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Hydrogen sulfide oxidation in novel Horizontal-Flow Biofilm Reactors (HFBRs) dominated by an Acidithiobacillus and a Thiobacillus species

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Keywords: H$_2$S oxidation, gas treatment, Horizontal Flow Biofilm Reactor, *Acidithiobacillus, Thiobacillus*.

Abstract

Hydrogen Sulfide (H$_2$S) is an odorous and highly toxic gas commonly encountered in various commercial and municipal sectors. Three novel, laboratory-scale, Horizontal-Flow Biofilm Reactors (HFBRs) were tested for the removal of hydrogen sulfide (H$_2$S) gas from air streams over a 178-day trial at 10°C. Removal rates of up to 15.1 g [H$_2$S] m$^{-3}$ h$^{-1}$ were achieved during the trial, demonstrating that HFBRs are a suitable technology for the treatment of H$_2$S-contaminated airstreams at low temperatures. Bio-oxidation of H$_2$S in the reactors led to the production of H$^+$ and sulfate (SO$_4^{2-}$) ions resulting in acidification of the liquid phase. Reduced removal efficiency was observed when a loading rate of 15.1 g [H$_2$S] m$^{-3}$ h$^{-1}$ was applied to the HFBRs. The addition of NaHCO$_3$ to the liquid nutrient feed (SWW) during this period resulted in improved H$_2$S removal. The bacterial community in the HFBRs was investigated by sequencing and fingerprinting the 16S rRNA genes. Bacterial diversity was low, likely due to the harsh environmental conditions prevailing in the systems, and the HFBRs were dominated by two species from the genus *Acidithiobacillus* and *Thiobacillus*. Even so, there were significant differences in microbial community structure between distinct zones in the HFBRs due to the influence of alkalinity, pH and SO$_4$ concentrations. Despite the low operating temperature, this study indicates that HFBRs have excellent potential to biologically treat H$_2$S contaminated airstreams.
Introduction

Hydrogen sulfide (H₂S) is an odorous and highly toxic gas that is commonly encountered in wastewater treatment. It can be generated when standing wastewater in sewers or clarifiers become septic; during anaerobic treatment; or during decomposition of solid organic matter [1, 2]. The emission of odours from wastewater treatment plants make their presence less acceptable to the general public and results in complaints to environmental protection agencies and to local authorities [3]. Legislation in Europe requires that waste and wastewater treatment facilities avoid excessive emissions of odours (Directive 2006/12/EC). Many gases emitted from wastewater treatment plants, such as hydrogen sulfide (H₂S), can cause significant odour nuisance and can be toxic.

Methods for remediating odorous gas emissions from the waste and wastewater sectors include physical, chemical and biological techniques. Physical and chemical techniques include scrubbing, absorption, membrane separation and iron oxide oxidation [4]. However, in recent years biological technologies, and in particular biofiltration, have been promoted as providing the most effective methods of air pollution control [6, 7]. This is due to the inherent advantages which include low capital, and maintenance, costs; energy efficiency; reduced, or eliminated, requirements for chemicals; and good long-term performance [8, 9, 10]. Several bioreactor configurations have previously been used for the treatment of H₂S-contaminated airstreams, including biofilters, bioscrubbers and biotrickling filters. Removal rates for biofilm-based reactors of between 5 and 169 g H₂S m⁻³ [reactor volume] h⁻¹ at residence times of less than 1 min have been reported [1, 2, 11, 12, 13, 14, 15]. Horizontal-Flow Biofilm Reactor (HFBR) technology has previously been used to treat methane-contaminated airstreams [16, 17] and were also shown to be capable of H₂S removal in a short proof of concept trial [18]. This study investigated H₂S removal in HFBRs in more detail than previously reported. The unique design of the HFBR allows for intensive analysis of the liquid and gas phases, and thus supports performance optimisation and control.

The study was done at 10°C, which is typical of ambient air and wastewater temperatures in Northern Europe, whereas most H₂S removal trials to date have been at mesophilic
conditions. Temperature has been shown to affect H\textsubscript{2}S removal rates in both lab and full-scale bioreactors, with optimum temperatures of 30-40°C [19, 20]. This is unsurprising as most known H\textsubscript{2}S oxidisers have an optimum growth temperature of 28-35°C [21], with the notable exception of \textit{Thermothrix azorensis} with an optimum growth temperature of 76-78°C [22]. Low-temperature treatment reduces the energy requirements needed to heat the HFBRs, which therefore reduces operating costs. Operation at lower temperatures also facilitates more extensive mass transfer of contaminants to the aqueous phase with increased H\textsubscript{2}S solubility [24]. To our knowledge this is the first trial to investigate H\textsubscript{2}S removal in a bioreactor operated at 10°C for a prolonged period.

The sulphur cycle is a complex biogeochemical cycle in which oxidised and reduced states of sulphur are transformed both biologically and chemically [26]. In the biological sulphur cycle, H\textsubscript{2}S is oxidised by bacteria to elemental sulphur (S\textsubscript{0}) or sulfate (SO\textsubscript{4}\textsuperscript{2-}) [26]. Several bacterial species have been identified as effective H\textsubscript{2}S-oxidizers, and bioreactors with immobilised cultures of \textit{Thiobacillus denitrificans} [27], \textit{Thiobacillus thioparus} [2] and \textit{Acidithiobacillus thiooxidans} [28] have all been used for H\textsubscript{2}S removal. Indeed, an alternative to pure culture inoculation of bioreactors for H\textsubscript{2}S abatement is to use activated sludge, as described by Gabriel and Deshusses [29] and Moussavi, Naddafiz [30]. H\textsubscript{2}S-oxidisers isolated from activated sludge have been immobilised on inorganic media by Jiang, Yan [31], Kim, Rene [12] and Duan, Koe [32]. The advantage of using activated sludge as a seed biomass is that it is readily available and likely contains a mixed microbial community capable of many biological processes, including H\textsubscript{2}S oxidation.

The main metabolic end-products of H\textsubscript{2}S oxidation in a biofilm reactor are sulfate and elemental sulphur (S\textsubscript{0}), and oxidation often results in a reduction of pH in the system [7, 11, 33]. Microbial diversity in engineered systems may be reduced with the production of H\textsuperscript{+} and SO\textsubscript{4}\textsuperscript{2-} ions during sulfide oxidation [32, 34]. H\textsubscript{2}S oxidation in bioreactors has been recorded at pH ranging between 1 [35] and 10 [36] with some studies reporting an optimum pH of 6 [34], while other studies found maximum oxidation rates as low as pH 3 [35]. The pH of the system also impacts on microbial community development [21]. Buffering of the pH can be achieved by dissolution of alkaline materials intrinsic in the bed media, such as calcium carbonate. Where inorganic bed media are used, manual addition of alkalinity in the form of CaCO\textsubscript{3} or NaHCO\textsubscript{3}, can be employed to buffer the pH [1, 34].
As the microbial community underpins bioreactor performance, an insight into the microbial ecology of bio-oxidation systems is necessary to understand, model and manage the reactions, processes and populations involved. The configuration of the HFBRs enables for depth resolved microbial community analysis as well as detailed gas and liquid profiling, therefore providing the links between environmental factors and their impact on microbial population dynamics. In this study, the performance of HFBRs used to treat H₂S-contaminated air at various loading rates and concentrations was investigated using gas and liquid phase analyses. The bacterial diversity of the HFBRs was investigated, and community structure and rates of H₂S oxidation were compared in different zones of the HFBRs.

Materials and methods

HFBR set-up and operation

Three HFBR units (R1, R2 and R3) were constructed by stacking sixty plastic sheets, each containing integrated frustums, vertically one above the other, to form a sealed reactor (Fig. 1). The HFBRs were configured so that the sheets could be easily separated for visual inspection and biofilm sampling. The working volume of each HFBR was 20 l and the top plan surface area (TPSA) of the plastic media was 0.04 m², giving a total media plan area of 2.4 m². Sample ports were distributed along the vertical profile of each HFBR, at sheets 4, 12, 21, 30, 40, 50, and at influent and effluent ports, allowing for gas and water measurements across seven specific zones (Z1-Z7). Z1 was located between the influent and first sample port; Z2 between the first and second ports; etc. The HFBRs were located in a temperature-controlled laboratory and operated at 10°C. Pure H₂S was mixed separately with compressed air to compose desired influent gas concentrations. Mass flow controllers (Bronkhorst High Tech BV), flow meters (Key Instruments) and pressure regulators (DruVa) were used to control gas flow rates and gas mix proportions as required. Flow and loading parameters are given in Table 1.

Insert Table 1 here.

Insert Figure 1 here.
An air mixture containing the H$_2$S gas was introduced above Sheet 1, at the top of the reactor and flowed horizontally across each sheet before moving to the sheet below. Nutrients were added to the liquid phase of each of the reactors in the form of a synthetic wastewater (SWW) mixture. The composition of the SWW (Table 2) was adapted from [37] to represent wastewater typically found at municipal wastewater treatment plants, with all organic carbon omitted so that autotrophic growth could be favoured. The SWW was pumped intermittently (10 min h$^{-1}$) onto each HFBR using a peristaltic pump in a step-feed manner with 75% of the SWW (4.5 l d$^{-1}$) pumped onto Sheet 1 and the remaining 25% of the SWW (1.5 l d$^{-1}$) onto Sheet 30. The step-feed facilitated more even distribution of nutrients through the units. As with the air mixture, the liquid phase flowed over each sheet before dropping to the next sheet below; therefore, the HFBRs did not operate as submerged reactors. The HFBRs were seeded with activated sludge from a local wastewater treatment plant (Mutton Island WWTP, Galway City, Ireland), which is operated at ambient temperature. To ensure that biofilm formation was successful, the activated sludge was added to the top sheet of the HFBRs and recycled with the SWW for two weeks before the H$_2$S supply was turned on.

*Insert Table 2 here.*

**Sampling and analytical methods**

The study was divided into five phases (Phases I - V lasting 35, 48, 43, 37 and 15 days, respectively) of varying loading rates and gas air mixture flow rates (Table 1). Influent, effluent and profile samples of the gas mixtures were taken from each reactor. Profile samples were taken from the six ports located along the vertical profile of each HFBR. H$_2$S concentrations were measured using a GasAlert Extreme Hydrogen Sulfide Detector (0-500 ppm detection range) GAXT-H-2-DL (BW Technologies). Liquid phase Total Organic Carbon (TOC), Total Nitrogen (TN) and Total Phosphorous (TP) concentrations were evaluated using a Biotector TOC TN TP Analyser (Biotector, Cork, Ireland). Liquid phase ammonium-nitrogen (NH$_4$-N), nitrite-nitrogen (NO$_2$-N), nitrate-nitrogen (NO$_3$-N), sulfate sulphur (SO$_4$-S), phosphate-phosphorous (PO$_4$-P) concentrations and alkalinity were determined using a Konelab 20 Nutrient Analyser (Fisher Scientific, Waltham, Massachusetts) and pH was measured using a multiple probe (HI9828, HANNA). The total biomass from each zone in the HFBRs was collected on day 180 and transferred to sterile containers. The biomass from each individual zone was homogenised by rigorous shaking.
and stored at -20°C for downstream molecular analyses. The Total Suspended Solids (TSS) and the Volatile Suspended Solids (VSS) of the biomass from each zone was calculated using Standard Methods [38].

**DNA extraction and quantitative-PCR (qPCR)**

DNA was extracted from biofilm samples (0.1 g) from each of the zones of each HFBR (i.e. 21 samples) on Day 180 using a Maxwell 16 Tissue DNA Purification Kit and a Maxwell 16 Research Instrument System (Promega). All extracts were visualised on a 1% agarose gel containing SYBR Gold under UV light, and quantified spectrophotometrically using a nanodrop (Thermo Scientific, UK).

Bacterial 16S rRNA genes were quantified by qPCR using a LightCycler 480 instrument (Roche) and the same primers (338f and 805r), probe (TaqMan 516f-ROX), reaction concentrations and cycling conditions described by Yu, Lee [39]. Concentrations of genes were expressed as gene copies m⁻²TPSA, where the TPSA is the top plan surface area of HFBR sheets.

**Clonal library construction and gene sequencing**

A composite HFBR DNA sample was prepared by combing an equal volume (2 µl) of each of the 21 DNA samples. Bacterial 16S rRNA genes were PCR-amplified using the 338f and 805r primers in reaction volumes of 50 µl and with the same reaction, and cycling conditions as described by Yu, Lee [39]. The resulting PCR amplicon was purified using Wizard SV Gel and PCR Clean-Up System (Promega), cloned using TOPO TA Cloning Kit and transformed into TOP10 cells as per manufacturer's instructions (Invitrogen). A total of 192 clones were sequenced by Macrogen (Seoul, South Korea) using an ABI3730xl DNA Analyzer.

Mothur software was used to screen for chimeras and identify unique operational taxonomic units (OTUs), at evolutionary distance of 3%, in the library [40]. Reference sequences were downloaded from the GenBank database and aligned with representative sequences from the library using ClustalX2 [41]. Phylogenetic trees were constructed using Mega5 [42] by neighbour-joining analyses with bootstrap method for 1000 replications using Jukes-Cantor model.
Temperature gradient gel electrophoresis (TGGE) fingerprinting

Bacterial 16S rRNA genes from each of the 21 DNA samples were amplified using primers 338f [39] and 534r [43], where the reverse primer included a GC clamp (CGCCCGCCGCGCCGCGCCGCCCCGGCCGCCCCC), according to the PCR conditions described previously. TGGE fingerprinting of the amplicons was performed using a Maxi System (Biometra, Göttingen, Germany). Denaturing gels composed of 6% acrylamide, 20% deionized formamide, 2% glycerol, 8 M urea with polymerisation agents TEMED (0.09%, v/v) and APS (0.016%, v/v), were run at 130 V for 16 h in Tris-acetate-EDTA buffer in a temperature gradient of 54-61°C. The temperature gradient used was determined by a previous perpendicular TGGE run. PCR banding patterns were visualised by silver staining as described by Bassam, Caetano-Anollés [44]. Statistical analyses of the banding patterns, and cluster and non-metric Multi-Dimensional Scaling (MDS) analyses, were done using the Primer6 software [45].

Results and discussion

Effect of retention times and gas loading on HFBR performance

The HFBR performance data from each of the five phases (Phase I – V) are summarised in Figure 2 and Table 3. Maximum H2S removal rates of 15.1 g H2S m⁻³ h⁻¹ were achieved in the HFBRs during Phase IV. During Phase I each of the HFBRs experienced an acclimation phase of seven days, when the H2S removal efficiency increased from 40% to 100%. Similar acclimation periods, usually lasting 6-30 d have previously been observed [7, 32] in H2S-oxidising systems and generally occur as the microbial community adapts to operating conditions and H2S availability. Following the acclimation, the median H2S removal was over 99% (Fig. 2; Table 3). After the loading rate was increased, an adjustment period of one week was observed at the beginning of Phase II, when average removal efficiencies were lower (86%, 95% and 92% for R1, R2 and R3, respectively; Fig. 2) and less consistent (standard deviations were 14%, 6% and 8% for R1, R2 and R3, respectively). However, H2S removal efficiency was consistently >98% for the remainder of Phase II. Adaptation periods can be expected following increased H2S loading rates before steady state values are observed [12, 46]. The HFBRs adapted to the next increment applied to the flow regime at the beginning of Phase III more rapidly than in Phase II (Fig. 3) and H2S removal efficiencies of 100% were
consistently observed during the remaining of Phase III. During Phase IV, initial removal rates remained high, although, after seven days, reduced performance was observed (Fig. 2). Variable performance occurred in the HFBRs for approximately 20 d. The drop in removal efficiency may have been due to the low pH experienced during this period (Fig. 2) or limited by carbon availability, coupled with the high H₂S loading rates. When the alkalinity in the HFBR was increased on Day 151 by adding additional NaHCO₃ to the SWW, the RE recovered to 100% almost immediately. Although 100% RE was achieved during Phase II and Phase III under similar pH conditions, the H₂S loading rates were lower. The optimum pH for biological H₂S oxidation can vary depending on the microbial community present [21] and in some cases it has been shown to be at pH values of 3-7 [32, 34, 47]. However some H₂S oxidising organisms, such as *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*, have optimum growth at pH 1.5-3.5 [48] and pH 2.0-2.5 [49]. Alternatively, the system may have been limited by inorganic carbon availability. Autotrophic H₂S-oxidising bacteria require an inorganic carbon source for the oxidation of H₂S and the addition of NaHCO₃ provided an extra carbon load into the HFBRs. The exact mechanism of how the NaHCO₃ improved the removal efficiency of the HFBRs is unclear, however, performance did stabilise following adjustment of the alkalinity (Fig. 3) and was above 99.7% for the remainder of the trial.

Although the H₂S loading and removal rates achieved during this trial were lower than has been previously reported in other bioreactor studies, to our knowledge all other studies have been performed at higher temperatures. The advantage of operating the HFBRs at 10°C in temperate environments such as Northern Europe is that there is no need to actively heat the reactors, which reduces energy costs. The critical loading rate (defined as the maximum load at which 95% removal occurs) was not determined in this trial following the amendment to the SWW during Phase IV and it is possible that higher removal rates may have been possible if the H₂S loading rate was increased.

*Insert Figure 2 here.*

*Insert Table 3 here.*
Depth-resolved gas and liquid phase analyses of the HFBRs showed a close relationship between H₂S removal from the gas phase and accumulating SO₄ in the liquid phase, accompanied by a reducing pH (Fig. 3). During Phases I and II, most H₂S removal occurred by Sheet 30 (Fig. 3). Increased H₂S loading rates during Phases III-V resulted in higher concentrations of remaining H₂S at lower sheets (sheets 30-60). Increased exposure of biofilm in the lower zones of the HFBR to H₂S resulted in the development of H₂S-oxidising activity. The H₂S removal rate in specific zones in the HFBRs, which was based on the total TPSA of all of the sheets in respective zone, was up to 0.21 g [H₂S] m⁻² [sheet area] h⁻¹, depending on loading rate and location in the reactor. The top zones in the HFBRs showed highest H₂S oxidation rates throughout the trial although in the latter phases of the trial the activity of the lower zones increased (Fig. 4) and contributed to overall H₂S oxidation in the systems. In a previous biotrickling filter study by Dhusa, Sambi [50] it was shown that at high concentrations of H₂S, the diffusional transfer rate of H₂S from gas to biofilm was high, resulting in high biological removal rates; concomitantly at lower gaseous H₂S concentrations the diffusional transfer and biological removal rates were lower. Similarly, in this study, highest H₂S removal rates were observed in locations with highest concentrations of H₂S.

Concentrations of sulfate in the liquid phase increased with depth in each of the HFBRs in each of the five phases (Fig. 3) and accumulation in the HFBRs coincided with the oxidation of H₂S. Higher concentrations of sulfate were produced in the latter phases with increased H₂S loading rates. In most cases a reduction in sulfate production was observed after Sheet 30, due to lower rates of H₂S oxidation. During Phases IV and V, sulfate concentrations were in the range of 2 – 3 g [SO₄²⁻] l⁻¹. Sulfate accumulation can also impair performance directly by inhibiting sulfide oxidisers in the biofilm [34] and this has previously been observed to occur in the range of 2-15 g [SO₄²⁻] l⁻¹, depending on loading regime, bioreactor configuration and operational pH [2, 34, 51]. Long-term inhibition was not apparent in R1-R3 as the performance recovered during Phase IV when pH was increased by NaHCO₃ buffering, despite high sulfate concentrations in the liquid phase. This may also be related to the flow of fresh SWW through the reactor (6 l d⁻¹), which limited the sulfate accumulation by flushing from the system in the liquid effluent. Indeed, it was shown by Duan, Koe [32] that periodic media washing can be employed to flush toxic sulphur compounds from the liquid phase. Sulfate in the liquid effluent can then be precipitated out using CaCO₃ or CaO to form
CaSO₄, to be reused for reclamation of, for example, alkali soils, rather than being recirculated and eventually converted back to H₂S in a wastewater treatment plant [34].

_H₂S conversion to sulfate_

The conversion ratio of H₂S to sulfate was calculated based on influent and effluent measurements of H₂S and sulfate (Fig. 5). Measurements were recorded several times weekly over the duration of the trial. H₂S removal correlated with effluent sulfate (correlation coefficient, 0.92), but there was a large proportion of unaccounted sulphur representing an average of 0.89 g [S] d⁻¹ (33% of influent sulphur). The disparity was likely due the production of sulphur forms other than sulfate, such as elemental sulphur and sulfide, which were not measured in this study. Large deposits of elemental sulphur were observed throughout the HFBRs when biofilm samples were collected on the last day of the trial.

The conversion of H₂S to sulfate in this trial (67%) was similar to the rates (60%) reported by Jin, Veiga [34], while Kim, Rene [12] reported conversion rates of 38%. Other studies have reported much higher conversion rates (100%) dependent on the pH and oxygen availability [19]. As the H₂S loading rate was increased during the trial and the H₂S was removed from the gas phase, the effluent sulfate concentration also increased (Fig. 2). This was evident at each of the loading rates, with the exception of Phase II when effluent sulfate concentrations were similar to those measured during Phase I. Sulfate is considered a desirable end-product with respect to environmental sustainability as it can be easily precipitated from the liquid.
stream, while the excessive production of alternative end-products, such as elemental sulphur, can lead to problems including reactor clogging [52].

**Bacterial community composition of the HFBRs**

Total clone library coverage was 83%, while overall bacterial diversity was low. The library was dominated by two clones from the class Gammaproteobacteria, which were related to the *Acidithiobacillus* and *Thiobacillus*, and which constituted 34% and 23% of the library, respectively (Fig. 6). *Acidithiobacillus* is associated with very acidic environments and has been detected in hydrogen sulfide-rich caves where the organism grows in biofilms at pH 0-1 [53]. *Acidithiobacillus* spp. are well known H2S oxidisers and pure cultures have been used to inoculate H2S-removing bioreactors [51, 54, 55]. In other systems, where mixed microbial consortia were used as seed biomass, *Acidithiobacillus* has been identified as the predominant species, including in an acidic biotrickling filter [56] and an acidic biofilter [54]. *Thiobacillus* spp. have also been identified as important H2S-oxidisers in biological systems and some species, including *Thiobacillus denitrificans* [27] and *Thiobacillus thioparus* [2], have been used as immobilised cultures for H2S removal. Critically, both *Acidithiobacillus* and *Thiobacillus* are capable of H2S oxidation under low pH conditions as found in the HFBRs, and they appeared to be important members of the bacterial community.

Insert Figure 6 here.

**Biomass distribution and bacterial gene copy concentrations in HFBR**

Biomass concentrations varied between zones in the HFBRs with zones 5-7 having the lowest density of biofilm in each HFBR (Fig. 7). Bacterial gene copy concentrations were highest in the top three zones of each of the HFBRs (Fig. 7). Z4 (sheets 21-30) had the lowest density of bacterial gene copies of any zone across the three HFBRs. After the step-feed (sheet 30), the gene concentrations in Z5-Z7 increased, although the levels detected were always lower than in zones Z1-Z3. The addition of SWW to the HFBRs at the step-feed may have provided additional nutrients, which had already been consumed in the upper zones of the HFBRs, or it may have diluted inhibitory compounds present in the liquid stream, thus stimulating
bacterial growth. The increase in bacterial gene copies indicates the benefit of providing a step-feed in the HFBRs. Based on Pearson’s coefficient there was no correlation between bacterial 16S rRNA gene copies and weight of biomass (p=0.38); however, there was a stronger correlation between 16S rRNA gene copies and DNA concentration (p=0.80). The lack of correlation between gene copies and biofilm mass may be due to the presence of dead cells, or extra cellular polymeric substances (EPS) which can accumulate in H2S oxidising biofilms [57]. Although the correlation between bacterial gene copies and DNA concentration is stronger, the number of 16S gene copies in a bacterial cell can vary between 1-15, [58], indicating that biomass weight is not an accurate proxy for bacterial gene copies.

*Insert Figure 7 here.*

**Environmental variables driving microbial diversity**

All zones (Z1-Z7) within a given HFBR were compared to determine the similarity of the bacterial communities and the effect of the environmental variables on community structure. Comparison of the environmental variables measured from the gas and liquid phases in the three HFBRs using permutational MANOVA (Euclidean distance) showed that, although the R1-R3 were operated in triplicate, the environmental conditions in the HFBRs were significantly different (p=0.004). However, a pair-wise test of the individual HFBRs showed that R2 and R3 were not significantly different (p=0.42), while R1 was different to R2 (p=0.01) and R3 (p=0.016). Variables measured included NO2, NO3, NH4, P, organic carbon, inorganic carbon, SO4, H2S concentrations, alkalinity and pH.

Based on the presence or absence of bands from the TGGE fingerprinting, and using permutational MANOVA (Bray-Curtis distance), the bacterial communities in the three HFBRs were observed to not be significantly different (p=0.247). Interestingly, even though the three HFBRs experienced different environmental conditions the same overall bacterial community was present in all three. However, there was some differences in the bacterial community structures observed with depth in the HFBRs (Fig. 8), indicating that spatial orientation in the reactors impacted on community assembly and development. Maestre, Rovira [59] also observed variation in community composition with depth in a biotrickling filter treating H2S. The bacterial community structure showed greater than 55% similarity
between zones, with some zones showing 100% similarity. The bacterial communities in the HFBRs were influenced by the environmental conditions in the reactors (Fig. 8). In each HFBR, Z1-3 showed highest similarity to each other and community structure was impacted by alkalinity and pH of the SWW. Similarly, the bacterial communities of Z4-Z7 shared similarity but were mainly impacted by sulfate and TOC. Other environmental variables had an impact on the bacterial community structure although none impacted across all three HFBRs. The high level of similarity between the three HFBRs may have been due to the extreme conditions, such as very low pH, high sulfate concentrations and limited organic carbon. These conditions provided an inhospitable environment that required a specialised consortium of bacteria resulting in a constricted community with limited diversity and dominated by *Acidithiobacillus* and *Thiobacillus* spp.

*Insert Figure 8 here.*

**Conclusions**

The HFBRs are a suitable technology for the treatment of H₂S-contaminated airstreams at 10°C, with removal rates of up to 15.1 g [H₂S] m⁻³ h⁻¹ and removal efficiencies of >99% achieved. Bio-oxidation of H₂S in the reactors led to acidification of the liquid phase and the production of a sulfate rich effluent. The addition of NaHCO₃ to the HFBRs resulted in improved H₂S removal at loading rates of 15.1 g [H₂S] m⁻³ h⁻¹. Bacterial diversity was low and the HFBRs were dominated by *Acidithiobacillus* and *Thiobacillus*. Differences in the microbial community structure between HFBR zones were influenced by alkalinity, pH and SO₄ concentrations, thus pointing to opportunities for microbial resource management and optimisation of HFBR performance.

**References**

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Table 1. Operational parameters of the laboratory-scale HFBRs over the five experimental phases.

<table>
<thead>
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<td>119</td>
<td>144</td>
<td>109</td>
</tr>
<tr>
<td>Empty Bed Residence Time (s)</td>
<td>265</td>
<td>112</td>
<td>60</td>
<td>51</td>
<td>40</td>
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<tr>
<td>Trial Duration (days)</td>
<td>35</td>
<td>48</td>
<td>43</td>
<td>37</td>
<td>15</td>
</tr>
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</table>
Table 2. Composition of synthetic wastewater, adapted from Odegaard and Rusten [37]

<table>
<thead>
<tr>
<th>Component</th>
<th>(g l⁻¹)</th>
<th>Component</th>
<th>(g l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaHCO₃ (Days 1-66)</td>
<td>1.2</td>
<td>MgSO₄.7H₂O</td>
<td>0.05</td>
</tr>
<tr>
<td>NaHCO₃ (Days 67-150)</td>
<td>2.5</td>
<td>FeSO₄.7H₂O</td>
<td>0.002</td>
</tr>
<tr>
<td>NaHCO₃ (Phase 151-178)</td>
<td>5</td>
<td>Urea</td>
<td>0.03</td>
</tr>
<tr>
<td>CaCl₂.6H₂O</td>
<td>0.003</td>
<td>MnSO₄.H₂O</td>
<td>0.002</td>
</tr>
<tr>
<td>Na₂HPO₄.12H₂O</td>
<td>0.1</td>
<td>NH₄Cl</td>
<td>0.06</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.025</td>
<td>KHCO₃</td>
<td>0.05</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.025</td>
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<td></td>
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</table>
Table 3. Average H₂S loading rate, concentration and H₂S removal efficiencies over the five experimental periods (PI-PIV). Standard deviations shown in parentheses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PI</th>
<th>PII</th>
<th>PIII</th>
<th>PIV</th>
<th>PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂S Loading Rate (g m⁻³ h⁻¹)</td>
<td>3.0</td>
<td>5.6</td>
<td>10.5</td>
<td>15.1</td>
<td>14.5</td>
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<tr>
<td>Influent Concentration (ppmv)</td>
<td>150</td>
<td>118</td>
<td>119</td>
<td>144</td>
<td>109</td>
</tr>
<tr>
<td>H₂S Removal (%) R1</td>
<td>99.1</td>
<td>97.8</td>
<td>99.6</td>
<td>95.3</td>
<td>99.7</td>
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<td></td>
<td>(2.4)</td>
<td>(7.3)</td>
<td>(1.9)</td>
<td>(7.0)</td>
<td>(0.4)</td>
</tr>
<tr>
<td>H₂S Removal (%) R2</td>
<td>98.8</td>
<td>99.4</td>
<td>99.9</td>
<td>98.1</td>
<td>99.8</td>
</tr>
<tr>
<td></td>
<td>(3.5)</td>
<td>(2.5)</td>
<td>(0.3)</td>
<td>(5.5)</td>
<td>(0.4)</td>
</tr>
<tr>
<td>H₂S Removal (%) R3</td>
<td>99.9</td>
<td>98.8</td>
<td>99.8</td>
<td>97.7</td>
<td>99.7</td>
</tr>
<tr>
<td></td>
<td>(0.4)</td>
<td>(3.9)</td>
<td>(1.1)</td>
<td>(5.8)</td>
<td>(0.4)</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1. Schematic of HFBR configuration and flow regime of the gas and liquid streams.

Figure 2. (a) H$_2$S loading rate (represented by solid line) and removal efficiency; (b) alkalinity concentration (represented by solid line) in influent synthetic wastewater (SWW) and pH of effluent; (c) effluent SO$_4$ concentration from the three HFBRs during each of the experimental phases, I-V. R1, R2 and R3 depicted by open circles, closed squares and closed circles, respectively.

Figure 3. (a) Average gas phase concentrations of H$_2$S in R1 during Phases I-V at each of the seven depth-resolved sampling ports; (b) Average pH of liquid phase in R1 during Phases I-V at each of the seven depth-resolved sampling ports; (c) Average liquid phase concentrations of SO$_4^{2-}$ in R1 during Phases I-V at each of the seven depth-resolved sampling ports. For clarity, only data from R1, which was typical of all 3 HFBR units, are shown.

Figure 4. Average in situ H$_2$S oxidation rates of the seven oxidation zones (Z1-Z7) from across the three HFBRs during Phases I-V.

Figure 5. Sulfur mass balance of three HFBRs based on influent and effluent measurements of H$_2$S and sulfate.

Figure 6. Neighbour-joining phylogenetic tree of the 16SrRNA gene sequences from the bacterial clone library. Reference sequences were downloaded from GenBank and their accession numbers are given. Numbers at the branch nodes are bootstrap values derived from 1,000 replicates. The open star represents the sequence SGH2S27 and the solid star represents sequence SGH2S14, which make up 23% and 34% of the clone library, respectively.

Figure 7. Quantification of (a) bacterial 16S rRNA genes, and (b) biomass, from different zones in R1, R2 and R3 on the last day of the trial.

Figure 8. Non-metric multidimensional scaling (MDS) plots of bacterial community structure showing correlation with environmental variables in (a) R1, (b) R2, (c) R3 and (d) in the three HFBRs. Direction and length of arrows indicate the correlations of the respective environmental variables. Only environmental variables with correlation of >0.5 are shown.