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Effects of different environmental and operating conditions on sulfate bioreduction in shake flasks by mixed bacterial culture predominantly *Pseudomonas aeruginosa*

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ABSTRACT

The performance of an acclimatized mixed microbial consortium obtained from a wastewater treatment plant was investigated for its potential to reduce SO_4^{2-} under different operating conditions. The effect of pH, temperature, initial SO_4^{2-} concentration, suspended and attached growth reactor configuration, and different carbon sources was investigated in this study. SO_4^{2-} was completely utilized within 15 d of reactor run from an initial concentration of 1,000 mg/L utilizing lactate as the sole carbon source. Compared to other carbon sources tested in this study, lactate proved to be better for SO_4^{2-} reduction by the mixed consortium. The optimum conditions for SO_4^{2-} reduction by the enriched mixed culture were found to be 30˚C and pH 7.0. The 16S rDNA analysis of the acclimatized consortium showed that *Pseudomonas aeruginosa* was the predominant strain in the mixed culture. For the first time, mixed culture with predominantly *P. aeruginosa* has been reported to be involved in SO_4^{2-} reduction except one limited report on the SO_4^{2-}-reducing capacity of the bacteria of genus *Pseudomonas*.

Keywords: Sulfate reduction; Mixed microbial culture; *Pseudomonas aeruginosa*; SRB; Lactate

1. Introduction

Biological SO_4^{2-} reduction (BSR) is one of the most common and efficient methods for the treatment of SO_4^{2-}-rich wastewaters in the present scenario. Various industrial activities such as mining and mineral processing, pulp and paper industries, production of explosives, scrubbing of flue gasses, petrochemical industries, food processing (molasses, seafood, edible oil), and pharmaceutical industries [1] discharge SO_4^{2-}-rich wastewaters. BSR takes place in anaerobic environment by a specialized group of micro-organisms known as sulfate-reducing bacteria (SRB) resulting in the conversion of sulfate to hydrogen sulfide as the end product. Different parameters such as pH, temperature, and carbon source affect the growth and activity of SRB to a great extent. The activities of the SRB are affected by the pH of the environment where they exist. Some species are sensitive to acidic waters [2], while some have extreme acid-tolerance capacity [3]. Mixed SRB cultures are much more tolerant to extreme conditions as compared to pure cultures as reported by Postgate [4]. Specific SRB require a certain temperature range for their growth and activity. SRB are broadly classified as

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mesophiles (growth temperature <40 °C), moderate thermophiles (growth temperature 40–60 °C), and extreme thermophiles (growth temperature >60 °C) based on their optimum growth temperature. Thus, temperature also forms an essential parameter to determine the efficiency of SO_4^{2-} reduction. Lens et al. [1] reported that SRB are very diverse in their carbon source utilization and metabolic activities. Generally, the SO_4^{2-} -rich wastewaters require the addition of an external carbon source as electron donor in order to achieve the required stoichiometric COD/SO_4^{2-} ratio of 0.67 achieving theoretical possible removal of SO_4^{2-} [5]. The carbon and energy sources provide energy for the growth and maintenance of SRB and thus form a very necessary part in the biological SO_4^{2-} reduction process. Different bioreactor configurations have been reported in the literature for anaerobic reduction of SO_4^{2-}. BSR can be achieved with freely suspended bacterial cells as well as immobilized cells. Cell washout in continuous bioreactors employing freely suspended cells can be prevented by operating at low flow rate and high residence time. However, in the case of an immobilized or attached cell bioreactor, it is possible to operate the reactor at a high flow rate without cell washout. More resistance to extreme conditions such as low pH and high metal concentrations is provided by the biofilm formed in the immobilized cell bioreactors [6].

The aim of the present study was to determine the performance of SO_4^{2-} removal by a mixed bacterial consortium. The optimization of the different parameters such as pH, temperature, and carbon source was also investigated to obtain better efficiency of the enriched microbial consortium. The performance of the mixed consortium under different environmental and operating conditions for SO_4^{2-} reduction was also investigated. In addition, phylogenetic analysis of the predominant strain in the mixed consortium was also done.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals and reagents used in the study were either of analytical reagent grade or laboratory reagent grade. Magnesium sulfate procured from Merck, India, was used as the source of SO_4^{2-} in all the experiments. The synthetic feed was prepared by addition of the following composition per liter of distilled water: 0.5 g KH_2PO_4, 0.1 g K_2HPO_4, 0.3 g NH_4Cl, 0.75 g $NaHCO_3$, and trace metal solution containing 0.03 g $NaMo_4{\cdot}2H_2O$, 0.05 g $CaCl_2{\cdot}2H_2O$, 0.02 g $CoCl_2{\cdot}6H_2O$, 0.05 g KCl, 0.05 g $MgCl_2{\cdot}6H_2O$, 0.01 g $NiSO_4{\cdot}6H_2O$, 0.05 g sodium ascorbate, and 0.05 g sodium thioglycollate. Dextrose was used as the carbon source unless otherwise mentioned. Sodium lactate (60% solution), procured from Loba Chemie, India, was used when lactate was the carbon source.

2.2. Analytical methods

All the samples collected in 15-mL centrifuge tubes were centrifuged at 8,000 rpm for 5 min to separate the biomass before being analyzed for residual SO_4^{2-} and COD in the treated effluent. pH was monitored with the help of digital pH meter (Thermo Scientific, Orion 3 Star, USA). SO_4^{2-} was determined by the turbidimetric method [7]. Before analyzing SO_4^{2-}, the sample was pretreated with NaOH (6N) and zinc acetate (1M) to fix the generated sulfide [8]. Chemical oxygen demand (COD) of samples was measured by closed reflux methods following the procedure recommended in the standard methods [7]. The COD samples were acidified with a few drops of H_2SO_4 and purged with N_2 gas instead of stirring, to release the sulfides present as gaseous sulfide, so as to reduce the sulfide interference on the COD value [9]. The bacterial culture for field emission scanning electron microscope (FESEM) was fixed with 2.5% glutaraldehyde and then washed three times with distilled water. The washed sample was then sequentially dehydrated using acetone with concentrations varying from 30 to 100% in 15% increments, with 10 min exposure time. After dehydration, dried sample was gold sputtered and examined under FESEM (Zeiss Sigma, USA).

2.3. Enrichment, isolation, and identification of predominant species of the mixed consortium

Mixed culture biological sludge, collected from a wastewater treatment plant located at IIT Guwahati, India, was used as a seed sludge for inoculating purpose and was acclimatized. The mixed microbial culture was grown in the medium with low initial concentration of SO_4^{2-}. Initially, required amount of $MgSO_4{\cdot}7H_2O$ was added to get 100 mg/L SO_4^{2-} which was steadily increased to 1,000 mg/L during the acclimatization phase. To maintain the anaerobic condition in the culture flasks, oxygen free nitrogen was purged at regular intervals. Fresh medium was replaced every week and oxygen free nitrogen was purged after medium replacement. The culture was then acclimatized over a period of two-and-a-half months to reduce SO_4^{2-} starting from 100 mg/L up to a concentration of 1,000 mg/L. One percent of the microbial culture was subcultured twice using a fresh

medium for isolating the predominant species present in the acclimatized mixed consortium. The culture was also plated on a solid medium and incubated in an anaerobic jar containing the media with 1.5 g/L agar containing an Anaerogas pack (Hi Media) to provide anaerobic environment. The isolated pure bacterial strain, which was named r3, was sent to Genie (Bangalore, India) for 16S rDNA sequence analysis and later, the result was submitted to GenBank database to carry out the similarity search for nucleotides by online basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/blast/). The neighbor-joining phylogenetic tree was constructed using robust phylogenetic analysis for the non-specialist [10] to represent the relationship between the strain r3 (JN600615.1) and related genera.

2.4. Influence of different parameters affecting SO_4^{2-} reduction

Several parameters affecting SO_4^{2-} reduction were investigated through batch and fed batch experiments, including the effect of pH, temperature, initial SO_4^{2-} concentration, suspended and attached growth system, and different carbon sources (Table 1). The batch reactor sets used in the study of the effect of pH, temperature, and initial SO_4^{2-} concentration were named as BR1, BR2, and BR3, respectively.

2.4.1. Effect of pH, temperature, and initial sulfate concentration

All experiments in this study were performed in triplicate sets of 150-mL Erlenmeyer flasks containing 100 mL of media with dextrose as the carbon source. About 200 mg/L of the acclimatized culture was added as inoculum in these reactors.

Media containing 1,000 mg/L SO_4^{2-} with COD/SO_4^{2-} ratio of 1.5 were adjusted to seven different pH values namely 4, 5, 6, 7, 8, 9, and 10 separately using 0.1 M HCl and 0.1 M NaOH solutions. After the pH adjustment was done, each flask was purged with nitrogen gas for 2 min to maintain anaerobic condition in the culture flasks. The effect of temperature on SO_4^{2-} reduction was also studied at five temperature ranges starting from 20 to 40°C. Similarly, the effect of initial SO_4^{2-} concentration on the reduction capacity of the mixed culture was investigated with five different SO_4^{2-} concentrations ranging from 500 mg/L to 1,200 mg/L maintaining COD/SO_4^{2-} ratio of 1.5 and initial pH of 7. All the flasks were incubated at 28°C in an incubator shaker at an arbitrarily chosen agitation speed of 140 rpm and samples were withdrawn at regular time intervals, centrifuged, and analyzed for residual SO_4^{2-} concentration.

The results were fitted to the following zero-order-rate equation which gave the best fit and SO_4^{2-} degradation rate coefficient (k_r) was estimated for different pH, temperature, and initial sulfate concentration.

$$C_0 - C = k_r t$$

where, C_0 and C are the SO_4^{2-} concentrations at initial and at time "t", respectively.

2.4.2. Suspended and attached growth system

Two 250-mL batch reactors $R1$ and $R2$ were operated simultaneously for around 70 d to study SO_4^{2-}

Table 1
Operating conditions of the reactors used in the different experiments

Mode of operation	Purpose of operation	Carbon source	Influent			
			SO_4^{2-} conc. (mg/L)	COD/SO_4^{2-}	HRT (d)	Temp. (°C)
Batch (150 mL Erlenmeyer flasks)	Effect of pH (BR1)	Dextrose	1,000	1.5	8	28
	Effect of temperature (BR2)		1,000	1.5		20, 25, 30, 35, 40
	Effect of SO_4^{2-} conc. (BR3)		500, 700, 800, 1,000, 1,200	1.5		28
Fed-batch (250 mL conical flasks)	Effect of suspended ($R1$) and attached growth ($R2$)	Dextrose	1,000	1.5, 1.2, 1	7.5, 5	30
	Effect of different carbon sources	Dextrose, acetate, formate, ethanol, lactate	1,000	1.5	6	
	Reactor performance ($R3$)	Lactate	1,000, 1,200	1.5, 1.2, 1	7.5, 5	

and COD reduction efficiency of the acclimatized sludge under different operating conditions. *R*1 was a suspended growth reactor, while *R*2 was a packed-bed system where about 5 g of polyurethane foam (PUF) cubes of approximately 2.5 cm × 1.5 cm × 1.5 cm sizes were used as a supporting material for microbial growth. Both *R*1 and *R*2 were kept in an incubator shaker at a constant temperature of 30 ± 0.2°C and 140 rpm. About 436 mg/L of the acclimatized sludge was added to each reactor. Both the reactors were started with an initial SO_4^{2-} concentration of 1,000 mg/L and COD of 1,500 mg/L. The study was done in fed batch mode maintaining a HRT of 7.5 d in Phase -I, by replacing 100 ml of treated wastewater with equal volume of fresh feed every 3 d interval. The HRT was further reduced to 5 d in Phase-II by replacing 150 mL of treated wastewater every 3 d interval keeping all the other conditions same. The COD in both the reactors was reduced stepwise to 1,200 mg/L in Phase-III and then finally to 1,000 mg/L in Phase-IV.

2.4.3. Effect of different carbon sources for SO_4^{2-} reduction

The ability of the acclimatized mixed bacterial consortium to utilize different carbon sources for SO_4^{2-} reduction was investigated in the present study. About 10% v/v of the enriched culture was added as inoculum in these experiments. The initial pH of the media was set to 7, temperature 30°C, and agitation speed 180 rpm. The different carbon sources used were acetate, dextrose, ethanol, formate, and lactate. The study was done in the fed batch mode maintaining an HRT of 6 d by replacing 50% of fresh feed every 3 d with the simultaneous removal of equal volume of the media. Nitrogen gas was purged for about 2 min after the replacement of feed each time, so as to remove any dissolved oxygen to maintain anaerobic conditions. Each of the carbon sources equivalent to 1,500 mg/L COD was added with an initial SO_4^{2-} concentration of 1,000 mg/L.

The results were fitted to the linear form of Monod's equation to determine the biokinetic coefficients of the most effective carbon source obtained in this study for SO_4^{2-} reduction.

$$\frac{\theta X}{S_0 - S_e} = \frac{K_s}{k} \cdot \frac{1}{S_e} + \frac{1}{k}$$

where θ = HRT (d), S_0 = initial substrate concentration (mg/L), S_e = final substrate concentration (mg/L), X = biomass concentration (mg/L), K_s = half velocity constant (mg/L), and k = maximum substrate utilization rate (d^{-1}), respectively.

2.4.4. Fed batch reactor with lactate as carbon source

After the study of the effect of different carbon sources, a fed batch reactor (*R*3) of 250 mL capacity was operated with lactate as carbon source. About 50 mL of the acclimatized sludge was added to the reactor. The initial SO_4^{2-} concentration in the reactor was 1,000 mg/L, and COD was 1,500 mg/L, and was operated at an HRT of 7.5 d in Phase-I. In Phase-II, the HRT was reduced to 5 d. The COD in the reactor was reduced stepwise to 1,200 mg/L in Phase-III and then finally to 1,000 mg/L in Phase-IV. The SO_4^{2-} concentration was further increased to 1,200 mg/L in Phase-V so as to reduce the COD/SO_4^{2-} ratio to 0.8 to study its effects on SO_4^{2-} and COD removal.

3. Results and discussion

3.1. Enrichment, isolation, and identification of predominant species of the mixed consortium

Enrichment of the mixed consortium was carried out by adding gradually increasing amount of SO_4^{2-} in synthetic wastewater from 100 to 1,000 mg/L. The detailed enrichment phase presented in Fig. 1 shows that the SO_4^{2-} removal rate was improved at each and every stages of acclimatization.

Morphological characterization of the mixed consortium performed using FESEM revealed that the cells were of various sizes and shapes from small rods to large rods (Fig. 2). Most of the rods were between 1.336 × 0.308 μm and 0.858 × 0.328 μm in size. The predominant strain was isolated from the enriched mixed consortium and designated as strain r3. Partial 16S rDNA sequencing result showed that strain r3 had

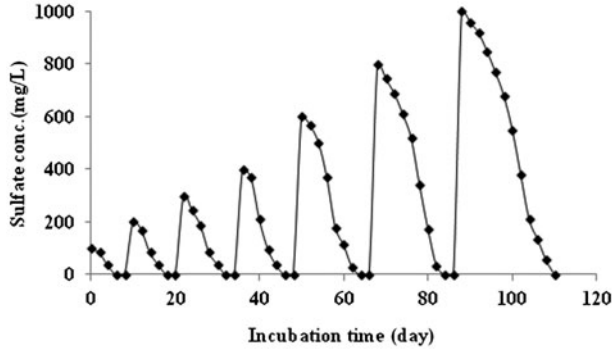

Fig. 1. Sulfate reduction profile during the acclimatization period.

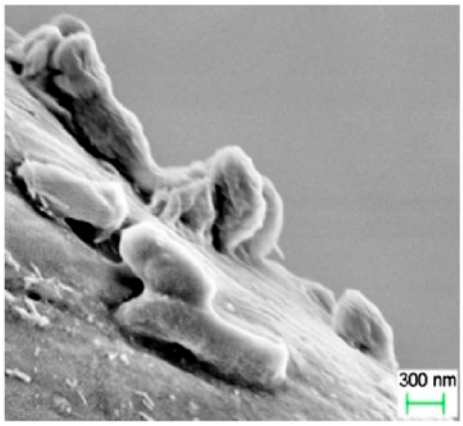

Fig. 2. FESEM image of the mixed culture.

1,525 base pairs (bp). The sequence was submitted to the GenBank with JN600615.1 as the accession number of the strain r3. Gene analysis by online BLAST tool indicated that the isolate contains sequences that are specific to the members of the γ subdivision of the family *Proteobacteria*. The phylogenetic tree shown in Fig. 3 was prepared using neighbor-joining method based on near-full-length 16S rDNA gene sequences recovered from the isolated strain and other sequences obtained from the GenBank database (GenBank accession number has been indicated with the generic name in the tree). The high-bootstrap support of the tree derived from the 16S rDNA analysis demonstrated that strain r3 is a typical member of the *Pseudomonas aeruginosa* species and has the closest relation (99%) to *P. aeruginosa* strain RI-1(JQ773431.1). For the first time, mixed culture with predominantly *P. aeruginosa* has been reported to be involved in SO_4^{2-} reduction except one limited report on the SO_4^{2-}-reducing capacity of the bacteria of the genus *Pseudomonas* [11].

3.2. Influence of different parameters affecting SO_4^{2-} reduction

3.2.1. Effect of pH, temperature, and initial SO_4^{2-} concentration

Fig. 4 shows SO_4^{2-} reduction profile by the mixed consortium over the pH range 4.0–10.0 with initial concentration of 1,000 mg/L SO_4^{2-} which indicates that the culture could substantially reduce SO_4^{2-} in this pH range; the degradation efficiency was considerably less at pH 4.0, 5.0, and 10.0 with values ~3.7, ~9.1, and ~7.5%, respectively. The maximum degradation (~68.6%) of SO_4^{2-} was observed when the initial pH was 7.0 within 8 d with a k_r value of 87.86 mg/L/d. Other researchers also observed that SRB require a pH in the range of 5–8 for survival and outside this range, the rate of microbial SO_4^{2-} reduction generally declines [12]. Low pH (<5) normally inhibits SO_4^{2-} reduction and increases the solubility of metal sulfides [13]. Although, the presence and survival of SRB at pH even less than 3.0 [3] and more than 10 [14] are reported, higher SO_4^{2-} reduction rates have only been achieved at pH close to 8.0 where a volumetric activity was 25 SO_4^{2-} g/L/d [15].

With respect to the effect of temperature on SO_4^{2-} reduction in the range 20–40°C, maximum degradation of 69.57% was observed at 30°C (Fig. 5) with a k_r value of 91.83 mg/L/d. Results indicated that at 30 and 35°C, SO_4^{2-} reduction by the culture was sufficiently high; on the other hand, reduction efficiencies were very less at 20 and 40°C (inset of Fig. 6). Van Houten

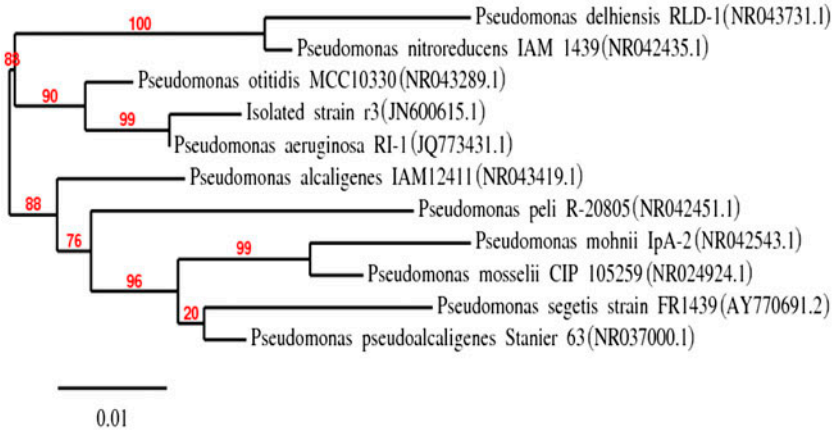

Fig. 3. Phylogenetic relationships between the isolated strain r3 (JN600615.1) and other related strains based upon the analysis of the aligned regions of 16S rDNA gene sequences.

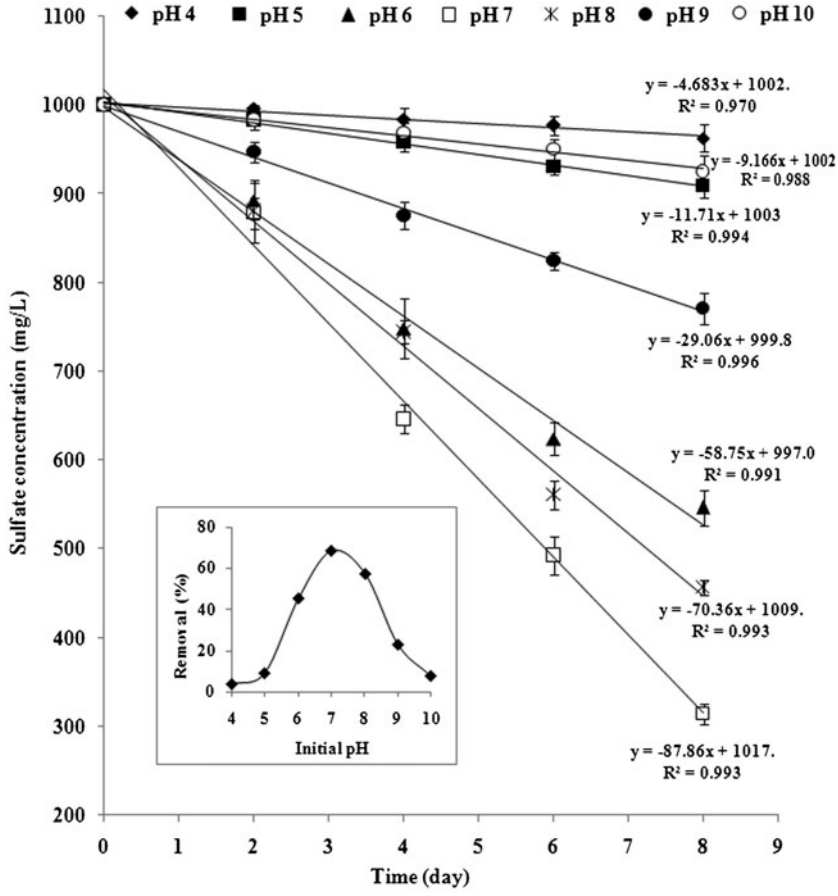

Fig. 4. Effect of pH on SO_4^{2-} reduction by the mixed consortium.

et al. [15] reported that SO_4^{2-} reduction increases when the reaction temperature is increased from 20 to 32°C when a mesophilic SRB culture was used. Moosa et al. [16] conducted batch experiments with a mixed culture consisting of acid producers, methane producers, and SO_4^{2-} reducers and reported that the SO_4^{2-} reduction rate was increased with an increase in the reaction temperature from 20 to 35°C. Further increase in the temperature to 40°C led to the inactivity of bacteria. From the results obtained in the present study, it can be inferred that the mixed culture, which is predominantly *P. aeruginosa* species, is a mesophilic bacterial consortium preferring 30°C and pH 7.0 for SO_4^{2-} reduction.

Time profile of SO_4^{2-} reduction by the mixed culture at different initial SO_4^{2-} concentrations is shown in Fig. 6. It is clear from the profile that the time taken by the mixed culture to degrade SO_4^{2-} to a certain lower concentration was dependent upon its initial concentration. From an initial SO_4^{2-} concentration of 500 mg/L it took around 4 d, while from 1,200 mg/L it took more than 8 d to reduce SO_4^{2-} below 150 mg/L. The maximum k_r value of 98.13 mg/L/d was observed with the initial SO_4^{2-} concentration of 1,200 mg/L (Fig. 6).

3.2.2. Suspended and attached growth system

Performance of the reactors *R*1 and *R*2 is shown in Fig. 7(a) and (b), respectively. Both the reactors were started with a HRT of 7.5 d, initial SO_4^{2-} concentration of 1,000 mg/L, and COD of 1,500 mg/L. At a HRT of 7.5 d in Phase-I, *R*1 showed a maximum SO_4^{2-} reduction of almost 84%, while that of the reactor *R*2 was around 73%. This result indicated that both the reactors were able to effectively reduce SO_4^{2-} with the efficiency of *R*1 was slightly higher than that of *R*2. The performance of R1 increased to more than 98% on SO_4^{2-} reduction in Phase-II, Phase-III, and even in Phase-IV when the COD was reduced to 1,000 mg/L. This may be due to the constant contact between the biomass and substrate in the case of the suspended

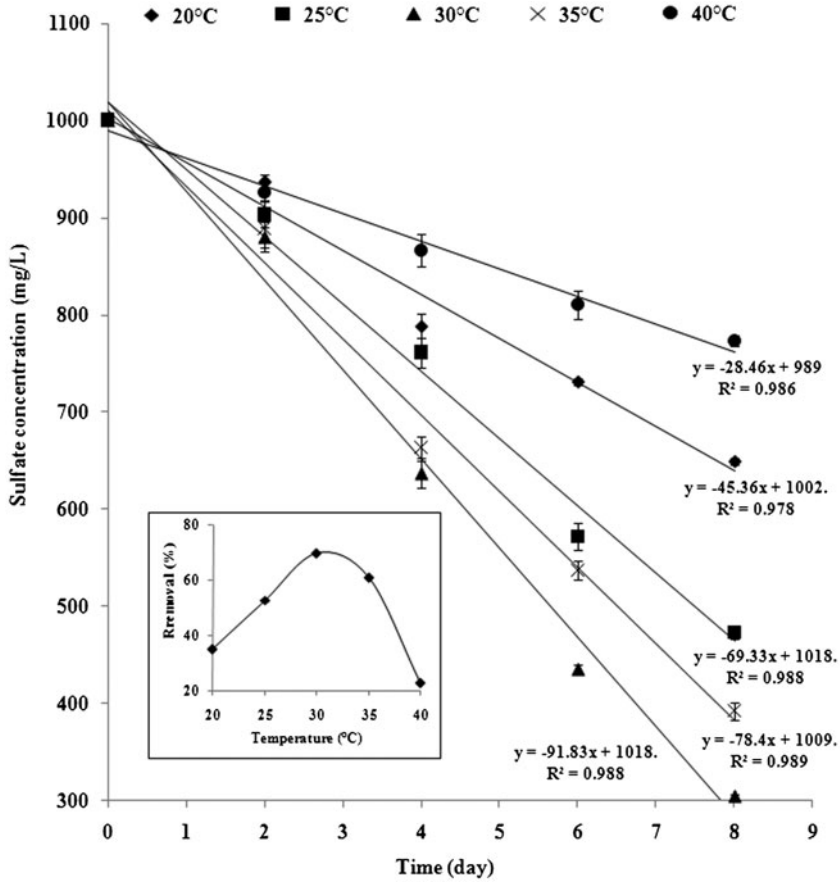

Fig. 5. Effect of temperature on SO_4^{2-} reduction by the mixed consortium.

growth system due to continuous mixing. However, in the case of *R*2, the SO_4^{2-} reduction efficiency improved from around 78% in Phase-II to around 88% in Phase-III which is inferior in comparison with that of *R*1 under the same operating conditions. The lower efficiency may be attributed to the lesser growth rate of attached micro-organisms in the packed-bed system (*R*2) due to diffusion limitations as compared to that of the suspended micro-organisms in the reactor *R*1 [17,18]. However, *R*2 gave similar efficiency with that of *R*1 in Phase-IV once the biofilm was established. When HRT was reduced from 7.5 to 5 d in both the reactors (Phase-II), SO_4^{2-} reduction rate increased in both *R*1 and *R*2. This result indicates that with an increase in the SO_4^{2-} load due to the decrease in HRT, SRB were able to outcompete the methanogens for COD utilization which correlates with the finding that at low feed rates, the methanogenic bacteria as well as the SRB degraded the COD, as COD removal can take place through the methanogenic as well as the SO_4^{2-} reduction pathways [19]. Although SO_4^{2-} reduction efficiency was improved in Phase-II, a high amount of COD was left out in the effluent. In order to compensate for this, the influent COD was further reduced to 1,200 mg/L in both the reactors in Phase-III. The SO_4^{2-} reduction was not noticed even with the reduction of COD concentration in both reactors *R*1 and *R*2. In Phase-IV, the influent COD was further decreased to 1,000 mg/L maintaining an influent COD/SO_4^{2-} ratio of 1.0. In this phase, SO_4^{2-} was completely removed and very less COD was left out in the effluent. No further study was carried out with further decrease in the COD concentration.

3.2.3. Effect of different carbon sources for SO_4^{2-} reduction

Among the five carbon sources used, the mixed consortium showed the maximum SO_4^{2-} reduction by utilizing lactate as the sole carbon source. On the other hand, minimum SO_4^{2-} reduction was observed where acetate was used as carbon source, the preliminary results of which have been discussed in another paper [20]. It may be because lactate promotes the growth of

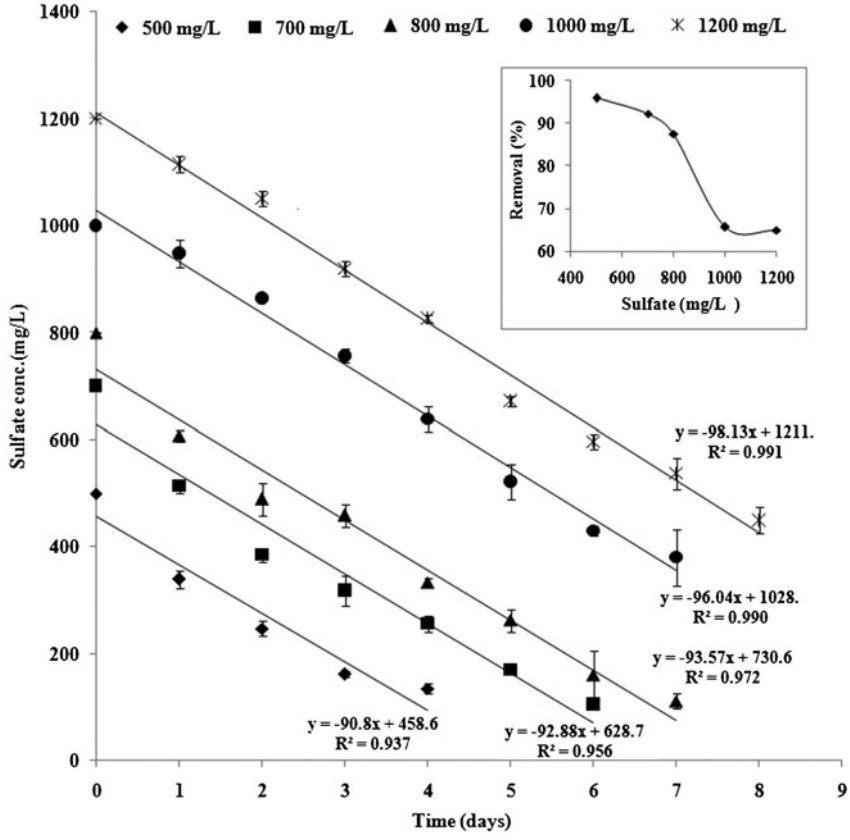

Fig. 6. Effect of initial SO_4^{2-} concentration on SO_4^{2-} reduction by the mixed consortium.

a wide variety of SRB, consequently increasing microbial diversity and treatment system resilience [21,22]. The lowered efficiency of the mixed consortium to reduce SO_4^{2-} using acetate could be due to the inability of the SRB to completely oxidize acetate even with excess SO_4^{2-} levels [23].

The biokinetic coefficients determined for the SO_4^{2-} reduction with the different carbon sources are given in Table 2. The maximum substrate utilization rate (k) for lactate which gave the maximum SO_4^{2-} removal efficiency was 0.063 d^{-1} and the half velocity constant (K_s) was found to be 386.17 mg/L.

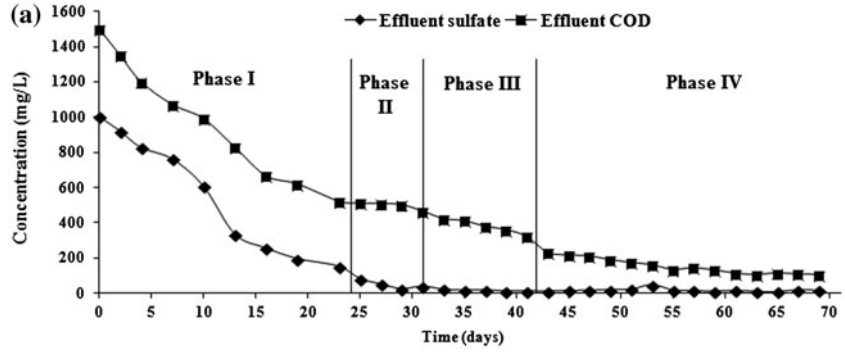

Fig. 7. (a) $SO42-SO_4^{2-}$ reduction profile in reactor $R1$ with initial $SO42-SO_4^{2-}$ = 1,000 mg/L. Phase-I: HRT = 7 d; COD = 1,500 mg/L; Phase-II: HRT = 5 d; COD = 1,500 mg/L; Phase-III: HRT = 5 d, COD = 1,200 mg/L; Phase-IV: HRT = 5 d; COD = 1,000 mg/L. (b) $SO42-SO_4^{2-}$ reduction profile in reactor $R2$ with initial $SO42-SO_4^{2-}$ = 1,000 mg/L. Phase-I: HRT = 7 d; COD = 1,500 mg/L; Phase-II: HRT = 5 d; COD = 1,500 mg/L; Phase-III: HRT = 5 d, COD = 1,200 mg/L; Phase-IV: HRT = 5 d; COD = 1,000 mg/L.

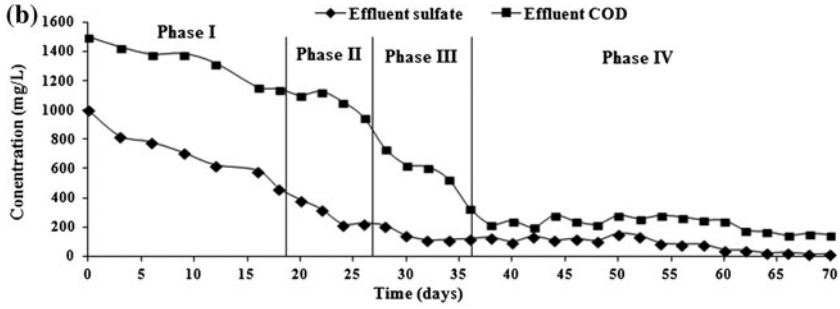

Fig. 7. (*Continued*).

Table 2
Half velocity constant (K_s) and the maximum substrate utilization rate (k) determined for SO_4^{2-} reduction with the five carbon sources

Electron donor	K_s (mg/L)	k (d^{-1})	R^2
Lactate	386.17	0.063	0.996
Formate	215.8	0.042	0.979
Ethanol	302.01	0.035	0.929
Dextrose	532.59	0.055	0.971
Acetate	142	0.027	0.907

3.2.4. Fed batch reactor with lactate as carbon source

After the study on the effects of different carbon sources on SO_4^{2-} reduction, the reactor *R*3 was fed with lactate which was found to be the most efficient carbon source. The performance of *R*3 is shown in Fig. 8. The reactor was started with an HRT of 7.5 d in the initial Phase-I with initial SO_4^{2-} concentration of 1,000 mg/L and lactate COD of 1,500 mg/L. At this HRT, *R*3 showed a maximum SO_4^{2-} reduction of around 90% after 18 d of reactor operation. This result indicated that SO_4^{2-} reduction was effectively going on in the reactor *R*3 where lactate was provided as the sole carbon source and the removal efficiency was better as compared to reactors *R*1 and *R*2 with dextrose as the carbon source. The SO_4^{2-} removal was almost complete in Phase-II (HRT = 5 d). This might be due to an increase in the SRB population utilizing lactate as the carbon source with an increase in SO_4^{2-} load due to decrease in the HRT. As a large amount of COD was left out in the effluent in Phase-II, influent COD was reduced from 1,500 to 1,200 mg/L in Phase-III. With this reduction in the COD/SO_4^{2-} ratio, the SO_4^{2-} removal efficiency was not hampered. So in the next phase (Phase-IV), the COD/SO_4^{2-} ratio was further decreased to 1.0 by decreasing the influent COD to 1,000 mg/L. The removal efficiency of SO_4^{2-} was not reduced even with this COD concentration and still, some amount was left out in the effluent. So in the next phase (Phase-V), the influent SO_4^{2-} concentration was increased to 1,200 mg/L to reduce the COD/SO_4^{2-}

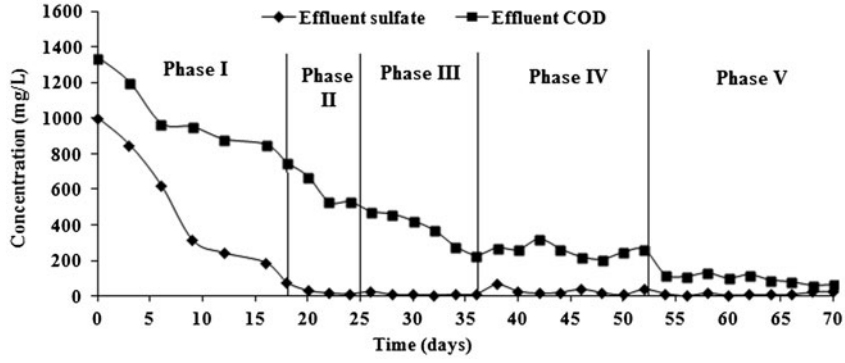

Fig. 8. SO_4^{2-} reduction profile in the reactor *R*3 with lactate as the carbon source. Phase-I: HRT = 7.5 d, SO_4^{2-} = 1,000 mg/L, COD = 1,500 mg/L; Phase-II: HRT = 5 d, SO_4^{2-} = 1,000 mg/L, COD = 1,500 mg/L; Phase-III: HRT = 5 d, SO = 1,000 mg/L, COD = 1,200 mg/L; Phase-IV: HRT = 5 d, SO_4^{2-} = 1,000 mg/L, COD = 1,000 mg/L; Phase-V: HRT = 5 d, SO_4^{2-} = 1,200 mg/L, COD = 1,000 mg/L.

ratio to 0.8. There was no further decrease in SO_4^{2-} removal, which was noticed even in this phase. In addition to this, the effluent COD concentration was reduced and very less COD was left out in the effluent.

4. Conclusions

The indigenous mixed microbial culture, isolated from the treatment plant, was highly effective in reducing SO_4^{2-} from synthetic wastewater. The phylogenetic analysis of the mixed consortium revealed *P. aeruginosa* as the predominant strain which is reported in detail for the first time to be involved in SO_4^{2-} reduction. The optimum conditions of the mixed culture for SO_4^{2-} reduction were observed to be 30˚C and pH 7.0.

In the fed batch mode, the suspended growth reactor *R*1 was found to be more effective than the attached growth reactor *R*2 for SO_4^{2-} reduction in the initial phases. However, once the biofilm was established in *R*2 with further operation, its removal efficiency was improved. This shows that attached growth reactors may require a longer start-up period as compared to the suspended growth systems. Eventually in Phase-IV of *R*1 and *R*2, almost complete SO_4^{2-} removal could be achieved under similar conditions.

Among the various carbon sources tested in the study, lactate showed maximum biodegradation of SO_4^{2-}. The performance of reactor fed with lactate as carbon source (R3) showed a much better efficiency for SO_4^{2-} reduction as compared to the reactors *R*1 and *R*2 which used dextrose as the carbon source. Almost complete SO_4^{2-} removal was observed even when the COD/SO_4^{2-} was reduced to 0.8, which shows that lactate was effectively utilized by the mixed consortium as the carbon source.

From the results obtained in the study, the potential of the mixed microbial culture can be implicated further in SO_4^{2-} removal from the polluted wastewater.

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