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Hydrogenotrophic Sulfate Reduction in a Gas-Lift Bioreactor Operated at 9°C

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The viability of low-temperature sulfate reduction with hydrogen as electron donor was studied with a bench-scale gas-lift bioreactor (GLB) operated at 9°C. Prior to the GLB experiment, the temperature range of sulfate reduction of the inoculum was assayed. The results of the temperature gradient assay indicated that the inoculum was a psychrotolerant mesophilic enrichment culture that had an optimal temperature for sulfate reduction of 31°C, and minimum and maximum temperatures of 7°C and 41°C, respectively. In the GLB experiment at 9°C, a sulfate reduction rate of 500–600 mg l^{-1} d^{-1}, corresponding to a specific activity of 173 mg SO_4^{2-} g VSS^{-1} d^{-1}, was obtained. The electron flow from the consumed H_2-gas to sulfate reduction varied between 27% and 52%, whereas the electron flow to acetate production decreased steadily from 15% to 5%. No methane was produced. Acetate was produced from CO_2 and H_2 by homoacetogenic bacteria. Acetate supported the growth of some heterotrophic sulfate-reducing bacteria. The sulfate reduction rate in the GLB was limited by the slow biomass growth rate at 9°C and low biomass retention in the reactor. Nevertheless, this study demonstrated the potential sulfate reduction rate of psychrotolerant sulfate-reducing mesophiles at suboptimal temperature.

Keywords: Sulfate reduction, low temperature, gas-lift bioreactor, hydrogenotroph, homoacetogenesis

Sulfate-reducing bacteria (SRB) can be utilized to treat acidic wastewaters that contain high metal and sulfate concentrations [13] and to recover valuable metals from wastewaters [10]. For a full-scale sulfidogenic treatment plant, the electron donor is the largest individual operational cost [3]. At present, one of the most feasible electron donors for sulfate reduction is hydrogen or synthesis gas [7, 8], but alcohols [14] and fermentation industry wastewater [3] are also used. A promising option is methane, which would halve the operational costs related to the electron donor, thus making biological sulfate reduction a lower-cost option than gypsum precipitation [3]. However, the slow growth rate of anaerobic methane oxidizers that couple methane oxidation to sulfate reduction prevents their practical application [18].

Another factor in the operational costs is the heating of a bioreactor and waste stream to the optimal temperature of the SRB. Consequently, treatment of cold wastewater streams containing metals and sulfate without heating would advance the treatment of acid mine drainage (AMD). Low-temperature sulfate reduction has potential for large-scale applications, but currently there is a paucity of information on suitable electron donors and the sulfate reduction rates that can be obtained at suboptimal temperatures. Moreover, the economics of sulfate reduction at low temperatures have not yet been addressed. The choice of electron donor and the effective biomass retention have been shown to be critical for low-temperature sulfate reduction [1, 20]. Ethanol was found to be a suitable electron donor at 8°C, but acetate produced from ethanol oxidation was not further utilized by the inoculum [20]. Therefore, we chose to use hydrogen-based substrates (H_2, formate) as electron donors for low-temperature sulfate reduction. A previous study by our group [1] showed that a low-temperature (9°C) sulfate-reducing fluidized-bed bioreactor fed with formate was capable of long-term stable treatment of AMD, but the sulfate reduction rate remained low compared with the rates reported for reactors operated at 30–35°C (Table 1).

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Table 1. Sulfate reduction rates and specific sulfidogenic activities reported for gas-lift bioreactors (GLB) including earlier fluidized-bed bioreactor studies [1, 20] with the inoculum used in the present study.

Reactor type	Electron donor	T (°C)	SRR (g SO_4^{2-} l^{-1} d^{-1})	Specific sulfidogenic activity (mg SO_4^{2-} g VSS^{-1} d^{-1})	Reference
GLB	H_2	30	30	nr	[7]
GLB	Synthesis gas	30	10	nr	[8]
GLB[a]	H_2	30	4.9	38000	[2]
GLB[b]	Synthesis gas	30–35	14.9	nr	[9]
Fluidized-bed[c]	Ethanol, acetate	8	0.3	48[d]	[20]
Fluidized-bed[c]	Formate	9	0.8–1.4	230	[1]
GLB	H_2	9	0.5	173	Present study

[a]At pH 5; [b]Full-scale reactor; [c]Experiments made with the same inoculum as the present study; [d]Calculated based on the VS measured for reactor carrier material; nr=not reported.

As formate is not an industrially feasible electron donor, H_2 remains as the electron donor of choice. To date, the use of H_2 has not been reported for low-temperature sulfate reduction. When sulfate-reducing gas-lift bioreactors were fed with H_2/CO_2 at 30°C, methane and acetate production also occurred [24]. Therefore, it was also necessary to study acetogenesis in the H_2/CO_2-fed sulfate-reducing bioreactor operated at low temperature.

Gas-lift bioreactors (GLB) fed with H_2/CO_2 have been successfully used in sulfate reduction with mesophilic microorganisms at neutral [7] and low pH [2]. The GLB concept and the use of hydrogen as electron donor have already been proven to be industrially applicable [7–9], whereas the feasibility of low-temperature sulfate reduction has not been demonstrated. Therefore, the aim of the present study was to study the viability of low-temperature sulfate reduction in a bench-scale H_2/CO_2-fed GLB at 9°C. Moreover, the temperature range of sulfate reduction of the low-temperature sulfate-reducing enrichment used in the present study [1] was assessed with a temperature gradient assay. Earlier lab-scale studies with this same low-temperature sulfate-reducing enrichment culture [1, 20] focused on its microbiology, biomass growth, and potential for AMD treatment.

Materials and Methods

Temperature Gradient Assay

To assess the temperature range of sulfate reduction by the inoculum originating from a 9°C fluidized-bed bioreactor [1], a temperature gradient assay was performed. A temperature gradient incubator (Terratec Asia Pacific Pty Ltd, Tasmania, Australia) was used. The 25-ml anaerobic batch tubes contained 15 ml of inoculum and 10 ml of modified Postgate medium with a 1:1 ratio of sulfate and formic acid. The modified Postgate medium (pH 7–7.5) contained $Na_2SO_4 \cdot 10H_2O$ 7 g/l, $MgSO_4 \cdot 7H_2O$ 18.5 g/l, NH_4Cl 0.1 g/l, KH_2PO_4 0.06 g/l, Resazurin 0.5 mg/l, yeast extract 0.2 g/l, ascorbic acid 0.1 g/l, sodium thioglycolate 0.1 g/l, and formic acid 5.8 g/l. The studied temperature range was 5–42°C. The tubes were analyzed for pH (Hamilton Slimtrode pH electrode and WTW pH 3301i pH meter), dissolved sulfide (DS), and biomass-bound nitrogen for 3 days.

Design of the GLB Experiment

The GLB temperature was maintained at 9°C and the pH was kept at 7.5 with a pH controller throughout the experiment. The GLB was operated in batch mode for the first 3 days, after which the hydraulic retention time (HRT) of 5 days was applied (days 3–11). The HRT was then decreased to 2 d (days 12–22) and then 1 d (days 23–78) (Fig. 3D). The sulfate loading rate was 0.58 g l^{-1} d^{-1} on days 3–11, 1.5 g l^{-1} d^{-1} on days 12–22, 3 g l^{-1} d^{-1} on days 23–68, and 1.1 g l^{-1} d^{-1} on days 69–78. The gas feed to the reactor was increased as the liquid HRT was decreased. The gas mixture fed to the GLB contained 90% H_2 (flow rate 17–22 l/d) and 10% CO_2 (flow rate 0.4–2.5 l/d). No external sulfide stripping was applied to the gas recycled back to the GLB.

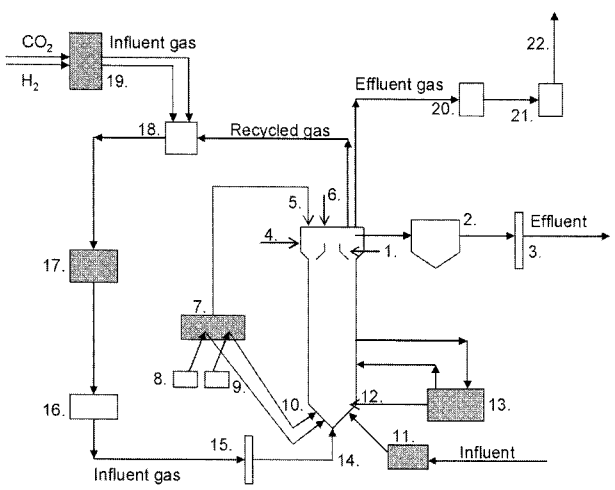
Growth Medium and Inoculum

The growth medium contained Na_2SO_4 0.7 g/l, $MgSO_4 \cdot 7H_2O$ 6.5 g/l, NH_4Cl 0.1 g/l, and KH_2PO_4 0.06 g/l in tap water. The start-up medium used for the first 5 days also contained 0.4 g/l $CH_3COONa \cdot 3H_2O$ to support biomass growth. The reactor was inoculated with 1 l of low-temperature sulfidogenic enrichment culture originating from a formic-acid-fed fluidized-bed bioreactor [1]. Pumice particles (Ø 0.2–0.5 mm, density ca. 2,440 kg/m^3; Aquavolcano, Aquatech, Papendrecht, The Netherlands) similar to the ones used in a previous study with a H_2/CO_2-fed bioreactor [8] were used as the carrier material to support biomass growth.

Reactor Configuration

The gas-lift bioreactor configuration was as shown in Fig. 1, and was as described in detail in Bijmans *et al.* [2]. The apparatus consisted of a double-walled glass reactor (active volume 4 l) equipped with an internal three-phase separator and an external settler unit. The GLB temperature was controlled by circulating cooling liquid inside the GLB double wall from a water bath (Julabo HE equipped with F25 cooling element; Seelbach, Germany). The pH was controlled with an Endress+Hauser LiquisysP control unit (Endress+Hauser Holdings A.G., Reinach, Germany) that was connected to a Schott sulfide resistant H63 electrode (Schott A.G., Mainz, Germany). The redox (oxidation or reduction potential, ORP) was measured with a QIS electrode (Type O14/NS/12x250/DJ/KNO_3/1M/BNC) connected to a radiometer read-out unit (Type PHM210, Meterlab; Radiometer Analytical SAS, Lyon, France).

H_2 and CO_2 were fed to the GLB with a KNF-Verder N840.3FT.18 gas membrane pump (KNF Neuberger Inc., Freiburg, Germany) *via* a Teflon sparger (hole diameter 0.4 mm, 168 holes). The recycle gas



1. Three-phase separator
2. Cooled settler
3. Water lock
4. Liquid level sensor
5. pH Electrode
6. Redox electrode
7. pH controller
8. Acid for pH control
9. Base for pH control
10. Acid/Base from pH controller
11. Influent pump
12. Temperature sensor
13. Temperature control
14. Influent gas feeding
15. Gas flow control
16. H_2S stripping unit
17. Vacuum pump
18. Gas mixing vessel
19. Gas feeding control
20. H_2S removal from effluent gas
21. Effluent gas flow measurement
22. Effluent gas composition measurement

Fig. 1. Configuration of the gas-lift bioreactor used in this study.

flow was adjusted to 4 l/min using a Sho-Rate gas flow meter (Type R2-15-C; Brooks Instruments, Veenendaal, The Netherlands). Gas dosing was controlled with a Brooks thermal mass flow controller (type 5850E), connected to a Brooks control unit, both from Brooks Instruments (Veenendaal, The Netherlands). The maximum gas flow was 30 l/min for H_2 and 7.5 l/min for CO_2. The growth medium was pumped to the GLB with a Stepdos 08RC liquid membrane pump (FEM 08TT.18RC; KNF-Verder, Freiburg, Germany). All tubings were made of PTFE (Schott A.G., Mainz, Germany) and the connection parts were from Serto A.G. (Fuldabrück, Germany).

Physicochemical Analysis

The composition of the GLB effluent gas was measured with gas chromatography, where H_2, N_2, CO_2, and CH_4 were analyzed with an online gas analyzer (Advance Optima; ABB Automation Product GmbH, Frankfurt am Main, Germany). Sulfate was analyzed with ion chromatography as described in Sipma *et al.* [22]. Volatile fatty acids were analyzed on a Hewlett Packard Series II GC according to Weijma *et al.* [23]. Dissolved sulfide (DS) was analyzed using a Dr. Lange sulfide kit LCK-653 and biomass-bound nitrogen with a Dr. Lange kit LCK-238. For the Dr. Lange analyses, a Xion 500 spectrophotometer was used (Hach Lange GMBH, Düsseldorf, Germany). The total suspended solids (TSS) and volatile suspended solids (VSS) were determined by filtering known amounts of GLB liquid through a glass fiber filter (Whatman GF/A, Kent, U.K.). For TSS analysis, filters were dried for 1–2 h at 105°C and then for 1 additional hour at 550°C for the VSS analysis [14]. The total solids (TS) and volatile solids (VS) of the GLB carrier material were determined to estimate the amount of biomass bound to the carrier material. The analysis was performed according to Finnish Standard SFS 3008 [21]. The VS and TS values were measured from day 22 onwards, following installation of a sampling tube for carrier material sampling.

Data Analysis

The sulfide production and biomass growth in the temperature gradient assay were fitted to the Ratkowsky equation [19] with Matlab 8 (The Mathworks Inc., Natic, MA, U.S.A.) as described by Franzmann *et al.* [6]. The specific sulfate reduction activity per gram of biomass was calculated as described in Bijmans *et al.* [2]. The proportions of HS^- and H_2S of the DS at the reactor pH were calculated using sulfide pK_a 7.28 at 9°C [15]. The biomass concentration was estimated from the biomass-bound nitrogen results using the general formula $CH_{1.8}O_{0.5}N_{0.2}$ for biomass [4].

RESULTS

Temperature Gradient Assay

The temperature gradient assay was performed to assess the cardinal temperatures of the sulfidogenic enrichment culture [1]. The cardinal temperatures for sulfide production were T_{opt} 30.9 (±0.2) °C, T_{min} 7.2 (±1.4) °C, and T_{max} 40.9 (±0.3) °C (Fig. 2A). For the biomass growth fitted to the Ratkowsky equation, the cardinal temperatures were T_{opt} 27.0 (±0.2) °C, T_{min} 3.0 (±2.3) °C, and T_{max} 41.7 (±3.1) °C (Fig. 2B).

Reactor Performance

The sulfate reduction rate (SRR) increased to 500–600 mg l^{-1} d^{-1} during the first 32 days of GLB operation (Fig. 3A, Table 2). During the reactor experiment, the specific sulfidogenic activity varied from 58 to 173 mg SO_4^{2-} g VSS^{-1} d^{-1} (Table 2). The electron flow to sulfate reduction increased from 5% to 52% during the experiment (Fig. 3B, Table 2). The low

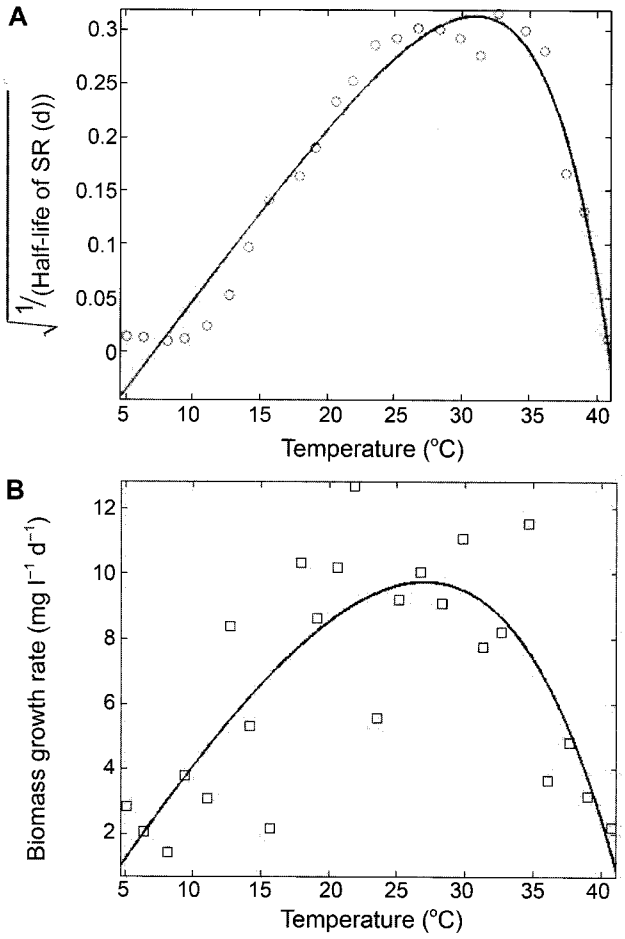


Fig. 2. The results of the temperature gradient assay fitted to the Ratkowsky equation.
A. The half-life of sulfate reduction (SR) calculated from sulfide production. **B.** Biomass growth rate.

percent electron flow from added H_2 to sulfate reduction resulted from the excess of H_2 gas feeding to the GLB that was necessary for the GLB operation and measurement of the gas consumption. Therefore, part of the added H_2 left the GLB unused. The SRR, specific sulfidogenic activities, and proportions of the electron flow at a HRT of 1 day were as presented in Table 2. The drops of the SRR were due to malfunctioning of the equipment (*e.g.*, pH control on days 33–35 and breakage of the sampling port on day 53), and also biomass wash-out.

The suspended biomass (VSS) and TSS present during the start-up phase were flushed out of the GLB when an HRT of 1 day was applied from day 23 onwards.

The TSS increased slightly after day 23, whereas the VSS increased slowly towards the end of the experiment (Fig. 3C). The carrier bound biomass (VS) remained at a constant level of 50 mg/g carrier material (Fig. 3C), constituting 98% of the total GLB biomass. The high shear forces in the GLB caused breakdown of the pumice, and the loss of the carrier material affected the biomass retention. The biomass accumulation in the GLB was close to zero or negative from day 30 onwards (data not shown), indicating loss of biomass. The breakdown of the pumice during the experiment was seen as increased TSS whereas VSS remained low, and filling-up of the external settler with crushed pumice particles.

The acetate concentration was highest at an HRT of 5 days, and reached a concentration of 560–720 mg/l. The acetate concentration in the GLB decreased simultaneously with the HRT (Fig. 3D) and as the VSS were flushed out of the GLB. The fraction of the electrons flowing to acetate production steadily decreased from 15% to 5% during the experiment (Fig. 3B). Methane was not produced. The ratio of electrons directed to sulfate reduction versus acetate production increased from 1.2 to 5.5 during the experiment, so the acetate production in the GLB ceased and the SRB competed more effectively for the hydrogen (Table 2).

The DS concentration of the GLB liquid increased to 73–103 mg/l and remained at this level. The concentration of H_2S (pH 7.5, 9°C) of the total DS was 32 mg/l (Fig. 3E). The liquid DS concentration was less than the total sulfide production, as part of the sulfide was continuously stripped from the liquid to the gas phase and left the GLB with the effluent gas. The redox in the GLB varied from −480 to −

Table 2. The sulfate reduction rate, specific activity, electron flow to sulfate reduction, and acetate production (of the consumed H_2) in a gas-lift bioreactor (GLB) at hydraulic retention time of 1 day.

Day no.	Sulfate reduction rate (g l^{-1} d^{-1})	Specific activity (mg SO_4^{2-} g VSS^{-1} d^{-1})	Electron flow to sulfate reduction (%)	Electron flow to acetate production (%)	Unused electrons[a] (%)	Electrons used for SR vs. Ac
23	0.3	86	10.6	3.3	86.1	3.2
31	0.5	144	31.5	12.0	56.6	2.6
39	0.2	77	12.1	9.8	78.1	1.2
45	0.2	58	8.7	4.7	86.6	1.9
58	0.5	154	44.2	8.2	47.6	5.4
73	0.6	173	52.2	5.8	47.8	9
78	0.3	86	27.5	5.0	71.5	5.5

[a]Unused hydrogen leaving the GLB. SR=sulfate reduction; Ac=acetate production.
The ratio of electrons consumed in sulfate reduction versus acetate production is also presented; this value indicates how much more electrons are directed to sulfate reduction than to acetate production.

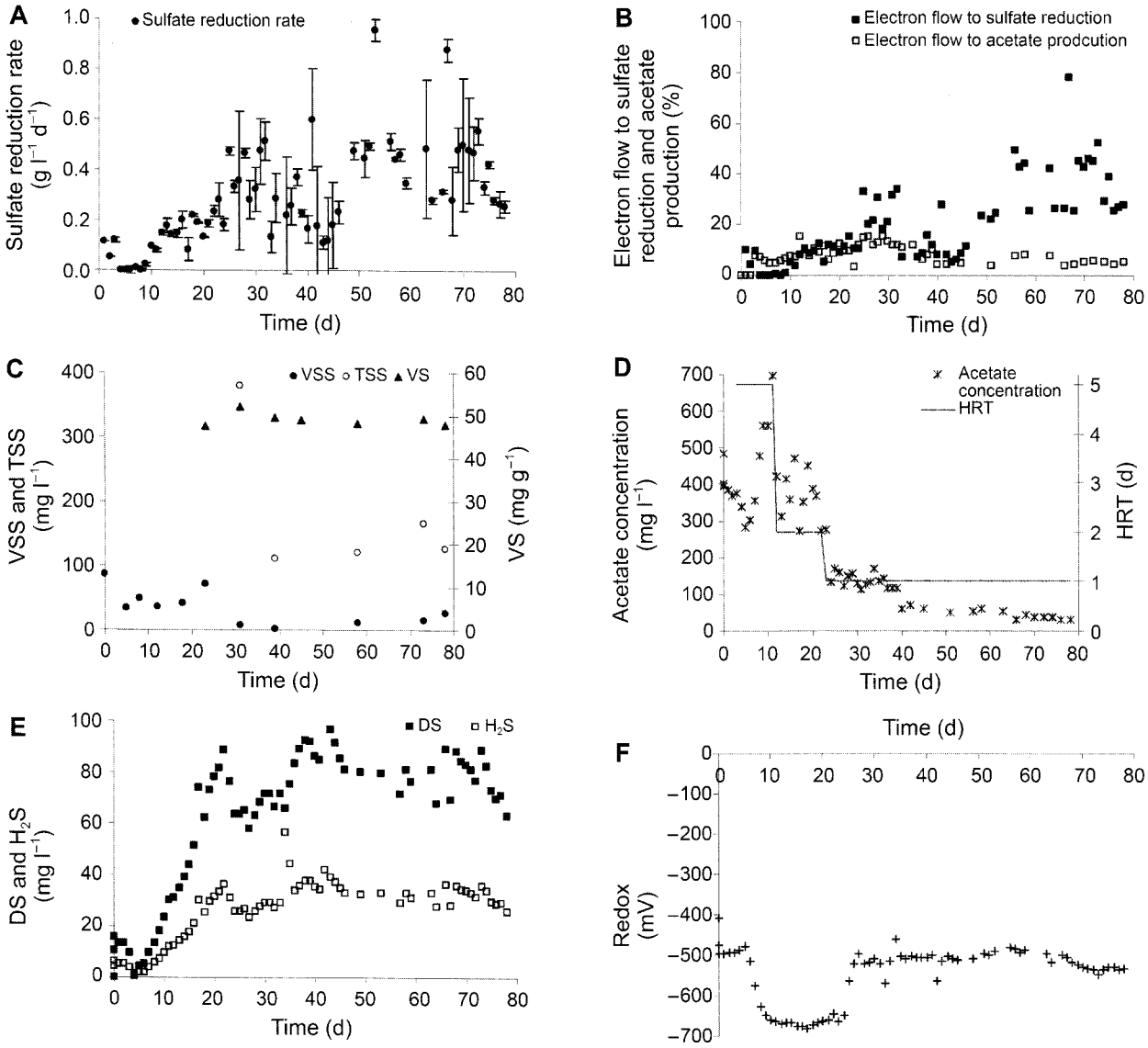


Fig. 3. The results of the H_2/CO_2-fed gas-lift bioreactor (GLB) run at 9°C.
(A) The sulfate reduction rate (SRR) (●), **(B)** percentage electron flow to sulfate reduction (■) and acetate production (□), **(C)** volatile suspended solids (VSS ●) and total suspended solids (TSS ○) in the GLB liquid and VS (▲) of the GLB carrier material, **(D)** the acetate concentration (*) versus hydraulic retention time (HRT —), **(E)** dissolved sulfide (DS ■) and H_2S (□) concentrations, and **(F)** the redox (+) are presented. The error bars in **A** indicate the standard deviation of the SRR measurements.

680 mV, being the lowest when the HRT was 2 days. When operating the GLB at an HRT of 1 day, the redox stabilized at around −500 mV (Fig. 3F).

DISCUSSION

Temperature Dependence of Sulfate Reduction

The optimal temperatures of the enrichment culture used in this study were 31°C for sulfate reduction and 27°C for biomass growth. These data together with Fig. 2A–2B demonstrate that the enrichment culture consisted of psychrotolerant mesophilic bacteria. The optimal temperature for the activity and biomass growth was slightly different as biomass measures the growth of all bacteria in the enrichment culture and sulfide production measures the activity of only SRB. It has been shown that psychrophilic and psychrotolerant SRB have the highest sulfidogenic activity above their optimum growth temperature [16]. The psychrotolerant strain *Desulforhopalus vacuolatus* isolated from cold marine sediments [11, 12] had the highest sulfate reduction rate at 28°C, although the growth T_{opt} was 18°C and growth ranged between 0–24°C. A growth range of 0–35°C (T_{opt} 29–32°C) was reported for the mesophilic *Desulfobacter hydrogenophilus* isolated from marine sediment [25].

Reactor Performance

This study demonstrates the suitability of the H_2/CO_2-fed GLB for the direct treatment of AMD at temperatures as low as 9°C. Sulfate reduction rates at suboptimal temperatures for an industrially proven reactor system (GLB) [7–9] have not previously been reported. The SRR of the present study are compared with results reported in other studies in Table 1. The SRR and specific activity of the inoculum used in this study with formate [1] and ethanol [20] measured for fluidized-bed bioreactors were similar to those measured in the present study, showing the level of the sulfate reduction rates that can be obtained at 9°C with the psychrotolerant mesophilic enrichment culture. The sulfate reduction rates measured at 9°C are nevertheless significantly lower than the SRRs measured at 30°C using a similar reactor configuration.

The ratio of electrons used for sulfate reduction versus acetate production increased during the experiment. Thus, more electrons were directed to sulfate reduction towards the end of the experiment. The competiveness of homoacetogens over methanogens at non-H_2 limiting conditions at temperatures below 10°C has been reported for incubations of tundra soil samples [17]. A study of the competitiveness of homoacetogens and sulfate reducers for hydrogen in gas-lift reactors (pH 7, 30°C) showed that H_2 was consumed at a higher rate by homoacetogens than by methanogens, and that SRB did not outcompete the homoacetogens [24]. The authors suggested that the heterotrophic SRB depended on the homoacetogens to produce the acetate as a carbon source. The inoculum used in the present study was dominated by a *Desulfomicrobium* sp. [1] that requires acetate as carbon source. Therefore, in the present study, the acetate is the likely carbon source, rather than an electron donor for sulfate reduction. As the homoacetogens produce the carbon source, acetate addition to the GLB would not be necessary, which decreases the operational costs [24]. In a H_2-fed GLB (pH 7, 30°C), SRB outcompeted homoacetogens for hydrogen when excess sulfate was provided [5], and acetate production ceased independently of the applied sludge retention time, as was also observed in this study. In the present study at 9°C, no methane was produced.

Pumice has previously been used successfully in gas-lift reactors with gas flow rates twice as high as used in this study [8] and no carrier breakdown was reported. In the present study, pumice could not be used at HRTs lower than 1 day because of crushing. In low-temperature bioreactors, effective biomass retention becomes essential owing to the slow growth rate of the biomass. Metal precipitates facilitated the retention of the suspended biomass in an FBR [1], and this may also be achievable in the GLB.

As temperature decreases, the proportion of H_2S of the total DS increases owing to the increase of the H_2S pK_a [15], resulting in higher H_2S concentrations at 9°C than at 30°C with the same total DS concentration. Therefore, the toxic effects of H_2S arise at lower total DS concentrations. At decreased temperature, bacteria usually remain viable, regaining their activity as the temperature rises. This is opposite to the case for increased temperature, which may cause severe damage to the cells reducing their viability.

The temperature gradient assay showed that the optimum temperature of the enrichment culture used in this study was 31°C, demonstrating the psychrotolerant nature of the mesophilic enrichment culture. The results of the GLB experiment showed that the sulfate reduction rate by this psychrotolerant mesophilic SRB enrichment culture increases to 500–600 mg l^{-1} d^{-1} in a well-mixed GLB system fed with H_2 and CO_2. In this study, the low-temperature sulfate reduction at 9°C was limited by biomass retention in the GLB. The results of the present study and our previous study [1] indicated that the sulfate reduction rate at 9°C can be improved if the biomass is provided with a sufficient growth period. Alternatively, the biomass growth phase could be performed at elevated temperature, before use in low-temperature AMD treatment.

When bioreactors are operated at suboptimal temperatures, the low sulfate reduction rates result in extended treatment times. The SRR in such conditions could be improved *via* better biomass retention, a more versatile microbial community, or by operating the reactor at a slightly elevated temperature. The low SRR obtained sets the requirements for a low-temperature reactor operation as (1) longer reactor start-up phases, (2) larger reactor volumes, and (3) a long process recovery time in case of biomass loss. However, low-temperature reactors have lower operational costs owing to lack of heating, and savings in the heating costs alone could make low-temperature bioreactors economically feasible.

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