

Implication of Molasses as Electron Donor for Biological Sulphate Reduction

Ali Hussain*, Javed Iqbal Qazi, Hafiz Abdullah Shakir

Department of Zoology, University of the Punjab, Lahore, 54590, Pakistan

Abstract Treatment of metals and sulphate rich effluents using sulphate reducing bacteria (SRB) has become a popular bioremediation process. Implication of an economical growth substrate for the bioremediation processes is a need of the day. The present study reports usage of molasses as an economical carbon source for biological sulphate reduction. Maximally about 20% sulphate was reduced using this substrate as electron donor in a 60 days trial of anaerobic incubation. The findings of this study will be very helpful in developing economical and environmental friendly bioremediation process(es) addressing removal of metals and sulphates from concerned industrial effluents.

Keywords Carbon source, Economical bioremediation, Electron donor, Molasses, Sulphate reducing bacteria

1. Introduction

Application of dissimilatory sulphate reducing bacteria (DSRB) for the concomitant detoxification of metals and sulphate loaded industrial effluents has become an attractive biological technique and much popular in the last few years[1,2]. These bacteria generate sulfide using multifarious simpler organic molecules and various forms of sulphates as electron donors and acceptors, respectively[2-5]. This biogenic sulfide precipitates metals efficiently thus, concomitantly removing sulphate and metals from the wastewaters.

Economical biosulfidogenesis thus, requires implication of an economical carbon source. In this context, various types of organic wastes have been employed as economical carbon sources and include leaf mulch, mushroom compost, sawdust, sewage sludge, vegetal compost, whey, wood chips and other agricultural wastes[6-11].

Economical bioremediation is a need of the day and it is well known that usage of organic wastes as growth substrates in the biotreatment of sulphate rich effluents economizes the process to possible extent. In this context, the present study was designed to investigate the effectiveness of molasses as growth substrate in the treatment of artificially prepared sulphate rich wastewater while recruiting sulfidogenic microbes.

2. Materials and Methods

2.1. Isolation of Pure Cultures of SRB from Wastewater Samples

Sulphate reducing bacteria were isolated and pure cultured using media B and E, respectively from the samples collected from Hadiara drain, Pakistan after Postgate[12]. Eight sulfidogenic bacterial isolates were pure cultured following the procedure.

2.2. Batch Experiments

These were performed in triplicates in serum bottles of 120 ml capacity using artificially prepared sulphate rich wastewater which was actually a modification of Postgate growth medium and comprised of sulphate (2 g/L) in the form of Na_2SO_4 and 2% molasses (w/v). In control experiments, sodium lactate was used as electron donor for cultivating SRB. The inoculum size used was 5% (v/v) harbouring around 1.7×10^6 C.F.U/ml. pH of the medium was adjusted at 7.0 ± 0.5 in each experiment. Diffusion of oxygen in inoculated media was prevented by adding a layer of autoclaved liquid paraffin (about 3-5 mm thick). The inoculated bottles were sealed with fine rubber stoppers and aluminium crimps and incubated at 30°C for 60 days.

2.3. Analytical Procedures

After every 10 days, 5 ml samples were withdrawn with the help of a sterilized syringe and filtered using a fine quality Whatman filter paper. pH and SO_4^{2-} were measured in each experiment. pH was measured with the help of a digital pH meter, while, SO_4^{2-} was estimated after Cha *et al.*[13].

2.4. Statistical Analysis

Statistically the data were analysed using GLM

* Corresponding author:

alihussainpu@yahoo.com (Ali Hussain)

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procedures and means were compared using Duncan's Multiple Range test with the help of SAS 9.1. Differences

between means were considered significant at $P < 0.05$.

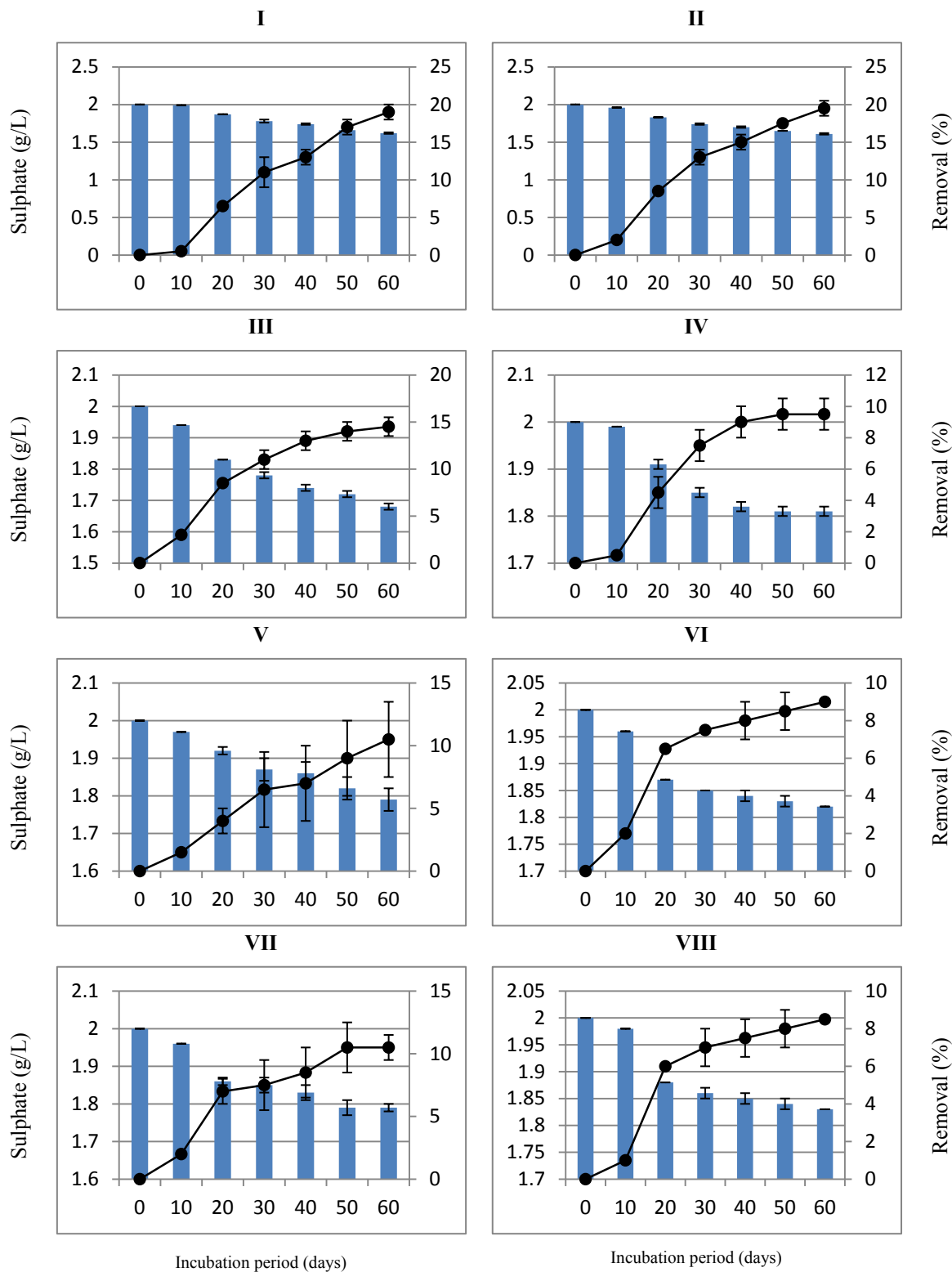


Figure 1. (I–VIII). Sulphate reducing potential of the HAQ1, HAQ2, HAQ3, HAQ4, HAQ5, HAQ6, HAQ7 and HAQ8 bacterial isolates shown in Figs. I, II, III, IV, V, VI, VII and VIII, respectively using molasses as growth substrate

Table 1. Pattern of biological sulphate reduction following periodic incubation of 10 days using molasses as growth substrate

Bacterial isolate	Incubation period (days)						
	0	10	20	30	40	50	60
HAQ1	2.00 ± 0.00	1.99 ± 0.00 ^a	1.87 ± 0.00 ^b	1.78 ± 0.02 ^b	1.74 ± 0.01 ^{bc}	1.66 ± 0.01 ^c	1.62 ± 0.01 ^c
HAQ2	2.00 ± 0.00	1.96 ± 0.00 ^d	1.83 ± 0.00 ^c	1.74 ± 0.01 ^b	1.70 ± 0.01 ^c	1.65 ± 0.00 ^c	1.61 ± 0.01 ^c
HAQ3	2.00 ± 0.00	1.94 ± 0.00 ^e	1.83 ± 0.00 ^c	1.78 ± 0.01 ^b	1.74 ± 0.01 ^{bc}	1.72 ± 0.01 ^d	1.68 ± 0.01 ^d
HAQ4	2.00 ± 0.00	1.99 ± 0.00 ^{ab}	1.91 ± 0.01 ^a	1.85 ± 0.01 ^a	1.82 ± 0.01 ^a	1.81 ± 0.01 ^{bc}	1.81 ± 0.01 ^a
HAQ5	2.00 ± 0.00	1.97 ± 0.00 ^c	1.92 ± 0.01 ^a	1.87 ± 0.03 ^a	1.86 ± 0.03 ^a	1.82 ± 0.03 ^{abc}	1.79 ± 0.03 ^{ab}
HAQ6	2.00 ± 0.00	1.96 ± 0.00 ^{bc}	1.87 ± 0.00 ^b	1.85 ± 0.00 ^a	1.84 ± 0.01 ^a	1.83 ± 0.01 ^{abc}	1.82 ± 0.00 ^a
HAQ7	2.00 ± 0.00	1.96 ± 0.00 ^d	1.86 ± 0.01 ^b	1.85 ± 0.02 ^a	1.83 ± 0.02 ^a	1.79 ± 0.02 ^c	1.79 ± 0.01 ^{ab}
HAQ8	2.00 ± 0.00	1.98 ± 0.00 ^{bc}	1.88 ± 0.00 ^b	1.86 ± 0.01 ^a	1.85 ± 0.01 ^a	1.84 ± 0.01 ^{ab}	1.83 ± 0.00 ^a

Values represent sulphate concentration (g/L) and are means ±S.E. of three replicates. Those not sharing a common alphabet within a respective column are significantly different from each other. Single factor analysis of variance at $P < 0.05$

3. Results and Discussion

The present study reports the sulphate reduction potentials of eight sulfidogenic bacterial isolates using molasses as growth substrate. The bacterial characterization and their classification is described elsewhere (unpublished data). All the sulfidogens showed round about similar results in terms of sulphate reduction [Figure 1 (I-VIII)]. In general, more sulphate reduction occurred in the initial (between 10 to 30 days) than the final phases (between 40 to 60 days) of the anaerobic incubations. Similar trend of sulphate reduction has also reported by Martins *et al.*[2] while studying efficiency of food industrial effluents in biological sulphate reduction processes.

All the bacterial isolates showed delayed lag phases which might be due to complexity of the growth substrate as reported by Beaulieu *et al.*[14]. Sulphate reduction rates were at their peaks between 10 to 20 days and after 30 days of incubation, sulphate reduction rates started to decrease (Table 1). Utilization of simpler molecules by SRB is a well known feature[6,15-18]. Ample availability of simpler molecules in the early stages and inadequate availability in the later stages due to their exhaustion might became the reason of lesser sulphate reduction rates in the final halves of anaerobic incubations. Here, the results were again consistent with those of Martins *et al.*[2]. However, detailed biochemical analyses of the SRB growth and sulfidogenesis promoting molecules and their consumption rates are required to verify these explanations.

Efficiency of sulphate reduction remained poor using molasses as electron donor. A satisfactory sulphate reduction by mixed cultures of SRB using molasses as carbon source has been reported[19-21]. In the present study, absence of fermenting agents (*Lactobacilli*) that were used by the above researchers was the most probable reason of lesser sulphate reduction. In addition, presence of significant proportions of highly volatile fatty acid and other non-biodegradable contents (products of caramelization) in molasses might have

further inhibited the sulphate reducing activity of SRB as also reported by other researchers[22,23].

In control experiments, maximum (100%) sulphate reduction was observed using sodium lactate as electron donor. All of the added sulphate was reduced in the starting 10 days of incubation. This efficient sulphate reduction was most probably due to simplicity of lactate molecules and neutral pH of the media as have been reported earlier[2,24].

In the present study, maximally about 20% sulphate was reduced in a 60 days trial of anaerobic incubation. Sulphate reduction rates can be enhanced further by using bioactivated bacterial consortia. According to Beaulieu *et al.*[14] bioactivation of bacterial consortia with an easily available simple organic source (e.g. lactate) and then replacing it with any organic waste or a mixture of organic waste can lead to better sulphate reduction rates. It is also noticeable that multiple organic wastes perform better than a single waste[9,18,25] and the above information demand further studies on these lines.

4. Conclusions

The present study arrived at the conclusion that molasses can serve as a growth substrate for passive biotreatment of sulphate rich wastewaters. As the present experimental trial was carried out only for 60 days to just assess the electron donating potential of molasses, however, the results could be improved if it will be allowed to operate for a longer time period. The efficiency of sulphate reduction can be enhanced further using mixed cultures of SRB as well as by mixtures of molasses with other organic wastes (carbon sources).

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