbon source for the mixed consortium.

Key Words: Sulfate Reducing Bacteria (SRB), Biological sulfate reduction, Electron donors, Mixed consortium, Carbon source

INTRODUCTION

The presence of sufficient amount of electron donors is essential for biological treatment of sulfate rich wastewater as 1 mole of sulfate for the complete reduction requires 0.67 moles of COD stoichiometrically. In the case of sulfate rich wastewaters with high COD or organic matter, an external electron source is not required as the same can be utilized as electron donors. However in COD deficient wastewaters addition of external carbon sources is a must in order to achieve complete reduction. Thus, the availability of carbon forms a critical limiting factor for the microbial activity. Preferably lower molecular-weight organic compounds are utilized by SRB as electron donors. Sewage sludge, leaf mulch, wood chips, animal manure, vegetal compost, sawdust, mushroom compost, whey and other agricultural waste serve as some natural electron donors. In addition, synthetic small molecular weight organic compounds like lactate, acetate, propionate, pyruvate and butyrate, ethanol and other alcohols are also used as electron donors. Nearly all of these compounds are known to be fermentation products of anaerobic bacterial degradation of carbohydrates, proteins and other constituents of dead biomass. Molasses, which contains a high amount of sucrose, has also been used as electron donor. Diversity of SRBs in their carbon source utilization and the metabolic activities have also been reported. The energy for the growth and maintenance of SRB
provided by the carbon source to carry out sulfate reduction based on the reaction (1)\(^\text{17}\).

\[
\text{SO}_4^{2-} + 4\text{H}_2\text{O} + 8\text{e}^- \rightarrow \text{S}^{2-} + 8\text{OH}^- \quad (1)
\]

Therefore, the ability of the mixed consortium to utilize different carbon sources for sulfate reduction was investigated in the present study. The study was done in the fed batch mode maintaining a Hydraulic Retention Time (HRT) of 6 days, replacing 50% of fresh feed every 3 days with the simultaneous removal of 50% of the media so that there is minimum inhibition of sulfate reduction due to non availability of carbon sources.

**MATERIAL AND METHODS**

All the chemicals used in the present study were either of Analytical Reagents (AR) grade or Laboratory Reagents (LR) grade. Double distilled water was used for the preparation of any standard solution as well as biological media. The wastewater was synthetically prepared by addition of the following components (g/l) in double distilled water: 0.5 g \(\text{KH}_2\text{PO}_4\), 0.1 g \(\text{K}_2\text{HPO}_4\), 0.3 g \(\text{NH}_4\text{Cl}\), 0.4 g yeast extract and 2.57 g \(\text{MgSO}_4\cdot 7\text{H}_2\text{O}\). Different carbon sources used as electron donor in the present investigation were acetate, dextrose, ethanol, formate and lactate. Each of the carbon sources was added at an initial concentration of 1500 mg \(\ell^{-1}\) as COD with initial sulfate concentration of 1000 mg \(\ell^{-1}\). The feed solution was purged with nitrogen gas to remove any dissolved oxygen present. The study was done in the fed batch mode in 150 ml Erlenmeyer flask with 100 ml as working volume, maintaining a Hydraulic Retention Time (HRT) of 6 days, replacing 50% of fresh feed every 3 days with the simultaneous removal of 50% of the media so that there could be minimum or no inhibition of sulfate reduction due to lack of carbon sources. About 10 % v/v of the enriched culture was added as the inoculum in these experiments. In all these experiments the initial pH of the media was set to 7.2, 30°C temperature and 180 rpm.

**RESULTS AND DISCUSSION**

Among the five carbon sources used, the mixed consortium showed the maximum reducing ability by utilizing lactate as the sole carbon source. A maximum removal efficiency of 98% was observed when lactate was used as the carbon source. It may be because lactate promotes the growth of a wide variety of sulfate reducing bacteria consequently increasing microbial diversity and treatment system resilience\(^\text{18,19}\). Sulfide toxicity has also been reported to decrease when lactate serves as the carbon source for biological sulfate reduction\(^\text{20,21}\). Only around 55% removal efficiency was observed in the case of acetate as the sole carbon source after even 15 days of fed batch operation. The poor efficiency of the mixed consortium to reduce sulfate using acetate could be due inability of the SRB to completely oxidize acetate even with excess sulfate levels\(^\text{22}\). SRB are generally poor competitors of Methanogenic Archaea (MA) for acetate. Sugar is an effective electron donor that is easily degraded under anaerobic conditions. The anaerobic degradation pathway of sugar such as glucose, fructose, dextrose etc. is also similar to that of other organic compounds in which hydrogen is the interspecies\(^\text{23}\). Dextrose is also utilized effectively for in our experiment for sulfate reduction. Most sulfate reducers using hydrogen as carbon source (e.g. *Desulfobulbus propionicus*, *Desulfovibrio baarsii*) are able to grow on formate\(^\text{13}\). Indeed, formate utilization is indicative of the presence of hydrogenotrophic sulfate-reducing bacteria\(^\text{24}\). High sulfate removal efficiency in the experimental results indicates the presence and growth of sulfate reducers.

The probable reaction mechanisms of sulfate reduction by the mixed consortium utilizing the five different carbon sources are given below.

For acetate,

\[
\text{CH}_3\text{COO}^- + \text{SO}_4^{2-} \rightarrow \text{HS}^- + 2\text{HCO}_3^-
\]

\(\Delta G^\circ = -47.3\text{ KJ/reaction}\)

For dextrose,

\[
\text{C}_6\text{H}_12\text{O}_6 + 3\text{SO}_4^{2-} \rightarrow 3\text{HS}^- + \text{HCO}_3^- + 3\text{H}^+
\]

\(\Delta G^\circ = -452.5\text{ KJ/reaction}\)

For ethanol,

\[
2\text{C}_2\text{H}_5\text{OH} + \text{SO}_4^{2-} \rightarrow 2\text{CH}_3\text{COO}^- + \text{HS}^- + \text{H}^+ + 2\text{H}_2\text{O}
\]

\(\Delta G^\circ = -132.7\text{ KJ/reaction}\)

For formate,

\[
4\text{HCOO}^- + \text{SO}_4^{2-} + \text{H}^+ \rightarrow \text{HS}^- + 4\text{HCO}_3^-
\]
\[ \Delta G^\circ = -146 \text{ KJ/reaction} \]
\[ \text{CH}_3\text{CHOHCOOH} + \text{H}_2\text{SO}_4 \rightarrow \]
\[ 2\text{CH}_3\text{COOH} + 2\text{CO}_2 + \text{H}_2\text{S} + 2\text{H}_2\text{O} \]

For lactate,
\[ \Delta G^\circ = -159.6 \text{ KJ/reaction} \]

Sulfate Reduction Efficiency (SRE) was calculated according to the equation
\[ \text{SRE} = \frac{(S_{in} - S_{ef})}{S_{in}} \times 100\% \], in which \( S_{in} \) and \( S_{ef} \) was the concentrations of sulfate in influent and effluent, respectively. (Fig. 1 and Fig. 2)

**Fig. 1**: Sulfate reduction with different carbon sources

**Fig. 2**: COD degradation with different carbon sources
CONCLUSION

Lactate was observed to be the most efficient carbon source to the mixed consortia which was utilized for the reduction of sulfate. The other carbon sources such as dextrose, formate, ethanol and acetate were also utilized by the mixed culture but highest removal efficiency was there in the case of lactate.

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DECLARATION BY THE AUTHOR(S)

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