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Microbial Consortia from Taptapani Hot Water Springs for Mining Effluent Treatment

[1]Poonam Nasipuri, [2]Gauri G. Pandit,
[3]Ashoke Ranjan Thakur and [1]Shaon Ray Chaudhuri
[1]Department of Biotechnology, West Bengal University of Technology,
BF-142, Sector-1, Salt Lake, Calcutta-700064, India
[2]Environmental Assessment Division, Bhabha Atomic Research Center,
Trombay, Mumbai-400085, India
[3]Vice Chancellors Office, West Bengal State University Barasat,
Berunanpukuria, P.O. Malikapur, North 24 Parganas-700126, India

Abstract: Problem statement: Soluble sulfate load in effluent is a crucial problem from mining industries. The study involved isolation of efficient Sulfate Reducing Bacterial (SRB) consortium from hot water spring for bioremediatrion of sulfate contaminated waste water. **Approach:** The enriched bacterial consortium was isolated in medium DSMZ 16695. The sulfate reduction efficiency was measured by turbidometric method. The K_m and R_{max} value of the consortium was determined. It was immobilized in 10L bioreactor and the sulfate reduction was measured in presence of media and mine effluent. **Results:** The consortium was found to reduce 2000ppm of sulfate in 36h under optimum condition. The K_m of 1.1530 ppm and R_{max} value of $0.030h^{-1}$ was obtained under optimum conditions of pH-7.5 at 40°C with 2% inoculum. Consortium immobilized under ambient condition in a 10L packed bed reactor yielded about 21-41% reduction of soluble sulfate in synthetic medium prepared using effluent water and tap water respectively. **Conclusion:** This study reports for the first time the use of efficient SRB consortia from hot water spring for bioremediation of mine effluent.

Key words: Sulfate reducing bacteria, sulfate remediation, packed bed bioreactor, taptapani hot water spring, microbial consortium

INTRODUCTION

Sulfate concentration is found to rise in natural water due to various anthropogenic activities. It is released into the environment due to various industrial activities (Nasipuri *et al*., 2010; Liamleam and Annachhatre, 2007) like mining procedure and atmospheric deposition in the form of acid rain (Bull *et al*., 2001). Increase in sulfate concentration in ground water causes various adverse effects like laxative effect (Zuhair *et al*., 2008) skin in problem, dehydration, as well as unpleasant taste of water. Lowering of ground water table due to agricultural reasons helps the oxygen to invade the anoxic layer oxidizing the iron sulfides to Fe (III) hydroxides and sulfate (Zak *et al*., 2006).The sulfate leaches to the ground water leading to increase in sulfate concentration (Zak *et al*., 2006). It had also been reported that sulfate pollution could lead to eutrophication of surface water (Zak *et al*., 2006). Increased sulfate concentration causes phosphate mobilization aggravating phosphate eutrophication that can inhibit the growth of different plant species (Geurts *et al*., 2008). Chemical means of decontaminating the soluble sulfate like reverse osmosis, distillation and ion exchange have various drawbacks including the high operational cost (Preez and Maree, 1994). Alternative way of solving the problem is by using Sulfate Reducing Bacteria (SRB) for bioremediation of both sulfate and metals.

SRB use sulfate as their terminal electron acceptor reducing it to hydrogen sulfide as the end product through dissimilatory sulfate reduction pathway (Rabus *et al*., 2006). The hydrogen sulfide thus produced has high affinity for divalent metals reducing them to insoluble metal sulfide which makes them technologically important not only for soluble sulfate removal but also for metal removal (Nasipuri *et al*., 2010, Hammack and Edenborn, 1992; Colleran *et al*.,

Corresponding Author: Shaon Ray Chaudhuri, Department of Biotechnology, West Bengal University of Technology, BF-142, Sector-1, Salt Lake, Calcutta-700064, India Tel: +913323210731 ext 108; +919831034236 Fax: +913323341030

1995; Hoa *et al*., 2007; Biswas *et al*., 2008). Thus the reduction of sulfate by SRB is the most important step for both metal and sulfate removal from waste water (Baskaran and Nemati, 2006; Alvarez *et al*., 2007; Hsu *et al*., 2010). Organic barriers like Zero Valent Iron (ZVI) plays an important role here by favoring the growth of SRB and that in turn produces sulfide which helps in metal removal (Fiore and Zanetti, 2009). SRB are found to be distributed widely in nature due to their various interactions like geochemical transformation, turnover of organic matter, cycling of the nutrient, bioremediation, biocorrosion (Barton and Tomei, 1995; Fauque, 1995). Although they are found in varied geographical habitat they have similar characteristics (George *et al*., 2008).

Hot water springs all over the world are reported to harbor thermophilic bacteria (Al-Quadan *et al*., 2009) and SRB are frequently found due to the high content of sulfate (Fathi and Al-Kahtani, 2009; Ghanem and Ghannam, 2010; Dillon *et al*., 2007). However studies on presence of SRB from hot water springs of India are rare (George *et al*., 2008). The present study reports the sulfate reduction efficiency of the SRB consortium (G-16695), isolated from hot water springs of Taptapani, Orissa in a packed bed bioreactor under ambient condition using both synthetic media and mining effluent water.

MATERIALS AND METHODS

Sampling site and sampling procedure: Water and soil samples were collected from Taptapani (84º40'E and 19º50'N) hot water spring (Orissa) in India and carried in sterile plastic containers to the laboratory at ambient temperature for further analysis.

Enrichment, cultivation and morphological characterization: Medium DSMZ-16695 as per the specifications of German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) (http://www.dsmz.de/microorganisms) was used for enrichment and cultivation of the consortia from hot water spring. 2% inoculum of water was directly added to the anaerobic media. For generation of anaerobicity sealed serum vials were taken and reducing agents like sodium dithionite and sodium bi-carbonate were added prior to purging with nitrogen. Soil sample was diluted in sterile water and the particulate matter was allowed to settle down. The supernatant was then used as inoculum at a similar concentration (2%). The vials were incubated at 40°C for 24 h and the growth and sulfate reduction was measured at 12 h interval until complete reduction was observed.

Sulfate measurement by turbidometric method: The turbidometric method reported by Nasipuri *et al*. (2010) was followed for measurement of sulfate concentration.

Determination of sulfate reduction kinetics: Monod (1949) kinetics was calculated to understand the substrate dependent microbial activity demonstrated by the consortium according to the formula:

$$t/(\ln Cc/Cco) = (\mu+1)/k + \mu/k(\ln Ca_0/Ca)/(\ln Cc/Cc_0) \quad (1)$$

Where:

t = Time
Ca = Substrate concentration at time‘t’
Ca_0 = Initial substrate concentration
Cc = Cell OD_{660} at time‘t’
Cc_0 = Initial cell OD_{660}
k = Maximum rate of sulfate utilization.
μ = $Km/(Ca_0 + Y_{A/C}\, Cc_0)$; where
$Y_{A/C}$ = The slope between Ca and Cc
Km = Half saturation constant

Finally the rate of the reaction can be calculated as:

$$R= (R_{max}*S)/ (K_m+ S) \quad (2)$$

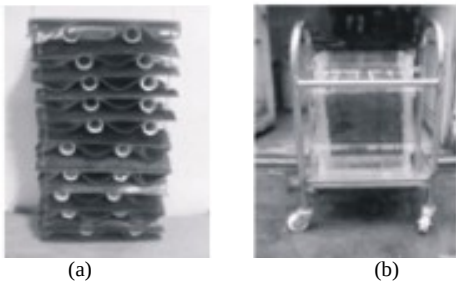
Where:

R = Rate of sulfate utilization
R_{max} = Maximum rate of sulfate utilization; equivalent to ‘k’ of Eq. 1
K_m = Half saturation constant
S = Substrate concentration; Equivalent to Ca of Eq. 1

The reason for taking Eq. 1 is due to the large time gap (12 h) between two sampling.

Here substrate is replaced by the sulfate concentration. The graph was plotted from the above equation with (lnCao/Ca)/(lnCc/Cco) on the X axis against t/(lnCc/Cco) on the Y axis. The slope and intercept were calculated from the graph and subsequently R_{max} and K_m was determined.

Immobilization of Consortium in 10L packed bed bioreactor: In order to be used continuously as a bioremedial package, immobilization was a better choice than suspension culture due to two reasons. Firstly it reduces the cost as well as effort of addition of fresh inoculum with each cycle making the operation less cumbersome. Secondly the biofilm developed would be more robust as compared to suspended cells against inhibitors that might be present in the environmental effluent.



(a) (b)

Fig. 1: Immobilization matrix for packed bed reactor. (a) Photograph representing the arrangement of the packed plastic sheets used for immobilization; (b) Photograph of the bioreactor with packed bed matrix inside

Thus immobilization was tried out on packed corrugated sheets (Fig. 1a) placed inside 10 L bioreactor (Fig. 1b). For charging of the bioreactor, 10 L medium was inoculated with 10% culture and the corrugated sheets were kept submerged for 24 h. The culture was decanted after 24 h and the sheets were left for another 6 h to facilitate adhesion of the cells onto the surface. Fresh medium was added and the percentage of sulfate reduction was studied at 24 h interval. The anaerobicity was not maintained externally but left to the continuous generation of H_2S by SRB. The medium was decanted every 24 h and replaced by fresh medium to facilitate the microbial growth and reduction. The entire operation was conducted under ambient temperature in absence of nitrogen purging. Similar procedure was repeated with mining effluent where medium was prepared eliminating sulfate salt and replacing distilled water by mining effluent water.

RESULTS

Characterization of the consortia: The consortium was found to reduce~2000 ppm of soluble sulfate from both synthetic media and waste within 36 h. The K_m of 1.1530 ppm and R_{max} value of 0.030h^{-1} was found (Fig. 2). The community morphology of the consortia showed the predominance of bacilli followed by vibrio and cocci. Molecular analysis of 25 clones (GQ503829-GQ503853) revealed maximum similarity with thermophilic isolates like *Clostridium thermopalmarium* and *Clostridium thermobutyricum*. This is expected since the consortium was enriched from hot water spring (Al-Quadan *et al*., 2009). The OTU saturation curve indicated completeness of the study while the Shannon diversity index of 0.950 and equitability index of 0.590 represented less diversity as well as non even distribution of members of the consortium (Al-Quadan *et al*., 2009). This is also expected as selective media was used for the purpose.

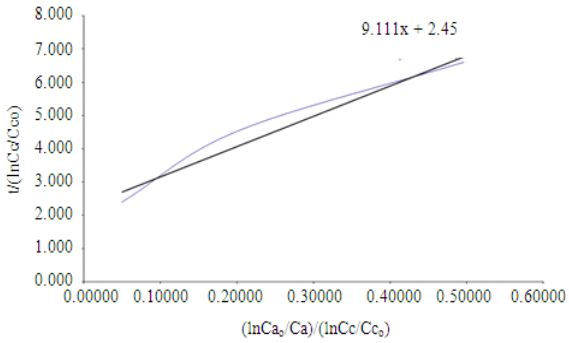


Fig. 2: Graph representing the plot for determination of K_m and R_{max} for the consortium where the intercept is (μ +1)/k and slope is μ/k

Immobilization of the consortium: Immobilization of living microorganisms and enzymes has been described by several investigators (Nasratun *et al*., 2009; Salemi, 2010; Yusilawati *et al*., 2010; Fedorak *et al*., 1985; Friday and Portier, 1991; Durham *et al*., 1994b) as being useful not only for the production of chemicals for industrial use but also for degradation of chemical pollutants by bacteria where they colonize on the immobilized surface and form biofilms. They are also found to be advantageous over suspended-cell growth (Friday and Portier, 1991; Durham *et al*., 1994a; 1994b; Shimp and Pfaender, 1982; Ehrhardt and Rehm, 1985) for bioremedial purpose. Bacteria immobilized in gels like alginate are surrounded by a gel network, which strongly limits their movement but their metabolic activity is not hampered. However due to accumulation of gas alginate beads tend to rupture after some time. Immobilization inside packed bed bioreactor had been reported with rice straw (Tao *et al*., 2010) as well as waste paper. Bioreactors has been well reported for removal of pollutants (Arbabi *et al*., 2009). Here the immobilization was tried out on corrugated sheets. Since the aim of the work was construction of simplest form of bioreactor no mechanical agitation was given to inhibit oxygenation (Mohamed *et al*., 2009), to mimic the native application as would prevail in the treatment plant, no nitrogen was purged and it was allowed to grow under ambient temperature (~30°C). As evident from Fig. 3a and b there was 41% reduction in medium while 21% in modified media made from effluent water under ambient condition.

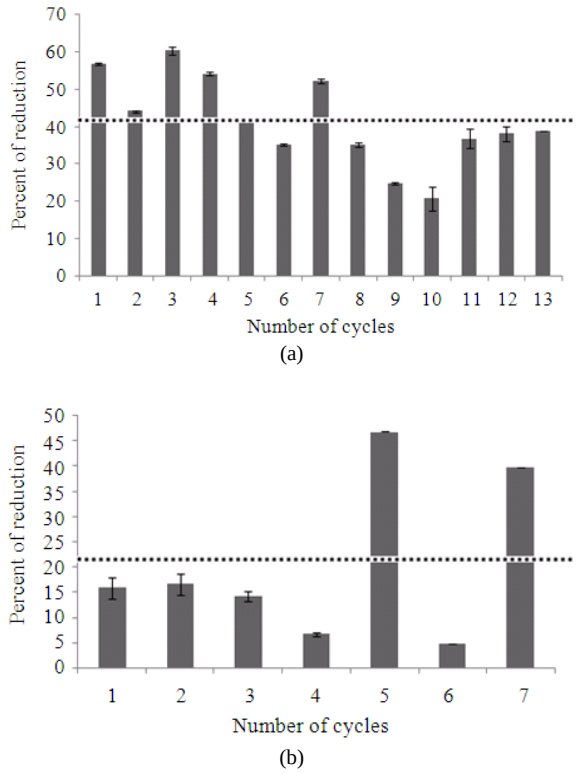


Fig. 3: Sulfate reduction in packed bed reactor. (a) Bar diagram representing the percentage of sulfate reduction after every 24 h in 10 L packed bed reactor in presence of medium DSMZ-16695 with recharging each 24 h. X axis represents the number of cycles of recharging with fresh medium while the straight dotted line represents the average sulfate reduction per day; (b) Bar diagram representing the percentage of sulfate reduction in 10 L packed bed bioreactor in presence of modified medium made using waste water instead of distilled water. X axis represents the number of cycles (of 24 h each) for which the reactor was charged with modified media. The straight dotted line represents the average sulfate reduction per day

DISCUSSION

The consortium isolated from hot water spring was found to reduce sulfate efficiently from 2000ppm in 36h when grown under anaerobic condition at optimum temperature (40°C) in sealed serum vials. Upon immobilization it was found to work better in synthetic medium as compared to waste water which may be due to the presence of inhibitors in the mine effluent. It was found to reduce 41 and 21% of sulfate on an average per day from media and waste water respectively as compared to 66% (in 24h) reduction under optimum growth condition (anaerobic, pH 7.5, temperature 40°C) (Al-Quadan *et al*., 2009). The K_m of 1.1530 ppm and R_{max} value of 0.030h^{-1} was within the range reported for different SRB (Ingvorsen and Jørgensen, 1984; Ingvorsen *et al*., 1984; Sonne-Hansen *et al*., 1999; Roychoudhury and Dean, 2006). The K_m value was much lower as compared to other hot water spring isolates reported earlier (Roychoudhury, 2004) while found to be comparable to fresh water SRB (Roychoudhury and Dean, 2006; Ingvorsen *et al*., 1981). However since the value of these variables was dependent on the initial sulfate concentration (Zuhair *et al*., 2008; Moosa *et al*., 2005) the comparison may not be quite valid. This is the first report of isolation of efficient SRB consortium from the hot water spring of Taptapani at Gunupur in India, for treatment of mine effluent.

CONCLUSION

Bioremediation by bacteria has been well reported since it's both cost effective and efficient in terms of acclimation time (Nasipuri *et al*., 2010; Adeyemi, 2009; Mukherjee *et al*., 2010; Olajide and Ogbeifun, 2010). The results clearly depicts that bioremediation of soluble sulfate can be done with this novel consortium. However since different metals may have inhibitory effect on the microbial growth (Deeb and Altalhi, 2009) the composition of the waste water may determine the sulfate reduction efficiency of the consortium.

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