



## Development of enhanced sulphidogenesis process for the treatment of wastewater having low COD/SO<sub>4</sub><sup>2-</sup> ratio

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### ABSTRACT

An upflow hybrid sulphidogenesis reactor of 1.75 L volume was developed (at oxidation–reduction potential (ORP) =  $-225 \pm 25$  mV) using flocculent extended aeration process sludge (selected based on screening study at COD/SO<sub>4</sub><sup>2-</sup> ratio = 1) for enhanced sulphidogenesis and COD removal. The reactor was subjected to various loading rate studies at a hydraulic retention time (HRT) of 1 day with COD/SO<sub>4</sub><sup>2-</sup> ratio of 1.3. At loading rate of 2.5 kg COD/(m<sup>3</sup> day), excellent performance with more than 97% removal of sulphate was achieved within bottom 40% volume of the reactor. At a higher loading rate of 3.75 kg COD/(m<sup>3</sup> day), there was a decrease in both sulphate (70–75%) and COD (50%) removal efficiencies. A controlled and continuous air injection (0.19 L/(L min)) given at 40% volume of the reactor affected sulphide oxidation inside the reactor and enhanced the sulphate reduction in the reactor. The specific sulphate reduction capacity of mixed culture drawn from the bottom part of the reactor was 0.35 kg SO<sub>4</sub><sup>2-</sup>/(kg VSS day). The results of this study showed that enhanced sulphidogenesis with sulphide inhibition control can maintain sulphate-reducing bacteria (SRB) in anaerobic reactor at low COD/SO<sub>4</sub><sup>2-</sup> ratios between 1 and 2, with efficient simultaneous removal of COD and SO<sub>4</sub><sup>2-</sup>. The sulphide generated in the system can be recovered as elemental sulphur and/or oxidized back to sulphate.

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### 1. Introduction

Sulphate bearing waste streams is generated by many industrial activities such as sea food processing, fermentation, tanning, paper production and edible oil refining [1–3]. Most of these wastewaters also contain substantial amount of organic matter (OM). Discharge of high sulphate or COD bearing wastewater into the natural water bodies is not advisable, and they need appropriate treatment. Many conventional methods are available for the removal of COD and sulphate using aerobic and/or anaerobic processes.

Although aerobic systems have high COD removal efficiency, sulphate removal is not possible in them. Mostly anaerobic treatment methods, incorporating sulphate-reducing bacteria (SRB) and methane-producing bacteria (MPB), are employed for treatment of wastewaters containing both COD and sulphate. However, SRB have kinetic and thermodynamic advantage over MPB in utilizing organic matter, which leads to the production of sulphide compounds [4]. At high concentrations, these sulphide compounds are toxic to MPB [1,3,5]. So far, no sustainable method has been devel-

oped for selective inhibition of SRB to drive the anaerobic process towards methanogenesis [6].

The COD/SO<sub>4</sub><sup>2-</sup> ratio in effluents appears to be a key factor in the regulation of the competition between methanogenic and sulphate-reducing bacteria [2,4,7]. In practice, anaerobic treatment is successful for wastewaters with COD/SO<sub>4</sub><sup>2-</sup> ratio exceeding 10 [8]. For such wastewaters, the H<sub>2</sub>S concentration in the anaerobic reactor will never exceed the critical value of sulphide inhibition due to the stripping effect of biogas produced. However, process failures of anaerobic reactors for methane generation have been reported [6] when the COD/sulfate ratio of the wastewater was less than 10. Dilution of H<sub>2</sub>S concentration, decrease of unionized H<sub>2</sub>S concentration by pH control, selective inhibition of SRB, addition of ferric salts to precipitate sulphides, separation of H<sub>2</sub>S production and methanogenesis and ORP based oxygenation for sulphide control by injecting controlled oxygen to recycle biogas line are commonly employed sulphide inhibition control measures [6,9,10]. However, many of these methods are not economically feasible or sustainable. For example, separation of H<sub>2</sub>S production and methanogenesis may be costly because it requires an additional reactor and accessories, which increases the complexity of treatment system [11].

Enhanced biological sulphate reduction (sulphidogenesis) process appears to be a promising alternative for treating low COD/SO<sub>4</sub><sup>2-</sup> ratio bearing effluents. In sulphidogenesis process, the

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reduction of 1.5 kg  $\text{SO}_4^{2-}$  consumes 1 kg COD [12,13], and thus both COD and  $\text{SO}_4^{2-}$  are removed in the process. Advantages of enhanced sulphidogenesis are anoxic removal of organic matter, process stability, no VFA accumulation, reduced biomass generation and high alkalinity generation inside the process [6]. SRB can grow using countless organic compounds as sources of energy for their metabolism and most of the species can oxidize these compounds completely to  $\text{CO}_2$  [14]. The SRB comprised of complete oxidizers and incomplete oxidizers. The complete oxidizers directly convert OM (after VFA formation) to  $\text{CO}_2$ , whereas incomplete oxidizers with the help of acetate utilizing SRB convert OM (after VFA formation) to  $\text{CO}_2$ . Possibilities of using biologically generated sulphide as a resource is well demonstrated and described by Hulshoff Pol et al. [6]. For COD/ $\text{SO}_4^{2-}$  ratios less than 1, sulphidogenic systems can be developed with complete COD removal by SRB alone [15]. However, sustainability of sulphidogenesis for COD/ $\text{SO}_4^{2-} \leq 1$  was not demonstrated because of severe sulphide inhibition. Sipma et al. [16] reported a maximum sulphate reduction efficiency of 50% at COD/ $\text{SO}_4^{2-}$  of 1.33 in thermophilic sulphate reduction in UASB reactors under acidifying conditions (pH 5.8–6.1). Though acidogens and SRB are not very sensitive to low concentrations of free  $\text{H}_2\text{S}$  [17,18], SRBs are inhibited at high sulphide concentrations [11,19]. Studies focussing on enhanced sulphidogenesis for OM removal in effluents with low COD/sulfate ratio are scarce in literature. The objective of this work was to develop an enhanced sulphidogenesis process, with a simple sulphide inhibition control, for biological treatment of sulphate bearing effluents having low COD/sulfate ratio with possibility of partial recovery of sulphate present in effluent as elemental sulphur.

## 2. Materials and methods

### 2.1. Sulphidogenesis medium

Sodium sulphate and sucrose were used as sources of sulphate and organic carbon, respectively. Predetermined amount of sulphate and sucrose were taken in each study based on required COD/ $\text{SO}_4^{2-}$  ratio. The other compounds of sulphidogenesis medium used in this study were:  $\text{NaHCO}_3$ , 0.7 g;  $\text{NH}_4\text{Cl}$ , 0.04786 g/g sucrose;  $\text{K}_2\text{HPO}_4$ , 0.014 g/g sucrose;  $\text{MgCl}_2$ , 0.08 g/g sucrose; dissolved in 1 L of distilled water. Trace elements were added to the medium. The composition of trace element solution was (in g/L):  $\text{H}_3\text{BO}_4$ , 0.0005;  $\text{ZnCl}_2$ , 0.0005;  $(\text{NH}_4)_6\text{MO}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ , 0.0005;  $\text{NiCl}_2\cdot 6\text{H}_2\text{O}$ , 0.0005;  $\text{AlCl}_3\cdot 6\text{H}_2\text{O}$ , 0.0005;  $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$ , 0.0005;  $\text{CoCl}_2\cdot 4\text{H}_2\text{O}$ , 0.0005;  $\text{NaSeO}_3\cdot 5\text{H}_2\text{O}$ , 0.001;  $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ , 0.0005;  $\text{CaCl}_2$ , 0.0003;  $\text{FeCl}_2$ , 0.000015 and EDTA, 0.000015. All chemicals were analytical reagent (AR) grade, supplied by 'Qualigens' (India). Clean 'Borosil' (India) make glassware were used for preparation of reagents and volume measurements.

### 2.2. Analytical techniques

All physical–chemical parameter analyses were conducted as per Standard Methods [20].

Sulphate was analyzed by Ion chromatography (DIONEX, USA) with ED50 electrochemical detector and the results were processed by in-built 'Chromleon' software. The samples for sulphate and sulphide analyses were immediately fixed with 2N zinc acetate in alkaline condition to avoid sulphide oxidation. The fixed samples were centrifuged at  $5000 \times g$  for 5 min and supernatant was analyzed for sulphate and precipitate was analyzed for total sulphides by the iodometric method. The COD analysis of centrifuged sample (without zinc acetate) was carried out after acidifying the sample to pH less than 1 with sulphuric acid for releasing sulphides as  $\text{H}_2\text{S}$  by stirring and then fixed with potassium dichromate. The closed reflux method of COD digestion was carried out by HACHS COD digester (Loveland, USA). ORP was measured using double junction platinum ORP electrode connected to a calibrated CyberScan pH (1100) meter in mV mode (EUTECH Instruments, Singapore). ORP electrode was calibrated using Quinhydrone 86.

### 2.3. Screening of biomass for sulphidogenesis process

Sludges collected from different sources (Table 1) were used for screening study in anoxic condition at COD/ $\text{SO}_4^{2-}$  ratios of 0.7 and 2.5. Batch experiments were carried out in 250 mL capacity bottles (Duran make, Germany), with airtight rubber septum having gas releasing arrangement through a water seal. Total feed volume was 200 mL. Batch reactors were operated for 2 weeks, at temperature of 30–32 °C, with mixing by means of a magnetic stirrer (Remi Equipments Ltd., India). Start-up conditions are given in Table 1. Further, the biomass from batch reactors with COD/ $\text{SO}_4^{2-}$  ratio of 2.5 was subjected to a feeding with COD/ $\text{SO}_4^{2-}$  ratio of 1.0, keeping other experimental conditions same as in preliminary screening tests. Based on the results from screening tests, the potential biomass was selected for further development of sulphidogenesis in batch and continuous reactors. A separate batch reactor was developed at a COD/ $\text{SO}_4^{2-}$  ratio of 1.0 with operation in a feed and draw mode for 2 months, and 100 mL sludge from this batch reactor was used for seeding the continuous reactor.

### 2.4. Continuous reactor studies

#### 2.4.1. Upflow hybrid reactor

Schematic of the hybrid reactor used for developing sulphidogenesis process is shown in Fig. 1. A reactor of 1.75 L volume was made from an acrylic cylinder, with an internal diameter of 5 cm and a height of 105 cm. The bottom 25 cm (volume = 0.50 L) of the reactor was not filled with any packing medium to provide space for suspended growth of biomass. The remaining 1.25 L of reactor volume was filled with plastic rings (with inside thin

**Table 1**  
Start-up conditions during screening of biomass for sulphidogenesis process

Reactor tag	Type of biomass	Total VSS (g)	COD/ $\text{SO}_4^{2-}$ ratio	Initial pH
SR1	UASB reactor sludge employed for treatment of sewage	3.7	0.7	7.5 ± 0.05
SR2	UASB reactor sludge employed for treatment of sewage	3.7	2.5	7.5 ± 0.05
SR3	Cow dung based sludge from biogas plant	3.8	0.5	7.5 ± 0.05
SR4	Cow dung based sludge from biogas plant	3.8	2.5	7.5 ± 0.05
SR5	Flocculent extended aeration process sludge employed for treatment of tannery effluent (EAP-Tannery)	0.6	0.7	7.5 ± 0.05
SR6	Flocculent extended aeration process sludge employed for treatment of tannery effluent (EAP-Tannery)	0.6	2.5	7.5 ± 0.05

Initial COD = 2500 mg/L.

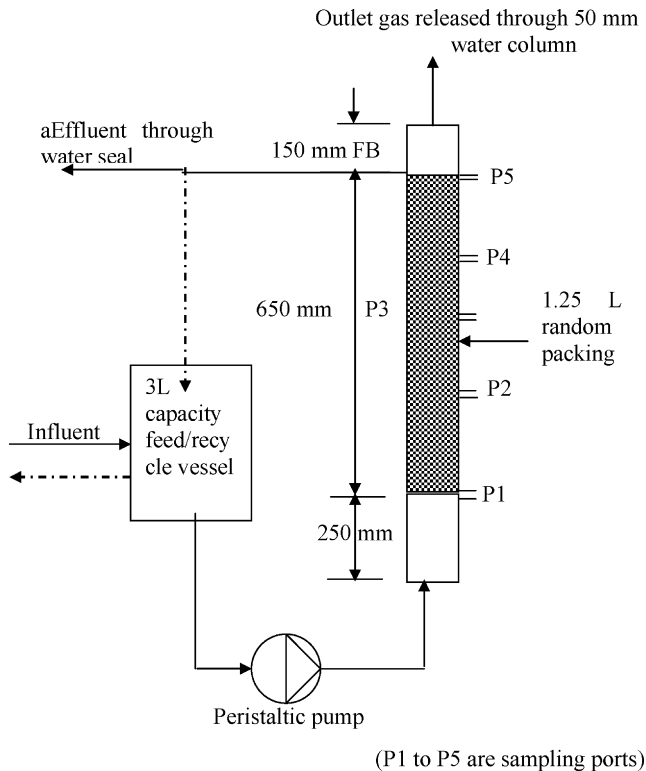


Fig. 1. Schematic of upflow hybrid sulphidogenesis reactor (broken lines show the recycling operation during start-up and acclimatization).

partition wall at  $90^\circ$  apart, mean diameter = 2 cm, height = 1.7 cm, bulk density =  $150 \text{ kg/m}^3$ ; porosity = 90%, area available for microbial attachment =  $2935 \text{ cm}^2/\text{L}$  of reactor volume) as medium for biomass attachment. The packing media was supported by a circular disc fixed to the reactor at 25 cm height from the bottom. The circular disc had uniformly distributed 0.5 cm circular holes for equal distribution of effluent to media. The reactor was provided with 5 sampling ports (P1–P5) at 25 cm, 40 cm, 55 cm, 70 cm and 90 cm from the bottom of the reactor, respectively. The last sampling port was used as effluent outlet too. The anoxic condition inside the reactor was maintained by providing water seals to outlet gas and effluent pipe. The outlet gas pipe was dipped in 50 cm water column in a separate bottle so that gas produced is released while maintaining anoxic condition inside the reactor. The effluent was taken out through a U tube containing effluent (as water seal) to effluent collection or recycle vessel as per the case. The effluent recycling to the reactor through feed vessel was carried out only during start-up and acclimatization phase. Feeding was from the bottom of the reactor through a 1 cm brass nozzle. This bottom nozzle was connected via PVC tubing through a calibrated peristaltic pump (Miclins, India) to the feed vessel.

#### 2.4.2. Start-up and acclimatization

The start-up of the reactor was done using extended aeration process flocculent sludge employed for treatment of tannery effluent. The reactor was initially started with 1 L extended aeration process flocculent sludge mixed with 100 mL mixed culture of sulphate-reducing bacteria (developed from same extended aeration process sludge at COD/SO<sub>4</sub><sup>2-</sup> ratio of 1.0 in a batch reactor). The mixed biomass had total volatile suspended solids (VSS) of 8.33 g. Initially, the reactor was fed with sulphidogenesis medium having a COD/SO<sub>4</sub><sup>2-</sup> ratio of 1.8, with an initial COD of 2500 mg/L (pH  $7.5 \pm 0.1$ ). For this, 2 L of sulphidogenesis medium was fed to the

reactor, after purging with oxygen free nitrogen for 5 min, and the effluent was recycled to the hybrid reactor through a closed feed vessel (3 L capacity Borosil bottle) to develop the SRB culture. The reactor was operated in an upflow mode of recycling (2.8 h HRT) during the start-up and acclimatization period. Whenever reactor was fed with the fresh feed, an equivalent amount of effluent was wasted. The oxidation–reduction potential (ORP) maintained inside the reactor was  $-225 \pm 25 \text{ mV}$ . Fresh feed was added to the system nine times during the start-up phase (1–50 days). pH was adjusted by adding NaHCO<sub>3</sub> to the recycle vessel as and when required during the period of start-up and acclimatization. Operational details of continuous reactor are presented in Table 2. During second and third phases of acclimatization feeding was done on alternate days (days 61–120) with COD/SO<sub>4</sub><sup>2-</sup> ratio of 1.8 and 1.3, respectively. After 60 days, feed/recycle vessel was stuffed with cotton to have uncontrolled diffusion of oxygen to the vessel, so that sulphide in the effluent could oxidize to form sulphur film.

#### 2.4.3. Loading rate studies

After 120 days, from the starting of the reactor, it was put in to continuous mode of operation at COD and SO<sub>4</sub><sup>2-</sup> loading rates of  $2.5 \text{ kg COD}/(\text{m}^3 \text{ day})$  and  $1.9 \text{ kg SO}_4^{2-}/(\text{m}^3 \text{ day})$ , respectively. There was no recycling of effluent during loading rate studies. The effluent was collected in an effluent collection vessel stuffed with cotton to have uncontrolled diffusion of oxygen to the vessel, so that sulphide in the effluent could oxidize to form sulphur film. Subsequently the loading rates of COD and SO<sub>4</sub><sup>2-</sup> were increased to  $3.75 \text{ kg COD}/(\text{m}^3 \text{ day})$  and  $2.85 \text{ kg SO}_4^{2-}/(\text{m}^3 \text{ day})$ , respectively. The reactor was operated at this increased loading rate during the period 149–190 days without any sulphide inhibition control.

#### 2.4.4. Sulphide inhibition control

A simple sulphide inhibition control was applied after 191 days, by injecting air through the second sampling port (P2). Air was supplied from a portable air compressor at the rate of 0.2 L/min through a stainless steel syringe from 191st to 205th day. Air flow rate was controlled at the rate of 0.1 L/min for the period 206th to 216th day, and later increased to 0.2 L/min for further operation. The air flow rate was measured using a Rotameter (100–1000 mL/min range, Placka make, India). The reactor was operated until 220 days.

#### 2.4.5. Sample port analysis

Samples collected from sampling ports were analyzed during each loading phase, after attaining a steady state, in order to get the COD and SO<sub>4</sub><sup>2-</sup> removal efficiency along the height of the reactor. Samples were analyzed for pH, COD, SO<sub>4</sub><sup>2-</sup> and sulphides. During the final phase of loading at  $3.75 \text{ kg COD}/(\text{m}^3 \text{ day})$  (203rd day), the sludge from the bottom part of the reactor (anoxic zone) was subjected to batch study in 100 mL serum bottles in sulphidogenesis medium under anoxic conditions to determine specific sulphate reduction rate of mixed SRB culture. Initial conditions in the batch reactor were: pH 7.5, COD = 2500 mg/L, SO<sub>4</sub><sup>2-</sup> = 1900 mg/L, mixed liquid suspended solids (MLSS) = 2160 mg/L, mixed liquid volatile suspended solids (MLVSS) = 1470 mg/L. Also, the same sludge was used for morphology identification by scanning electron microscopy (SEM) technique. The sludge for SEM was fixed with 2.5% glutaraldehyde in 20 mM K<sub>2</sub>HPO<sub>4</sub>/KH<sub>2</sub>PO<sub>4</sub> buffer at a pH of 7.4, and then washed with the same phosphate buffer for three times. Then the sludge specimen was sequentially dehydrated using ethanol with concentrations varying from 30% to 100% in 20% increments, with 20 min exposure time. After dehydration, dried sample was sputtered with platinum in an ion auto fine coater (JEOL, JFL-160, Japan) and then examined under a scanning electron microscope (JOEL, JSM-6360, Japan).

**Table 2**  
Operational schedule of continuous reactor

Sl. no.	Operational phase	Period of operation (days)	Feed COD (mg/L)	Feed $\text{SO}_4^{2-}$ (mg/L)	COD/ $\text{SO}_4^{2-}$ ratio	Operation mode and HRT
1	Start-up and acclimatization	1–50	2500	1390	1.8	Effluent recycle to feed vessel, HRT = 2.8 h
2	Shut down	51–60	–	–	–	–
3	Second phase of acclimatization	61–90	2500	1390	1.8	Effluent recycle to feed vessel with sulphide inhibition control, HRT = 2.8 h
4	Third phase of acclimatization	91–120	2500	1900	1.3	Effluent recycle to feed vessel with sulphide inhibition control, HRT = 2.8 h
5	Loading studies					
i	First phase of loading study (loading rate: 2.5 kg COD/(m <sup>3</sup> day))	121–148	2500	1900	1.3	In daily continuous operation, no recycle and no sulphide inhibition control, HRT = 1 day
ii	Second phase of loading study (loading rate: 3.75 kg COD/(m <sup>3</sup> day))	149–190	3750	2850	1.3	In daily continuous operation, no recycle and no sulphide inhibition control, HRT = 1 day
iii	Third phase of loading study (loading rate: 3.75 kg COD/(m <sup>3</sup> day))	191–220	3750	2850	1.3	In daily continuous operation, no recycle and with sulphide inhibition control, HRT = 1 day

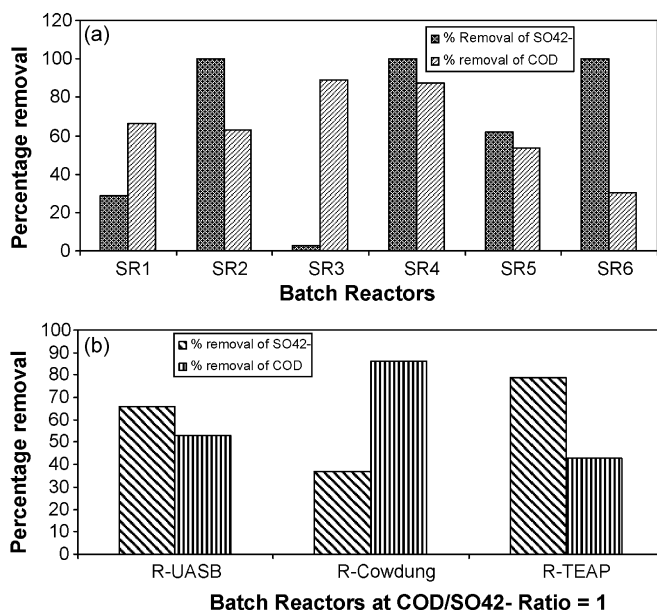
### 3. Results and discussion

#### 3.1. Screening of biomass for sulphidogenesis process

Three types of sludges, each with two different COD/ $\text{SO}_4^{2-}$  ratios, were screened for sulphidogenesis process. Results from this study are presented in Fig. 2a. It can be seen from these preliminary screening results that all sludges were able to reduce sulphate completely when COD/ $\text{SO}_4^{2-}$  ratio was 2.5. However, for a COD/ $\text{SO}_4^{2-}$  ratio of 0.7 (stoichiometric ratio without consid-

ering carbon requirement for growth of SRB), extended aeration process flocculent sludge employed for treatment of tannery effluents showed better performance (62% of sulphate reduction) than the other anaerobic sludges. The lesser performance of anaerobic sludges especially cow dung sludge might be as a result of competition of anaerobic bacteria and methanogenic bacteria for available COD. From Fig. 2a, it is evident that higher COD removals occurred with anaerobic sludges at a COD/ $\text{SO}_4^{2-}$  ratio of 0.7. Better performance of extended aeration flocculent sludge for sulphate reduction might be due to the presence of sulphate-reducing bacteria already exposed to sulphides present in tannery effluent during the extended aeration process and the availability of more OM (COD) as a result of sludge hydrolysis of this sludge in anaerobic and/or sulphidogenic conditions. In presence of sulphide enhanced sludge hydrolysis was reported [21]. The stoichiometry of COD removals in correspondence with sulphate removals in SR5 and SR6 were not matched as a result of sludge hydrolysis and increase of COD. The tannery effluent normally contains high concentrations of sulphate (1000–3000 mg/L), sulphides (20–200 mg/L) and COD (3000–6000 mg/L) [22,23]. Such high concentrations of sulphate and COD with flocculent nature of activated sludge might contain aero-tolerant SRBs in the floc core. There are reports about the presence of large numbers of sulfate-reducing bacteria in the oxic zones and near the oxic–anoxic boundaries of sediments and in stratified water bodies, microbial mats and termite guts [24]. Community structure analyses and microbiological studies in such systems have shown that the SRB populations in those zones are adapted to oxygen [24].

Further optimization in screening was carried out by sequential batch operation of promising batch reactors at a COD/ $\text{SO}_4^{2-}$  ratio of 1.0. At this ratio, it was expected that SRB might predominate methane-producing bacteria [25,26] and there could be sufficient carbon available for growth also. Fig. 2b shows the results of screening at COD/ $\text{SO}_4^{2-}$  ratio of 1.0. In contrast to expectation, it was observed that anaerobic sources of sludge showed higher COD removal compared to sulphate reduction in the similar way as



**Fig. 2.** Percentage removals of sulphate and COD during screening of different sources of biomass (COD/ $\text{SO}_4^{2-}$  ratios of reactors in (a) are given in Table 1). (a) Initial screening of biomass for sulphidogenesis. (b) Percentage removal at COD/ $\text{SO}_4^{2-}$  ratio = 1.

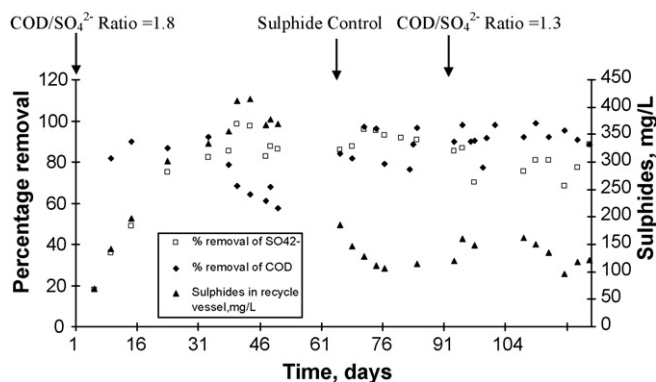


Fig. 3. Performance of sulphidogenesis continuous reactor during start-up and acclimatization in recycling operation (changes in operation are indicated by vertical arrows).

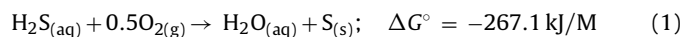
obtained in screening study at a COD/SO<sub>4</sub><sup>2-</sup> ratio of 0.7. Again, the biomass screened from extended aeration process sludge employed for treatment of tannery effluents gave better sulphate reduction compared to cow dung and UASB sludges. However, the sulphate reduction was not complete and lesser compared to result obtained at a COD/SO<sub>4</sub><sup>2-</sup> ratio of 0.7. Here it is to be noted that the sludge used in optimization study was from batch reactors where 100% sulphate reduction occurred. These sludges had a prior exposure to high concentration of sulphides (312 mg/L) at the end of batch study. During optimization study also, there was competition among sulphate reducers and methanogens for available COD in cases of UASB sludge and cow dung sludge where as competition was less intense in case of EAP sludge. Also, the amount of COD available by sludge hydrolysis could be less during optimization study as the sludge from first batch reactor (already undergone hydrolysis) was used. The less performance of EAP sludge at COD/SO<sub>4</sub><sup>2-</sup> ratio equal to one might be due to combined effect of sulphide toxicity for more prolonged time (during screening test and optimization test) and less availability of organic compounds from sludge hydrolysis during optimization test. The sulphide concentration inside R-TEAP reactor was 616 mg/L. There are reports of inhibition of SRB by high concentrations of sulphide generated in the system [11,19].

### 3.2. Start-up and acclimatization of sulphidogenesis process in continuous reactor

Performance of sulphidogenesis reactor during different phases of operation is presented in Table 3. Fig. 3 shows the performance of sulphidogenesis reactor during start-up. Although more than 80% of COD reduction was achieved in the reactor within 10 days, it took 30 days to achieve more than 80% sulphate reduction. The initial high removal of COD might have accomplished by non-

SRB. This could be due to prior non-acclimatization of the start-up sludge. As mentioned earlier, the seed sludge was collected from an aerobic plant. As the sulphidogenesis process progressed, COD removal reduced from 80% to 58% (40–50 days) corresponding to increase of sulphide concentration above 300 mg/L. This decrease in COD reduction might be attributed to sulphide inhibition to methanogens and non-SRBs [1,17] as the sulphide bearing effluents were recycled through the hybrid reactor. There was marginal deterioration in performance as far as sulphate reduction is concerned, and this might be due to the sulphide inhibition to SRB [11,19,27].

In order to develop and acclimatize required microbial culture for sulphidogenesis process (60–120 days), a simple sulphide inhibition control strategy was adapted in recycle vessel. Small amount (uncontrolled) of air was allowed to enter the recycle vessel to induce sulphide oxidation. This strategy worked satisfactorily and there was generation of sulphur film on liquid surface of recycle vessel. The sulphur thus generated could be easily separated using a fine mesh filter. The filtered sulphur sludge had excellent settling properties to the extent of 90% volume reduction in 3 min. Fig. 4 shows the pictures of sulphur film generation in recycle vessel, separated sulphur sludge in tap water before settling and settled sulphur sludge within 3 min. This sulphide oxidation was expected to be a combination of chemical and biological oxidation as per Eq. (1). However, further studies were not carried out to identify microorganisms responsible for such sulphur film formation.



It was found that as the time progressed, the simple sulphide inhibition control strategy improved the COD removal in corresponds to lesser concentration of sulphides. This improved COD removal may be due to the combined action of SRB and non-SRB including MPB. It was also observed that further reduction of COD/SO<sub>4</sub><sup>2-</sup> ratio from 1.8 to 1.3 (90–120 days) decreased the sulphate reduction where as the COD removal improved marginally. Freese and Stucky [28] reported that, for COD/SO<sub>4</sub><sup>2-</sup> ratios between 1 and 2, there is a possible shift to sulphidogenesis as predominant process. In an anaerobic baffled reactor with sucrose as carbon source, they have achieved more than 80% sulphate reduction for COD/SO<sub>4</sub><sup>2-</sup> ratio of 2.5 and more than 50% sulphate reduction for COD/SO<sub>4</sub><sup>2-</sup> ratio of 1.0. However, the present results show that simple sulphide inhibition control strategy may be used to sustain a good percentage of COD and SO<sub>4</sub><sup>2-</sup> removal (Fig. 3) from effluents with low COD/SO<sub>4</sub><sup>2-</sup> ratio, with possibility of sulphur recovery. The demerit of sulphur generation as per the demonstrated process in this study is the lack of proper process control to limit the sulphide oxidation at the level of elemental sulphur. A little extra amount of air can drive the reaction to other oxidized forms of sulphur. Another disadvantage of this inhibition control is the recycling cost associated in process operation. However, results from development stage of the present study indicated that it is possible to maintain SRB in anaerobic reactor at COD/SO<sub>4</sub><sup>2-</sup> ratios between 1 and 2, with more than 85% of COD removal.

Table 3  
Performance history of continuous reactor

Sl. no.	Operational phase	Period of operation (days)	Outlet pH	Outlet COD (mg/L)	Outlet SO <sub>4</sub> <sup>2-</sup> (mg/L)	%Removal of COD	%Removal of SO <sub>4</sub> <sup>2-</sup>
1	Start-up and acclimatization	1–50	8.2–7.9	195–307	855–30.2	92.2–67.7	35.9–97.7
2	Shut down	51–60	–	–	–	–	–
3	Second phase of acclimatization	61–90	7.8–7.2	595–83	188–65	76.3–96.6	85.9–95.1
4	Third phase of acclimatization	91–120	7.7–7.2	286–27	599–213	88.6–98.9	68.5–88.7
5	Loading studies						
i	First phase of loading study	121–148	7.1–6.7	483–1414	250–0	80.7–43.4	86.8–100
ii	Second phase of loading study	149–190	6.8–6.0	1173–2063	25.7–853	68.7–45	99.7–70
iii	Third phase of loading study	191–220	6.5–8.0	926–201	708–214	75.3–94.6	75.1–92.5

All values are range of minimum and maximum values obtained during each operational phase.

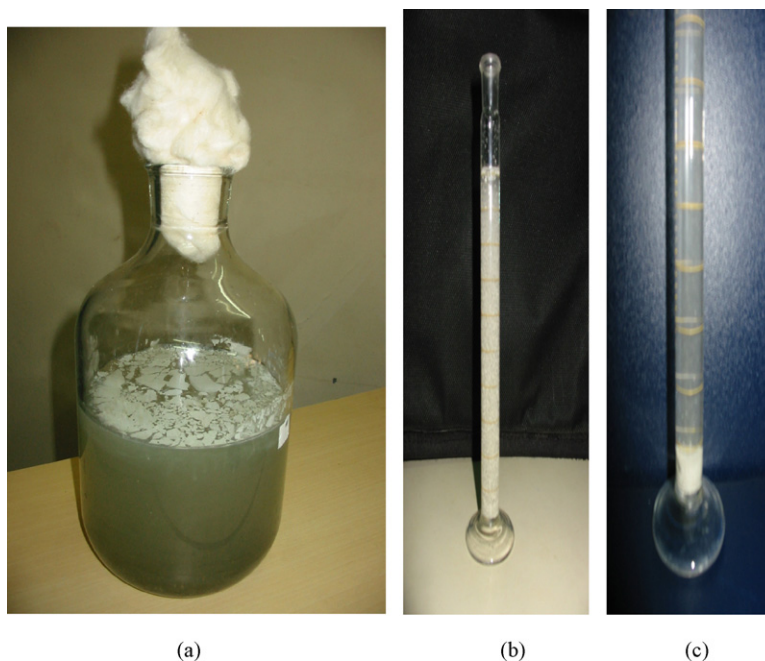


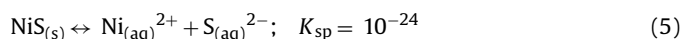
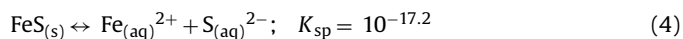
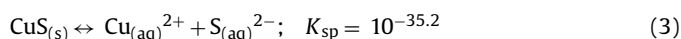
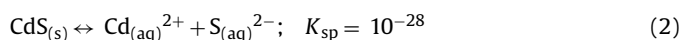
Fig. 4. (a) Sulphur generation inside the effluent collection/recycle vessel, (b) separated sulphur film in tap water and (c) settled volume (1/10th) of sulphur in 3 min.

### 3.3. Loading rate study

Loading rate study was carried out in three phases, with different organic loading (at COD/SO<sub>4</sub><sup>2-</sup> ratio of 1.3) and operational strategies. Results from this study are discussed in the following sections.

#### 3.3.1. Phase 1

Loading study in phase 1 continued from 121 to 148 days, with an organic loading of 2.5 kg COD/(m<sup>3</sup> day). Fig. 5 shows the operational characteristics and the performance during the loading study in continuous operation without recycling. Fig. 5a shows the effluent pH and total aqueous sulphides concentration during the operational period. There were fluctuations in both sulphides concentrations and pH during the initial 10 days, where sulphidogenesis was not in a steady state. From Fig. 5b, it is clear that during 131–148 days; sulphidogenesis process reached a steady state and consistently achieved more than 98% of sulphate reduction. Stoichiometrically, the total sulphide produced can be to the extent of 621 mg/L (from 1900 mg/L SO<sub>4</sub><sup>2-</sup>) at 98% reduction of sulphate. But, the aqueous total sulphide concentrations inside the reactor were in the range of 515–534 mg/L during this period. A part of remaining fraction of total sulphide (14–17%) might have escaped from the system through gas phase as there was strong rotten egg smell of H<sub>2</sub>S from the bottle containing water seal kept for the gas releasing from the reactor. The concentration of H<sub>2</sub>S in gas phase was not monitored. Some amount of sulphides might have formed as polysulphides and a small fraction of sulphides might have formed as metal precipitates and remained inside the reactor. Since the concentration of trace elements added were not very high the possibility of substantial removal of sulphide as metal sulphide is not significant in the present system. The following equilibrium reactions show the feasibility of certain metal sulphide precipitation [29].



where  $K_{sp}$  is the solubility product constant of the reaction.

Though above equilibrium relations show easy precipitation of metal sulphides, in practice complete removal of metals is not possible as some residual metal sulphide concentration will remain in aqueous phase. Such low concentrations of metals can satisfy the micronutrient requirement of the bacterial culture in the reactor.

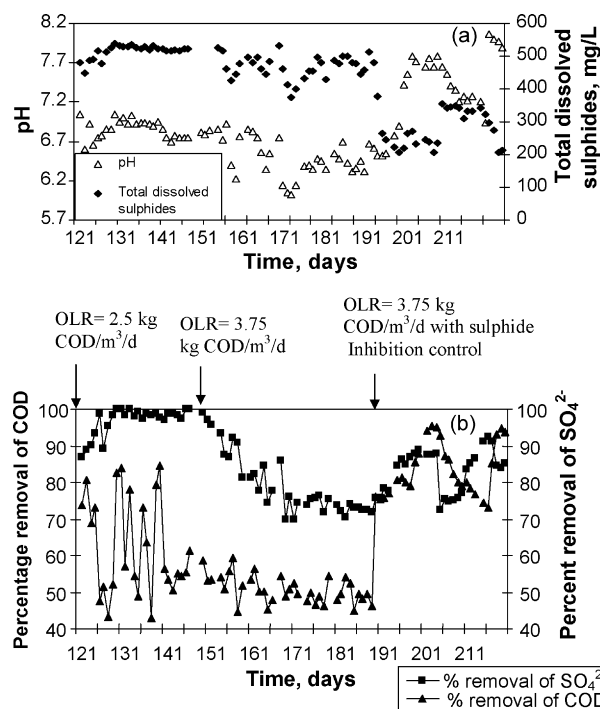


Fig. 5. Operational characteristics and performance during organic loading rate (OLR) study of sulphidogenesis reactor at COD/SO<sub>4</sub><sup>2-</sup> ratio of 1.3 and HRT of 1 day (changes in operation are indicated by vertical arrows). (a) Variation of pH and total dissolved sulphides. (b) Removal efficiency in the reactor.

$\text{H}_2\text{S}$  produced as a result of sulphate reduction would be in equilibrium with sulphides in the aqueous phase as per the following equations [3]:



The above equilibrium reactions show that when pH decreases more sulphides would be in free  $\text{H}_2\text{S}_{(\text{g})}$  form and chances of escape of  $\text{H}_2\text{S}$  through the gas phase would be more. It can be seen from the trend of pH and total dissolved sulphides concentration (Fig. 5a) that the above fact was true in the present study until 190 days of operation.

Fig. 5b shows the removal efficiency of sulphate and COD in the system. It was observed that COD removal efficiency varied between 43% and 84% during 131–140 days of continuous operation, whereas sulphate reduction was consistently more than 98%. As per sulphidogenesis stoichiometry, theoretical maximum removal of COD possible by sulphidogenesis is approximately 52% during this period. The reason for deficiency of COD removal less than 52% occurred in few days is unknown. However, extra removal of COD (above 52%) during this period might be due to non-SRBs and methanogens present in the system with competition with SRB. However, the competition by SRB could not be sustained by MPB after 140 days as evident from sudden drop in COD removal efficiency to 50–60%. During this period, the total aqueous sulphides concentration was above 500 mg/L at a pH less than 6.8. At lower pH conditions, free  $\text{H}_2\text{S}$  would be more and it can penetrate the cell wall of bacteria easily and inhibit bacterial cells including SRB [6,17,26]. However, MPB is relatively more sensitive to sulphide toxicity.

Fig. 6a shows the percentage removal of sulphate and COD along the height of the reactor on 146th day of continuous operation. It was found that approximately 97% sulphate reduction occurred within the bottom 40% volume of the reactor. There was 100% removal of sulphate by the time effluent reached the top of the reactor. However, the COD removal efficiency remained at 60–62% throughout the reactor. The theoretical COD requirement for sulphate reduction was approximately 52% of the COD removal in the system, without considering the carbon requirement for growth of SRB. Extra 8–10% of COD removed might have been used for the growth of anaerobic mixed culture. The effective loading possible by sulphidogenesis process was found to be  $4.75 \text{ kg SO}_4^{2-}/(\text{m}^3 \text{ day})$  at COD/ $\text{SO}_4^{2-}$  ratio of 1.3, with an effective HRT of 0.44 day (considering bottom 40% volume of reactor). Accordingly, the volumetric sulphate reduction obtained in this study was within the reported values of  $0.8\text{--}10.4 \text{ kg SO}_4^{2-}/(\text{m}^3 \text{ day})$  in literature [30,31].

### 3.3.2. Phase 2

Loading study in phase 2 continued from 149 to 190 days, with an organic loading rate of  $3.75 \text{ kg COD}/(\text{m}^3 \text{ day})$ . As 97% of sulphate reduction occurred within bottom 40% volume of the reactor when loading was  $2.5 \text{ kg COD}/(\text{m}^3 \text{ day})$ , both COD and sulphate (keeping COD/ $\text{SO}_4^{2-}$  ratio of 1.3) concentrations were increased further to achieve  $3.75 \text{ kg COD}/(\text{m}^3 \text{ day})$  and  $2.85 \text{ kg SO}_4^{2-}/(\text{m}^3 \text{ day})$ , respectively. At increased loading, there was a drop in sulphate reduction from 99% to 80% during the period 149–163 days (Fig. 5b). Sulphate reduction efficiency reduced further as the time of operation progressed and reduced to a value of 70–75% as the system attained steady state. Since steady state was achieved, this loading is considered as the maximum loading to achieve at least 70% sulphate reduction in a wastewater with a COD:sulphate ratio of 1.3. The present results are in agreement with the results reported by Freese and Stucky [28] without sulphide inhibition control.

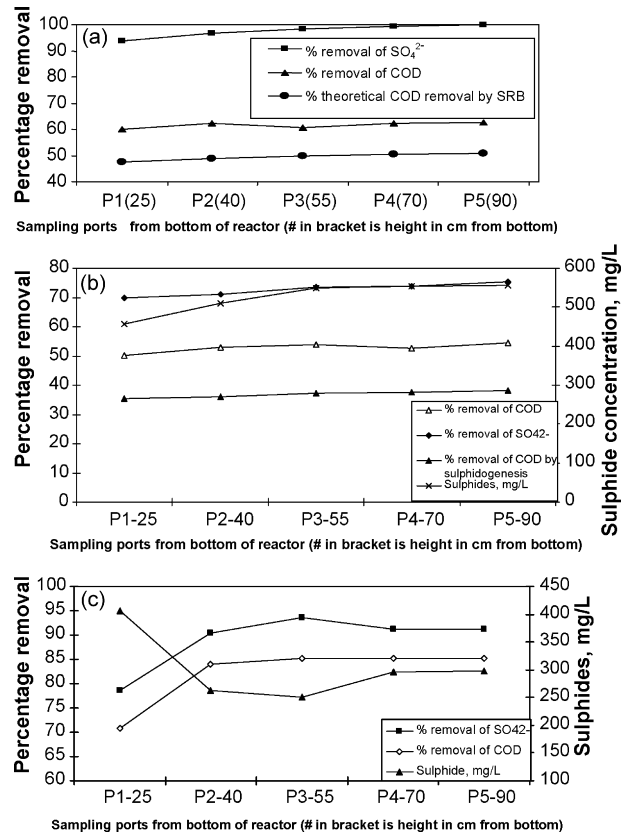


Fig. 6. Sample port analysis under various conditions (COD/ $\text{SO}_4^{2-} = 1.3$ ). (a) Percentage removal along the depth of reactor on 146th day without sulphide inhibition control, COD loading rate of  $2.5 \text{ kg}/(\text{m}^3 \text{ day})$ . (b) Percentage removals along the depth of reactor with sulphide concentration on 179th day without sulphide inhibition control at OLR of  $3.75 \text{ kg COD}/(\text{m}^3 \text{ day})$ . (c) Percentage removals along the depth of reactor with sulphide concentration on 217th day after implementing sulphide inhibition control at OLR of  $3.75 \text{ kg COD}/(\text{m}^3 \text{ day})$ .

Fig. 6b shows sample port analyses on 179th day of operation (at COD loading of  $3.75 \text{ kg}/(\text{m}^3 \text{ day})$ ) where sulphidogenesis process was affected by sulphide inhibition. From the results, it is obvious that sulphate reduction was affected at high sulphate loading though major part of sulphate removal occurred within the bottom 40% volume of the reactor. Such high percent removal of sulphate at the bottom of the reactor might be due to action of the high concentration of suspended biomass at the bottom with high substrate concentration. Considering major part of sulphate reduction was taking place within bottom 40% volume of reactor, the effective sulphate loading possible was  $7.125 \text{ kg SO}_4^{2-}/(\text{m}^3 \text{ day})$  at 9.6 h HRT. However for this loading, sulphate removal efficiency achieved at steady state was only 75% without sulphide inhibition control. The performance of the present system in terms of sulphate reduction was comparable to the performance of high rate reactors [32]. Percentage removal of total COD obtained in the experiment and the theoretically possible percentage removal of COD (as per sulphidogenesis stoichiometry) showed similar trends.

During phase 2, the pH in the reactor dropped from 6.8 to 6.0 (Fig. 5a) and this resulted in the formation of more free  $\text{H}_2\text{S}$  from sulphide generated in the system. Here, it is to be noted that at a pH of 6.0 and at  $30^\circ\text{C}$ , approximately 93% of total sulphide could be present as free  $\text{H}_2\text{S}$ , compared to a value of 60% at a pH of 6.8 at similar conditions [13]. The concentration of free  $\text{H}_2\text{S}$  during this period could be varying between 476 and 518 mg/L. High concentration of free  $\text{H}_2\text{S}$  (more than 250 mg/L)

is highly toxic to all bacteria including sulphate-reducing bacteria compared to ionic form of sulphides [6]. Earlier studies have indicated that sulphate reduction can proceed well when pH is maintained above 6.0 [18]. From the above discussion, it may be inferred that the gradual decrease in performance in terms of sulphate reduction in phase 2 could be due to sulphide inhibition at higher sulphate loading rate at a COD/SO<sub>4</sub><sup>2-</sup> ratio of 1.3.

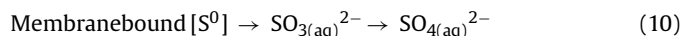
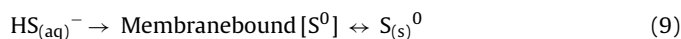
### 3.3.3. Phase 3

Loading study in phase 3 continued from 191 to 220 days, with an organic loading of 3.75 kg COD/(m<sup>3</sup> day). As mentioned earlier, a simple sulphide inhibition control strategy was adopted during this period by injecting controlled air through sample port P2. Due to this inhibition control, both sulphate and COD removal efficiencies substantially improved during the period 191–205 days (Fig. 5b). Sulphide concentration in the effluent reduced to less than 250 mg/L during the period 195–205 days as shown in Fig. 5a. The injected air supply might have oxidized part of sulphide to sulphate and/or other oxidized forms of sulphur and reduced the sulphide inhibition in the system. Oxidized form of sulphur thus formed might have been consumed by SRB for further COD removal in the zones closer to air injection. By this simple sulphide inhibition control, it was possible to maintain aerotolerant SRB and sulphide oxidizing bacteria (SOB) to work symbiotically within the same reactor. SRB and SOB could grow as biofilm over support media and in the process, better removal of COD can be achieved. Application of such bacterial symbiosis for wastewater treatment (COD removal) by attached growth system (in a multistage reversing flow bioreactor) was earlier demonstrated by Tare and Sabumon [33].

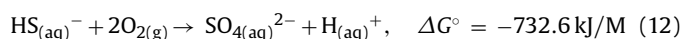
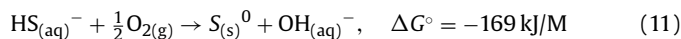
The overall reactions involved in sulphate reduction (by complete oxidizing SRB) and sulphide oxidation of the present system are given below.



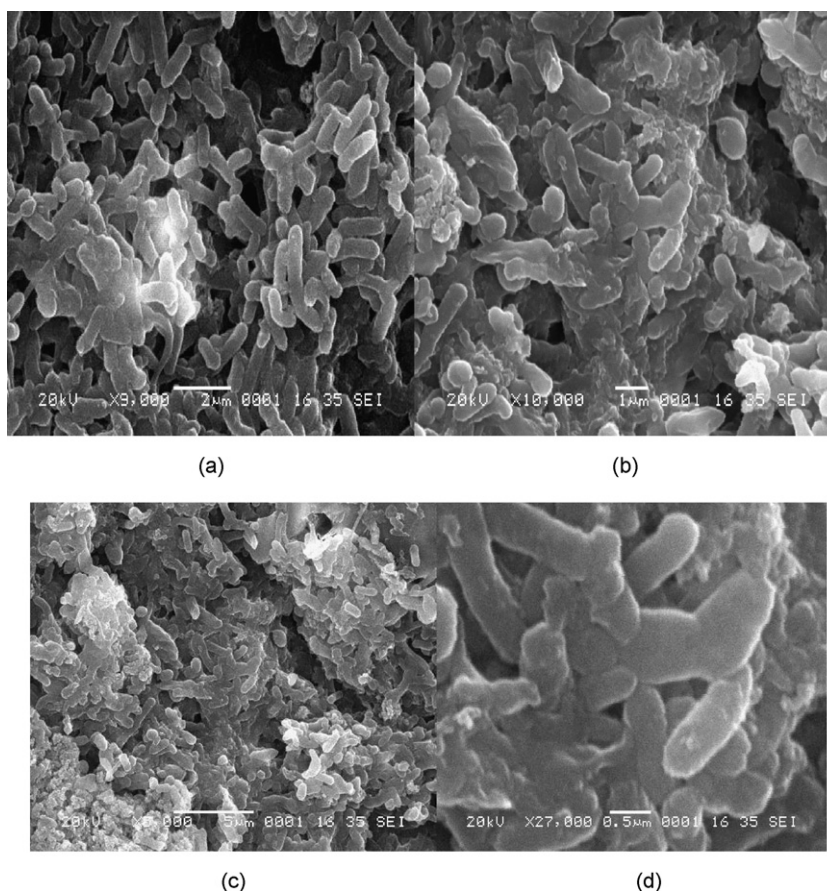
The sulphide formed as a result of sulphate reduction can be chemically oxidized to elemental sulphur as per Eq. (1) in presence of air. However, Buisman et al. [34] and Visser et al. [35] reported that the main products of biological sulphide oxidation are elemental sulphur and sulphate and these reactions (Eqs. (9) and (10)) are independent of the pH in the range of 6.5–9.0 and faster compared to chemical oxidation of sulphide.



There was formation of pale yellowish color sulphur formation in biofilm of the reactor and this could be due to sulphur formed as per reactions of sulphide oxidation described above. The precise control of sulphide oxidation to elemental sulphur is difficult because of thermodynamic advantage of sulphide oxidation to sulphate as evident from the following equations [36]:



It is to be noted that (from Eqs. (11) and (12)) for oxidation of sulphide to elemental sulphur, 1 M sulphide consumes 0.5 M O<sub>2</sub> and for oxidation of sulphide to SO<sub>4</sub><sup>2-</sup>, 1 M sulphide consumes 2 M O<sub>2</sub>, respectively. Also, most of the SRBs can utilize the above



**Fig. 7.** SEM images showing morphology of mixed culture taken from bottom of the sulphidogenesis reactor on 203rd day. (a) 9000 times magnification. (b) 10,000 times magnification. (c) 5000 times magnification. (d) 27,000 times magnification.



mentioned oxidized sulphur compounds as electron acceptors for sulphate reduction using organic compounds as electron donors for sulphidogenesis. In the present simple sulphide inhibition control, by supplying a very less quantity of air (0.19 L/(Lmin)), the COD removal efficiency was increased from 45% to more than 85%. Here it is to be noted that only stoichiometric requirement of oxygen for part of sulphide oxidation need to be met as the reactor at air injection location was a fixed bed unlike in suspended growth system. The oxygen diffused to bottom portion of the reactor from air injection point helped in oxidizing sulphides to remove toxicity from bottom zone of the reactor and there was enhanced sulphate reduction between P1 and P2 (Fig. 6c). The simple sulphide inhibition control technique presented requires minimum level of energy to achieve better COD removal as there was no need of energy requirement to keep the biomass in suspension.

There was increase of pH during this period as a result of sulphide oxidation to elemental sulphur (Fig. 5a). However, contribution of COD removal by facultative/heterotrophic aerobic bacteria also could not be ruled out. It is very difficult to estimate COD removal by sulphidogenesis process alone from the stoichiometry as sulphate reduction and sulphide oxidation were occurring simultaneously in the controlled aeration habitat (above 40 cm from bottom of reactor and below air injection point).

The control of aeration (maintaining the air flow rate at 0.1 L/min) to keep the sulphide concentrations above 250 mg/L (206–216 days) reduced the COD removal efficiency of the system (Fig. 5b). Increase of air supply to 0.2 L/min (217–220 days) in order to keep the sulphide concentration below 250 mg/L increased the removal efficiency of COD (Fig. 5b). So the above results shows that sulphide inhibition control is required to keep the total sulphide below 250 mg/L inside the reactor for better removal of both sulphate and COD. Fig. 6c shows sample port analysis on 217th day of operation when process was under sulphide inhibition control. It is obvious from these results that the proposed sulphide inhibition control strategy was effective in increasing sulphate and COD removal efficiencies to more than 85% efficiency, with an HRT of 1 day.

Fig. 7 shows the morphology of mixed culture drawn from the bottom of hybrid sulphidogenesis reactor by SEM image. The predominant mixed culture was found to be rod shaped, though cocci and spiral shaped bacteria were also present. The specific sulphate reduction rate by this mixed SRB culture was found to be 0.35 kg SO<sub>4</sub><sup>2-</sup>/(kg VSS day) and was found to be within the range of reported values (0.08–0.79 kg SO<sub>4</sub><sup>2-</sup>/(kg VSS day)) [30,31].

Complete treatment of effluent with lower COD/SO<sub>4</sub><sup>2-</sup> ratio to meet stringent dischargeable regulation might not be possible using the developed system in a single reactor. Therefore, a down stream aerobic polishing unit is essential to oxidize the remaining sulphides and COD present in the effluent after partial recovery of elemental sulphur.

#### 4. Conclusions

Flocculent type activated sludge employed for treatment of tannery effluents can be used as a potential biomass for development of sulphidogenesis process for treating wastewaters with low COD/SO<sub>4</sub><sup>2-</sup> ratio (1.3). The sulphate reduction loading achieved in the developed upflow hybrid sulphidogenesis reactor was comparable with high rate reactors. The specific sulphate reduction capacity of mixed culture developed was found to be 0.35 kg SO<sub>4</sub><sup>2-</sup>/(kg VSS day). Adoption of a simple sulphide inhibition control strategy improved COD removal efficiency substantially. The sulphide generated in the system can

be recovered as elemental sulphur and/or oxidized back to sulphate.

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