Research article

Biological removal of iron and sulfate from synthetic wastewater of cotton delinting factory by using halophilic sulfate-reducing bacteria

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ABSTRACT

Industrial and agricultural wastewater treatment, which has the potential to cause serious risks to human health and the environment, has special importance at the lowest cost and highest efficiency such as biological processes to treat wastewater. The purpose of the study was removing iron and sulfate from very saline synthetic wastewater by means of halophilic sulfate-reducing bacteria. This process was performed under anaerobic conditions to change wastewater to a chemical fertilizer to use in saline and alkaline soils. Three halophilic SRBs were isolated and purified from wastewater of the cotton delinting factory by Postage C medium which supplemented with sodium chloride and magnesium chloride hexahydrate. The highest NaCl tolerance strain (HSR973) was allocated to *Desulfovibrio halophilus* sp. This experimental study was conducted in a fluid bed reactor at anaerobic conditions. Diluted concentrations of cotton linters wastewater containing 50–400 ppm iron were added to the reactor. After the bacteria fixation to different iron concentrations, the maximum removal efficiency of iron and sulfate was achieved 85.3 % and 78.4 % at the optimum retention time of 24-hours respectively. Sulfate concentration in samples decreased to about 20 % of initial concentration after 24-h retention time. The highest production of H_2S at optimum operational conditions was about 228 ml l^{-1}. The reduction of sulfate and iron biological precipitation by anaerobic rector presented high performance. This removing accompanied with the alkalinity increase during the process which could be improved condition for acidic wastewater treatment. The produced iron sulfide sludge was not suitable for use as a chemical fertilizer due to its lack of complete separation. However, the total sludge produced was able to be consumed in saline and alkaline soils for various purposes after additional treatment.
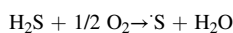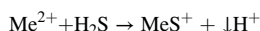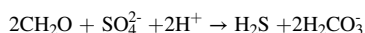
1. Introduction

Industrial wastewater that contains high levels of pollution, such as heavy metals and sulfate, has the potential to cause serious risks to human health and the environment (Jong and Parry, 2003; Kieu et al., 2011). Therefore, offering practical solutions with the lowest cost and the highest efficiency to eliminate them is very important (Jong and Parry, 2003; Dave et al., 2010). Sulfate-Reducing Bacteria (SRBs) play an important role in achieving this goal and preventing harmful environmental effects (Castilloa et al., 2012; Hsu et al., 2010). The bacteria of the sulfur cycle are classified into two general categories, including sulfate reducing bacteria (SRBs) and sulfide-oxidizing bacteria (SOBs). SOBs play an important role in reducing sulfide and SRBs in reducing sulfate and heavy metals from the environment (Tang et al., 2009). SRBs are chemoheterotrophic bacteria (use simple organic compounds as a source of carbon) and absolute anaerobic (Kieu et al., 2011; Martins et al., 2009). Removal of heavy metals by the SRBs occurs in three general stages. In the first step, sulfate as the final receptor of the electron is reduced to sulfide by these bacteria. In the second step, the sulfide due to sulfate reduction reacts with heavy metal and forms a metal deposition and finally, in the third step, an additional sulfide is converted to sulfur (˙S) by oxidizing sulfide bacteria or manually adding the oxidizing agent (Baskaran and Nemati, 2006; Luptakova and Kusnierova, 2005). The reactions are included (Pagnanelli et al., 2012) :

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$$2CH_2O + SO_4^{2-} + 2H^+ \rightarrow H_2S + 2H_2CO_3$$

$$Me^{2+} + H_2S \rightarrow MeS^+ + \downarrow H^+$$

$$H_2S + 1/2\ O_2 \rightarrow \dot{S} + H_2O$$

Although more than 100 SOB strains have been isolated from soda lakes (Sorokin et al., 2006), so far only four species of SRB, i.e., *Desulfonatronovibrio hydrogenovorans* (Zhilina et al., 1997), *Desulfonatronum lacustre* (Pikuta et al., 1998), *Desulfonatronum thiodismutans* (Pikuta et al., 2003), and *Desul fonatronum cooperativum* (Zhilina et al., 2005) have been isolated, and they are all low-salt-tolerant alkaliphiles.

Although iron is an essential nutrient for plants, its accumulation within cells can be toxic. Plants, therefore, respond to both iron deficiency and iron excess by inducing expression of different gene sets (Connolly and Guerinot, 2002). The overload of iron may cause severe health problems such as liver cancer, diabetes, cirrhosis of the liver, diseases related to the heart and central nervous system, infertility etc, in human (Behera et al., 2012). Iron toxic soils, which show visual symptoms of toxicity contain 105.1–569.5 ppm DTPA-Fe (Mitra et al., 2009).

Kashmar cotton delinting factory is one of four factories in Iran, which is the most important cotton seed provider in the country with 8.2 Ton h^{-1} delinting capacity. Delinting of cotton seeds is done to reduce seed adhesion during sowing, increase the emergence of seeds, the possibility of mechanized planting and disinfect of seeds to prevent the spread of pests and diseases. In the chemical methods to delinte cotton, acid is used to eliminate cotton linters. In this method, the sulfuric acid 14% (v/v) contacts the surface linters of the seed and eliminates the cotton linters completely (Hamidi and Mobser, 2005). The biggest problems of Kashmar cotton delinting factory liquid waste were high salinity and very low pH. Moreover, high concentration of sulfate (2882.0 mg l^{-1}) and iron (953 ppm) were somewhat problematic too. But, it was assumed that all these problems after a simple pretreatment were solvable with biological treatment methods.

Biological methods are more attractive than physicochemical methods due to some advantages such as removal of low concentrations of heavy metals (Jalali and Baldwin, 2000) and production of denser sludge (with the high sedimentation ability), low volumetric sludge production (Cabreraa et al., 2006), and high stability of metal deposits (Jalali and Baldwin, 2000; Kieu et al., 2011). Generally, biological methods for removing metal ions and sulfate are two types. Inactive methods, which include wetlands and anaerobic ponds (Rodrigueza et al., 2012; Tang et al., 2009) and bioreactor methods, which include up-flow anaerobic packed bed reactor (UAPB), up-flow anaerobic sludge blanket (UASB) reactor, fixed bed reactor (Pagnanelli et al., 2012; Kieu et al., 2011), and reactor with semi-continuous mixing tanks (Kieu et al., 2011). Biological reactors, although require a lot of maintenance, but with advantages such as the persistent and simultaneous removal of sulfur and heavy metals (Rodrigueza et al., 2012; Tang et al., 2009; Hsu et al., 2010) and the possibility of recycling valuable metals (Rodrigueza et al., 2012; Jalali and Baldwin, 2000; Tang et al., 2009), require a little space, comfortable control and better prediction of the process (Rodrigueza et al., 2012) are now considered the most appropriate method (Tang et al., 2009).

The purpose of this study was the possibility of making a fertilizer by halophilic sulfate-reducing bacteria purified from wastewater of cotton delinting factory and to determine their optimal conditions for maximum efficiency in the biological removal of iron and sulfate in the simple anaerobic fluid bed reactor (AFBR) which was made simplify and affordable in the laboratory.

2. Materials and methods

2.1. Isolation and identification of bacteria

2.1.1. Sampling

Kashmar cotton delinting factory, commonly has at least two types of waste: 1) solid waste (cotton linters waste), 2) liquid waste (acidic wastewater). The characteristics of these two types of waste have been shown in Tables 1 and 2. The liquid waste of this factory was a serious problem for the environment. However, it has good potential to convert to a liquid fertilizer (Table 2) especially in saline and alkaline soils of this region due to alkaline soil pH with low micronutrients uptake by plants.

2.1.2. Source of strains

Strain HSR 973 was isolated from a sample of liquid waste of cotton delinting factory. It was sampled by means of a Plexiglas-core sampler in September 2018. The main characteristics of the wastewater have been described in Table 2. The total salinity and pH were 4.93 dS/m and 2.3 in a ratio of 1:250.

2.1.3. Media, culture conditions and isolation

For enrichments and isolation, the culture medium was prepared according to Pfennig et al. (1981) and supplemented with 10% NaCl and 1 % $MgCl_2.6H_2O$. The final medium used for tests and maintenance of strain HSR 973 contained per liter of distilled water: Na_2SO_4, 3 g; KCl, 0.3 g; NH_4Cl, 0.3 g; NaCl, 70 g; $MgCl_2 . 6H_2O$, 3 g; KH_2PO_4, 0.2 g; $CaCl_2.2H_2O$, 0.15 g; $NaHCO_3$, 2.5 g; $Na_2S. 9H_2O$, 0.1–0.2g; vitamin solution (Pfennig et al., 1981), 4 ml; trace element solution (Widdel and Pfennig, 1984), 1 ml; sodium lactate, 10–20 mmols. The pH was adjusted to 6.9–7.1. The isolation was performed by the streak plate method as described elsewhere (Magot et al., 1992). Isolation and purification of isolates from wastewater sample were done by its specific medium culture (Pfennig et al., 1981). For isolation, One thousand micro-liter (1000 µL) of this wastewater was spread on the specific agar media culture as a pour-plate method. Medium cultures were incubated in the proper temperature (37 °C) for 5–7 days. To ensure the purity of the isolates, bacteria were sub-cultured several times. Purified isolates were preserved by a liquid nitrogen method for long time preservation (Horikoshi, 1999). For the nitrogen preservation of purified isolates, 15% glycerol was added to 20 ml of fresh liquid medium of each isolates and after thorough mixing, one milliliter part of it was transferred to 1.5 ml Eppendorf tube and was placed in the freezer at -80 °C for 1 h. Finally, after this period,

Table 2. The characteristics of liquid waste (cotton linters wastewater).

Parameter	Unit	Wastewater	Parameter	Unit	Wastewater
EC (1:250)	(dS m^{-1})	4.93	Na^+	meq l^{-1}	22.0
pH (1:250)	-	2.3	S.A.R	-	2.9
CO_3^{-2}	meq l^{-1}	0.0	Fe	ppm	953
HCO_3^-	meq l^{-1}	0.0	Mn	ppm	8.4
Cl^-	meq l^{-1}	0.0	Zn	ppm	2.7
SO_4^{-2}	mg l^{-1}	2882.0	Cu	ppm	0.4
K^+	meq l^{-1}	112.5	Pb	ppm	1.3
Ca^{++}	meq l^{-1}	19.8	Cd	ppm	0.2
Mg^{++}	meq l^{-1}	13.2	B	ppm	2.5

Table 1. The characteristics of solid waste (cotton linters waste).

Type	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	B	Pb	Cd
Unit	%						ppm						
Linters waste	0.40	0.01	0.49	0.19	0.06	0.05	167	45	22	0.0	4	0.0	0.0

the samples were transferred into the freezer at -20 °C for long-term preservation.

2.1.4. Identification of strains

In order to determine the definite bacterial strain, 16S rDNA genetic identification method was used. For extraction of DNA, a company extraction kit (Dena Zist Asia Company[1]) was used. In order to ensure extraction of DNA, 3 µl of DNA was transferred to 1% buffered gel electrophoresis wells, and then electrolyzed with 120 V and 30 min separation process. The electrophoresis product was transferred to the indicator device. The extracted DNA was stored at a temperature of -20 °C. To amplify the 16S rDNA region, universal primers fD1 (5′-AAG GAG GTG ATC CAG CC-3′) and rD1 (5′-AGA GTT TGA TCC TGG CT C AG-3′) were used (Nathani et al., 2014). Thermal reaction of PCR with the thermal program was performed (Assareh et al., 2012). Amplification conditions included: initial denaturation at 94 °C for 2 min, 30 cycles of denaturing at 94 °C for 45 s, annealing at 52 °C for 60 s, and primer extension at 72 °C for 30 s. A final elongation step for 4 min at 72 °C completed the amplification. The PCR product with the size of the markers was observed on agarose gel electrophoresis (1%) in the area of 800 bp. PCR products were transported to Macrogen South Korea Company for sequencing. Sequencing results were compared by BioEdit and Chromas pro software and they were identified by the National Center for Biotechnology Information (NCBI) and the sequences were blasted and the degree of similarity with the species in the genetic databases was examined.

2.2. Bioreactor construction

This research is an experimental study on a laboratory scale which was done with synthetic wastewater. This system was designed and made at the laboratory scale after studying on biogas production systems and energy production bioreactors, which work with organic residues by anaerobic digestion processes and chemistry applications in new technologies (Hajizadeh et al., 2017). This system has two 10.5-liter tanks connected by an interconnecting tube at the elevation where shows 8 L scaled. The system consists of an inlet pipe for entering the wastewater and a sampling tap to sample the input wastewater at the required time. Materials enter from the bottom of the first tank and overflow from this tank to the second tank through the interconnect tube. Finally, the material exit from the bottom of the second tank by a second sampling tap (Figure 1). The necessary predictions have been made in all parts of the device for depletion and sampling. Two transparent container have been installed to determine the amount of gas produced by the anaerobic bacteria activity in two different heights. The gas which was produced by bacteria entered to the lower container that filled with distilled water, pure H_2SO_4 and sodium sulfate to prevent of H_2S exit. The upper container was scaled as bacteria gas production entering into the lower container that it caused the liquid moved to the upper container and then it will be measurable. Finally, a tap has been installed at the top of the upper container for depletion and exhaust gas. The device is installed in water bath to regulate the activity temperature of the microorganisms indirectly and permanently. The device is capable of setting the temperature in the range of 0–85 °C. Thermal energy supply is provided by a heating element that can be controlled.

2.2.1. Bioreactor setup

The 1 to 5 diluted liquid waste (cotton linters wastewater) with sterilized distilled water was used to set up and activate the anaerobic reactor. About 800 ml of this wastewater was diluted with 4 L of distilled water (useful capacity of the reactor) and was added to the main body of the reactor. Then, 50 ml of liquid fresh medium containing strain HSR

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[1] www.denazist.ir.

973 was added to bioreactor. Finally, the system was checked for correct operation and anaerobic conditions maintenance.

2.3. The process of experiment

Bioreactor setup was carried out in 20 days (in 72 h periods). This period was determined based on the formation of a suitable biofilm layer and maximum gas production (about 228 ml L^{-1}). After system preparation and activation, iron concentrations were added in 50, 100, 150, 200, 250, 300, 350 and 400 ppm to matching Halophilic SRBs to iron concentrations. The liquid wastewater was diluted with sterilized distilled water to make 50, 100, 150, 200, 250, 300, 350 and 400 ppm iron concentrations in 1: 20 ratio. At the beginning of the operation, a concentration of 50 ppm of iron was prepared and used for 24 h. In the following days, the concentration was increased to 100, 150, 200, 250, 300, 350 and 400 ppm. However, due to the sudden decrease in efficiency, after increasing the concentration from 350 to 400, the concentration of 350 mg L^{-1} was chosen as the concentration of the stabilization-process. In order to investigate the effect of the retention time and achieve the minimum retention time, along with the acceptable efficiency, the operations in this concentration at the retention times of 20, 16, 12, 8 and 4 h were also carried out in twice repeated, and their average was used as actual efficiency.

2.4. Sampling and testing parameters

All parameters were evaluated in accordance with the standard method book (water and wastewater tests (method 3500) (standard methods for the examination of water and wastewater, 1998). In order to measure the dissolved parameters and sediment analysis, 50 ml of the effluent from the reactor center was extracted in each sample, and after filtration of 0.45 micron membrane, were used for the analysis of the determined parameters. The amount of input sulfate to the system and the residual sulfate were determined by a turbidimetric method with a spectrophotometer (PG9000 model). The amount of residual iron was analyzed using atomic absorption (PG9000 model). In addition, the amount of sulfite (SO_3) and sulfide (^{-2}S) by iodometric method, total suspended solids (TSS) by gravimeters, organic suspended solids (VSS) by frying and weighting methods, alkalinity by potentiometric method, electrical conductivity (EC) by electrical conductivity meter (EW-35414-00), pH with a pH meter (EW-35414-00), and ultimately chemical oxygen demand (COD) by oxidation by potassium dichromate was determined.

3. Results

The amount of sulfate injection was adjusted so that the concentration of sulfate in the solution after the stabilization stage was gained to the 1580 mg L^{-1}. In attention to the different inputs of iron and sulfate (Table 3), it was observed that in the 24-hour retention time, the average percentages of iron and sulfate removal were 85.35 % and 78.4 %, respectively (Figure 2A and B compares the average of both removals).

Study of electrical conductivity showed a different trend (Figure 2C). At first, the amount of EC increased, but after the 72-hour retention time, it suddenly decreased in the wastewater. The downward trend was very slow.

Maximum average of COD reduction for the 72-h retention time was about 17.11%, which was reduced to 5.8% in the 4-h retention time (Figure 2D).

The rate of alkalinity increase (with input alkalinity of 1675 mg l^{-1}) varied with different periods of retention time. It was increased by 47.61 % over the 24-hour period and 11.52 % at 4-h. Table 3 and Figure 2E showed the increasing of alkalinity in the wastewater of the reactor outlet. Input VSS and TSS values after the adaptation of microorganisms at different iron concentrations, was about 140 and 3166 mg l^{-1} respectively in it. The mean of VSS and TSS reduction with the mentioned

Figure 1. Bioreactor pictures made for the biological removal of iron and sulfate from wastewater.

Table 3. The mean of the input and output results of iron, sulfate, VSS, TSS, alkalinity, COD, electrical conductivity and H_2S from the bioreactor during microbial adaptation period.

retention time	Fe		SO_4			COD		ALK	
(h)	mg/L								
	in	eff	in	eff	eff	in	eff	in	eff
4	350	107.95	2882	32.06	924.1	3500	3297	1675	1482
8	350	96.95	2882	31.14	897.7	3500	3231	1675	1014
12	350	88.6	2882	27.96	806.06	3500	3108	1675	1072.5
16	350	71.4	2882	26.76	771.3	3500	3121	1675	1027.5
20	350	67.1	2882	22.98	662.34	3500	3066	1675	980
24	350	51.25	2882	21.61	623	3500	2998	1675	877.5
48	350	51.15	2882	21.55	621.1	3500	2936	1675	828.5
72	350	51	2882	21.53	620.7	3500	2901	1675	618.5

in: influent, eff: effluent.

input concentrations for different time periods are given in Table 4. As shown, the removal rate at the retention time 24-hours for VSS and TSS were about 77.0, 89.9 %, and for the retention time-4 hours, were 52.3 and 96.2 %, respectively. Figures 2G and H showed the VSS and TSS removal rates, respectively.

4. Discussion

4.1. Iron and sulfate removal

Comparison of the findings of this study and the results of other studies confirmed the biological removal of metal such as iron, copper, etc. and sulfate. The system composed of two pretreatment units of dispersed alkaline substrate reactors and one unit of passive biochemical reactor was found the most efficient (Fe and SO_4^{2-} removal of 99 % and 77 %, respectively) (Rakotonimaro et al., 2017). Jong and Parry (2003) studied the amount of sulfate reduction and heavy metals in up-flow anaerobic packed bed reactor by sulfate reducing bacteria and removed about 82 % of iron and sulfate. The removal pattern of Fe showed a trend in Fe solubility in the form of metal sulfide. The decreasing removal pattern of Fe can be due to absorption other metals by sulfate. In another study, which was performed in two separate reactors without SRBs and the other with SRB bacteria inoculation, it has been shown that the rate of formation of copper sulfide deposits in a reactor containing SRB bacteria was faster and associated with a higher copper removal efficiency (99.93 %) (Jalali and Baldwin, 2000). Therefore, it can be expected that sulfate-reducing bacteria play a major role in the treatment of heavy metals in the form of metal sulfide. According to studies conducted on the

kinetics of chemical reactions, excess iron removal can be due to very low solubility of iron sulfide deposits (Machemer and Wildeman, 1992). Another reason is probably the high sulfide tendency to react with iron and other heavy metals. The removal rate of iron and sulfate was decreased gradually to less than one day. The retention time of iron and sulfate was 67.93 % and 69.15 % in 4-hours respectively. Other studies also confirm this trend (Jong and Parry, 2003; Jalali and Baldwin, 2000). Comparison of iron removal percentages at different times, was displayed that the amount of iron removal at a retention time of 20-hours was about 80 % and for 16 and 12-hours RT were about 79.6 % and 74.6 % and finally for the 8-hours, it was 13 % less than the removal rate compared to 24-hours. This result indicated that the effective removal efficiency was obtained for the 24-retention time and even 16-hours (Figure 2A). It should be noted that the amount increase of metal sulfide deposits can cause eclipse in the reactor bed and as a result of it by less availability of bacteria to suns substrate, it can be reduces the ability of sulfate reducing by this bacteria and subsequently reduced the system efficiency. As shown in Figure 2B and A in the low retention time there was no difference between the amount of removal of sulfate and iron but, the elimination of these two is almost equal in the higher retention time. The probable cause of this phenomena could be due to the longer retention time that makes the bacteria have a higher chance for sulfate recovery.

In this study, an anaerobic reactor in a laboratory scale was used to evaluate the performance of halophilic sulfate-reducing bacteria in removing Iron and sulfate. Using the lactate as a carbon source and the concentration of input sulfate at about 2500–3000 mg l^{-1} after a period of bacterial adaptation (for 20 days), the removal rates of iron and sulfate at higher retention times was observed higher than the low retention times.
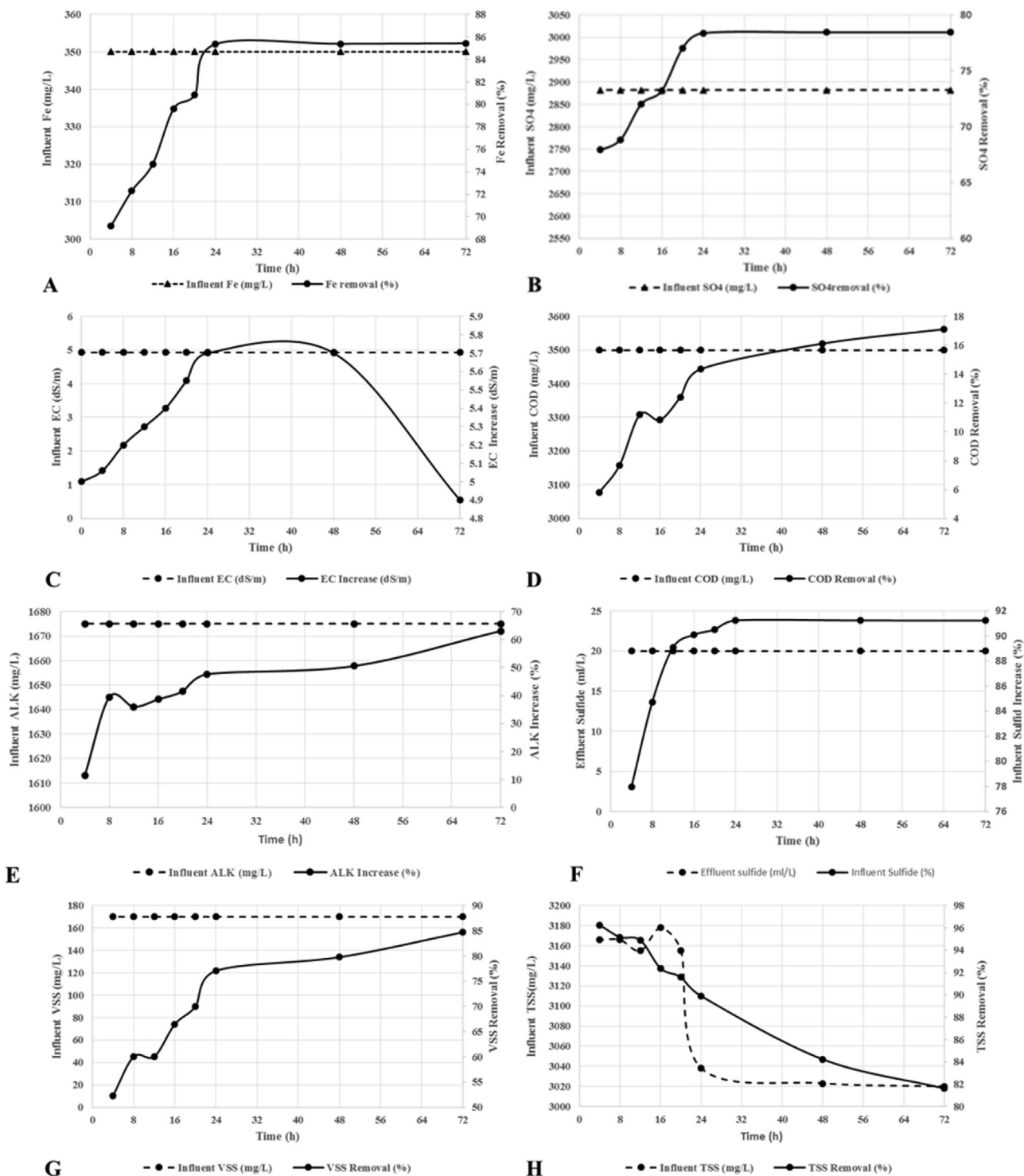
Figure 2. Input concentrations and iron (A), sulfate (B), EC (C), COD (D), alkalinity (E), H₂S (F), VSS (G) and TSS (H) removal efficiency at different retention time.

4.2. COD removal

Considering that the only source of COD is lactate, some reduction of COD to lactate decomposition is attributed to SRBs, which is converted to acetate. These bacteria cannot use acetate as a carbon source. Therefore, acetate decomposition requires presence of methane-producing bacteria (MPBs). On the other hand, in the growth media of the anaerobic reactor, due to the high value of sulfate (COD: $SO_4 = 1.21$) reduction of sulfate, is overcome on methane action, so methanogens bacteria cannot active

well, so less COD removal was obtained (McCartney and Oleszkiewicz, 1993). Of course, the values obtained are related to the total COD, so in the tested samples, there is a probability of suspended COD producing microbial mass, which increases the output COD. However, using this system, the acceptable percentage removal for COD is not achieved. Figure 2D shows the very low removal rate of COD. Other studies confirmed it. Singha et al. (2011) using a small, laboratory-bioreactor inoculated with sulfate-reducing bacteria for the simultaneous treatment of sulfate, chromium (VI) and COD from actual wastewater,

Table 4. The mean of the input and output results of iron, sulfate, VSS, TSS, alkalinity, COD, electrical conductivity and H₂S from the bioreactor during microbial adaptation period.

| retention time	VSS		TSS		EC		H₂S	S	SO₄	SO₄ reduction
(h)	mg/L				dS/m		ml/L	mg/L		
	in	eff	in	eff	in	eff	eff	eff	eff	eff
4	170	89	3166	3289.2	4.93	5.06	90.8	163.1	590	1716
8	170	102.1	3166	3326.5	4.93	5.2	131.1	205.7	760	1892
12	170	102.1	3155	3324.2	4.93	5.3	183.5	193.7	882	2016
16	170	113	3178	3440.3	4.93	5.4	202	168	850	2065
20	170	119	3155	3444.4	4.93	5.55	210.8	118	713	2089
24	170	131	3038	3379.4	4.93	5.7	228.3	92.8	766	2116
48	170	135.6	3023	3589	4.93	5.7	228.3	-	-	-
72	170	144	3020	3700	4.93	4.9	228	-	-	-

in: influent, eff: effluent.

reported COD removal rate more than 36.2 %. Henry and Prasad (2000) also used SRBs and methanogens for landfill treatment and compared two ways to recover sulfate and methane production, and they achieved 70 % COD removal (in high litter sludge) whereas, the SRBs efficiency in removal of it was only about 20 % in COD: Sulfate ratio of 1.6. Therefore, it was concluded that the pathway for the recovery of sulfate is not suitable for Landfill treatment (Henry and Prasad, 2000).

4.3. Alkalinity changes

The increase in alkalinity showed that alkaline produced by SRBs in the process of sulfate recovery, which production amount was depended on the retention time. Very short retention time reduced reactor capacity in alkalinity production. The produced alkalinity could neutralize the acidity of the solutions to the system, so it can also be used to treat high acidity wastewaters.

4.4. Sulfate balance

Increasing activity of bacteria increased the amount of sulfate reduction and increased sulfide concentration (Table 4). Although the amount of precipitated iron sulfide was not measured, the stoichiometry of sulfate removal based on sulfur, and the amounts of hydrogen sulfide, sulfite (SO_3^-) and various forms of sulfide produced in the reactor outlet indicate that significant amounts of sulfur in the form of insoluble metal sulfides, was is separated from the aqueous medium and deposited.

$$SO_4 = H_2S\ (g) + SO_3 + (H_2S + HS + S) + MS$$
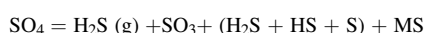
4.5. Electrical conductivity

The trend of electrical conductivity changes, unlike most of the parameters which were tested in this research, was not very satisfying at the 24-retention time. However, at the higher retention time, (72 h) the amount of EC showed a higher decrease that was probably due to more TSS sedimentation at the 72 RT compare to 48 RT.

4.6. Total solid suspended (TSS) and volatile suspended solids (VSS)

Although the volatile suspended solids were not added to the reactor, it is likely that the source of VSS increasing is due to the absorption of lactate by suspended solids. Some of the VSS in the outlet solution may be due to the presence of microbial masses. However, the low total VSS in the outlet samples may indicate the compromise of the system, none significant bacterial mortality and sticking to the growth platforms. TSS removal trend by HSRBs bacteria was not proper in this research so that

with the passage of time (4, 24 and 72 h) the amount of reduction in total suspended solids decreased (96.2, 89.8 and 81.6 % respectively).

5. Conclusion

The iron and sulfate removal efficiency was acceptable and its rate was observed superior at the higher retention time. This study also showed that the pathway for sulfate reduction due to the competition of HSRBs with methane substrates for COD removal is not suitable. Therefore, this system can be used for sulfate and iron-based acid waste products such as Kashmar cotton delinting factory wastewater due to alkalinity production. At the same time, biological methods such as the present study are applicable to a higher range of heavy metals and electrical conductivity than the common SRBs which may be due to the type of microorganism. But, the range of heavy metals and EC should be determined by further studies. The produced iron sulfide sludge was not suitable for the goal of this study as a chemical fertilizer due to its lack of complete separation. However, the total sludge produced was able to be consumed in saline and alkaline soils for various purposes. This method can only be used as a pretreatment of high-risk acidic wastewater of cotton delinting factories and needs further study to improve the separation of iron sulfide from the bed bioreactor.

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