Immobilization of sulfate and thiosulfate-reducing biomass on sand under haloalkaline conditions

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HIGHLIGHTS
- Sand as carrier material increased biomass retention and S volumetric conversion rates.
- Attachment of biomass to sand was weak and confined to cavities in the sand particles.
- The addition of sand led to acetate production by facilitating growth of acetogens.
- Produced acetate increases biomass growth, especially of the attached biomass.
- Formate had a central role in the bioreactor as electron donor for sulfate reduction.

GRAPHICAL ABSTRACT

ABSTRACT

Biological sulfate and thiosulfate reduction under haloalkaline conditions can be applied to treat waste streams from biodesulfurization systems. However, the lack of microbial aggregation under haloalkaline conditions limits the volumetric rates of sulfate and thiosulfate reducing bioreactors. As biomass retention in haloalkaline bioreactors has not been studied before, sand was chosen as a biomass carrier material to increase cell retention and consequently raise the volumetric rates. The results showed that ~10 fold higher biomass concentrations could be achieved with sand, compared to previous studies without carrier addition. The volumetric rates of sulfate/thiosulfate reduction increased approximately 4.5 times. Biomass attachment to the sand was restricted to cavities within the sand particles. Acetate produced by acetogenic bacteria from H2 and CO2 was used as carbon source for biomass growth, while formate that was also produced from H2 and CO2 enhanced sulfate reduction. The microbial community composition was analyzed by 16S rRNA gene amplicon sequencing, and Tindallia related bacteria were probably responsible for formate formation from hydrogen. The community attached to the sand particles was similar to the suspended fraction, but the relative abundance of sequences most closely related to Desulfohalobius cæus was much higher in the attached fraction compared to the suspended fraction (36% and...
1. Introduction

Sulfate and thiosulfate reduction are widespread microbiological processes that occur under anoxic conditions. These processes can be applied to treat sulfate and thiosulfate-rich waste streams (Qian et al., 2019; Liu et al., 2018; Muyzer and Stams, 2008). For example, gas biodesulfurization systems under haloalkaline conditions produce sulfate and thiosulfate rich waste streams with high salinity (≥0.5 M NaCl) and a pH value >8.5 (Janssen et al., 2009). In this case, haloalkaliphilic sulfate- and thiosulfate-reducing microorganisms that thrive under these extreme conditions are required to treat the waste streams. The microorganisms convert sulfate and thiosulfate to sulfide with a suitable, low cost electron donor such as hydrogen (Liu et al., 2018). The sulfide is then recycled back to the biodesulfurization reactor, where it serves as electron donor for the sulfide-oxidizing biomass. These classes of sulfate- and thiosulfate-reducing microbes can be found in natural environments such as soda lakes, where pH values up to 11 are commonly found and the salinity can reach saturation (Sorokin et al., 2011a). Sediments from soda lakes have been successfully used to inoculate a bioreactor to treat sulfate and thiosulfate-rich streams at haloalkaline conditions (Sousa et al., 2015b).

Even though sulfate and thiosulfate conversion to sulfide has been previously shown in a gas-lift bioreactor operated at haloalkaline conditions, the reported lack of biomass aggregation poses a challenge (Sousa et al., 2015a). Without biomass aggregation, the hydraulic retention time (HRT) determines the biomass concentration in these bioreactors. When applying an HRT of 2.5 days in a sulfate-reducing bioreactor, the sulfate reducing capacity was significantly decreased compared to higher HRTs. Similarly, in a thiosulfate reducing bioreactor operated with a HRT of 1.7 days, the thiosulfate reduction capacity also decreased (Sousa et al., 2017). This indicated that the system was limited by biomass growth. Therefore, retaining biomass in the bioreactor is a key factor to improve the sulfate and thiosulfate conversion capacity.

When biomass self-aggregation as flocks or granules does not occur, the use of carrier materials or membranes can improve the cell retention (Saxena et al., 2018). Haloalkaline environments have unique physical-chemical characteristics that may affect aggregation, such as reduced effect of electrostatic charges, difference in hydrophobicity of cell surfaces and low divalent ions concentration (Grant et al., 1990; Otto et al., 1999; van Loosdrecht et al., 1987). Sand and pumice were already used as carrier materials or membranes to improve the cell retention (Muyzer and Stams, 2008). Sand and pumice were previously used for sulfate-reducing bioreactors operated at neutral and acidic conditions and the biomass successfully attached to these silica based carrier materials (Christensen et al., 1996; van Houten et al., 1995). Biofilm growth was also observed on the glass wall of a sulfate-reducing bioreactor operated at haloalkaline conditions (Sousa et al., 2015a). Small batch studies with the haloalkaliphic sulfur oxidizing bacterium *Thiobacillus versutus* immobilized on magnetic nanoparticles revealed that the cells had the same sulfur oxidation capacity as free cells but could be reused at least eight times ( Xu et al., 2015; Mu et al., 2017). However, studies on sulfate or thiosulfate converting gas-lift bioreactors at haloalkaline conditions using carrier material are not reported. In addition, detailed information on microbial aggregation and attachment at haloalkaline conditions in natural environments is scarce (Sousa et al., 2015b). In the current study, sand was chosen as a carrier material for biomass retention in a H2 fed, sulfate- and thiosulfate-converting gas-lift bioreactor operated at haloalkaline conditions. The bioreactor performance and biomass retention were investigated along with investigating the attached and suspended microbial communities.
2.5. Experimental design

The bioreactor was filled with medium and flushed overnight with 10 ml min⁻¹ H₂ and a gas recirculation at 5 l min⁻¹ to lower the redox potential. During reactor operation, H₂ was first supplied at 5 ml min⁻¹, but was increased during the experiment to maintain an overpressure in the bioreactor (Fig. 1). Different influent and gas recirculation flows were tested and adjusted to prevent washout of sand particles with the effluent. Finally, the gas recirculation was set to approximately 7 l min⁻¹.

The operation was performed under non-sterile conditions. Inoculation of the reactor was defined as time zero. A batch run was performed to start-up the bioreactor and to confirm that the biomass was still capable of performing sulfate and thiosulfate conversion to sulfide (Table 1; start-up). The start-up batch run was continued until both sulfate and thiosulfate were below 10 mM. After the start-up phase, five continuous experiments were performed (Table 1; runs 1 to 5) as well as two experiments, where formate and acetate were spiked. Sodium formate (100 mM increase) and sodium acetate (100 mM increase) were directly injected into the bioreactor at the beginning of each test. The tests were performed in duplicate for formate and a single test for acetate.

One reactor was used throughout the experiments and the previous experiments performed in the same reactor, but without carrier material (Sousa et al., 2015a; Sousa et al., 2017) served as the control experiments for present work.

2.6. Analytical procedures

All samples were taken from the bottom of the completely mixed reactor as described previously (Sousa et al., 2015a, 2015b) Liquid samples for volatile fatty acids, sulfate, thiosulfate and sulfide analysis, along with gas samples were prepared and analyzed as described previously (Sousa et al., 2015a). For biomass measurements, the sand-attached biomass (45 ml) was separated from the suspended biomass by settling for 30 s and both fractions were transferred to a new 50 ml tube. One ml of sand was collected in an Eppendorf tube and the samples were washed three times with a carbonate/bicarbonate buffer with lower salinity (LS buffer; pH 9, 0.5 M Na⁺ instead of 1.5 M Na⁺). In the washing steps, sand was separated from the buffer by settling for 30 s. Two ml of the suspended biomass was centrifuged (10 min, 10,000 g) and was washed three times with LS buffer. Finally, the total nitrogen content was determined using a cuvette test (LC238, Hach Lange, Düsseldorf, Germany).

Biomass particle size for the suspended fraction was measured using laser measurement in a particle size and shape analyzer (Eyetech, Doner technologies, Or Akiva, Israel) with the Dipa 2000 software (Doner technologies, Or Akiva, Israel). Measurements were performed in triplicate for 120 s while stirring continuously. The morphology of the microorganisms was routinely examined using light microscopy (DMI6000B, Leica, Biberach, Germany). Biomass particle size for the suspended fraction was measured using a JEOL JSM-6480LV Scanning Electron Microscope (JEOL Benelux, Nieuw-Vennep, The Netherlands).

2.7. DNA extraction

Biomass samples were fixed in 2.5% (w/v) glutaraldehyde overnight at 4 °C. The fixed samples were separated and washed following the same procedure for the attached and suspended fractions described above for biomass measurement. The samples were then dehydrated for 20 min in a series of ethanol solutions (10%, 25%, 50%, 75%, 90%, and twice 100%) and dried in a desiccator overnight. The samples were coated with gold and analyzed in a JEOI JSM-6480LV Scanning Electron Microscope (JEOl Benelux, Nieuw-Vennep, The Netherlands).

2.8. DNA extraction

Suspended and sand attached biomass fractions (10 ml) were separated by settling for 30 s. The suspended fraction was centrifuged (10 min, 7500 g), and the supernatant of the sand-attached biomass

Table 1

<table>
<thead>
<tr>
<th>Operational characteristics of bioreactor runs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Start-up</td>
</tr>
<tr>
<td>Run 1</td>
</tr>
<tr>
<td>Run 2</td>
</tr>
<tr>
<td>Run 3</td>
</tr>
<tr>
<td>Run 4</td>
</tr>
<tr>
<td>Run 5</td>
</tr>
<tr>
<td>Formate spike</td>
</tr>
<tr>
<td>189–194</td>
</tr>
<tr>
<td>Acetate spike</td>
</tr>
</tbody>
</table>

¹ Not applicable.
² Total S includes sulfate and thiosulfate in mol of S.

Fig. 1. Activities in the bioreactor during all operational periods: (A) Sulfate loading and volumetric reduction rates; (B) Thiosulfate loading volumetric reduction rates; and (C) H₂ supply and consumption rates.
was removed after settling. Both fractions were then washed three times with buffer LS and finally re-suspended in 2 ml of buffer LS and stored at -80 °C. Total genomic DNA was extracted using the PowerBiofilm™ DNA Isolation Kit (MoBio, Carlsbad, CA, USA) following the manufacturer’s instructions.

2.9. Sequencing and phylogenetic analysis

For sequencing, suspended biomass samples from day 0 (inoculum), 53, 97, 113, 179, and 197 as well as biomass attached to sand samples from day 179 and 197 were used. A fragment of the V3-V5 region of 16S rRNA gene of bacteria was amplified using primers 341F and 805R (Herlemann et al., 2011). The PCR protocol was performed according to Hugenholtz et al. (2014) and amplicons were sequenced on the Illumina platform according to Lindh et al. (2015) at the Science for Life Laboratory, Sweden (www.scilifelab.se). The sequence data was processed with the UPARSE pipeline (Edgar, 2013) and annotated against the SINA/SILVA database (SILVA 119; Quast et al., 2013). Afterwards, the data was analyzed using Explicet 2.10.5 (Harris et al., 2013).

The 16S rRNA gene amplicon data are deposited at the NCBI database with Bioproject number PRJNA613432.

2.10. Calculations

Calculations were performed and followed the following assumptions:

1. Thermodynamic calculations were performed using eQuilibrator online tool (Flamholz et al., 2012) using the actual conditions in the bioreactor: pH 9, 1.5 M ionic strength, 0.825 M HCO₃⁻, and 1 atm H₂. For formate production from H₂ and HCO₃⁻, calculations were made based on Eq. (1). For sulfate reduction using H₂ or formate as electron donors, calculations were made based on Eqs. (2) and (3).

\[
 \begin{align*}
 H_2 + HCO_3^- &\rightarrow HCO_2^- + H_2O \\
 4H_2 + SO_4^{2-} + H^+ &\rightarrow HS^- + 4H_2O \\
 4HCO_2^- + SO_4^{2-} + H^+ &\rightarrow HS^- + 4HCO_3^- 
\end{align*}
\]

2. The bioreactor liquid volume was constant during the operation, which means that the liquid flow that goes into the bioreactor is equal to the liquid flow exiting the bioreactor.

3. Accumulation of sulfur compounds in the bioreactor, by incorporation in biomass and formation of sulfide precipitates, was not taken into account in the calculations as it plays a minor role due to the high sulfate and thiosulfate concentration in the influent.

4. S volumetric conversion rate was calculated by the sum of the volumetric conversion rates of sulfate and thiosulfate based on mol of S atoms.

5. The N molar fraction value of 0.2 was used to calculate biomass concentration based on total N, following the biomass molecular formula: C₁H₁.₈O₀.₅N₀.₂.

3. Results and discussion

3.1. Bioreactor performance

In this study, we tested if sand as a biomass carrier material in a H₂ fed sulfate and thiosulfate-converting bioreactor operated at haloalkaline conditions improves reactor performance.

The use of sand allowed the operation of the gas-lift bioreactor at an HRT of one day, which is similar to studies using anaerobic filters filled with sediments (Zhou and Xing, 2015). Previously described sulfate and thiosulfate-reducing bioreactors fed with H₂ at haloalkaline conditions did not contain aggregated biomass (Sousa et al., 2015a; Sousa et al., 2017). Consequently, the minimum HRT achieved without a decrease of biomass concentration and reduction activity was 3.3 days for the sulfate-reducing bioreactor and 1.7 d for the thiosulfate-converting bioreactor. Operation of the bioreactor with sand as carrier material at lower HRT led to higher S volumetric conversion rates, 85 ± 3 mmol l⁻¹ d⁻¹ during Run 5, compared with previous published results (Table 2).

Thiosulfate was completely converted to sulfide throughout the whole reactor operation, while 93 ± 2% of the sulfate was converted in the stable phase of Run 5 (Figs. 1 and 2). These sulfate conversion efficiencies were similar to other systems using different bioreactors and electron donors (Table 2). To exclude the sulfide toxicity effect as cause for incomplete sulfate reduction, in run 5 the loading of sulfate and thiosulfate was doubled. This resulted in higher concentrations of sulfide and higher sulfate and thiosulfate reduction rates, and the conversion efficiencies of sulfate and thiosulfate remained in the same range as in previous runs. Sulfide can enter cells of sulfate reducers and other bacteria rather easily as H₂S at pH 6.0 and lower (Reis et al., 1992), while at hypersaline and highly alkaline conditions it is mainly present as HS⁻ which cannot enter cells (Mora-Naranjo et al., 2003). This explains that there is no sulfide toxicity up to 100 mM, but does not clarify why sulfate is not completely reduced. An explanation for this limitation in sulfate reduction might be the sulfate uptake system of microorganisms. Tarpgaard et al. (2011) showed that some sulfate reducers possess two different sulfate uptake systems, one low affinity and another high affinity for low sulfate concentrations (< 0.2 mM). If the high affinity system is not switched on, or not present in the microorganisms, this might explain why the conversion of sulfate is incomplete.

3.2. Biomass attachment

The swift and efficient sulfate and thiosulfate conversion points to a positive effect of adding sand as a carrier material for biomass when compared with results without using sand (Table 2). This is caused by an overall increase of biomass, which is ~10 times higher than without using carrier material. However, most biomass was suspended and not attached to the sand particles (Fig. 3). Microorganisms were only detected in sand particle cavities, where they were protected from shear. In fact, already around day 130 (in the middle of run 4), it can be seen in Fig. 1 A, B and C that sulfate reduction rates were rather low, and

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**Table 2**

<table>
<thead>
<tr>
<th>Reactor type</th>
<th>This study</th>
<th>Sousa et al., 2015a</th>
<th>(Zhou and Xing, 2015)</th>
<th>Sousa et al., 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrier material</td>
<td>Sand separator</td>
<td>Gas lift with 3 phase separator</td>
<td>Gas lift with 3 phase separator</td>
<td>None</td>
</tr>
<tr>
<td>e⁻ donor</td>
<td>H₂</td>
<td>H₂</td>
<td>Sulfate</td>
<td>Formate/Sulfate/CO₂</td>
</tr>
<tr>
<td>Carbon source</td>
<td>CO₂</td>
<td>CO₂</td>
<td>CO₂</td>
<td>H₂</td>
</tr>
<tr>
<td>pH</td>
<td>9</td>
<td>9</td>
<td>9.5</td>
<td>9</td>
</tr>
<tr>
<td>Na⁺ conc. (M)</td>
<td>1.5</td>
<td>1.5</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>35</td>
<td>35</td>
<td>37</td>
<td>35</td>
</tr>
<tr>
<td>HRT (d)</td>
<td>1</td>
<td>3.3</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>S volumetric conversion rates (mmol L⁻¹ d⁻¹)</td>
<td>85 (± 3)</td>
<td>18</td>
<td>85 (± 0.2)</td>
<td>57 (± 0.8)</td>
</tr>
<tr>
<td>Side products</td>
<td>Formate/Acetate</td>
<td>Formate</td>
<td>Acetate</td>
<td>Formate</td>
</tr>
<tr>
<td>Biomass conc. (mg L⁻¹)</td>
<td>127 (± 41)</td>
<td>7 (± 3)</td>
<td>N.D.</td>
<td>14 (± 2)</td>
</tr>
</tbody>
</table>
sulfate removal was also low (see Fig. 2A). The whole performance of the reactor was poor, except for the thiosulfate conversion. The reason for the bad performance could be attributed to the high load applied just before. Sulfate reduction was severely reduced and the suspended biomass concentration decreased. Unfortunately, we do not have microbial community results for Run 4 because the samples were of insufficient quality.

Obviously, gas recirculation of 7 L min⁻¹ that may have prevented the microbes from attaching to the outside of the sand (Fig. 4). At neutral pH and non-saline conditions using a similar bioreactor, no microbial attachment to basalt (non-porous) was observed, while attachment to pumice (porous) occurred (van Houten et al., 1994). However, in that study the biomass growing on the pumice pores, colonized and covered the whole particle, which is not observed in the current study. The main difference between that earlier work and the current study is the haloalkaline conditions. As discussed previously, aggregation and attachment at haloalkaline conditions is more difficult for microorganisms (Ismail et al., 2010; Sousa et al., 2015a; Sousa et al., 2017). The aggregation is weak and vulnerable to shear forces that strongly indicates that the combination of high pH and high salinity, characteristic for soda lakes, affects the biofilm formation process.
To investigate if there is a dedicated microbial community attached to the sand particles, the composition of the suspended and attached biomass to sand was studied at the end of run 5. Fig. 5 shows the relative abundance of different 16S rRNA gene sequences on the sand, which reveals that microorganisms most closely related to members of the Desulfovahalobiaceae had a higher relative abundance than in suspended biomass. The Desulfovahalobiaceae family consists of sulfate and thiolsulfate-reducing bacteria, that were also present in the inoculum (Sousa et al., 2015a; Sousa et al., 2017). The microbial community data show that the relative abundance of bacteria categorized as “others” (abundance <0.5%) was higher in the attached fraction. Some of these rare microorganisms that are more abundant in the attached fraction might produce compounds, such as vitamins, that are beneficial to the other microorganisms in the bioreactor. This was previously shown for other different microbial communities (Seth and Taka, 2014).

3.3. Effect of acetate on biomass growth and attachment

Acetate production in the bioreactor had an effect on biomass concentration, sulfate reduction and methane production. This is demonstrated by the increase of these parameters following an increase in acetate from the end of run 4 until the end of run 5 (Fig. 2). Additionally, the acetate-spiking test confirmed that indeed acetate enhances growth of the biomass, specifically of the attached fraction of biomass (Supplementary Fig. A3).

Heterotrophic growth (e.g. using acetate as carbon source) is energetically more efficient as it requires less energy input, compared to autotrophic growth (Oren, 1999). Stimulating effects of acetate on biomass growth were reported for pH neutral conditions where sulfate reducers were dependent on acetate produced from H2 and CO2 by acetogens (Weijma et al., 2002). Such an interaction might also have occurred in the current study. Most of the sulfate reducers present in the biomass affiliated, based on their 16S rRNA gene, most closely to the Desulfovahalobiaceae family (Fig. 5). Within this family, most of the sulfate reducers were closely related to bacteria belonging to the Desulfonatronovibrio genus that indeed require acetate as carbon source (Sorokin et al., 2011b).

The formation of small microbial aggregates (not attached to sand) was observed after the acetate increase and these aggregates became more abundant during run 5 (Supplementary Figs. A1 and A2). This indicates that acetate cross feeding might play a crucial role in microbial aggregation in the bioreactor. The microbial aggregates size stabilized at approximately 10 μm in diameter and the maximum size observed was approximately 30 μm in diameter. The shear forces and the haloalkaline conditions in the airlift bioreactor might have prevented further increase in aggregate size.

3.4. Formate as an electron donor

Formate production from H2 and HCO3− was one of the main reactions taking place in the bioreactor (Fig. 2). From a thermodynamic point of view, although the Gibbs free energy change is low (Supplementary Fig. A3), this reaction is favorable at the conditions used in this study. Production of formate was shown previously, where it was proposed to be linked to the dominance of Tindalia related bacteria (Sousa et al., 2015a; Sousa et al., 2017). Tindalia related bacteria were detected in high relative abundance in a 12 L bench-scale haloalkaliphilic bioreactor that was continuously operated for the treatment of high concentrations of sulfate (Mu et al., 2019). The bioreactor was fed with glucose as substrate, that was first converted into ethanol, lactate, acetate and formate in the lower region of the bioreactor by fermentative microbews, and then sulfate reducers metabolized the organic acids coupled to sulfate reduction, both in the lower as well as in the higher regions of the bioreactor. Formate was detected in all regions of the bioreactor as well as in the effluent. In the current study, the dominant bacteria in the bioreactor were closely related to the Clostridiaceae family, and about 99% of these were closely related to the Tindalia genus. The 16S rRNA gene sequences were 98% similar to the 16S rRNA gene sequences related to Tindalia identified previously (Sousa et al., 2015a; Sousa et al., 2017). In contrast, Tindalia related bacteria were not dominant in haloalkaline sulfate-reducing bioreactors fed with formate, ethanol, glucose, and lactate as electrons donors and no formate production was observed in any reactor where formate was absent (Zhou et al., 2015). Tindalia related bacteria may have been present at low abundance, as they were detected in the study by Mu et al. (2019), that used the sludge from Zhou et al. (2015) as inoculum for their studies. These differences suggest the hypothesis that Tindalia related bacteria in the bioreactor use H2 and are possibly responsible for the formate production.

Formate production from H2 and HCO3− occurred even though the Gibbs free energy (ΔG°) per reaction is lower than sulfate reduction and methane or acetate production.

Fig. 5. Bacterial 16S rRNA gene relative abundance from a defined day from each run of the bioreactor. All samples were collected and sequenced in duplicate, represented by “A” and “B”. OTUs with less than 0.5% abundance were grouped as “others”.

\[ \text{Formate production from } H_2 \text{ and } HCO_3^- \]
In soda lakes of the Kulunda Steppe (Altai, Russia), formate is the preferred electron donor for sulfate and thiosulfate reduction when compared to H₂ (Sorokin et al., 2010). The results from formate spike injections showed enhanced sulfate reduction with higher formate concentrations (Fig. 7). The calculated ΔG for sulfate reduction with formate is less favorable than with H₂ (Fig. 6). However, the poor solubility of H₂ at high pH might lead to low in situ concentrations of dissolved H₂. A lower dissolved H₂ concentration results in a less negative ΔG of sulfate reduction using H₂ making this less favorable than formate driven sulfate reduction.

4. Conclusions

This study describes that sand as carrier material in H₂ fed sulfate- and thiosulfate-reducing bioreactors at haloalkaline conditions increases biomass retention and S volumetric conversion rates. Biomass attachment to the sand was restricted to cavities within the sand particles where microorganisms are protected from shear forces. Acetate increases biomass growth, especially the growth of attached biomass. Addition of acetate could be used as a strategy to increase overall biomass growth and biomass retention. Formate that is formed from hydrogen and CO₂ has a central role in the bioreactor as electron donor for sulfate reduction.

Fig. 6. Thermodynamic models at pH 9 and 0.825 M HCO₃⁻: (A) ΔG values for formate production following Eq. (1), at different formate concentrations and H₂ was assumed to be constant at 1 atm and (B) ΔG for sulfate reduction using H₂ and formate during the first formate spike experiment [E1]. Measured concentrations of sulfate, sulfide, formate, H₂ and CO₂ were used for the calculations.

Fig. 7. (A) Formate, acetate and (B) sulfate concentration together with (C) hydrogen consumption and methane production during the two formate spiking experiments (“E1” and “E2”). 100 mM formate was injected in to the continuously operated bioreactor on day 182 (E1) and day 189 (E2).

CRediT authorship contribution statement

João A.B. Sousa: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. Andrea Bolgár: Investigation, Formal analysis. Stephan Christel: Methodology, Investigation, Formal analysis, Writing - review & editing. Mark Dopson: Supervision, Methodology, Formal analysis, Writing - review & editing. Martijn F.M. Bijmans: Supervision, Project administration, Funding acquisition, Writing - review & editing. Alfons J.M. Stams: Supervision, Project administration, Funding acquisition, Writing - review & editing. Caroline M. Plugge: Conceptualization, Formal analysis, Supervision, Funding acquisition, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsicotem.2020.141017.

References


