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Sulphate reduction and zinc precipitation from wastewater by sulphate-reducing bacteria in an anaerobic moving-liquid/static-bed bioreactor

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ABSTRACT

Sulphate and zinc removal from a synthetic wastewater by sulphate-reducing bacteria was evaluated using an anaerobic moving-liquid/fixed-bed bioreactor packed with ceramic media. The system was initially augmented by adding sewage sludge as a source of the bacteria. Calcium sulphate dihydrate as a source of sulphate and sodium lactate as a source of carbon were added to the reactor. The system was operated under batch and anoxic conditions with inlet zinc concentrations from 30 to 110 mg/L at different retention times (4–24 h). After adaptation of the system and reaching a steady-state, for a zinc inlet load of 100 mg/L, a maximum removal of 98.7% was attained with a retention time of 24 h. With an optimum inlet sulphate concentration (1,500 mg/L) and with a retention time of 24 h, a maximum sulphate removal efficiency of about 89.2% was achieved. However, these values were declined by decreasing the retention time. The system showed low capacity to COD removal, e.g. at a COD/SO_4^{2-} loading ratio of 2.26, the effluent COD was eliminated by 35%. The amount of hydrogen sulphide (H_2S) produced among the processes, the concentrations of sulphide (S^{2-}) and sulphite (SO_3^{2-}) in the effluent were also measured.

Keywords: Biological treatment; Industrial wastewater; Sulphate-reducing bacteria; Zinc precipitation; Sulphate removal

1. Introduction

Wastewaters containing heavy metals are considered as a serious threat to humans and the environment [1,2]. The main sources of sulphate and heavy metals such as zinc are the effluents of plating, mining, metal smelting, battery manufacturing and electronic industries [3–7], the effluent from the tanning industry and wastewaters from scrubbers of power plants [8,9]. Zinc itself has less environmental hazard, but if it reacts with oxygen or acids, it can be converted to potentially toxic compounds such as zinc chloride which can create

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environmental hazards. Excessive exposure to zinc chloride can cause respiratory disorders. Long-term ingestion of excessive amount of zinc or zinc oxide can cause immunological and cardiovascular effects. Excessive ingestion of zinc can lead to anaemia due to the displacement of iron [3]. Therefore, to reduce such risks and sterile effluents from industries, chemical and biological methods have been widely. Primarily, chemical methods are absorption, adsorption, reverse osmosis, neutralization with alkali, solvent extraction, etc. Commonly, alkali materials such as lime, limestone, etc. are added to raise the pH and thereby convert the heavy metal ions into insoluble forms which can be easily precipitated and removed from the wastewater. This method produces more sludge due to the use of alkali and demands high expenses [10–14]. In contrast, biological methods do not have these drawbacks. They have recently become popular due to low sludge production and sludge without the need for re-treatment [11,13]. Biological methods are divided into two categories: active and passive biological methods. The major advantages of active methods (up-flow anaerobic sludge blanket (UASB) reactor, anaerobic filters and fluidized bed reactors) are predictability of the process and recovery of the precious metals [15,16]. Passive methods can include aerobic wetlands and permeable reactive barriers [15,17]. The basic phenomena of biological methods are alkalinity production and deposition of metals in the form of metallic sulphides [18,19]. In this method, sulphate-reducing bacteria (SRB) reduce the sulphate to sulphide under anaerobic condition, then sulphide ions react with metal ions to form insoluble metal sulphide [20–22]. SRBs are routinely found in industrial effluents [23], and their advantage is simultaneous reduction of sulphate and heavy metals from wastewater [8,18]. The main reactions of sulphate reduction and zinc sulphide production are as follows [24,25]:

$$CH_3CH(OH)COOH + 0.5\,SO_4^{2-} \rightarrow CH_3\,COOH + CO_2 + 0.5\,H_2\,S + H_2O \quad (1)$$

$$0.5\,CH_3\,COOH + 0.5\,SO_4^{2-} \rightarrow CO_2 + 0.5\,H_2\,S + H_2O \quad (2)$$

$$Zn^{2+} + H_2\,S \rightarrow ZnS + 2H^+ \quad (3)$$

Although the main mechanism of metals removal by SRBs is bio-precipitation of metals as metal sulphides, the possibility of metal ions adsorption on the outer surface of bacteria and sediment adsorption on the extracellular exopolymer of bacteria are the mechanisms that promote the removal of metals in these methods [18]. In recent years, several researches in the field of industrial wastewater treatment have been conducted on sulphate and heavy metal removal [26–35]. However, detailed information is needed in terms of achieving a proper operational condition and efficient application of the system for highly contaminated sources. In the present study, the design of a batch bioreactor was developed by concentrically locating a cylindrical perforated tube inside the reactor equipped with a magnetic mixer. This novel configuration of the reactor provides better mixing and distribution of the solution among the media. Using this laboratory scale moving-liquid/fixed-bed batch system, the deposition of zinc ions with elevated concentrations (up to 110 mg/L) and sulphate reduction by SRBs at different retention times (4–24 h) were evaluated.

2. Materials and methods

2.1. SRBs source, reagents and solutions

Sludge from the secondary sedimentation tank of a municipal wastewater treatment plant was used as a source of sulphate-reducing bacteria. Approximately, 100 ml of the sludge was diluted with 5-L distilled water (capacity of the reactor). To provide substrate for better growth of SRBs, supplementary materials including NH_4Cl (1 g/L), $MgSO_4{\cdot}7H_2O$ (2 g/L), K_2HPO_4 (0.5 g/L), $CaCO_3$ (1 g/L), $CaCl_2{\cdot}2H_2O$ (0.1 g/L), NaCl (2 g/L), $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ (trace) were added to the reactor inlet solution. Calcium sulphate dihydrate ($CaSO_4{\cdot}2H_2O$) was applied as a source of sulphate (final electron acceptor) and sodium lactate was used as an energy source (electron donor) for SRBs. Lactate was chosen as a substrate because, according to the literature cited, growth yield is lower, and kinetics are slower for ethanol compared to lactate [36]. Sodium lactate solution was prepared by gradually adding NaOH 6 M into lactic acid 85% until reaching its pH of around 7.3 ± 1. Calcium sulphate was added to the solution with a concentration range to achieve a sulphate content of 1,000–1,500 mg/L. Sodium lactate of around 8 mg/L was added to the prepared inlet solution. The pH of the solution was adjusted to 7.3 ± 1 (suitable for the growth of SRBs) using NaOH 1 molar. Various concentrations of zinc in the inlet solution were attained by adding calculated amount of zinc chloride ($ZnCl_2$) to the solution.

2.2. Bioreactor characteristics

The bioreactor is shown schematically in Fig. 1. A cylindrical glass container 58 cm in height and 15-cm internal diameter having a capacity of about 10 L was used. Considering 8 cm from the top as a free board,

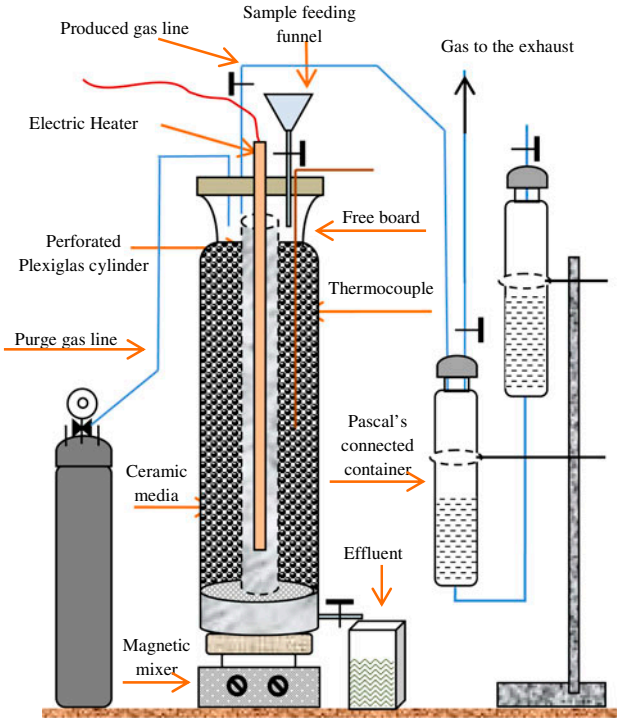

Fig. 1. Schematic diagram of the experimental anaerobic bioreactor for sulphate removal and zinc precipitation.

remaining height of 50 cm gives a practical volume of 8.84 L. A perforated stainless steel disc was installed 8 cm from the bottom of the reactor and a magnetic stirrer was used in the bottom. A cylindrical, perforated Plexiglas tube with 6-cm outer diameter was mounted on the disc inside the reactor concentrically (moving-liquid/static-bed bioreactor). This modification was applied in order to provide a better distribution of the solution within the porous area of the surrounding media and to prevent settling of the formed sediments. Reactor temperature was set at 32–35°C by an electric aquarium element and a thermocouple placed inside the internal tube of the reactor. Inside of main compartment and outer space of internal tube were filled with ceramic media leaving 8-cm free board at the top of the reactor. The media was a ceramic ring with a dimension of 20-mm height and 20-mm external diameter, a specific area of 325 m^2/m^3 and a density of 320 kg/m^3. The packed media had a solution capacity of 5 L with a porosity percentage of approximately 67%. The reactor was purged with a stream of nitrogen gas before and after the loading to prevent oxygen insertion. Any gases produced through the anaerobic process were collected via a Pascal's connected glass containers (Fig. 1) and the gas volume was roughly measured by solution displacement inside the containers. In other words, the volume of the gas entering the first bottle is equal to the volume of the solution entering the second bottle.

2.3. Experimental procedures

Synthetic wastewater containing the required substrate with pH adjusted to 7.4 ± 1 was introduced into the reactor (5 L each run). The experiments were carried out in three stages. First, to stabilize the system, the reactor was run for 20 d with 48-h retention time interval (10 runs) as a start-up period under anaerobic condition and constant temperature of 32°C (until a maximum gas production and biofilm formation with a suitable thickness was achieved). In the second stage, various concentrations of zinc including 30, 50, 70, 80, 90, 100 and 110 mg/L, along with adequate complementary substrate were individually introduced into the reactor with a retention time of 24 h. $ZnCl_2$ was added to the inlet solution as a source of zinc. As there was a possibility of partial sedimentation of zinc in the forms of hydroxide and carbonate in the solution, the supernatant was introduced into the reactor and its zinc concentration was considered as a zinc inlet concentration. To avoid any bacterial shock which may be occurred by sudden increase in the zinc loading rate, intermittent concentration between the defined values were also applied. This stage of the experiments was conducted in order to acclimatize the bacteria to the ascending zinc concentrations lasting 12 d. As the removal efficiency dropped with the upper range of inlet zinc concentration (110 mg/l), the tests were established for a constant inlet zinc concentration of 100 mg/l. In the third stage, the experiments were conducted in retention times of 4, 8, 12, 16 and 20 h, each in triplicate, and the average of the results were reported. There was no lapse of time between each retention time.

2.4. Analytical methods

All parameters were analysed according to the standard methods for the examination of water and wastewater, AWWA [37]. The concentration of zinc in the samples taken from the reactor inlets and outlets was analysed using a flame atomic absorption spectrometer (Perkin Elmer) after adjusting the solutions pH to less than 2. The instrument was already calibrated by introduction of three concentrations of zinc standard solution. Sulphate content of the solutions were analysed by turbidimetric method using a DR-5000 (Hack Co.) [37]. The calibration curve of the DR-5000 was attained by introducing different sulphate standard solutions to the system. Total

suspended solids (TSS) were analysed by gravimetric method. Volatile suspended solids (VSS) were measured by heating the filters used for TSS at 550˚C in a muffle furnace and gravimetric methods. Standard glass fibre filter was used for filtration of the samples (Whatman®, 0.45 μm). Other parameters which were also analysed include: alkalinity (potentiometric method), chemical oxygen demand (COD; oxidation with potassium dichromate) electrical conductivity (EC; electrical conductivity meter), sulphite, (SO_3^{2-}) sulphide (S^{2-}) (iodometric method) and pH (using a broad range pH meter). Analysis of a reference sample for zinc and sulphate concentration in triplicate showed the coefficient of variations less than 2.5% which confirms the quality of the analytical data.

3. Results and discussion

3.1. Zinc removal

Synthetic wastewater containing different concentrations of zinc from 30 to 110 mg/L was introduced to the reactor, each for 24 h and in triplicate. These ascending concentrations were applied for the microbial acclimatization. However, for 110 mg/l inlet zinc, the system did not tolerate and it led to the decrease in the removal efficiency. Thus, the inlet zinc level was fixed at 100 mg/L and the experiments were performed at descending retention times from 24 to 4 h (Table 1). According to Fig. 2(a), in experiments with 30 mg/L zinc in the inlet, 94% removal was observed, however, with 50 mg/L the elimination was reduced to 93.4%. This could be because of the shock imposed on the bacteria via increasing the initial concentration of zinc, or partial adsorption of zinc onto the ceramic media. While, continuing the experiments with inlet zinc of 70, 80, 90 and 100 mg/L was associated with a gradual removal increase to the extent that higher removal efficiency (98.7%) was obtained for the 100 mg/L inlet zinc. This could be due to the adaptation of micro-organisms to the zinc containing solutions by the passage of time.

These findings are consistent with the results of some previous studies. Azabou et al. evaluated the zinc precipitation potential of SRBs using an anaerobic system inoculated with activated sludge as a source of bacteria, and enriched with phosphogypsum as a source of sulphate. They detected less than 5% of average inlet zinc concentration (150 mg/L) in the reactor effluent (96% removal) [27]. In another study, using a continuous-flow reactor adopted for SRBs growth, removal efficiencies of four metals including zinc, cadmium, copper and nickel from aqueous solution were investigated [38]. They attained reduction percentages of 99% for the former three metals and 87% for nickel. In our study, by increasing the inlet concentration of zinc to 110 mg/L, the removal efficiency showed a slight drop and hit to 97.7%. This may be due to the beginning of zinc toxicity dosage on the micro-organisms. In another study conducted by Radhika et al. on zinc removal by *Desulfotomaculum nigrificans* (a type of SRBs), the zinc was completely eliminated with an inlet concentration of 12 mg/L [32]. However, on operating the system with inlet concentrations of 63, 108 and 210 mg/L for 40 d, a reduction of about 70% was achieved. They concluded that decrease in the removal efficiency could be attributed to the decline of bacterial population due to the high levels of zinc [32]. In this regard, there is also some contradictory reports in the literature. For instance, Min et al. carried out a study with 200–1,000 mg/L zinc inlet and showed that with inlet concentration of 600 mg/L, removal was over 99%, even at inlet concentration of 1,000 mg/L the removal efficiency was

Table 1
Removal efficiency and elimination capacity of the bioreactor for zinc and sulphate along with alkalinity and pH changes and H_2S production at various retention times

Retention time (h)	Zn removal efficiency (%)	Zn elimination capacity (g/m^3 h)	SO_4^{2-} removal efficiency (%)	SO_4^{2-} elimination capacity (g/m^3 h)	Alkalinity (mg/L)		pH Eff (±SD)	H_2S (ml/L) Eff (±SD)
					In	Eff (±SD)		
4	93.8	13.26	76.7	162.61	1,400	1,885 ± 99	7.2 ± 0.1	42 ± 3.6
8	95.9	6.78	79.1	83.85	1,400	2,092 ± 75	7.0 ± 0.1	70 ± 5.5
12	96.4	4.54	82.0	57.98	1,400	2,257 ± 108	6.9 ± 0.1	93.5 ± 14.1
16	96.6	3.41	84.2	44.65	1,400	2,320 ± 117	6.9 ± 0.1	100 ± 11.3
20	97.8	2.77	86.5	36.71	1,400	2,382 ± 136	6.8 ± 0.1	106 ± 14.2
24	98.7	2.33	89.2	31.53	1,400	2,434 ± 101	6.8 ± 0.1	108 ± 9.8

Notes: In: influent and eff: effluent.

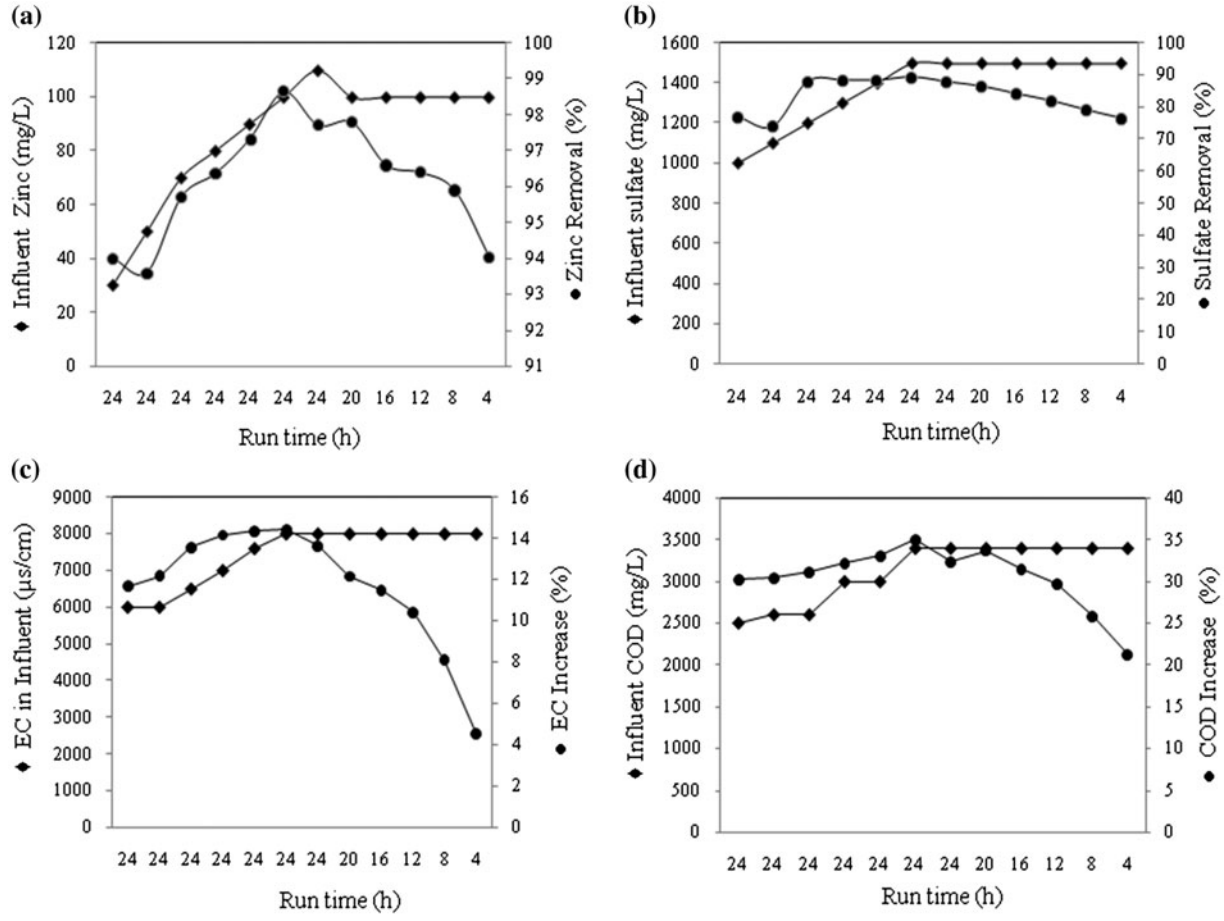

Fig. 2. Concentrations of inlet zinc (a) and sulphate (b) and their removal efficiencies, inlet level of EC and its increase (c), inlet COD and its decrease (d), at different retention times.

more than 95%. However, this high efficiency even at high concentrations of the inlet zinc would be due to the application of ISIS process (immobilized sludge bead with inner cohesive carbon source) [39].

To assess the system efficiency at lower retention times, the experiments were carried out with retention times less than 24 h using inlet zinc concentration of 100 mg/L. As shown in Fig. 2(a), decreasing the retention time to 20, 16, 12, 8 and 4 h, respectively, with the constant inlet zinc concentration caused a gradual decline in the removal efficiency. This could be attributed to the lack of sufficient time for the reduction of sulphate by bacteria. Considering the practical volume of 8.84 L for the reactor media and 5 L solution introduction to the reactor containing 100 mg/l zinc and 1,500 mg/l sulphate, the removal efficiency and elimination capacity for zinc at various retention times were calculated (Table 1). It can be seen in Table 1 that the majority of zinc removal occurred over the retention time of 4 h (93.8%) and operating the system at higher retention time, up to 24 h, the removal efficiency reached 98.7%.

3.2. Reduction of sulphate

Sulphate concentration of the reactor inlet begun from 1,000 mg/L along with inlet zinc level of 30 mg/L and was carried out three times at 24 h retention time. After that, the sulphate level was gradually increased to 1,500 mg/L in conjunction with the increase in zinc level from 30 to 100 mg/L, and the system was operated with these concentrations for a few days until the steady-state was achieved. Then, these concentrations were applied for all the experiments with retention times less than 24 h. Fig. 2(b) shows the inlet concentration of sulphate in relation to its reduction at different retention times. For the inlet concentration of 1,000 mg/L, its reduction efficiency was about 77%, however, for 1,100 mg/L, it decreased to 74%. This is probably

due to a bacterial shock that may have occurred by increasing the inlet zinc concentration from 30 to 50 mg/L. Among the processes, similar to the zinc removal pattern, the sulphate reduction efficiency was gradually increased with increase in its inlet concentration. Whereas, a maximum sulphate reduction efficiency of 89.2% was attained with a zinc inlet concentration of 100 mg/L (Table 1). However, when 110 mg/L zinc was introduced into the reactor with the inlet sulphate of 1,500 mg/L, the sulphate reduction declined to 88%. This is likely due to the increased concentrations of inlet zinc which may affect the bacterial populations.

Rodriguez et al. investigated the sulphate removal from acid mine drainage using slaughterhouse waste (to inoculate SRB) and ethanol as a source of carbon in a UASB reactor. With a COD/SO_4^{2-} ratio of 1, they achieved a sulphate removal of 85.6% [15]. In comparison, Li et al., reported lower reduction of sulphate (75%) when they applied two-stage UASB with a hydraulic retention time of 38 h for sulphate removal from acrylic fibre manufacturing wastewater [40]. The reason for lower reduction in some studies could be in one hand, the variation range of COD/SO_4^{2-} ratio, and on the other hand, the type and amount of substrate used as a carbon source. Whereas, working with a COD/SO_4^{2-} ratio of 0.67, Rodriguez et al. have reported a sulphate removal efficiency of 46.3% [15]. Probably, in lower ratios of COD/SO_4^{2-}, low carbon source (COD) could be available for bacteria, so bacterial growth cannot be enough to reduce sulphate. It is also possible that in environments with higher level of COD, the competition between methane-producing bacteria and SRBs is increased leading to low sulphate reduction by SRBs. The blockage of media by sulphide deposits and subsequent decrease in substrate delivery to the bacteria and different experimental conditions may cause differences in the efficiency of sulphate reduction.

In the present study, with the inlet zinc and sulphate concentrations of 100 mg/L and 1,500 mg/L, respectively, the sulphate reduction efficiency in retention times of 20, 16, 12, 8 and 4 h were investigated. Under these operational conditions, the sulphate reductions were 86.5, 84.2, 82, 79.1 and 76.7% for the mentioned retention times, respectively (Fig. 2(b) and Table 1). Probably, due to the insufficient time for sulphate reduction by bacteria, the amounts of effluent sulphate were increased with decreasing retention times. In a similar study, Chai and colleagues investigated the sulphate removal in a moving-bed anaerobic bioreactor fed with glucose as a carbon source. The results showed that by reducing the hydraulic retention time from 12 to 4 h, sulphate removal efficiency rapidly declined. With a maximum loading rate of sulphate (7.19 g/cm^3) for 260 d application, the sulphate removal efficiency reached 85–95% [41].

3.3. Changes in suspended solids (TSS, VSS) and electrical conductivity (EC)

By the changes in the concentrations of inlet zinc in different runs and by the passage of time, depending on the amount of organic matter and microbial density in the reactor, the TSS and VSS levels in the reactor effluent changed. VSS data are critical in determining the operational behaviour and biological concentration throughout the system. As Fig. 3(c) shows, with an increase in the concentration of inlet zinc and as the time passes, the VSS level in the effluent was gradually reduced. Whereas, with the inlet zinc level of 100 mg/L, a maximum VSS removal (81.1%) was obtained. This is probably due to the increased adaptability of bacteria to the concentration of zinc and the lack of significant bacterial death and their sloughing off from the media. While, in the experiments with an inlet zinc level of 110 mg/L, more VSS in the effluent was detected. This could be attributed to the bacterial death, biomass detachment from the media and reduced decomposition of organic matter. When VSS removal was examined at lower retention times with the optimum inlet zinc level (100 mg/L), the efficiency was dropped. For instance, at 4-h retention time, perhaps due to the lack of opportunity for the decomposition of organic matter by bacteria, the efficiency hovered around 59.2% (Table 2).

The TSS removal from the beginning of the experiments until achieving the optimum inlet zinc concentration fluctuated (Fig. 3(d)). However, under the steady-state condition and at different retention times, its removal percentages were very close together varying from 96.25 to 97.75%. Slight drop in the TSS removal efficiency occurred when the inlet zinc concentration increased to 110 mg/L which may reduce the performance of bacteria. In experiments at lower retention times and 100 mg/L inlet zinc, very little changes in TSS removal level occurred; the EC level which was set at 8,000 μS/cm in the reactor inlet, in all the experiments, showed a mild increase in the effluents that may be due to a decrease in the amount of suspended solids (Fig. 2(c)).

3.4. Alkalinity changes

Because of bicarbonate production and acidity reduction due to conversion of a strong acid (H_2SO_4) to a weak acid (H_2S) by SRB bacteria according to the

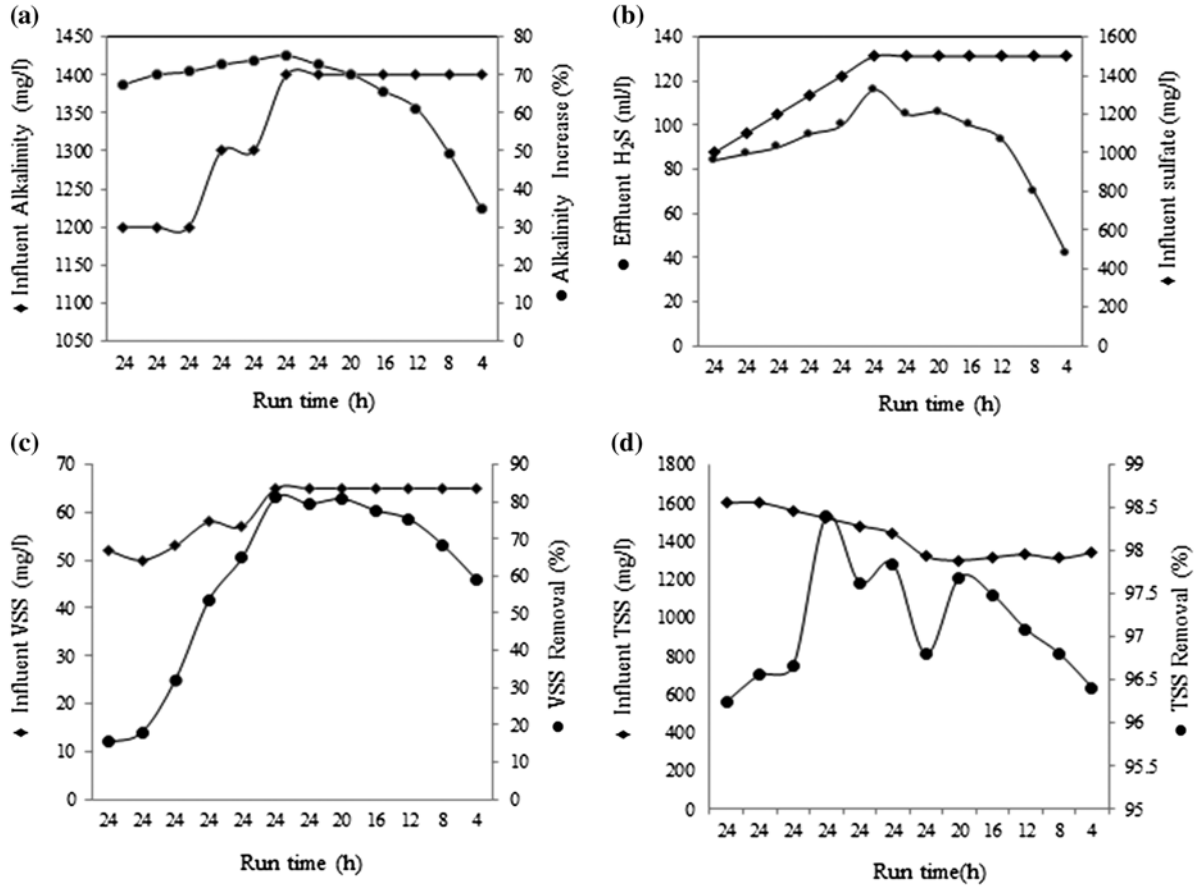

Fig. 3. Inlet alkalinity and its increase (a), the volume of H_2S gas produced compared to the influent concentration of sulphate (b), inlet VSS levels and its removal efficiency (c) and inlet concentrations of TSS and its removal efficiency (d), in relation to various retention times.

reaction (2), the alkalinity is increased [24,42]. Generally, biological activity such as denitrification, sulphate reduction, methane production and reduction of iron and manganese produces alkalinity [19]. Alkalinity of the reactor inlet and its increase in the effluent are shown in Fig. 3(a). When the experiments were conducted with an initial inlet zinc concentration of 30 mg/L and inlet alkalinity of 1,200 mg/L, an alkalinity increase in 67.5% was measured in the reactor effluent. As the pH of the inlet solution was adjusted to 7.3 ± 1, any increase in the alkalinity and changes in pH value could be attributed to the activity of SRBs. Conducting the experiments in the subsequent days with the same inlet, the alkalinity of the effluent increased to 70–70.8%. In the fourth and fifth sets of experiments, where the inlet alkalinity of 1,300 mg/L was introduced to the system, the alkalinity increase in the effluent was 72.69 and 73.84%, respectively. In the sixth stage of the experiment, with an inlet zinc of 100 mg/L and alkalinity of 1,400 mg/L, the alkalinity of the effluent increased by 75.1%.

Alkalinity increase with constant retention time (24 h) can be linked to the gradual increase in sulphate reduction (Fig. 2(b)) and subsequent production of sulphide and bicarbonate. While, lowering the alkalinity production may also be due to the reduction of bacterial population and their performance. When the experiments were conducted at lower retention times, up to 4 h, with optimum concentration of inlet zinc and sulphate, the alkalinity production was reduced probably for the same reasons described for sulphate.

3.5. COD removal

Among the experiments, consumption of organic matter by anaerobic micro-organisms reduced the COD level in the reactor effluent. However, there was

Table 2
Changes in the average concentrations (±SD) of COD, TSS, VSS and EC after running the bioreactor at various retention times

	COD (mg/L)		TSS (mg/L)		VSS (mg/L)		EC (μz/cm)	
Retention time (h)	In	Eff	In	Eff	In	Eff	In	Eff
4	3,400	2,677 ± 128	1,340	53.5 ± 22	65	26.5 ± 14	8,000	8,360 ± 71
8	3,400	2,520 ± 111	1,310	46.0 ± 17	65	20.5 ± 9.2	8,000	8,647 ± 98
12	3,400	2,390 ± 120	1,330	38.5 ± 20	65	16.0 ± 10.7	8,000	8,868 ± 92
16	3,400	2,328 ± 99	1,315	33.0 ± 12	65	14.5 ± 8.7	8,000	8,916 ± 118
20	3,400	2,256 ± 74	1,300	30.0 ± 19	65	12.5 ± 8.3	8,000	8,972 ± 102
24	3,400	2,227 ± 58	1,340	29.3 ± 15	65	12.3 ± 5.4	8,000	9,152 ± 133

Notes: In: influent and eff: effluent.

a lower COD reduction in all the experiments (Fig. 2(d)). Whereas, a maximum elimination of 32.3% occurred at 24-h retention time with 100 mg/L zinc, 1,500 mg/L sulphate and 3,400 mg/L COD in the reactor inlet (COD/SO_4^{2-} ratio of 2.26). Perhaps, a plausible reason is that lactate, the main source of carbon in this study, is converted to acetate by SRBs that cannot be broken down by these bacteria. On the other hand, methane-producing bacteria in spite of their ability in acetate decomposition are defeated in competition with SRBs due to the high concentration of sulphate. Hence, the COD removal of the present process would be less significant [43]. Nevertheless, total COD of the effluent was measured, but not its soluble fraction, so that possible presence of microbial mass can increase the effluent COD, which is consistent with literature. Henry and colleagues have assessed the removal rates of COD in a landfill leachate using both sulphate-reducing and methane-producing bacteria. They attained 70% COD reduction from which the contribution of sulphate-reducing bacteria was only 20% [44]. Studying the effects of sulphate level on anaerobic treatment of landfill leachate, Yilmaz et al. found that SRBs activity increased at the lower ratio of COD/SO_4^{2-} producing higher levels of sulphide and alkalinity which causes a sharp reduction in the total COD removal efficiency [45]. However, regarding this ratio there is still discrepancy in the literature. The type of substrate used as a carbon source may affect the efficiency. About 65% of lactate can be oxidized to CO_2, so we used a higher ratio than that was applied for ethanol substrate.

3.6. H_2S production

Throughout the bacterial sulphate-reduction processes, the sulphate in the solution is converted into various reducing forms of sulphur including sulphite (SO_3^{2-}), sulphide (S^{2-}) ions and hydrogen sulphide (H_2S) (Table 3). However, a significant amount of sulphur in the effluent appears in the form of insoluble sulphides bounded with metal ions. Fig. 3(b) shows the amount of H_2S produced (v/v) throughout the experiments in relation to the inlet sulphate concentration at different retention times. It is clear that the more the sulphate reduction, the higher the amount of H_2S production. With inlet zinc of 100 mg/L and inlet sulphate of 1,500 mg/L, maximum H_2S of 116 ml/L was produced. In experiments with 100 mg/L inlet zinc and with the same sulphate concentration in the

Table 3
Comparison between influent sulphate and effluent sulphur compounds concentrations at various retention times (sulphur balance)

Retention time (h)	SO_4^{2-} in influent (mg/L)	SO_4^{2-} reduction (mg/L)	SO_3^{2-} in effluent (mg/L)	S^{2-} in effluent (mg/L)	H_2S in effluent (ml/L)
4	1,500	1,150	406	221	42
8	1,500	1,186	457	238	71
12	1,500	1,230	482	260	93
16	1,500	1,263	494	268	100
20	1,500	1,298	525	249	106
24	1,500	1,338	577	75	116

inlet at lower retention times, the H_2S production was declined due to the lack of adequate time for better performance of the bacteria. Whereas, in the experiments with retention time of 4 h, the H_2S production declined to 42 ml/L (Table 3).

4. Conclusions

The laboratory scale moving-liquid/static-bed anaerobic bioreactor adopted for SRBs growth and operated at retention times of 24 h or less, showed a high potential of sulphate reduction and zinc precipitation in the form of metallic sulphide from synthetic aqueous solution. The novel configuration of the reactor in this study increased its efficiency compared to literature, providing a better solid–liquid contact and preventing the media blockage over time. Experiments were conducted with inlet zinc concentration ranges of 30–110 mg/L at residence time of 24 h, until gaining a steady-state condition. Maximum zinc removal efficiency of 98.7% was attained for the inlet zinc of 100 mg/L at 24-h contact time. Reducing the retention time decreased the removal efficiency slightly, however, it was not considerable, e.g. at the retention time of 4 h it reduced to 93.8%. Sulphate removal ability of the reactor at different retention times were evaluated with inlet zinc of 100 mg/L. At 24-h retention time and inlet sulphate concentration of 1,500 mg/La, maximum sulphate removal of about 89.2% was achieved. Reducing the retention time declined the efficiency, whereas, at 4 h, 76.7% of the sulphate was eliminated. The COD removal efficiency was not considerable (about 35%) due to the production of acetate from lactate, which is a limiting factor in the decomposition of organic matter by SRBs. Acetate production within a few hours of the reactor operation caused the pH to be reduced slightly. Increase in the alkalinity due to sulphate reduction and acetate oxidation intensified the pH again and thereby the formation of zinc sulphide precipitate occurred. Therefore, sulphate reduction by SRBs in a properly designed bioreactor is an effective method for the precipitation of heavy metals and sulphate removal from industrial wastewater.

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