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Sulfate reduction in a hydrogen fed bioreactor operated at haloalkaline conditions

João A.B. Sousa [a,b,*], Caroline M. Plugge [a], Alfons J.M. Stams [a], Martijn F.M. Bijmans [b]

[a] Laboratory of Microbiology, Wageningen University, Dreijenplein 10, 6703 HB Wageningen, The Netherlands
[b] Wetsus, Centre of Excellence for Sustainable Water Technology, Agora 1, 8900 CC Leeuwarden, The Netherlands

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ABSTRACT

Biological sulfate reduction is used as a biotechnological process to treat sulfate rich streams. However, application of biological sulfate reduction at high pH and high salinity using H_2 was not thoroughly investigated before. In this work the sulfate reduction activity, biomass growth, microbial community and biomass aggregation were investigated in a H_2-fed gas lift bioreactor at haloalkaline conditions. The process was characterized by low sulfate reduction volumetric rates due to slow growth and lack of biomass aggregation. Apparently, the extreme conditions and absence of organic compounds prevented the formation of stable aggregates. The microbial community analysis revealed a low abundance of known haloalkaliphilic sulfate reducers and presence of a *Tindallia* sp. The identified archaea were related to *Methanobacterium alcaliphilum* and *Methanocalculus* sp. The biomass did not attach to metal sulfides, calcite and magnesite crystals. However, biofilm formation on the glass bioreactor walls showed that attachment to glass occurs.

1. Introduction

Dissimilatory reduction of sulfate to sulfide is an important process in the biogeochemical cycling of sulfur and carbon (Jørgensen, 1982). Sulfate reducing bacteria (SRB) can oxidize hydrogen gas and various organic compounds using sulfate as electron acceptor (Muyzer and Stams, 2008; Rabus et al., 2013). Biological sulfate reduction is also used as a biotechnological process for treatment of sulfate- and heavy metal-rich effluents, wastes and waste streams (Muyzer and Stams, 2008). At pH neutral conditions high specific sulfate reduction rates have been achieved (van Houten et al., 1995; Van Houten et al., 2006). Also application of biological sulfate reduction at extreme conditions like treatment of acidic mining effluents or high salinity waste streams have been previously investigated (Vallero et al., 2005; Bijmans et al., 2008).

The application of biological sulfate reduction to treat waste streams with both high pH (>8.5) and high salinity (>1 M Na^+) was shown previously in a combined denitrification and sulfate reduction bioreactor (Zhou et al., 2014). Application of solely biological sulfate reduction at haloalkaline conditions, however, was never thoroughly investigated before. These conditions are present in biodesulfurization systems where sulfate reduction may be applied to treat sulfate-rich bleed streams (Janssen et al., 2009). These extreme conditions are present in nature, only in soda lakes which are characterized by high Na carbonate/bicarbonate alkalinity resulting in stable

* *Corresponding author*. Wetsus, Centre of Excellence for Sustainable Water Technology, 1, Agora 1, 8934 CJ Leeuwarden, The Netherlands. Tel.: +31 (0) 582843000.
E-mail addresses: joao.sousa@wetsus.nl, joaoasousa@gmail.com (J.A.B. Sousa).
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Nomenclature

C_x	Biomass concentration (g l^{-1})
C_{tn}	Total Nitrogen concentration (mol l^{-1})
M_x	Biomass molecular weight (g mol^{-1})
r_v	Volumetric activity (mmol l_r^{-1} d^{-1})
Ø	Flow rate (l d^{-1})
C_s	Sulfate concentration (mmol l^{-1})
V_r	Bioreactor volume (l)
μ	Suspended biomass growth rate (d)

Subscript

x	Biomass
r	Reactor
in	Influent
out	Effluent
tn	Total nitrogen

high pH of the brines around 10 (Sorokin et al., 2011a). In these lakes, where pH can reach up to 11 and salinity up to 4 M Na^+, sulfate reduction was shown to occur (Sorokin et al., 2010). The haloalkaliphilic SRB isolated from these lakes are represented by the three genera of lithotrophs (*Desulfonatronovibrio*, *Desulfonatronum* and *Desulfonatronospira*) and several VFA-utilizing SRB (Pikuta et al., 1998; Sorokin et al., 2008a, b, 2011b).

Information on *in situ* sulfate reduction activities in these extreme environments and optimal growth conditions for haloalkaliphilic SRB in pure culture already exists. Yet, there are no available studies on continuously operated bioreactors where haloalkaliphilic biomass is active. Information about volumetric and specific sulfate reduction activities, aggregation of biomass and adaptation are essential to successfully apply biological sulfate reduction under extreme haloalkaline conditions in biotechnological systems.

We investigated the performance of a lab scale H_2-fed gas lift bioreactor at haloalkaline conditions that could be applied to treat sulfate-rich bleed streams. The sulfate reduction activity rate, growth rate, structure of the microbial community and biomass aggregation were investigated during the bioreactor operation. The influence of the wall biofilm formation on the sulfate reduction activity and sulfide toxicity was also investigated. As there is no dedicated biomass available, inocula from combined natural and man-made high pH and high salinity sources were used to enrich for haloalkaliphilic hydrogenotrophic SRB.

2. Methods

2.1. Bioreactor set-up

A 4.4 l glass gas lift reactor with an internal 3 phase separator was used (Fig. 1). A water jacket connected to a thermostat bath (DC10-P5/U, Haake, Germany) was used to maintain the bioreactor temperature at 35 °C. The liquid feeding was performed by a membrane pump (Stepdos 08 RC, KNF-Verder, the Netherlands). The gas supply was controlled using H_2 and CO_2 digital mass flow controllers (F-201CV-020-AGD-22-V and F-201CV-020-AGD-22-Z, Bronkhorst, the Netherlands). For the gas recirculation a vacuum pump (Laboport®, KNF, Trenton, NJ) was used and the gas flow measured using a flow meter (URM, Kobold, the Netherlands). A pH and an oxidation-reduction potential sensor (CPS11D and CPS12D, Endress + Hauser, the Netherlands) connected to a controller (Liquiline CM44x, Endress + Hauser, the Netherlands) were used to monitor the conditions inside the reactor. The pH was controlled at pH 9 by supplying CO_2 through the mass flow controller.

2.2. Inoculum

The bioreactor was inoculated with 50 ml of biomass that was collected from a sulfate and thiosulfate (1/1 mol ratio) reducing gas-lift bioreactor with 3 phase separator fed with H_2 and CO_2, with the same set-up as described in Fig. 1. This bioreactor was operated for 3 months with 150 mM (total S loading) and the biomass was collected and washed with anoxic carbonate/bicarbonate buffer (pH 9, 1.5 M Na^+). The original inoculum used for this bioreactor is a mix of sediments and sludge listed in table A.1 in supplementary data. From each sediment 20 g (wet weight) and from each sludge type 10 ml were added to start the reactor.

2.3. Medium

A mineral medium that was buffered at pH 9 (±0.05) with sodium carbonate and sodium bicarbonate and with a total of 1.5 M Na^+ was used. The medium composition was as follows: Na_2CO_3 (33.6 g l^{-1}), $NaHCO_3$ (69.3 g l^{-1}), $KHCO_3$ (1 g l^{-1}), K_2HPO_4 (1 g l^{-1}), NH_4Cl (0.27 g l^{-1}), $MgCl_2.6H_2O$ (0.1 g l^{-1}), $CaCl_2.2H_2O$ (0.01 g l^{-1}) and 10 ml l^{-1} of vitamin solution (Wolin et al., 1963). Two trace (TE) element solutions with the following composition were mixed together and 1 ml added: TE1 - sodium EDTA (1000 mg l^{-1}), $FeCl_2.4H_2O$ (370 mg l^{-1}), H_3BO_3 (60 mg l^{-1}), $MnCl_2.2H_20$ (26 mg l^{-1}), $CoCl_2.6H_2O$ (40 mg l^{-1}), $ZnCl_2$ (10 mg l^{-1}), $CuCl_2$ (3 mg l^{-1}), $KAl(SO_4)_2.12H_2O$ (32 mg l^{-1}), $NiCl_2.6H_2O$ (31 mg l^{-1}); TE2 – NaOH (40 mg l^{-1}), $Na_2SiO_3.5H_2O$ (10 mg l^{-1}), $Na_2MoO_4.2H_2O$ (10 mg l^{-1}), $Na_2SeO_3.5H_2O$ (10 mg l^{-1}), $Na_2WO_4.2H_2O$ (10 mg l^{-1}). As electron acceptor, 7.1 g (50 mM) of sodium sulfate ($NaSO_4$) was added.

2.4. Experimental design

The bioreactor was filled with medium and flushed with hydrogen gas over night with gas recirculation to lower the redox potential. The hydrogen gas supply was set to 5 ml min^{-1}, the gas recirculation to 2.5 l min^{-1} and the pH control set at pH 9. Inoculation of the reactor was defined as time 0. A batch run was performed to start-up the bioreactor and verify the biomass sulfate reduction activity (Start-up, Table 1). Three continuous experiments (Run 1, 2 3) with different hydraulic retention times (HRT) were performed as described in Table 1. Two batch experiments, biofilm run and sulfide toxicity run, were performed to investigate the contribution of the bioreactor wall biofilm to the sulfate reduction activity and the toxicity of high sulfide concentrations (Table 1). In the biofilm run, the suspended biomass was removed leaving only the biofilm in the bioreactor and fresh medium was added. In the sulfide toxicity run, the biofilm was removed and only suspended biomass was used in the same