Iron and manganese removal from textile effluents in anaerobic attached-growth bioreactor filled with coirfibres

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Abstract A laboratory scale study on Fe and Mn removal in upflow anaerobic bioreactor of a working volume of 20 L with coir fibre as the filter medium was investigated for a period of 312 days. The maximum Fe and Mn levels considered were 10 and 5 mg/L respectively, which are the typical average values of textile effluents subsequent to the primary and secondary treatments. Ten sub-experimental runs were conducted with varying HRTs (5 days to 1 day), ratios of COD:SO$_4^{2-}$ (20 to 3.5), Fe levels (0.005 to 10 mg/L) and Mn levels (0 to 5 mg/L). COD:SO$_4^{2-}$ of 3.5 was identified as the optimum point at which sulphate reducing bacteria (SRBs) out competed methane producing bacteria (MPBs) and further reduction of this ratio caused total and/or significant inhibition of MPBs, thus building sulphate reducing conditions. The effluent contained Fe and Mn below the permissible levels (1.6 and 1.1 mg/L for Fe and Mn, respectively) stipulated by US National Pollution Discharge Elimination System (NPDES) for inland surface waters at HRTs higher than 3 days. Results of the mass balance showed more Fe accumulation (60%) in sediments whereas 27% in the filter media. An opposite observation was noticed for Mn.

Keywords Bioreactor; Fe; methane producing bacteria; Mn; sulphate reducing bacteria; textile effluents

Introduction

Due to various anthropogenic activities, heavy metals will reach water courses. Various industries are responsible for a range of heavy metal releases. Several surveys carried out by Gunawardhana (2004), have showed high Fe and Mn levels in ground water sources in the vicinity of textile processing and garment washing plants. The catalysts which consist of Fe and Mn compounds are the main source of Fe and Mn in textile effluents. Further Mn containing compounds are known to be good colouring agents. Another survey carried out by Asian Regional Research Program on Environmental Technology (ARRPET) Phase II, Department of Civil Engineering, University of Moratuwa, Sri Lanka (2004) confirmed the presence of Fe and Mn in ground water. It was also found that subsequent to primary and/or secondary treatment the effluents contained Fe and Mn at levels of 10 ± 3.0 and 5 ± 2.0 mg/L, respectively, which is well above the permissible level for effluents discharge into inland waters, (1.6 and 1.1 mg/L for Fe and Mn respectively, US NPDES).

However, existing methods to remove Fe and Mn are neither efficient nor effective especially when metals are present in low concentrations (less than 10 mg/L). Hence, it is of paramount importance to develop an efficient and effective technology to deal with low polluted textile effluents and/or effluents subsequent to primary and secondary treatment. Other than the low cost, the minimum involvement of experts in day to day operations makes the anaerobic treatment more attractive at the first glance. Other advantages include low energy consumption, low excess sludge production, enclosure of
odours and aerosols. High rate anaerobic digesters which retain biomass have high treatment capacity, hence site requirements are low (Perez-García et al., 2005). Unlike treating for organic loading using methane producing bacteria (MPBs), here it is intended to have sulphate reducing bacteria (SRBs), since sulphide species produced as a consequence of sulphate reduction will precipitate Fe and Mn as sulphides. Therefore, this study investigated the removal of Fe and Mn under sulphate reducing conditions in an up flow attached growth bioreactor.

Materials and methods
Experimental set-up
The experimental set-up (Figure 1) comprised an equalisation tank, an anaerobic upflow bioreactor containing coconut husk made coir fibre as the filter media and a gas collection unit. Working volume of the reactor was measured to be 20 L, and coir fibre provided a total surface area, approximately 16 m² (surface area per unit volume of reactor 800 m²/m³ at a void ratio of 80–90%). Coir fibre was extensively washed with a detergent (Teepol®) followed by tap water, distilled water and deionised water. Then the fibre was dipped in a 0.1 N NaOH solution. This was carried out in order to increase the adsorption capacity of the biomass (Guangyu and Viraraghavan, 2000). The pretreated coir fibre was kept in the reactor vertically under tension and prior to it the fibre was kept dipped in a sludge (for 12 h), obtained from a brewery wastewater treatment plant. Bacterial analysis of this sludge showed the presence of MPBs.

Operational procedure
Ten experimental runs were carried out with varying hydraulic retention times (HRT), COD loading rates, sulphate loading rates, Fe concentrations and Mn concentrations (Table 1). A synthetic wastewater containing 2.175 g of K₂HPO₄, 1.77 g of Na₂HPO₄, 2.25 g of MgSO₄·7H₂O, 0.17 g of NH₄Cl, 7.15 g of urea, 0.85 g of KH₂PO₄, 2.75 g of CaCl₂ and 60 g of starch was mixed with distilled water to make the final volume 1 L. By diluting 75 mL of it to 5 L using tap water, the COD was controlled in the feed within the range 500–600 mg/L. MgSO₄ concentration was increased to obtain the desired COD:SO₄²⁻ ratio. The FeSO₄·7H₂O and MnSO₄·7H₂O were used as the Fe and Mn sources. The HRT was varied from 5 days to 1 day.

![Figure 1 Schematic view of the experimental set up](https://iwaponline.com/wst/article-pdf/55/8-9/143/439896/143.pdf)
Chemical analysis
Sampling for all chemical analysis was carried out at intervals less than or equal to the HRT of each run. Analysis of COD, SO$_4^{2-}$ and alkalinity were carried out in accordance to Standard Methods for Examination of Water and Wastewater (1995). Total Fe and Mn analysis were conducted using a flame GBC 932 Plus Atomic Absorption Spectrometer after digestion using the aqua regia method (ASTM, 1991).

Gas production measurement
Water displacement method was used to measure the gas quantity.

Calculation of stress on the system
Increase in Fe concentration in the effluent was measured by taking the difference between concentrations before (average of the last two) and concentration after the increment, for the latter it is considered to be the first two readings coming after a lap of $y$ number of days ($y$ is the HRT), after the increment. This approach was considered since any change in the feed will be evident/effect only after at least a time gap equal to the HRT. This difference was then divided by the initial concentration and this value has been considered as the stress on the system.

Mass balance
The graphical expression of the Fe and Mn accumulation is the difference between the areas of temporal variation of feed and effluent making with the time axis (Figure 2) and this gives the accumulation in mg.day/L. For this purpose, a trapezoidal theorem was used and multiplied by the relevant flow rate in order to obtain the accumulation in terms of mg. Then, this theoretical accumulation was correlated to the total Fe and Mn found in coir fibre, aggregates, water and sediments.

Results and discussion
Temporal variation of Fe
The reactor start-up lasted for a period of 60 days ($R_0$) with operating conditions as shown in Table 1. Continuous monitoring of pH and alkalinity was carried during this period (data not shown) to keep the pH within the range of 6.5–7.5, since micro-organisms are known to function optimally in this pH range. Further at neutral pH, majority of reduced sulphate species will be in non-toxic form (Annachhatre and Suktrakoolvait, 2001).

### Table 1 Operational condition for the experimental run $R_i$

<table>
<thead>
<tr>
<th>Experimental run</th>
<th>Heavy metal concentration (mg/L)</th>
<th>HRT (days)</th>
<th>Duration (days)</th>
<th>COD:SO$_4^{2-}$</th>
<th>OLR* kgCOD/m$^3$day</th>
<th>SLR* kgSO$_4^{2-}$/m$^3$ day</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_0$</td>
<td>0.005</td>
<td></td>
<td>60</td>
<td>20</td>
<td>0.110 ± 0.011</td>
<td>0.005 ± 0.001</td>
</tr>
<tr>
<td>$R_1$</td>
<td>0.5 ± 0.2</td>
<td></td>
<td>5</td>
<td>45</td>
<td>0.110 ± 0.011</td>
<td>0.005 ± 0.001</td>
</tr>
<tr>
<td>$R_2$</td>
<td>1.2 ± 0.2</td>
<td></td>
<td>5</td>
<td>28</td>
<td>0.110 ± 0.011</td>
<td>0.005 ± 0.001</td>
</tr>
<tr>
<td>$R_3$</td>
<td>2.0 ± 0.3</td>
<td></td>
<td>5</td>
<td>32</td>
<td>0.110 ± 0.011</td>
<td>0.009 ± 0.001</td>
</tr>
<tr>
<td>$R_4$</td>
<td>4.1 ± 0.4</td>
<td></td>
<td>5</td>
<td>28</td>
<td>0.110 ± 0.011</td>
<td>0.014 ± 0.001</td>
</tr>
<tr>
<td>$R_5$</td>
<td>10.0 ± 2.0</td>
<td></td>
<td>5</td>
<td>35</td>
<td>0.110 ± 0.011</td>
<td>0.032 ± 0.003</td>
</tr>
<tr>
<td>$R_6$</td>
<td>10.0 ± 2.0</td>
<td></td>
<td>3</td>
<td>21</td>
<td>0.183 ± 0.016</td>
<td>0.053 ± 0.005</td>
</tr>
<tr>
<td>$R_7$</td>
<td>10.0 ± 2.0</td>
<td></td>
<td>2</td>
<td>13</td>
<td>0.275 ± 0.025</td>
<td>0.080 ± 0.008</td>
</tr>
<tr>
<td>$R_8$</td>
<td>10.0 ± 2.0</td>
<td></td>
<td>1</td>
<td>5</td>
<td>0.550 ± 0.050</td>
<td>0.160 ± 0.002</td>
</tr>
<tr>
<td>$R_9$</td>
<td>10.0 ± 2.0, 5.0 ± 1.0</td>
<td></td>
<td>3</td>
<td>45</td>
<td>0.183 ± 0.016</td>
<td>0.053 ± 0.005</td>
</tr>
</tbody>
</table>

*Organic loading rate (OLR), sulphate loading rate (SLR) and multiplying OLR and SLR by a factor of 12.5 will give loading rates in terms of respective surface loading rates.
NaHCO₃ was used to adjust the pH. During the period R₀, Fe concentration (data not shown) of the feed was kept in trace levels (approximately 0.005 mg/L) in order to provide it as a trace nutrient for micro-organisms (Pelczar et al., 2000). During this period effluent Fe concentration was negligible (data not shown). This almost 100% removal was mainly due to the adsorption of Fe²⁺ taking place on pre-treated coir fibres (Guangyu and Viraraghavan, 2000). During the R₀ period, it was very unlikely to have significant bacterial mediated metal removal since the seed sludge primarily contained organisms which are not known to fix metal ions.

During the initial operation of the reactor (R₀), good gas production with visible bubble generation was observed. This gas production seemed to be a result of the metabolism of MPBs in the bioreactor in view of the fact that coir fibre was dipped in a sludge (obtained from a plant designed to remove COD) enriched with MPBs. The high COD:SO₄²⁻ of 20 in the bioreactor helped maintain conditions favourable for MPBs (Omil et al., 1998; Annachhetre and Savorpak, 2001).

A 100-fold increase in Fe concentration in R₁ and further increase in R₂ (1.2 mg/L) showed the limits of adsorption capability for Fe with a slight increase of Fe in the effluent (Figure 2). In runs R₃ and R₄, the Fe concentrations were doubled together with reduction of COD:SO₄²⁻ ratios from 20 to 10 and then to 7. These runs showed remarkable Fe removal potential with gradual proliferation of SRBs.

According to Figure 2, the results showed that Fe removal was satisfactory for each run as the effluent Fe levels were approximately 40% lower than the US NPDES standard of 1.6 mg/L (though a slight increase in the effluent Fe levels was noticed at the beginning of each run). This removal seemed to be mainly attributed to SRB mediated sulphate reduction (subsequent to R₃). Further formation of black coatings (precipitates, which was identified as FeS) in sediments and in biofilms were noticed.

The proportionate increase in sulphate levels (Figure 3A) to the increase in Fe levels in the feed has accommodated the elevated Fe removal, thus keeping the Fe concentration in the effluent below the standard value. This is by producing more sulphides by reducing sulphates with the mediation of SRBs. For this to be a reality, there must be a significant SRB population in the bioreactor, which was augmented by MPB-rich inoculum.

![Figure 2 Temporal variations of Fe and Mn](https://iwaponline.com/wst/article-pdf/55/8-9/143/439896/143.pdf)
Competition between MPB and SRB
During the runs R0, R1 and R2 (COD:SO$_4^{2-}$ of 20) a high COD removal efficiency (60–80%, Figure 3C) and a high gas production in the external gas collection set-up (Figure 3D) were noticed, indicating that MPBs were dominating in the system which have been introduced by the inoculum. In contrast to COD reduction, sulphate reduction was 20–40% (Figure 3C). During the run R3 (COD:SO$_4^{2-}$ of 10), the COD removal and gas production was reduced while sulphate reduction increased. Further, the effluent started giving the characteristic rotten egg smell of H$_2$S. Considering these observations, R3 was categorised as the transitional period in which SRB out competed MPB at a COD:SO$_4^{2-}$ ratio of 10. This was manifested by the reduction in gas production with the production of aqueous H$_2$S (Lens et al., 2002). According to Annachhatre and Suktrakoolvait (2001), of the H$_2$S produced, approximately 70% will be in the non-toxic form of HS$^-$. Considering the neutral pH. The cumulative gas production reduced after the period R3 (approximately on the 155th day, Figure 3D). This was due to the dissolution of CO$_2$ portion of the collected gas and also insignificant or non-occurrence of gas production other than aqueous H$_2$S. A similar percentage removal of COD was observed for the runs R4, R5 and R6. Only sulphate reduction increased, also an increase in rotten egg smell was noticed. These observations suggested that competition of MPB and SRB is a function of COD:SO$_4^{2-}$ (or COD:S) ratio, where similar observations were reported by Annachhatre and Suktrakoolvait (2001).

Stress on the removal process for a given increase in Fe concentration
The stress caused by increments in Fe levels was minor for the runs R1 to R2 and R2 to R3, where it was calculated to be less than 25%. Further, the system recovered within a period of less than a week. The stress showed by increment R3 to R4 was moderate, which was approximately 53% and the system recovered within a period of 1 week as was observed in the early runs. This observation suggested that the bacterial biofilms in the coirfibres acclimatized to the Fe levels provided and the levels used in each run were not toxic to the bacterial biofilms. However, in the final increment of Fe from R4 to R5 there was a fairly high stress on the bacterial biofilms and simultaneously a fairly drastic

**Figure 3** (A) Temporal variation of sulphate; (B) temporal variation of COD; (C) percent removal of sulphate and COD with time; (D) cumulative gas production with time
reduction in SO$_2$\textsuperscript{2-} removal efficiencies (Figure 3C) was noticed suggesting that SRBs were mainly involved in immobilising Fe. Nevertheless, after 3 weeks the Fe levels in the effluent were comparable to the Fe levels in the effluent reported during previous runs (Figure 2) and the SO$_2$\textsuperscript{2-} removal efficiencies (Figure 3C) also increased, suggesting that the bacterial biofilms acclimatised to the Fe levels.

**Effect of HRT on Fe removal**

The results showed that there was no significant change in the temporal variation of Fe in the effluent (Figure 2) when the HRT reduced from 5 to 3 days (R$_5$ to R$_6$). A similar observation was noticed when the HRT reduced from 3 to 2 days (R$_6$ to R$_7$) and from 2 to 1 day (R$_7$ to R$_8$). Availability of free H$_2$S as a result of excess production of H$_2$S more than required for Fe precipitation in the early functioning of the reactor seemed to be the reason for no-change in Fe removal efficiencies even at a reduced HRT. This was evidenced by the characteristic rotten egg smell of H$_2$S in the reactor water. HRTs less than 3 days (i.e. 2 days and 1 day, runs R$_7$ and R$_8$) gave Fe in the form of Fe$^{3+}$ in the effluent. This suggests that low HRTs are not capable of maintaining proper anaerobic conditions. The effluents Fe$^{3+}$ content (which was 4–5% of total Fe in the effluent) found at low HRTs (R$_7$ and R$_8$), will certainly get increased if the system was operated maintaining same low HRTs for a long period. Inducement of aerobic conditions will certainly affect the sulphate reduction in the biofilm formed by anaerobic bacteria. Considering this phenomenon and the very low COD and sulphate reduction (Figure 3C), HRTs less than 3 days considered not to be suitable as a viable HRT for proper functioning of this anaerobic bioreactor. This phenomenon is true in the case of operating the reactor at Fe loading rates approximately higher than 3.34*10$^3$ mgFe/m$^3$day. Hence, HRT of 3 days was selected as the optimum HRT. It should be noted that at this HRT Fe removal was also significant enough to satisfy the US NPDES standard.

**Temporal variation of Mn**

Mn was added at this optimum HRT of 3 days straight at the level of 5.0 ± 1.0 mg/L. As the study progressed, a drastic reduction in Mn levels was noticed (Figure 2). After approximately 6 weeks of adding Mn, the effluent Mn content was measured to be around 1.1 mg/L (Figure 2), which is the US NPDES limit stipulated for inland surface waters. Moreover, the overall trend for the temporal variation of effluent Mn level was in a decreasing trend, indicating that anaerobic bioreactors in the sulphate reduction mode could be effectively used in the removal of Mn.

**Effect of binary metal system**

When two or more metals exist in a system there can be a competition among the metals to get fixed in the system, especially with respect to biosorption. If binding sites are limited then the metal having the low ionic radius has a better chance of occupying these limited sites (Horsfall et al., 2003). Further, it can repulse any bounded metals having large ionic radius from their binding sites and will get biosorbed. In this case, Fe$^{2+}$ has that ability of replacing Mn$^{2+}$ due to its smaller ionic radius. Considering the temporal variation of Fe, this was observed to be the case since the addition of Mn did not have an effect on effluent pattern of Fe (Figure 2). The superiority of Fe for the binding sites is not the only reason for this occurrence of unaltered trend in effluent Fe level. Other reasons include:

- availability of aqueous H$_2$S in excess for precipitating Fe as FeS as it is and therefore no constraint for the Fe fixation, since sulphate reduction is more likely to be the main metal removing mechanism, (thus H$_2$S is not a limiting factor at all);
• growth in the biofilm may provide more binding sites and/or there can be unutilised binding sites in the system.

**Metal removal mechanisms**

Though various mechanisms are possible, the predominant metal removal mechanism seems to be SRB mediated precipitation of Fe and Mn as sulphides. The black precipitate accumulated in the bottom of the reactor and on coir fibres were good indications for it. Biosorption and/or attachment of Fe and Mn to the filter media seemed to be another possible metal removal mechanism.

At the end of the study (312th day) a comprehensive mass balance was carried out considering Fe and Mn accumulation that occurred on coir fibre, sediments, water and aggregates. Considering the Fe and Mn accumulation occurred per unit weight (Table 2), coir fibre had accumulated substantial amounts of Fe and Mn than the aggregates. The main reason for this is the provision of high surface area per unit volume. Further considering the Fe and Mn accumulation along the reactor depth, the metal content in water is in a decreasing trend from the bottom to the top, due to metal removal taking place along the reactor column. Hence reactor height is an important factor in the overall metal removal process. However, the metal accumulation on coir fibre along the reactor is not subject to significant variation, thus proving the reduction in metal concentration is mainly due to the sulphate reduction, also indicating it is occurring in the full reactor profile.

For Fe, the total accumulation (Table 3) is in the ascending order of water, aggregates, coir fibre and sediment. Sediment and coir fibre contained approximately 87% of accumulated Fe. More importantly, more Fe has been found in sediments (60%), hence indicating sulphate reduction is the main removal mechanism for Fe. It should be noted that deposition of FeS (black precipitate) on coir fibre is possible, thus indicating Fe accumulation taking place on coir fibre is significantly contaminated. Thus, part of the biosorption on coir fibre needs to be credited to the sulphate reduction. On the other hand, for Mn, more accumulation was found to be on coir fibre, which was approximately 65%, whereas for sediments it was measured to be 26%. This low availability in sediment...
is likely due to the relatively high solubility (for MnS the solubilities are $3 \times 10^{-11}$ (pink) and $3 \times 10^{-14}$ (green) and for FeS it is $8 \times 10^{-19}$ at 25°C, Skoog et al. (2005) performed by MnS.

**Conclusions**

Results have revealed anaerobic bioreactors filled with coirfibres can be successfully used to remove Fe and Mn from textile effluents as a tertiary method. Fe removal of 90% was obtained under sulphate reducing conditions at the influent level of 10 mg/L. Maximum loading rate of $3.34 \times 10^3$ mgFe/m$^3$day removed metals without affecting sulphate reducing capability. Fe accumulation was prominent (60%) in sediments whereas for Mn similar percentages were found to be on the coir fibre. The developed bioreactor is a low cost solution for polishing textile effluents containing Fe and Mn under sulphate reducing conditions.

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**References**


