Abstract. The purpose of this laboratory pilot scale study at the Wastewater Technology Centre (WTC), Environment Canada, Burlington, ON was to investigate the anaerobic biological removal of H2S from biogas under real-time operating conditions. Biogas produced in a 538 Litre pilot anaerobic digester was continuously fed into a 12 Litre biotrickling filter containing plastic fibres as packing bed media. The process was monitored for several months. The biogas flowrate and H2S concentration ranged between 10 to 70 L/h and 1000 to 4000 ppmv respectively over the course of the test period. Nitrate rich wastewater from a pilot scale sequencing batch reactor effluent was used as the nutritive solution for the biotrickling filter. The paper presents the influence of several operational parameters such as biogas flowrate, hydrogen sulphide concentration and composition of nutrient solution on process performance. To date, our results show H2S removal rates up to 100% without adverse effects on the methane concentration of the biogas. No system deterioration was observed over long term operation. This non-conventional technology is very promising and could be considered for full scale applications.

Keywords. biofiltration, biogas, biotrickling, hydrogen sulphide

INTRODUCTION

Using biogas as a renewable energy source requires pre-treatment technologies to remove damaging low concentration contaminants present in the biogas, such as hydrogen sulphide (H2S) and siloxanes. These impurities have detrimental effects on cogeneration engines and microturbine units and should be eliminated before combustion (Syed et al., 2006). Due to the high cost of existing removal technologies, predominantly based on chemical and physical processes, biogas pre-treatment contributes significantly to the overall operation and maintenance costs of any energy recovery system (Syed et al., 2006; Monteith et al., 2005). New research in biogas purification is focused on biological processes which are attractive from an economical and technological point of view. Current biotechnologies for H2S removal from gas fluxes are based on an aerobic process (Soreanu et al., 2005) which has several identified major drawbacks in equipment and operating costs (Devvinny et al., 1999).

A new research direction in biogas treatment has been undertaken at WTC, using an anoxic process for H2S removal from biogas. This process is based on denitrification in wastewater, however, to date no study has been published using this process for anaerobic biogas biofiltration. H2S removal under anoxic conditions was successfully tested previously at the WTC on a synthetic biogas (Soreanu et al., 2005). The purpose of this laboratory pilot scale study was to test the process on biogas under real-time operating conditions and to further demonstrate the stability of the robustness of the system. The biological process takes place in a biotrickling filter under anoxic conditions (no O2 feeding), where specific bacteria such as Thiobacillus denitrificans can convert H2S to elemental sulphur and sulphate, according to reaction scheme (1). In this process, nitrate (NO3-) acts as an electron acceptor instead of oxygen (Syed et al., 2006; Prescott et al., 2003).
Anoxic biological reaction:

\[ \text{Microorganisms} \]
\[ \text{H}_2\text{S} + \text{CO}_2 + \text{Nutrients} + \text{Nitrate} \rightarrow \text{Cells} + \text{Sulphur} \text{ and } / \text{or} \text{ Sulphate} + \text{Water} + \text{Nitrite} \text{ and } / \text{or} \text{ Nitrogen} \quad (1) \]

This paper presents the main technical considerations for applying this process for biogas purification, including the influence of several operational parameters such as the biogas flowrate, \( \text{H}_2\text{S} \) concentration and nutrient solution composition. Biofilm characterisation is also presented.

**MATERIAL AND METHODS**

**Experimental installation**

The experimental laboratory pilot-scale set-up using digester biogas is presented in Figure 1. The reactor is a biotrickling filter made from a PVC column having a diameter of 15 cm and equipped with 12 L of packing bed media. The packing media is a commercial fiber filter (Duststop Air Filters Inc., Petrolia, Ontario, Canada), fabricated using polyester fiber with a polyvinyl chloride binder. Although this type of filter has not been previously investigated for this application, we found it to be promising as a packing material for biofilters due to its durability, inert properties and high fibre density which allows it to retain water. Water retention in the packing material is important for biological processes, since the gaseous contaminants are more accessible to microorganisms in the aqueous phase and more easily degraded at the biofilm-liquid-gas interface. This material is suitable for processes in which biofilm production is not excessive. Prior to the start of experimentation, the packing media was inoculated with effluent from the SBR (sequencing batch reactor).

![Figure 1. Experimental laboratory pilot-scale biotrickling filter using digester biogas](image)

1- gas flowmeter; 2 - gas equalization bottle; 3 - biotrickling filter; 4 - nutrient solution tank; 5 - peristaltic pump; 6 - pH electrode; 7 - manometer; 8 - thermocouple.

The biotrickling filter was operated with hydrogen sulphide-containing biogas continuously produced and collected from a 538 L pilot anaerobic digester periodically fed with municipal organic waste provided by the City of Toronto. Biogas flowrate was measured using a flowmeter (Cole-Parmer Instruments Inc., model PMR1-010296). The nutrient solution was fed at the top of the column, counter-current with the biogas, using a peristaltic pump (MasterFlex, model 77200-62) connected to a nutrient solution tank. Operational conditions and parameters for the biotrickling filter treating digester biogas are presented in Table 1. The \( \text{H}_2\text{S} \) loading rate was increased by mixing real biogas with synthetic biogas from an \( \text{H}_2\text{S} \) generation unit described in Soreanu et al. (2005).
Table 1. Operational conditions and parameters for the biotrickling filter treating digester biogas

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Conditions and parameters of operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type process</td>
<td>Anoxic</td>
</tr>
<tr>
<td>Packing bed</td>
<td>Plastic fibers</td>
</tr>
<tr>
<td>Microorganisms</td>
<td>Indigenous</td>
</tr>
<tr>
<td>Biogas source</td>
<td>Organic waste digester (538 L)</td>
</tr>
<tr>
<td>Biogas composition, % (v/v)</td>
<td>65% CH₄, 35% CO₂ (±5%)</td>
</tr>
<tr>
<td>Biogas flowrate, L/h</td>
<td>40±10</td>
</tr>
<tr>
<td>H₂S concentration in biogas, ppmv</td>
<td>1100(±300)</td>
</tr>
<tr>
<td>Room temperature, °C</td>
<td>23 (±2)</td>
</tr>
<tr>
<td>Nutrient solution flow rate, L/h</td>
<td>30 (±0.6)</td>
</tr>
<tr>
<td>Nutrient solution composition, g/L NO₃⁻ as N</td>
<td>0.6 (±0.03)</td>
</tr>
<tr>
<td>pH of nutrient solution</td>
<td>6-7</td>
</tr>
</tbody>
</table>

Nutrient solution

Pilot-scale SBR effluent was used as the nutrient solution for microorganisms. Sodium nitrate (NaNO₃) was added to supplement the electron acceptor source. Several initial concentrations were tested (0.3 to 1.8 g N-NO₃/L), without maintaining constant nitrate concentration during experimentation. The pH of each new nutrient solution was adjusted to approximately 6.5 immediately after the start of the experiment, using a 4% NaOH solution. According to Kelly and Wood (2000), the optimum pH for growth of *Thiobacillus denitrificans* is 6.8-7.4. Immediately after start-up, an auto-buffering effect was itself achieved and no further pH adjustment was necessary over long term operation (i.e. months) using the same solution. At this pH value, it can be concluded that the removal of H₂S from biogas was mainly due to biological processes and not as a result of other physical/chemical processes (e.g. absorption of H₂S from gas in solution at higher pH). The composition of the nutrient solution was checked daily.

Analytical / measurement methods

The biogas was analysed for H₂S, CH₄, CO₂, O₂ and N₂, using a GC/TCD (Agilent 3000A, model G280 gas chromatograph/thermal conductivity detector) coupled directly to gas sampling ports located upstream and downstream of the biofilter. The pH of the nutrient solution was determined directly in the nutrient solution tank, using a permanently immersed Orion pH electrode (model 912600) coupled to a digital pH controller (ETATRON DS, model PBX0922110BA). The nutrient solution was analysed for N-NO₃⁻, N-NO₂⁻ and N-NH₄⁺ using a Technicon TRAACS Autoanalyser, as well for SO₄²⁻, S₂O₃²⁻ using a Dionex ICS 2000 Ion Chromatograph, according to Standard Methods for the Examination of Water and Wastewater 21st Edition (2005).

The temperature was measured in the middle of packing bed, using a thermocouple thermometer (Digi-Sense, model 91100-40, type T probe), while the pressure drop was measured at the base of the reactor, using a digital manometer (SPER Scientific, model 840080).

Microscopy pictures of the biofilm were obtained using a microscope Olympus (Model BX61TRF) coupled to a photocamera. Plate experiments were performed on 50x15 mm *Thiobacillus denitrificans* Petri dishes (supplied by ACP Chemicals, Montreal, QC).

Experimental methodology

Continuous monitoring of the biogas produced during anaerobic digestion and subsequent biofiltration was achieved for approximately 3.5 months. After 3.5 months of operation, the H₂S loading rate was artificially varied under controlled conditions by changing the H₂S concentration and the biogas flowrate in order to determine the limits of the biofilter performance. The following biogas flowrates were tested: 10; 40; 70 L/h (± 3 L under controlled conditions). The concentration of H₂S in the biogas was varied as follows: 1000; 2000; 3000; 4000 ppmv (± 300). The nutrient solution was circulated counter-currently at a flowrate of 30L/h. Maximum process performance (i.e. H₂S removal) was maintained by the periodic addition of nitrate to the
nutrient solution. The following initial nitrate concentrations were tested: 0.3; 0.6; 1.6; 1.8 g N-NO₃/L. Control of the accumulation of elemental sulphur on the packing bed was achieved by occasional flooding of the column with nutrient solution without discharge of the solution.

The process performance was evaluated in terms of H₂S removal efficiency (RE, %), H₂S loading rate (IL, g H₂S fed/(m² biofilter · day)), biofilter elimination capacity (EC, g H₂S removed/(m² biofilter · day)), and nitrogen demand in the nutrient solution (g N-NO₃ consumed/g H₂S removed). These criteria were described in Soreanu et al. (2005).

RESULTS AND DISCUSSIONS

The technology developed is based on a dual biological anoxic process: H₂S oxidation and NO₃ reduction. These processes take place simultaneously, in the presence of denitrifying bacteria, according to the reaction scheme (1). The behaviour of the biotrickling filter under varied operational parameters is presented and discussed in the following paragraphs.

Influence of nutrient solution composition on process performance

The biofilter was operated for 178 days under nitrate limiting (c_Nitrate = 0) and non-limiting conditions. After day 103, several H₂S loading rates (IL) were also tested. Graphs illustrating the response of the biofilter to the changes in nutrient solution and H₂S loading rates are presented in Figures 2 and 3 (day 28-143).

Figure 2 shows the variation of H₂S removal efficiency (RE, %) and N-NO₃ concentration as a function of time. As can be seen, the biofilter was able to function for long term (day 41-128) without changing the nutrient solution, and simply by supplying nitrate. Nitrate dependence is demonstrated by the very fast response (usually max. 1 day) of the biofilter to the nitrate supply in the nutrient solution (RE decreases when nitrate concentration drops to zero and increases after nitrate addition is resumed). During this period, nitrite accumulation up to a maximum 750 mg N-NO₂/L was observed (results not shown), as an intermediate product of nitrate reduction to nitrogen. However, this phenomenon appears not to have an inhibitory effect on process performance, as has been mentioned previously in literature. According to Tiedje (1988) concentrations of 42 mg N-NO₂/L could be inhibitory for some denitrifiers. Moreover, the biofilter was able to function at a lower capacity using only nitrite as an electron acceptor when nitrate was limited (i.e. RE approximately 40-90%, Figure 2). Lower process performance with nitrite can be explained by its lower degradation rate in comparison to nitrate (Tiedje, 1988). In addition, the linearity pattern of nitrate consumption observed in Figure 2 can be used to predict the nitrate uptake in nutrient solution.
It can be also observed in Figure 2 (days 115-128) that RE diminished even when excess nitrate (i.e. 1800 mg N-NO\textsubscript{3}/L) was present, but this behaviour was not due to nutrient solution composition. It was due to an increasing H\textsubscript{2}S loading rate to beyond the reaction capacity in the bed (Figures 2 & 3). It can be observed (Figure 2) that maintaining the nitrate concentration around 200 mg N-NO\textsubscript{3}/L could assure maximum process performance for typical digester biogas conditions.

Typically, introduction of a new batch of nutrient solution did not affect process performance (day 28, 41 etc.), with the exception of day 128 when an adverse affect was observed. It is suspected that a very specific consortium of microorganisms had developed during long time operation of the biofilter using the same nutrient solution (day 41-128) and that a re-adaptation time of 2-3 days was necessary after introduction of the new solution. Nitrate demand usually fluctuated between 0.25 and 0.47 g N-NO\textsubscript{3}/g H\textsubscript{2}S removal, depending on the reaction conditions.

Figure 3 presents the variation of H\textsubscript{2}S loading rate (IL) and biofilter elimination capacity (EC) as a function of time. As can be observed, EC increases with IL and maximizes at approximately 300-350 g H\textsubscript{2}S/(m\textsuperscript{3}biofilter \cdot day). Decreases in EC during normal biogas conditions (day 63-68, 73-77, 88-103) were coincident with nitrate limiting conditions (Figure 2).

During experimentation, sulphate accumulates, maximizes and remains at approximately 2500 mg SO\textsubscript{4}/L, after which, elemental sulphur becomes the primary reaction product. This behaviour occurs when the nitrate source was limited (i.e. nitrite present) as well as during H\textsubscript{2}S overloading. Similarly, elemental sulphur was the major degradation product produced during high H\textsubscript{2}S loading under aerobic conditions according to Chung et al. (1996).

Figure 2. Variation of H\textsubscript{2}S removal efficiency as a function of time. Influence of nitrate on process performance (H\textsubscript{2}S Removal). Initial nitrate concentration (g N-NO\textsubscript{3}/L): (A) 0.6; (B) 0.3; (C) 1.8; (D) 1.6. Variation of IL is presented in the Figure 3.

Figure 3. Variation of H\textsubscript{2}S loading rate (IL) and biofilter elimination capacity (EC) as a function of time.

Day 28-103; 128-143: typical conditions for the real biogas (from WTC pilot digester); day 103-125: the H\textsubscript{2}S loading rate was artificially increased by addition of synthetic biogas to real biogas.

(Changes in nutrient solution are presented in the Figure 2).
Influence of biogas flowrate and H₂S concentration on process performance

The developed process is nitrate dependent; however it can be adapted for a large range of nitrate concentrations in order to achieve the maximal removal efficiency. When nitrate concentration is not a limiting factor, it became obvious that biogas flowrate and H₂S concentration were the most significant factors, as can be seen in the Figure 4. It was observed that increasing biogas flowrate (Q) and H₂S concentration results in decreasing of the RE to less than 80% under the following conditions: [40 L/h; > 3000 ppmv] and [70 L/h; > 2000 ppmv], respectively. For pilot-scale biogas digester conditions (40 L/h; 1000 ppmv H₂S) a maximum RE was recorded (>99%). According to Kim et al. (2002), an accumulation of S-degradation products in packing material over a long time could affect H₂S removal efficiency. Sensitivity of bacteria to increase of sulfide concentration is mentioned in McComas and Sublette (2001). After testing at adverse conditions, the operation of the biofilter was returned to function under normal biogas conditions in order to verify the viability of the microorganisms. Because the RE under typical conditions recovered quite fast after these tests, it can be concluded that decreasing of the RE at high biogas flowrate and H₂S concentration, respectively, was mainly due to insufficient biogas/solution/packing (biofilm) contact time, thus the mass transfer between these phases was limited.

Combined effects of biogas flowrate and H₂S concentration are presented in Figure 5. The ideal case where the RE is equal to 100% (i.e. IL=EC) is represented by the theoretical line. In the present experiments, EC increased with IL up to 300-350 g H₂S/(m³ biofilter · day), and remained constant in this range when IL further increased between 400-800 g H₂S/(m³ biofilter · day). From this figure it can be determined that the biofilter performance (i.e. maximal EC corresponding to maximum RE) seemed to stabilize around 270-300 g H₂S/(m³ biofilter · day).

In addition, no reduction in methane concentration or change in pressure across the biofilter was observed during the long-term operation of the biofilter. On occasion, the elemental sulphur that accumulated on the packing material could be removed by flooding the column with nutrient solution. This operation did not affect the biological process.

Microscopic investigations

A sample of biofilm was obtained by rinsing a small piece of packing material from the middle of the reactor with nutrient solution, and spread on an agar surface and incubated under anaerobic conditions at 29 °C (optimal temperature for *Thiobacillus denitrificans* is between 28 and 32°C, Kelly and Wood, 2000). After approximately 4–7 days, a consortium of microorganisms containing 4 colony types was identified. One of the colony types was found to be dominant (i.e. >70% of total colonies). These colonies did not grow under aerobic conditions and were not present in the laboratory control samples. Enumeration of these colonies showed a low-medium population density of 2.4 · 10⁵ cells/mL packing, that may be due to the anaerobic nature of the process (Prescott et al.,
Based on gram staining as well as optical and fluorescence microscopy (Figure 6), colonies of small size rod gram-negative bacteria (0.5–0.7 x 1-1.4 mm) were observed. These observations are consistent with *Thiobacillus denitrificans* which is known to be able to degrade H$_2$S under anoxic conditions (Syed et al., 2006).

**Figure 6.** Fluorescence microscopy (DAPI added to mark the bacteria) (x 1000)  
(DAPI=4',6-diamidino-2-phenylindole)

**CONCLUSIONS**

The developed technology is based on a dual anoxic biological process that achieves hydrogen sulphide removal from biogas in a biotrickling filter based on nitrate removal from amended wastewater. High H$_2$S removal efficiency (RE >99%) is obtained under real-time operating conditions. The influence of nitrate concentration, biogas flowrate and H$_2$S concentration has been demonstrated. Under the current experimental conditions, an EC of 270–300 g H$_2$S/(m$^3$biofilter · day), corresponding to the maximum RE was achieved. No reduction in methane concentration or change in pressure across the biofilter was observed. This technology is suitable for treating digester biogas. This technology may be adapted for treating gases at higher loading rates through further optimization of process parameters.

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