



# Biotreatment of zinc-containing wastewater in a sulfidogenic CSTR: Performance and artificial neural network (ANN) modelling studies

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## ABSTRACT

Sulfidogenic treatment of sulfate (2–10 g/L) and zinc (65–677 mg/L) containing simulated wastewater was studied in a mesophilic (35 °C) CSTR. Ethanol was supplemented (COD/sulfate = 0.67) as carbon and energy source for sulfate-reducing bacteria (SRB). The robustness of the system was studied by increasing Zn, COD and sulfate loadings. Sulfate removal efficiency, which was 70% at 2 g/L feed sulfate concentration, steadily decreased with increasing feed sulfate concentration and reached 40% at 10 g/L. Over 99% Zn removal was attained due to the formation of zinc-sulfide precipitate. COD removal efficiency at 2 g/L feed sulfate concentration was over 94%, whereas, it steadily decreased due to the accumulation of acetate at higher loadings. Alkalinity produced from acetate oxidation increased wastewater pH remarkably when feed sulfate concentration was 5 g/L or lower. Electron flow from carbon oxidation to sulfate reduction averaged  $83 \pm 13\%$ . The rest of the electrons were most likely coupled with fermentative reactions as the amount of methane production was insignificant. The developed ANN model was very successful as an excellent to reasonable match was obtained between the measured and the predicted concentrations of sulfate ( $R = 0.998$ ), COD ( $R = 0.993$ ), acetate ( $R = 0.976$ ) and zinc ( $R = 0.827$ ) in the CSTR effluent.

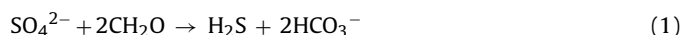
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## 1. Introduction

Mining and metallurgical industries produce large volumes of wastewater containing high concentrations of sulfate and metals (e.g. acid mine drainage (AMD)) [1]. Conventionally, hydroxide precipitation is the most commonly applied method for the treatment of metal-containing waters. The production of high quantities of sludge is the main disadvantage of the method. Also, sulfate removal is only possible when  $\text{Ca}^{2+}$  containing chemicals, such as lime, are used for neutralization. However, stringent discharge legislations will dictate more efficient sulfate removal and recovery of valuable metals from waters, which are possible with the use of active bioreactor processes [2].

In the treatment of AMD and metal-containing industrial wastewater, sulfate-reducing bioreactors are becoming an alternative to conventional chemical treatment (for a review, see [2]). With the supplementation of organic compounds, sulfate is microbially reduced to  $\text{H}_2\text{S}$  under anaerobic conditions and heavy metals form stable precipitates with produced  $\text{H}_2\text{S}$ . Moreover, produced bicarbonate increases the pH of the wastewater (Eqs. (1) and (2)). This way, metals and sulfate are concomitantly removed and pH can be

increased to neutral values in a single reactor (Eqs. (1) and (2)). The precipitate can be used for metal recovery [3,4]:



In the literature several studies have been conducted on the treatment of AMD using different attached growth reactor configurations [3–10]. The competition between sulfate-reducing bacteria (SRB) and methanogenic archaea (MA) can be controlled in cell suspension bioreactors based on their growth kinetics [11]. Cell suspension bioreactors also allow the recovery of metal-sulfide precipitates at the effluent of the reactor without accumulation within the bioreactor. Also, start-up time of cell suspension bioreactor may be shorter than attached growth reactor systems as the yield coefficient of biomass may be higher (due to lower sludge retention time (SRT)) and biomass granulation is not required.

The modelling of metal recovering bioprocesses is very important to optimize operational conditions of the reactor. However, it is very difficult to predict the performance of such a bioprocess with the classical approaches as the performance depends on several factors, such as wastewater composition, operational parameters of the reactor and the microbial community. When circumstances or processes are not understood well enough or parameter determination is unpractical, there is a distinctive advantage for black-box modelling [12]. Black-box models like artificial neural network

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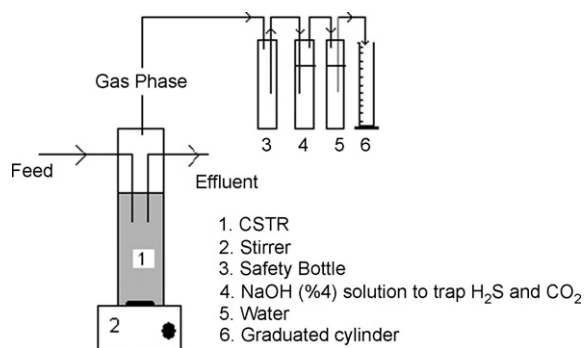


Fig. 1. Schematic diagram of CSTR.

(ANN) are very attractive. They do not require prior knowledge about the structure and relationships that exist between important variables. Moreover, their learning abilities make them adaptive to system changes.

In this context, this study aims at investigating sulfidogenic zinc removal from a simulated wastewater using a mesophilic ethanol-fed completely stirred tank reactor (CSTR). The reactor performance was investigated at different feed pH values and increased loadings of sulfate, ethanol and zinc, which is present in effluents from various industries, such as galvanization, electroplating, manufacture of batteries and other metallurgical industries. Also, the performance of the reactor was modelled by a popular neural network-back-propagation algorithm. To my knowledge, this is the first study on ANN modelling of zinc recovering sulfidogenic bioreactor.

## 2. Materials and methods

### 2.1. Bioreactor

A laboratory scale CSTR (Fig. 1) inoculated with an anaerobic digester effluent was used in the study. To maintain anaerobic conditions, the glass lids of the reactor and the other fittings were sealed after inoculation. The reactor was maintained in an incubator at 35 °C and it was mixed using a magnetic stirrer at 400 rpm. The working volume of the reactor was 500 mL. Hydraulic and sludge residence times were kept constant at 10 days throughout the study. To do this, the reactor was fed batch-wise with synthetic wastewater (Table 1) at a rate of 50 mL/day. After daily feeding, high purity N<sub>2</sub> gas was passed through the reactor to exclude oxygen gas coming with feed and to strip excess H<sub>2</sub>S. The stripped CO<sub>2</sub> and H<sub>2</sub>S were trapped in 4% NaOH solution. Produced methane was measured using a liquid displacement method (Fig. 1). Safety bottle was used to avoid the vacuum of NaOH to the bioreactor. Ethanol was used as a carbon and electron source stoichiometrically to reduce sulfate to hydrogen sulfide and oxidize ethanol and its major by-product, acetate, to CO<sub>2</sub>. Hence, throughout the study COD/SO<sub>4</sub><sup>2-</sup> ratio was kept at 0.67.

Table 2  
Operational conditions of the reactor

Parameter	Period I	Period II	Period III	Period IV	Period V	Period VI
Days	0–28	28–42	42–55	55–93	93–144	144–159
Feed sulfate concentration (mg/L)	2000	2000	3000	5000	10,000	10,000
Feed ethanol concentration (mg COD/L)	1360	1360	2010	3400	6927	6927
Feed zinc concentration (mg/L)	0	65	65	130–325	677	0
Feed pH	7.0–7.2	5.25–5.35	5.65–5.75	5.0–5.5	4.4–5.42	5.42

Table 1  
Composition of synthetic feed containing 2000 mg/L SO<sub>4</sub><sup>2-</sup>

Component	Concentration
MgSO <sub>4</sub> ·7H <sub>2</sub> O (mg/L)	2563
Na <sub>2</sub> SO <sub>4</sub> (mg/L)	1479
Ethanol (mg/L)	642
Yeast extract (mg/L)	50
KH <sub>2</sub> PO <sub>4</sub> (mg/L)	56
NH <sub>4</sub> Cl (mg/L)	110
Ascorbic acid (mg/L)	11
pH	4.4–7.2

For higher sulfate and ethanol concentrations, synthetic medium was concentrated.

### 2.2. Experimental procedure

The reactor performance was investigated at different feed organic, sulfate and zinc loadings for 159 days (Table 2). Firstly, the reactor was fed with an alkaline solution containing 2000 mg/L SO<sub>4</sub><sup>2-</sup> without zinc (Period I, days 0–28) to enrich sulfate-reducing bacteria. Then, the reactor performance was investigated at increased zinc, sulfate and organic loadings (Table 2).

Settleability of zinc sulfide particles was also studied when feed Zn concentration was 325 mg/L. For this purpose, reactor content was allowed to settle under quiescent conditions for 2 h. Then, total zinc concentration (soluble + particle) was measured in cleared water.

The reactor feed and effluent were sampled 3 to 4 times in 1 week for the measurement of pH, alkalinity, acetate, chemical oxygen demand (COD), sulfate, sulfide, soluble zinc, suspended solids (SS) and volatile suspended solids (VSS).

### 2.3. Modelling

In the ANN modelling of CSTR, the procedure given by Ozkaya et al. [13] was followed. A neural network is defined as a system of simple processing elements, called neurons, which are connected to a network by a set of weights (Fig. 2). The network is determined by the architecture of the network, the magnitude of the weights and the processing element's mode of operation [12]. At the start of training, the output of each node tends to be small. Consequently, the derivatives of the transfer function and changes in the connection weights are large with respect to the input. As learning progresses and the network reaches a local minimum in error surface, the node outputs approach stable values. Consequently, the derivatives of the transfer function with respect to input, as well as changes in the connection weights, are small [14].

In this work, a two-layer ANN with a tan-sigmoid transfer function for the hidden layer and a linear transfer function for the output layer were used. Fig. 2 shows the ANN structure used in the study. Feed pH, sulfate, Zn, COD and operation time were used as input parameters in ANN modelling to predict effluent sulfate, COD, acetate and Zn concentrations (Table 3).

The data were divided into training, validation and test subsets. Half of the data were used for training and one-fourth of the data was used for validation and tests.

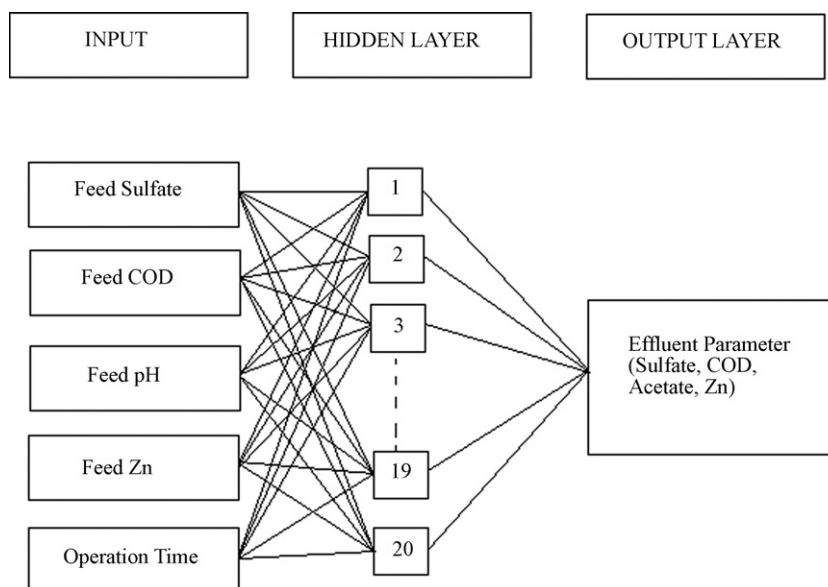


Fig. 2. The artificial neural network (ANN) structure for the prediction of CSTR effluent parameters.

### 2.3.1. Selection of back-propagation algorithm and neuron number

In this study, 12 back-propagation (BP) algorithms were compared to select the best fitting one. For all algorithms, a two-layer network with a tan-sigmoid transfer function within the hidden layer and a linear transfer function within the output layer was used. In the selection of BP algorithm, the number of neurons was kept constant at 20. The performance of the BP algorithms was evaluated with the root mean square error (M.S.E.) and determination coefficient ( $R$ ) between the modelled output and measured data set. After selecting best BP algorithm, which was Levenberg–Marquardt (trainlm) algorithm in this study, the number of neurons was optimized keeping all other parameters constant.

### 2.4. Analytical techniques

Before the measurement of sulfate, sulfide, soluble zinc and COD, samples were centrifuged at 4000 rpm for 5 min. Before centrifugation for sulfide measurement, sample pH was increased to around 10 not to cause any loss of sulfide. A turbidimetric method was used to measure sulfate concentrations [15]. COD, alkalinity, SS and VSS were also measured according to standard methods [15]. Before COD measurements, sample pH was decreased to below 2 with concentrated  $H_2SO_4$  and the sample was purged with  $N_2$  gas around 5 min to remove  $H_2S$  from the sample. Acetate concentra-

tion was measured following the procedure described by Alvarez et al. [16]. For soluble zinc measurements sample was first filtered through  $0.45 \mu m$  filter and then acidified with concentrated HCl to pH below 2. For total zinc concentration measurements, samples from settleability tests were first acidified with concentrated HCl to solubilise zinc particles. Then, samples were filtered through  $0.45 \mu m$  to remove biomass and other particles. Zinc concentration was measured with an atomic absorption spectrophotometer (Varian AA 140).

## 3. Results and discussion

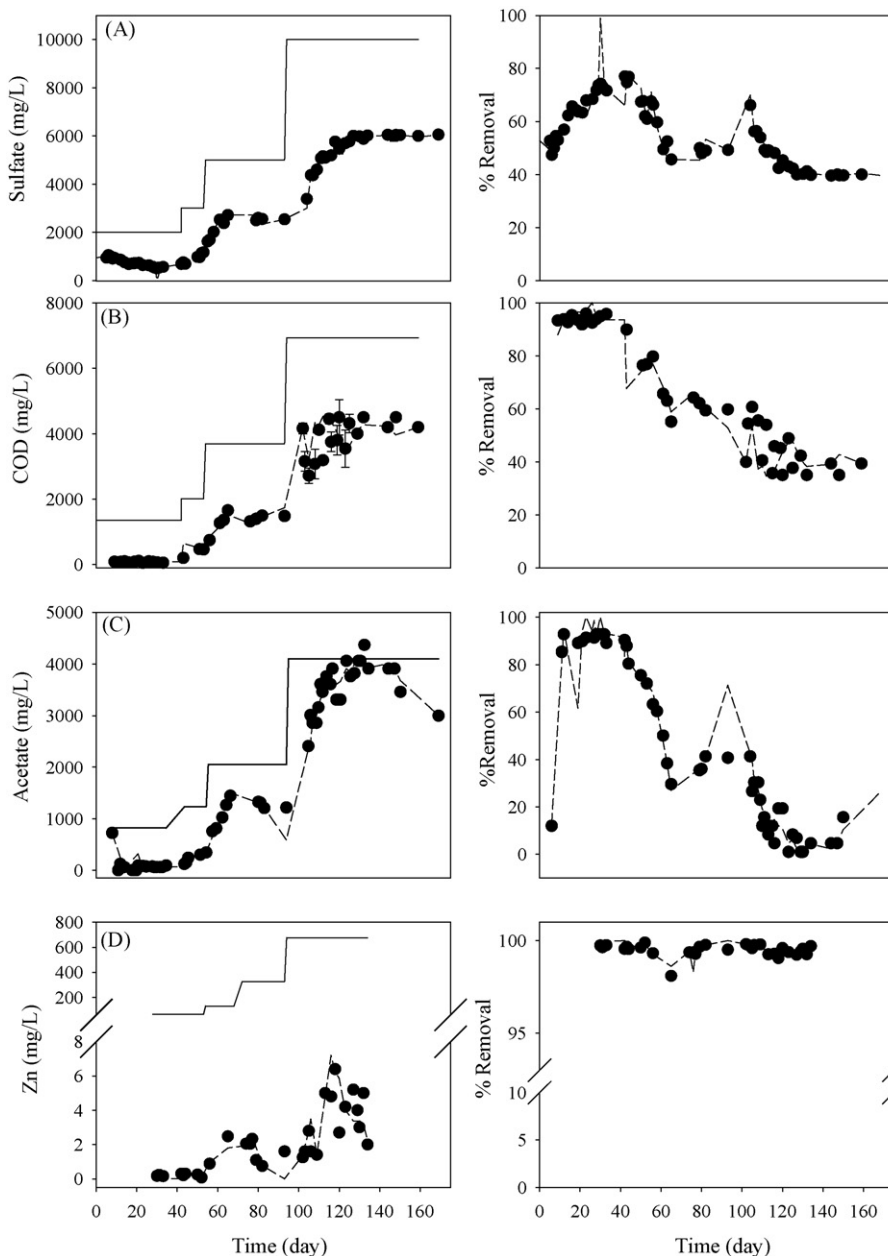
### 3.1. Performance of CSTR

The performance of the reactor throughout the study is summarized in Fig. 3 and Table 4. Between days 0 and 28 (Period I), reactor was fed with 2000 mg/L sulfate at pH 7.0–7.2 without zinc supplementation. For the first 10 days the sulfate removal efficiency was around 50% and it increased steadily to around 70%. When feed sulfate concentration was increased to 3000 mg/L (Period III), effluent sulfate concentration increased to 1156 mg/L, corresponding to 61% sulfate reduction. When feed sulfate concentration was increased to 5000 (Period IV) and 10,000 mg/L (Periods V and VI), effluent sulfate concentrations at the steady state averaged around 2500 and 6000 mg/L corresponding to 50 and 40% sulfate reduction, respectively (Fig. 3A and Table 4). As feed sulfate concentrations was increased from 2000 to 10,000 mg/L, sulfate reduction efficiency decreased from 70 to 40%, whereas removal rate increased from 140 to 400 mg/(L day). The specific sulfate reduction rate increased from 523 mg sulfate/(g VSS day) at Period I to 1077 mg sulfate/(g VSS day) at Period IV and decreased to 633 mg sulfate/(g VSS day) at Period V. The increase in specific degradation rate at Period IV should be due to the increased feed concentrations of ethanol and sulfate. The decrease of specific degradation rate at Period V may be due to high feed concentration of zinc. During the reactor operation, consistent, reliable and rapid equilibrium conditions were achieved, which are typical for CSTR type reactors [2].

Ethanol is an effective carbon source in the treatment of metal-containing wastewater by mesophilic SRB [5], whereas, relatively

Table 3  
Input and output parameters in artificial neural network modelling

Input parameters ( $P$ )	
$P_1$	Feed pH
$P_2$	Feed sulfate (mg/L)
$P_3$	Feed Zn (mg/L)
$P_4$	Feed COD (mg/L)
$P_5$	Operation time (day)
Output parameters ( $T$ )	
$T_1$	Effluent sulfate (mg/L)
$T_2$	Effluent COD (mg/L)
$T_3$	Effluent acetate (mg/L)
$T_4$	Effluent Zn (mg/L)



**Fig. 3.** Sulfate (A), COD (B), acetate (C) and zinc (D) removal performance. Lines and data points show feed and effluent concentrations, respectively. Dashed lines show ANN model predictions. Acetate feed concentration was calculated based on the oxidation of ethanol to acetate.

slower degradation rate of acetate may result in acetate accumulation (Eqs. (3) and (4)):



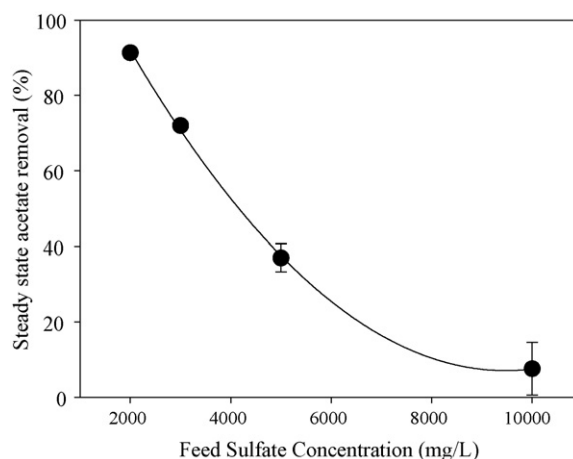
According to Eq. (3), 1 mol of ethanol consumption can produce 1 mol of acetate. Some SRB oxidize organic substrates completely to  $\text{CO}_2$ , while others oxidize them incompletely to acetate [17]. The main drawback of incomplete ethanol oxidation to acetate is an effluent with significant residual COD and less electron flow for sulfate reduction. Also, the acetate oxidation step in ethanol oxidation produces bicarbonate alkalinity (Eq. (4)), which neutralizes the acidity of wastewater. Hence, low acetate oxidation results in low production of alkalinity and sulfide, which may not

be enough for the neutralization of acidity and the precipitation of heavy metals (Eq. (5)). When feed sulfate concentration was 2000 mg/L, the effluent acetate concentration was  $70 \pm 10$  mg/L (Fig. 3C and Table 4). Assuming that ethanol was theoretically converted to acetate without further oxidation, effluent acetate concentration would be around 820 mg/L (Fig. 3C). Hence, acetate oxidation efficiency was higher than 90%. The effluent COD concentration also supported this result as the effluent COD concentration was  $80 \pm 20$  mg/L corresponding to 94% removal (Fig. 3B). When feed sulfate concentration was increased to 3000 mg/L, effluent acetate concentration increased to around 340 mg/L corresponding to 72% acetate removal (Fig. 3C). Similarly effluent COD concentration was around 470 mg/L corresponding to 77% COD removal efficiency (Fig. 3B and Table 4). When the sulfate concentration was increased to 5000 mg/L (Period IV), effluent acetate concentration averaged 1300 mg/L corresponding to around 36% removal

**Table 4**  
Steady-state performance of sulfidogenic CSTR

Parameter	Period I	Period II	Period III	Period IV	Period V	Period VI
Effluent sulfate (%) reduction)	613 ± 41 (67 ± 3)	543 ± 23 (73 ± 1)	1156 ± 45 (61 ± 1)	2545 ± 112 (49 ± 2.2)	5847 ± 189 (42 ± 2)	6025 ± 25 (40 ± 0.5)
Effluent COD (%) removal)	87 ± 18 (94 ± 1.5)	71 ± 13 (95 ± 1)	470 ± 28 (77 ± 3)	1448 ± 134 (61 ± 4)	4076 ± 360 (41 ± 6)	4350 ± 212 (37 ± 3)
Effluent Zn (%) removal)	–	0.21 ± 0.06 (99.7 ± 0.1)	0.22 ± 0.10 (99.6 ± 0.1)	1.79 ± 0.70 (99.25 ± 0.6)	3.7 ± 1.5 (99.2 ± 0.8)	–
Volatile suspended solid (mg/L)	265 ± 28	195 ± 80	201 ± 30	228 ± 19	656 ± 106	NM
Yield (mg VSS/mg sulfate reduced)	0.157 ± 0.03	0.135 ± 0.006	0.150 ± 0.005	0.093 ± 0.006	0.149 ± 0.03	NM

NM: not measured.



**Fig. 4.** Steady-state acetate removal performance at different feed sulfate concentrations.

(Fig. 3C). Similarly, effluent COD concentration averaged around 1500 mg/L corresponding to around 60% removal (Fig. 3B). Hence, high effluent COD concentration at high loadings was due to accumulation of acetate according to Eq. (3). When sulfate concentration was 10,000 mg/L (Periods V and VI), effluent acetate concentration increased to  $3800 \pm 300$  mg/L corresponding to only 8% acetate oxidation. Hence, steady-state acetate oxidation efficiency decreased with increasing feed sulfate concentration (Fig. 4). The maximum and minimum specific acetate oxidation rates of 443 and 46 mg acetate/(g VSS day) were attained at Periods III and V, respectively. Between days 134 and 144, the reactor was not fed and it was operated in a batch mode to follow the acetate oxidation in the absence of ethanol. During 10 days of batch operation no significant acetate oxidation, COD removal and sulfate reduction were observed. There may be three possible reasons of observing less acetate oxidation efficiency at 10,000 mg/L sulfate feed. One is high sulfate and organic loading. Second is relatively high dissolved sulfide concentration and the last one is high metal concentration (677 mg/L) in the feed. There are several studies on dissolved sulfide toxicity on sulfate-reducing bacteria (for a review, see Kaksonen and Puhakka [2]) and it is well known that acetate oxidation efficiency decreases at high sulfide concentration [18]. As for metal inhibition, Herrera et al. [19] reported that the metal-sulfide precipitates may adversely affect the activity of SRB and the mechanism of inhibition is that metal-sulfides act as barriers preventing the access of the reactants (sulfate, organic matter) to the necessary enzymes [7]. In order to understand the impact of zinc precipitate on acetate oxidation performance, the reactor was fed with 10,000 mg/L sulfate in the absence of zinc between days 144 and 159 (Period VI). Omitting of zinc from the reactor feed did not improve the acetate oxidation performance of the reactor. Hence, high concentration of heavy metal in the feed of the reactor may not be the reason of low acetate oxidation efficiency. Not observing acetate oxidation during 10 days of batch operation (between days 134 and 144) and in the absence of zinc (between days 144 and 159) may be the sign of wash-out of acetate oxidizing SRB during high sulfate and COD loadings. Hence, acetate oxidation is the limiting step of sulfidogenic treatment of acidic and zinc-containing wastewater in a CSTR.

High zinc removal efficiencies ( $\geq 99\%$ ) were obtained throughout the reactor operation (Fig. 3D). After day 93 (Period V) when sulfate and zinc concentrations were increased to 10,000 and 677 mg/L, respectively, no extra effort was put on removing excess dissolved sulfide due to high Zn concentration (677 mg/L) in the feed. Zinc removal efficiency between days 93 and 144 (feed Zn 677 mg/L) was  $99.2 \pm 0.8\%$  (Table 4). Although dissolved sulfide concentration



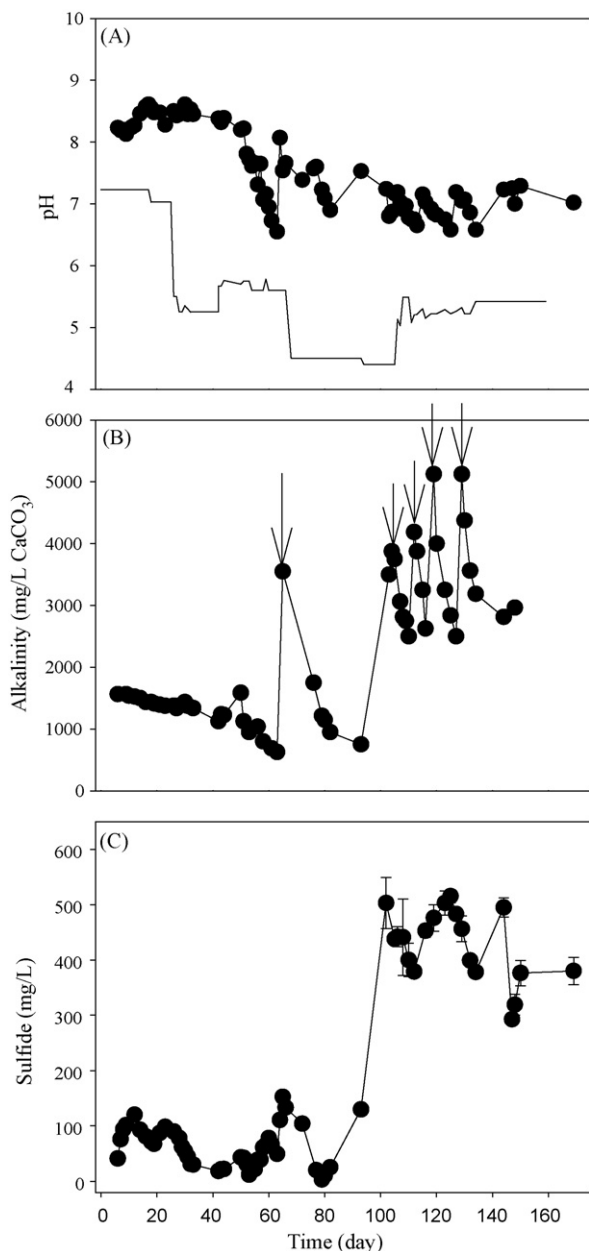


Fig. 5. pH (A), alkalinity (B) and dissolved sulfide (C) variations in CSTR (line (A) shows feed pH values and arrows show external alkalinity additions).

in the reactor was very high,  $428 \pm 63$  mg/L (days 93–144) (Fig. 5C), there was still measurable Zn concentration ( $3.7 \pm 1.5$  mg/L) at the effluent of the reactor. The reason of this observation may be the formation of small ZnS particles at high sulfide concentrations that can pass through the  $0.45 \mu\text{m}$  membrane filter [20]. Hence, at high sulfide concentrations measured Zn concentrations consist of both soluble Zn and ZnS particles that pass through the  $0.45 \mu\text{m}$  membrane filter.

The settleability of zinc-sulfide particles was also studied when feed zinc concentration was 325 mg/L. The total zinc concentration in cleared liquid after 2 h settling was only 17.8 mg/L, corresponding to 94.5% removal. Hence, zinc-sulfide particles can easily be recovered at the effluent using a settling tank.

Except start-up phase of the reactor (days 0–28), the feed pH was kept between 4.4 and 5.5. The effluent pH increased to neutral values due to alkalinity production from acetate oxidation when

sulfate concentration was 5000 mg/L or less. For example, feed pH value of 5.25 was increased to around 8.5 at the effluent when feed sulfate and zinc concentrations were 2000 and 65 mg/L (Fig. 5A). However, when sulfate concentration was 10,000 mg/L, alkalinity production decreased due to much less acetate degradation (Fig. 4) and external alkalinity addition was necessary to keep pH at neutral values (Fig. 5B). Alkalinity was added externally as bicarbonate salt ( $3000 \text{ mg CaCO}_3$ ) (arrows in Fig. 5B) when the reactor pH decreased to below 6.7. Hence, at high loadings, low acetate oxidation may result in pH decrease in the reactor. As most known SRB are very sensitive to even mild acidity [21], sulfate reduction-based reactors treating acidic and metal-containing wastewater should be monitored carefully.

After 1 week of reactor operation (days 0 and 7), methane production was not observed. This showed that MA was washed out due to low SRT (10 days) in CSTR or  $\text{H}_2\text{S}$  toxicity. Unionized hydrogen sulfide causes inhibition as only neutral molecules can permeate well through the cell membrane. It is known that MA is much more sensitive to sulfide compared to SRB [22]. With the elimination of methane producers, ethanol is available for sulfate reducers and fermentative bacteria. Electron flow from carbon oxidation to sulfate reduction throughout the reactor operation is presented in Fig. 6. The electron flow to sulfate reduction was calculated assuming that 0.67 mg COD is required to reduce 1 mg sulfate according to Eq. (1). Percent electron flow to sulfate reduction increased with increasing sulfate loading and it averaged  $83 \pm 13\%$ . The rest of the electrons were most likely coupled with fermentative reactions as methanogenesis was insignificant.

### 3.2. Biomass concentration and yield

The variations of VSS and SS concentrations through the reactor operation are presented in Fig. 7. The steady-state VSS values are also presented in Table 4. The ratio of VSS/SS was around 0.56 in the absence of zinc between days 13 and 28. When zinc concentration was increased to 65 and 677 mg/L, the VSS/SS ratio decreased to around 40 and 30%, respectively, due to metal precipitates in the reactor.

The variation of yield coefficient (Table 4) ( $0.1\text{--}0.16$  mg VSS/mg sulfate reduced) at different sulfate concentrations was statically insignificant and average yield coefficient was  $0.137 \pm 0.03$  mg VSS/mg sulfate reduced. Kaksonen et al. [4] reported  $0.053\text{--}0.074$  mg VSS/mg sulfate reduced in mesophilic fluidized bed reactors. In another study, Sahinkaya et al. [5] reported around 0.1 mg VSS/mg sulfate reduced in FBRs fed with ethanol and operated at 8 and  $65^\circ\text{C}$ . Relatively higher value in the present

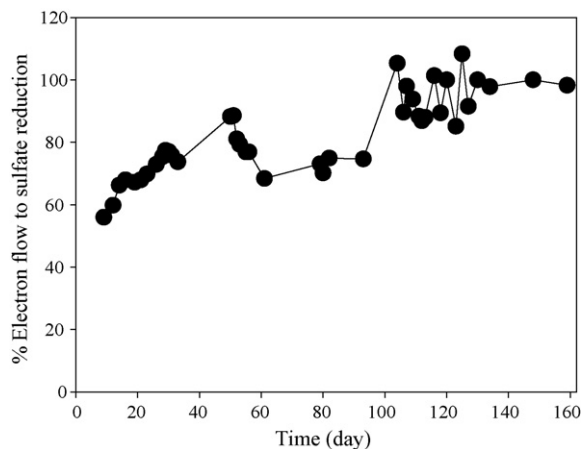


Fig. 6. Percent electron flow to sulfate reduction throughout the CSTR operation.

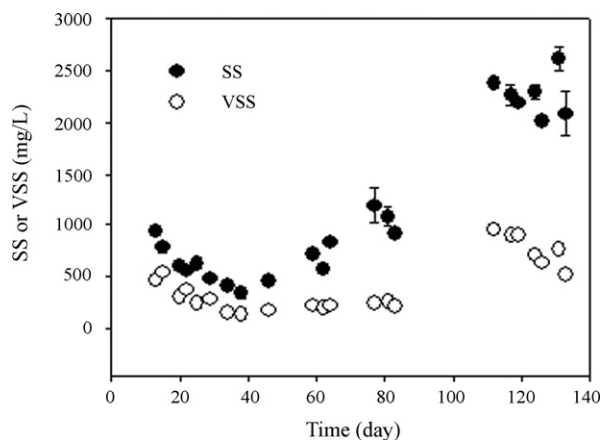


Fig. 7. Suspended (SS) and volatile suspended solids (VSS) variations in CSTR.

study is due to much lower sludge retention time (10 days) in CSTR compared to attached growth reactor systems.

### 3.3. Modelling results

The applicability of ANN was investigated to predict the performance of CSTR-based sulfate-reducing bioprocess treating zinc-containing (65–677 mg/L) wastewater. In the modelling study, the effluent sulfate, COD, acetate and zinc were predicted. First of all, 12 BP algorithms were compared to select the best fitting one. In the selection of BP algorithm, the number of neurons was kept constant at 20. Training results are provided in Table 5. The performance of the BP algorithms was evaluated with the root M.S.E. and determination coefficient ( $R$ ) between the modelled output and

measured data set. The best BP algorithm with minimum training error and maximum  $R$  was the Levenberg–Marquardt (trainlm) algorithm for all effluent parameters being modelled.

After selecting best BP algorithm, Levenberg–Marquardt (trainlm) algorithm, the number of neurons was optimized keeping all other parameters constant (Table 6). For all output variables, the squared mean error decreased for the training set with increasing neuron number. However after optimum neuron number, the squared mean errors increased or did not change significantly. The optimum neuron number was 20 for effluent sulfate, COD and acetate predictions, whereas, it was 40 for zinc prediction (Table 6). The optimum algorithm and neuron numbers were shown in bold in Tables 5 and 6.

Fig. 8 illustrates training, validation and test M.S.E. and the linear regression analysis between measured ( $T$ ) and predicted ( $A$ ) values for effluent sulfate, COD, acetate and zinc. The test and the validation set errors have similar characteristics, and it did not appear that any significant change in fitting occurred. The  $R$ -values were observed as 0.998, 0.993, 0.976 and 0.827 for sulfate, acetate, COD and Zn predictions, respectively. The time course variations of measured and the predicted data were as shown in Fig. 3 for both effluent concentrations and percent removals. The model data tracked the measured data closely for all output parameters (sulfate, acetate, COD and Zn).

The composition of the incoming wastewater may show great variations and the response of the bioreactor to unexpected overloads can be predicted using ANN. This may allow the operation engineer to take some measures to overcome possible process upsets caused by unpredicted changes in the incoming wastewater. Also model results can be used to optimize reactor's operational parameters to improve performance. Using the ANN predictions, alkalinity addition can be automated to maintain good process performance in a Zn recovering sulfidogenic bioreactor system.

Table 5

Comparison of back-propagation algorithms for predicting effluent sulfate, acetate, COD and zinc

BP algorithms	Sulfate		Acetate		COD		Zn	
	$R$	M.S.E. <sup>a</sup>	$R$	M.S.E.	$R$	M.S.E.	$R$	M.S.E.
<b>Trainlm</b>	<b>0.998</b>	<b><math>5.7 \times 10^{-26}</math></b>	<b>0.993</b>	<b>0.00172</b>	<b>0.976</b>	<b>0.00075</b>	<b>0.782</b>	<b><math>4.8 \times 10^{-18}</math></b>
Traincgp	0.997	0.0031	0.983	0.011	0.955	0.0398	0.594	0.1782
Traingd	0.856	0.1558	0.963	0.0449	0.928	0.1010	0.754	0.385
Traingda	0.991	0.0199	0.984	0.0150	0.971	0.0254	0.75	0.374
Traingdx	0.95	0.0455	0.77	0.533	0.914	0.0997	0.685	0.652
Trainrp	0.989	0.0068	0.99	0.0072	0.966	0.0199	0.72	0.2947
Trainscg	0.989	0.0056	0.985	0.0122	0.947	0.028	0.738	0.1290
Trainoss	0.986	0.0075	0.988	0.0120	0.976	0.01557	0.671	0.1597
Traincgf	0.997	0.0027	0.991	0.0100	0.937	0.04819	0.785	0.1046
Trainbfg	–	–	0.987	0.0084	0.978	0.0097	0.769	0.0627
Traingdm	0.42	3.793	0.828	0.769	0.434	1.822	0.398	1.344
Traincgb	0.989	0.0074	0.985	0.0082	0.969	0.0277	0.771	0.0611

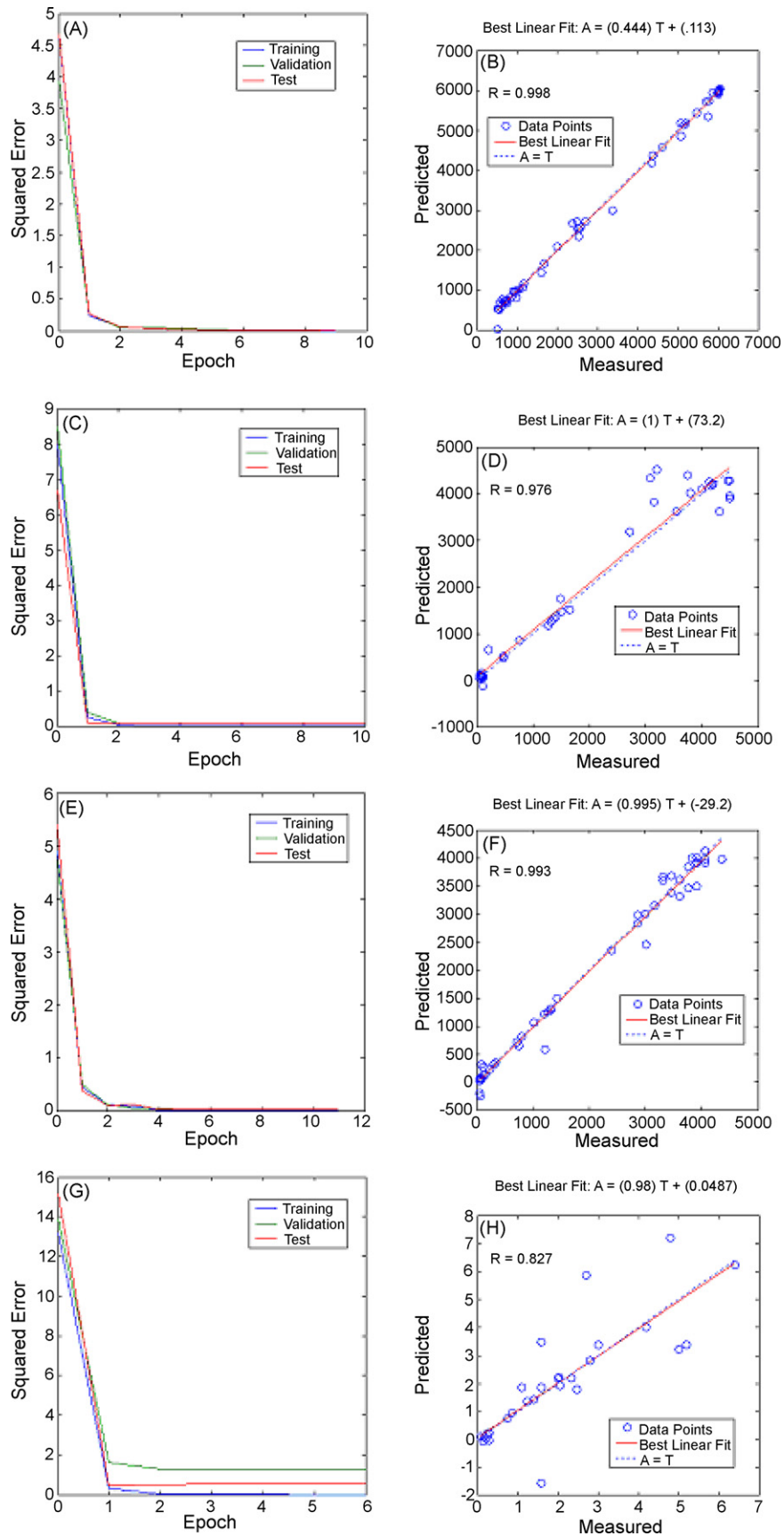
<sup>a</sup> Mean square error.

Table 6

$R$ -Values and mean square errors at different neuron numbers for predicting effluent sulfate, acetate, COD and zinc concentrations (Levenberg–Marquardt BP algorithm was used)

Neuron number	Sulfate		Acetate		COD		Zn	
	$R$	M.S.E. <sup>a</sup>	$R$	M.S.E.	$R$	M.S.E.	$R$	M.S.E.
3	0.991	0.00742	0.990	0.01058	0.899	0.0234	0.744	0.3317
5	0.998	$8 \times 10^{-5}$	0.990	0.0054	0.954	0.02168	0.758	0.3240
10	0.998	$2 \times 10^{-4}$	0.994	0.0030	0.974	0.00643	0.667	0.0561
15	0.998	$9 \times 10^{-5}$	0.987	0.0019	0.958	0.01404	0.656	0.00258
20	<b>0.998</b>	<b><math>5.7 \times 10^{-26}</math></b>	<b>0.993</b>	<b>0.00172</b>	<b>0.976</b>	<b>0.00075</b>	0.782	$4.8 \times 10^{-18}$
25	0.997	$7 \times 10^{-27}$	0.978	0.0006	0.946	0.00154	0.63	0.01509
30	0.985	$9 \times 10^{-25}$	0.986	0.0002	0.947	0.00011	0.797	$1.4 \times 10^{-27}$
40	0.927	$1.3 \times 10^{-7}$	0.987	0.0003	0.947	0.00037	<b>0.827</b>	<b><math>4 \times 10^{-31}</math></b>

<sup>a</sup> Mean square error.



**Fig. 8.** Training, validation, and test square mean errors (left column) and linear regression between the network outputs and the corresponding targets (right column) for Levenberge–Marquardt algorithm for effluent sulfate (A and B), COD (C and D), acetate (E and F) and zinc (G and H) predictions.



#### 4. Conclusions

In the study, ethanol supplemented CSTR fed with sulfate and zinc-containing wastewater enriched sulfate-reducing bacteria. MA was washed out within 1 week of operation and average electron flow to sulfate reduction was over than 80%. Sulfate removal efficiency was 70% at feed sulfate concentration of 2 g/L and it steadily decreased with increasing feed sulfate concentration and reached 40% at 10 g/L. COD removal efficiency at 2 g/L feed sulfate concentration was over than 94%, whereas, it steadily decreased due to the accumulation of acetate at higher loadings. Hence, acetate oxidation is the rate-limiting step in sulfidogenic treatment of metal and sulfate containing wastewater. The alkalinity produced from acetate oxidation increased wastewater pH remarkably when feed sulfate concentration was 5 g/L or lower. However, external alkalinity supplementation was required due to low acetate oxidation efficiency at higher loadings. The average yield coefficient throughout the study was  $0.137 \pm 0.03$  mg VSS/mg sulfate reduced. Throughout the study, over 99% Zn removal (precipitation rate was around 70 mg zinc/(L day)) was attained even at very high feed Zn concentrations (up to 677 mg/L) due to the formation of zinc sulfide precipitate. The designed, trained and validated artificial neural network model gave excellent to reasonable fits to the experimentally obtained sulfate, COD, acetate and zinc data.

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