A UASB bioreactor using silage as a carbon source to reduce sulfate
Winton Li and Susan A. Baldwin

ABSTRACT
Low cost-treatment for sulfate removal is required in many areas where potable water is scarce. The biggest challenge in biological treatment is finding an abundant low or no-cost carbon source. This work demonstrated for the first time that leachate from the agricultural byproduct silage can be used in an upflow anaerobic sludge-bed bioreactor to reduce sulfate for on-farm water treatment. The reactor ran continuously for approximately one year with an average silage leachate feed COD concentration of 4,471 ± 857 mg L⁻¹, and sulfate feed concentrations varying from 1,253 to 2,081 mg L⁻¹. The maximum sulfate reduction rate (SRR) of 9.75 ± 0.23 mmol (L day)⁻¹ was achieved at the high sulfate influent concentration and the amount of organics consumed was between 80–90%. Sulfide levels in the UASB bioreactor were consistently high for most of the experiment, averaging 516.6 ± 188.5 mg L⁻¹. Interestingly, during the last month of operation when sulfide concentrations were highest the SRR continued to increase. It was estimated that 36% of the silage leachate carbon was used directly for sulfate reduction.

Key words | cattle drinking water, silage, sulfate-reduction, UASB, water treatment

INTRODUCTION
The availability of suitable drinking water has always been a major concern for livestock farmers. Although cattle are known to tolerate sulfate levels as high as 3,000 mg L⁻¹, their health declines if they consume too much water of this concentration (Hibbs & Thilsted 1995; Beke & Hironaka 1994; Council 2001). Therefore, the Canadian Government recommends that sulfate concentrations in cattle drinking water be less than 1,000 mg L⁻¹. Despite this, many surface ponds and underground aquifers in the British Columbia (B.C.) interior and on the Prairies that farmers use as cattle drinking water sources have sulfate levels much higher than this (Brown 2007). As these aquifers are the only viable water source farmers have for their livestock, a suitable sulfate removal treatment process is needed to meet the demand for a healthy and productive herd. Although physical processes are effective for sulfate removal, the initial upfront purchase prices of the treatment units, as well as the ongoing costs of maintenance and chemicals make these methods too expensive for small scale farming operations (Baldwin 2004). Biological treatment processes, on the other hand, have much lower operating costs. For example, to treat 4,500 L of water per day, which is what is typically required for a small herd, with 1,000 mg L⁻¹ sulfate removal, nanofiltration costs $12, while a biological treatment process using ethanol as a carbon source costs only $1–$2 (Baldwin 2004). Several different biological reactors using ethanol and lactate or even acetate have been successful at reducing sulfate (Barnes et al. 1991; Nagpal et al. 2000; Sahinkaya et al. 2007; Nevatalo et al. 2009). Using defined carbon sources achieves high sulfate-reduction rates between 60–100 mmol (L day)⁻¹ with yields of 0.3–0.6 mol SO₄²⁻ reduced per mole ethanol or lactate consumed. Defined carbon sources for sulfate-reducing bacteria are still too expensive for farmers to use if they are purchased commercially. Producing ethanol on site is prohibited or subject to permitting, therefore an alternative, readily available on-farm carbon source for use in biological reactors is preferable.
Leachate from complex organics or wastewater high in dissolved organic compounds can be used to support sulfate reduction, albeit at lower rates than when defined carbon sources are used, as seen in bioreactors treating high sulfate wastes such as yeast wastewater (14.5 mmol SO$_4^{2-}$/L day$^{-1}$) (Krapivina et al. 2007) and in passive treatment of mine drainage (0.5–3.6 mmol SO$_4^{2-}$/L day$^{-1}$) (Waybrant et al. 1998; Zagury et al. 2006). However, the latter uses complex organics such as manure, woodchips or other plant and waste derived materials that could release chemical compounds unsuitable for livestock drinking water. Food industry wastes, such as cheese whey and wine waste have also been considered for supporting sulfate reduction (Martins et al. 2009), but typically cattle farms are not located close to the sources of these wastes. On the other hand, silage is a fermented product made from plant material such as alfalfa that is used as a feed for cattle. Lactic acid and other acidogenic bacteria are active during the ensiling process and they produce mostly lactic acid as well as butyrate, ethanol, soluble proteins and carbohydrates (Krizsan et al. 2007) all of which are potential carbon sources for sulfate-reducing bacteria (SRB). In this study the use of silage leachate as a carbon source for sulfate reduction in simulated Saskatchewan groundwater was demonstrated in an upflow anaerobic sludge blanket (UASB) bioreactor. The UASB design was used since this configuration has been very successfully applied to anaerobic digestion of liquid wastes and is used for sulfate reduction of contaminated ground water high in sulfate (Lettinga & HulshoffPol 1991). In addition, high concentrations of biomass are possible in an UASB due to granulation and build up of a sludge bed (Tiwari et al. 2006). In this study the performance of the bioreactor was assessed in terms of sulfate removal and utilization of the organic compounds in leachate used as a carbon source.

**METHODS**

**Reactor set-up**

A flow diagram of the reactor configuration is shown in Figure 1. The UASB bioreactor consisted of an 8.26 cm ID plexiglass cylinder with a working volume of 5 L. Simulated ground water and silage leachate entered at the bottom of the reactor through a conical inlet assembly with plastic balls and a dispersion plate to distribute the flow evenly. The effluent port was above a gas solid separation device built according to guidelines in Tchobanoglous (2003, pages 1010–1011). An inverted cone collected any gases produced in the bioreactor, which were directed to a NaOH solution and lime column to remove H$_2$S and CO$_2$. A portion of the effluent was recycled through the reactor column so as to bring the mean linear upflow velocity to 1 m h$^{-1}$ as recommended by Lettinga (1991). For part of the experiment, a N$_2$ gas stripper was installed in the recirculation line in an attempt to remove H$_2$S gas and lower the sulfide concentrations in the bioreactor. The final effluent was collected in an aerated tank. Sulfate feed was made up from four different sulfate salts to give a final concentration ratio of cations similar to that found in Saskatchewan groundwater: 21.5 parts Ca; 10.5 parts Mg; 3.5 parts Na and 1 part K. Silage leachate was prepared by soaking 148 g wet weight of alfalfa silage, obtained from the UBC Dairy Education and Research Centre, located in Agassiz, B.C., in 1 L of deionized H$_2$O for 30 min followed by filtering through a 0.45 μm membrane. Filtered leachate was autoclaved at 120 °C for 20 min so as to preserve the leachate. This recipe resulted in silage leachate with a chemical oxygen demand (COD) concentration of ≈10,000 mg L$^{-1}$. The flow rates were maintained at ≈0.5 mL min$^{-1}$ for silage leachate and ≈1 mL min$^{-1}$ for sulfate feed for most of the experiment. These flow rates were based on the residence time required to reduce 1,000 mg L$^{-1}$ sulfate assuming a sulfate reduction rate that was measured in batch tests using silage leachate (Brown 2007).

The bioreactor was seeded with 1 L of granulated sludge from an existing UASB bioreactor operated by Fleischmanns Yeast in Calgary, Canada. The sludge was added along with 1 L of SRB inoculum, obtained from a natural sediment (Lac DuBois, Kamloops, B.C.) known to contain highly active SRB (Brown 2007), as well as 1.7 L of silage leachate and 700 mL of water with sulfate salts. The bioreactor was filled with distilled water so as to provide a total of 5 L of reactor contents. The recycle pump was started to maintain an upflow velocity of 1 m h$^{-1}$ inside the UASB column. One month of acclimation time was needed to allow anaerobic conditions to establish and for...
SRB to enter exponential growth before continuous feed water was added. Initially the height of the sludge bed was just above Port 4, therefore liquid samples were taken from the Effluent Port, Port 1, Port 3, and the Influent Port. As the sludge bed grew, it eventually covered Port 3 and then samples were taken from only the Effluent Port, Port 2, and the Influent Port. With the addition of the sulfide stripper column, effluent concentration profiles came from samples taken from the Final Effluent Port. These samples were taken at various intervals ranging from a few hours during the batch start-up stage, to a week during the stable continuous stage. About once a month samples of granules were taken from Port 4 and analyzed for particle size distribution.

Chemical analysis

Sulfate and sulfide concentrations were measured using the standard methods $4500$-$\text{SO}_4^{2-}$ and $4500$-$\text{S}_2^{2-}$, respectively (APHA 2005). If samples were not analyzed immediately, they were preserved with 4% zinc acetate until analysis. Organic carbon compounds were characterized by measuring soluble chemical oxygen demand (COD) (APHA method 5220D), total organic carbon (TOC) (Shimadzu TOC-L), total carbohydrates (anthrone method of Ludwig (1956)), total protein (Bradford Assay (Bradford 1976)), volatile and organic acids (Waters IC-Pak Ion Exclusion 50A˚ 7 μm 7.8 × 300 mm HPLC Column on a Waters 2487 Dual λ Absorbance Detector). All samples were analyzed in duplicate or triplicate and the errors were less than 10% of the average value. For a few samples, alcohols and total phenols were quantified at a commercial laboratory using the US EPA method 8000 and the APHA method 5530D, respectively. Granule samples were viewed under a Leica visual microscope and photographed with a hand held digital camera for particle size distribution analysis using the software program ImageJ (Abramoff et al. 2004).

RESULTS

The goal of this project was to design and operate a laboratory-scale bioreactor to reduce sulfate in cattle drinking water using silage as the carbon source. Use of silage in sulfate reduction is new and innovative, and in this study the potential of silage as a carbon source was investigated by...
determining the sulfate reduction rates attainable and simultaneously measuring rates at which organic compounds in the silage leachate are used up. After an initial batch phase of 33 days, the bioreactor was operated continuously for 358 days. Within the first 10 days of batch operation, sulfate was reduced from 2,747 to 1,357 mg L\(^{-1}\). As well, COD and TOC decreased from 7,110 to 2,251 mg L\(^{-1}\) and 2,658 to 450 mg L\(^{-1}\), respectively. The pH was maintained between 7.5 and 8 through addition of NaOH when needed. Since the COD concentration leveled off indicating that no further utilization of the remaining organic compounds was occurring, additional fresh carbon source was added together with more sulfate. This resulted in further sulfate reduction of 1,289 mg L\(^{-1}\) and an increase in sulfide concentration to 327.7 mg L\(^{-1}\) at the end of the batch operation.

During the first 20 days of continuous operation several aspects of the feed system were improved. It was discovered that the sulfate water and silage leachate could not be mixed together before entering the USAB since bacterial growth would occur in the feed-line causing plugging. Therefore, they were mixed at a T-junction just before the reactor inlet. One common challenge with continuous bioreactors is preventing bacterial activity in the organic-rich feed. To overcome this the silage leachate feed tank was kept anaerobic by having a floating lid that maintained contact with the liquid surface so as to preclude contact with air. Lactic acid in the silage lowers the pH and acts as a preservative preventing further organic matter degradation. However, if silage comes into contact with oxygen in the air, aerobic microbes consume the lactate and other organic compounds resulting in silage spoilage. As an additional measure to limit microbial activity in the silage leachate feed, the feed tank and feed line were kept at 4 °C using a small refrigerator and a chiller. Thereafter, silage leachate and sulfate water feed rates were slowly ramped up and maintained at 0.53 ± 0.08 mL min\(^{-1}\) and 1.05 ± 0.07 mL min\(^{-1}\), respectively, for the rest of the experiment.

From Day 97 to Day 186 the UASB bioreactor successfully reduced sulfate as seen in the plot of sulfate concentrations in the feed and effluent over time in Figure 2. (Figure 2). The average sulfate concentration entering the reactor was 1,253 mg L\(^{-1}\) and the average effluent concentration was 53.36 mg L\(^{-1}\). According to the measured flow rates, the calculated mean hydraulic retention time was 2.2 days, which gave an average sulfate reduction rate (SRR) of 4.45 ± 1.32 mmol SO\(_4^{2-}\) (L day\(^{-1}\)). Up until that time, the sulfate feed water was made from NaSO\(_4\). However, cattle drinking water in Saskatchewan comes from underground aquifers where the dissolved solids consist of other cations as well as Na\(^{2+}\). As such, use of a simulated groundwater with a chemical makeup similar to that of the ground water used in Saskatchewan would provide valuable insights into how those chemicals affect the treatment process. For example, cations can influence the size of the UASB granules (Tiwari et al. 2006). Therefore, using a chemical analysis of groundwater provided by the Western Beef Development Centre (WBDF) [Larry Braul, personal communication, January 14, 2008], fresh simulated sulfate feed water was premixed using a combination of Ca, K, Na, and Mg sulfate salts as outlined previously. Thus, from Day 214 to Day 250 an average of 1,400 mg L\(^{-1}\) sulfate in the influent was reduced to an average of 14.77 mg L\(^{-1}\) (SRR = 6.26 ± 1.17 mmol SO\(_4^{2-}\) (L day\(^{-1}\)).

Some of the groundwater to be made potable for cattle is very high in sulfate (Weeth & Capps 1972; Beke & Hironaka 1991) and so the reactor performance was tested further by increasing sulfate concentration in the influent. After some instability due to a bad batch of silage and temporary loss of the silage leachate pump (leading to a peak in sulfate in the effluent for one month from day 267 (Figure 2)), the reactor finally stabilized and treatment performance improved steadily until the last week of operation when
sulfate was reduced from an average of 2,081 ± 21 mg L⁻¹ to an average of 50 ± 41 mg L⁻¹ in the effluent (SRR = 9.75 ± 0.23 mmol SO₄²⁻ (L day)⁻¹).

One of the major challenges in reducing large amounts of sulfate is the accumulation of sulfide, which can be inhibitory to SRB activity especially when sulfide is in the form of H₂S (Okabe et al. 1995, Kaksonen et al. 2004). From Day 97 to Day 256 total sulfide concentration in the effluent averaged 476 ± 115 mg-S L⁻¹ (Figure 3). On day 236 a sulfide stripper was introduced (Figure 1) where 1 L min⁻¹ N₂ was used in an attempt to remove H₂S and reduce the sulfide concentrations in the UASB. The impact of this stripper was seen as the sulfide concentration dropped to 260 mg-S L⁻¹ three days later. However, the purge gas carried over to the UASB and started to disrupt the sludge bed. The stripper was removed in order to improve the design. When sulfide concentration in the reactor feed was increased, the sulfide levels climbed reaching 805.2 mg-S L⁻¹ on Day 307. The re-installed stripper N₂ purge gas flow rate was increased to 200 mL min⁻¹, which resulted in decreasing the sulfide concentration to 482 mg-S L⁻¹. Various gas flow rates were experimented with over the next five days, but eventually solids built up within the stripper and it had to be removed. In the last week of the experiment, the sulfide concentration had reached an average of 936 ± 22 mg-S L⁻¹.

Sulfide in the effluent would still pose a health risk for cattle if they were to drink water directly from the UASB. Therefore, at the beginning of the experiment the UASB effluent solution flowed into a downstream tank (TK-03, Figure 1), which was aerated for the purpose of oxidizing the sulfides. If the O₂ supply rate is maintained at stoichiometric levels (molar ratio of O₂ to S of 0.4) then most of the sulfide should be oxidized to elemental S and not back to sulfate. After some experimentation with different methods, aeration was supplied with an aquarium pump to the effluent water just below a floating lid. After this, the sulfide levels in the tank stayed below 10 mg L⁻¹, with just a few exceptions. There was no need to seed the oxidation tank with sulfide-oxidizing bacteria (SOB), since in shake flask experiments Beggiatoa spp. and Thiotrix spp. appeared naturally without any inoculation in lightly aerated effluent from silage leachate-treated high-sulfate water (data not shown). The aerated effluent tank was operated for the first 91 days as a proof of concept.

Bulk carbon content in the UASB reactor feed was estimated with soluble chemical oxygen demand (sCOD), which is used often to quantify the strength of organics in wastewater. Since silage leachate comprises many different carbon compounds, the rate at which these different types of compounds are used up was measured by performing tests such as total organic carbon (TOC), total carbohydrates and organic acids. Total protein concentration was measured initially, but levels were found to be very low. Several different batches of silage were used during the experiment, but all material came from the same location.

In the initial days of influent sCOD measurement, the values fluctuated, varying between 2,067 mg O₂ eq. L⁻¹ and 4,604 mg O₂ eq. L⁻¹ (Figure 4). Once the system setup was changed to minimize biological activity in the feed line, the influent sCOD concentration stabilized at 4,270 ± 413 mg O₂ eq. L⁻¹. An upset occurred for about one month starting on Day 267 when the influent sCOD dropped down to 2,190 mg O₂ eq. L⁻¹. This reduction in nutrient supply to the reactor coincided with poor performance with respect to sulfate reduction as seen in Figure 2. On Day 301, the influent sCOD concentration was restored to an average of 4,629 ± 30 mg O₂ eq. L⁻¹ for the duration of the experiment. The final effluent sCOD concentration averaged around 551 ± 123 mg L⁻¹ for the first part of the experiment, when sulfate concentration in the feed was approximately 1,400 mg L⁻¹. Interestingly, the sCOD effluent concentrations were higher towards the end of the experiment (957 ± 99 mg L⁻¹).
The variations in TOC and carbohydrate concentrations mirrored the fluctuations in sCOD. After fixing the influent line clogging problems, the TOC concentration of the influent averaged 2,000 mg L$^{-1}$ (except for during the one month upset). Effluent TOC concentrations were approximately 250 mg L$^{-1}$, rising slightly towards the end of the experiment to around 325 mg L$^{-1}$. Carbohydrate concentrations in the influent averaged 315.9 ± 77 mg L$^{-1}$ until after day 323, when the concentration jumped to 626 ± 66 mg L$^{-1}$ for the remainder of the experiment. Effluent carbohydrate concentrations averaged 17.4 ± 9 mg L$^{-1}$ before day 323 and 66.6 ± 16.5 mg L$^{-1}$ after day 323. The only low molecular weight organic acids detected in the silage leachate feed were lactate and acetate. Concentrations of these two components in the influent differed between the pre day 323 period and thereafter. Lactate concentrations increased from 228 to 730 mg L$^{-1}$ whereas acetate concentrations remained more or less the same at 53.4 ± 0.1 mg L$^{-1}$. No lactate or acetate was detected in the effluent stream at any of the sampling times.

Silage leachate is known also to contain alcohols. Only a couple of influent and effluent samples were analyzed due to limited funds. Ethanol was the most abundant at 266 mg L$^{-1}$, followed by methanol, propanol and butanol, each at 20 mg L$^{-1}$. Likely, most alcohols were consumed in the bioreactor as none were detected in the effluent samples that were analyzed. Silage contained phenols some of which were decomposed by the reactor microbes since concentration decreased from influent (2.4 mg L$^{-1}$) to the effluent (0.69 mg L$^{-1}$).

The UASB design was chosen since the sludge bed retains a high concentration of biomass inside the reactor contributing to improved performance over time. Sludge bed volume increased steadily until day 238, when it reached a maximum of 2.7 L. The mean granule size was difficult to assess since there were always many very small particles, however the largest particle size seen and the number of particles over 1 mm increased with time (Table 1). When some granules were viewed under a visual microscope at 10× magnification, they appeared spherical in shape early on, becoming more agglomerated as the reactor aged (data not shown).

### DISCUSSION

In this study, silage leachate was used as a carbon source for biological sulfate reduction in a UASB reactor achieving a maximum rate of 9.75 ± 0.23 mmol SO$_4^{2-}$ (L day)$^{-1}$. To our knowledge this is the first report of a continuous flow SRB reactor using silage. The main impediment to wide spread implementation of biological sulfate reduction for treating large quantities of water is the cost of the carbon source. Silage is produced on the farm and since it is a fermentation product it contains organic acids that are preferred carbon sources for SRB. To assess the efficiency of silage utilization in the bioreactor the mass ratio of

<table>
<thead>
<tr>
<th>Day</th>
<th>Number of particles</th>
<th>Max size (mm)</th>
<th>Mean size (mm)</th>
<th>Median size (mm)</th>
<th>Percent &gt; 1 mm</th>
<th>Number &gt; 1 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>59</td>
<td>1.06</td>
<td>0.43</td>
<td>0.38</td>
<td>3.49</td>
<td>2</td>
</tr>
<tr>
<td>97</td>
<td>420</td>
<td>2.24</td>
<td>0.57</td>
<td>0.48</td>
<td>19.58</td>
<td>82</td>
</tr>
<tr>
<td>125</td>
<td>156</td>
<td>1.92</td>
<td>0.78</td>
<td>0.80</td>
<td>38.19</td>
<td>60</td>
</tr>
<tr>
<td>143</td>
<td>136</td>
<td>3.03</td>
<td>0.69</td>
<td>0.49</td>
<td>26.28</td>
<td>36</td>
</tr>
<tr>
<td>220</td>
<td>2,256</td>
<td>3.38</td>
<td>0.47</td>
<td>0.31</td>
<td>12.93</td>
<td>292</td>
</tr>
<tr>
<td>236</td>
<td>258</td>
<td>2.64</td>
<td>0.74</td>
<td>0.71</td>
<td>25.98</td>
<td>67</td>
</tr>
<tr>
<td>288</td>
<td>1,099</td>
<td>4.29</td>
<td>0.45</td>
<td>0.14</td>
<td>16.95</td>
<td>186</td>
</tr>
<tr>
<td>331</td>
<td>1,398</td>
<td>4.92</td>
<td>0.52</td>
<td>0.12</td>
<td>20.91</td>
<td>292</td>
</tr>
</tbody>
</table>
sCOD consumed to \( \text{SO}_4^{2-} \) reduced was examined. According to stoichiometry, 0.67 g of sCOD is required to reduce one gram of sulfate. Actual measured sCOD:SO\(_4^{2-}\) ratios were greater than this and varied from 1–6 g g\(^{-1}\). During the stable periods of operation (Figure 5) when the silage leachate influent sCOD was constant, the sCOD:SO\(_4^{2-}\) ratio decreased steadily from 3 to 1.8 g g\(^{-1}\). Over this period of time the sulfate concentration in the influent increased and the SRR improved while the silage leachate feed sCOD and the amount of COD consumed in the bioreactor remained approximately the same. A strong positive correlation \( (R^2 = 0.9867) \) was observed between SRR and the concentration of sulfate in the influent, excluding data from the period when low quality silage was used. Therefore, it appeared that at higher sulfate concentrations more of the carbon was used for sulfate-reduction rather than other microbial processes. One explanation for this is that high sulfate concentrations increase the growth rate of SRB, which build up in number in the sludge over time. Based on a comparison of the theoretical sCOD:SO\(_4^{2-}\) with the actual measured values, it was estimated that in the last 50 days of the experiment 36% of the COD consumed in the reactor went towards sulfate reduction.

On average, for the whole experiment, the amount of COD consumed was 84%. Not all carbon compounds in the silage leachate were biodegradable since, during the batch phase, COD concentration in the effluent leveled off at 2,551 mg L\(^{-1}\). The chemical composition of the silage did change slightly over time since higher concentrations of carbohydrates and lactate were measured in the influent after day 323. To see if carbohydrates are directly consumed in sulfate reduction, carbohydrate consumption rate was correlated with sulfate reduction rate. The hypothesis was that if carbohydrates are directly consumed in sulfate-reduction then as the SRR increased so should have the carbohydrate consumption rate. After the outliers corresponding to the reactor upset periods were removed a relatively strong correlation was observed \( (R^2 = 0.817) \), which suggests that carbohydrate consumption did contribute to the increase in sulfate reduction rate in the reactor. The same analysis could not be carried out for lactate, due to lack of enough data, but it is suggested that higher concentrations of lactate would also increase SRR.

Silage leachate proved to be very successful for supporting sulfate reduction and all of the carbon compounds that were measured were consumed to some extent in the reactor. Table 2 presents the consumption of each of the organic compounds as a percentage of the overall change.

![Figure 5](https://iwaponline.com/ws/article-pdf/11/2/229/416472/229.pdf)

**Table 2** Organic compounds consumed in the UASB and their COD and TOC equivalents

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration consumed (mg/L)</th>
<th>COD equiv. (mg/L)</th>
<th>Portion of COD consumed (%)</th>
<th>TOC equiv. (mg/L)</th>
<th>Portion of TOC consumed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>730</td>
<td>771.8</td>
<td>18.9</td>
<td>291.7</td>
<td>17.4</td>
</tr>
<tr>
<td>Acetate</td>
<td>53</td>
<td>56.5</td>
<td>1.4</td>
<td>21.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Ethanol</td>
<td>90.4</td>
<td>188.7</td>
<td>4.6</td>
<td>47.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Methanol</td>
<td>6.4</td>
<td>9.6</td>
<td>0.2</td>
<td>2.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Propanol</td>
<td>6.8</td>
<td>16.3</td>
<td>0.4</td>
<td>4.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Butanol</td>
<td>6.8</td>
<td>35.3</td>
<td>0.9</td>
<td>4.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>559</td>
<td>596.7</td>
<td>14.6</td>
<td>223.6</td>
<td>13.3</td>
</tr>
<tr>
<td>Total</td>
<td>1,674.9</td>
<td></td>
<td>41.1</td>
<td>594.6</td>
<td>35.5</td>
</tr>
</tbody>
</table>
in COD and TOC. The compounds that were measured accounted for only 41.1% and 35.5% of the total COD and TOC consumed, respectively. More than half of the biodegradable COD or TOC in the silage leachate was unaccounted for. Likely high molecular weight organic acids and soluble amides were some of these other compounds present also in silage leachate. Of the chemical compounds that were measured, lactate comprised the most COD and if all lactate was used for sulfate reduction it would have been responsible for 1,161 mg L$^{-1}$ or 55.8% of the sulfate reduced. The next largest amount of COD came from carbohydrates followed by ethanol. If all of the compounds in Table 2 were used for sulfate reduction, a total of 2,500 mg L$^{-1}$ would be possible, which is more than the 2,053 mg L$^{-1}$ that was actually reduced. However, it was likely that other microbes in the UASB competed with SRB for these readily available compounds. The 64% of COD that was not used for sulfate reduction, went towards increasing biomass as evidenced by the expanding sludge bed volume, or was consumed by other electron transfer processes.

Both lactate- and acetate-utilizing SRB may have been present, but methanogens also use acetate as a carbon source and they can coexist with SRB under some conditions. However gas bubbles in the reactor or in the solution of NaOH, which was part of the gas scrubbing train, were not observed at any time. The reactor pH was between 7.5 and 8, which meant that most of the sulfide (97–99%) was in the form of HS$^-$. Thus, the absence of gas bubbles contraindicated the presence of methanogens.

**CONCLUSIONS**

It was demonstrated that silage was effective for reducing sulfate in water destined for livestock on farms using a simple UASB bioreactor. The maximum sulfate reduction rate achieved was 9.75 ± 0.23 mmol SO$_4^{2-}$/L day$^{-1}$. Efficiency increased with sulfate concentrations up to 2,081 ± 21 mg L$^{-1}$ at which point 1.8 g of COD were used per gram of sulfate reduced. Under these conditions we estimated that 36% of the COD consumed in the bioreactor was used for sulfate-reduction. Sulfate removal was not compromised by the accumulation of sulfide, which reached levels as high as 936 mg L$^{-1}$. Therefore we have shown that silage is a readily available and inexpensive carbon source for on farm sulfate reduction.

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