Factors affecting biological sulphate reduction and the microbial consortia in continuously operated bioreactors

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Introduction

Sulphate-rich mine water must be treated before it is released into receiving water bodies

- Chemical and biological methods
  - Biologically using sulphate reducing microorganisms
  - Anaerobic bioreactors
  - Low redox, suitable pH, suitable electron donors
  - Ultimately cost-efficient, using waste material as electron donor for sulphate reduction
  - Other benefits, e.g. from precipitating metal sulphides

However, bioreactors are living things reacting to environmental factors, such as temperature, pH, redox potential, microbial community composition…
Sulphate reducing microorganisms and electron donors

- Two types of sulphate reducers
- Oxidicing electron donors to CO$_2$ – complete oxidizer
  - *Desulfarcula, Desulfobacter*
- Oxidizing electron donors to acetate – incomplete oxidizers
- May cause accumulation of acetate -> lowering of pH
  - *Desulfovibrio, Desulfuromonas*
- Lactate is one of the best electron donors and metabolized by a wide range of sulphate reducers
- However, lactate is too valuable to be economically feasible
- **Cheap and abundant substrates;**
  - manure, woodchops/hay/straw, crude glycerol and ethanol
The study

- We compared bioreactors run in South Africa (Mintek) and Finland (VTT) on different substrates
- Measured pH, redox, sulphate removal
- The microbial communities were investigated with high throughput amplicon sequencing of the taxonomical marker gene for the 16S ribosomal RNA subunit
- Sequence data was analyzed using bioinformatic tools (QIIME, greengenes 13_8 database, Phyloseq in R, PAST)
  - Salo M. 2017. MSc thesis. Tampere University of Technology. 
<table>
<thead>
<tr>
<th>Reactor</th>
<th>Bioreactor type</th>
<th>Operating T (°C)</th>
<th>Substrate</th>
<th>Added nutrients</th>
<th>Influent sulphate (g L⁻¹)</th>
<th>HRT (d)</th>
<th>Location (SA/FIN)</th>
<th>pH</th>
<th>Redox potential (mV)</th>
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</thead>
<tbody>
<tr>
<td>R1</td>
<td>Down-flow anaerobic flooded column</td>
<td>23</td>
<td>Woodchips, hay, manure</td>
<td>None</td>
<td>2.7</td>
<td>21</td>
<td>SA</td>
<td>8.04</td>
<td>-236</td>
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<tr>
<td>R2</td>
<td>Down-flow anaerobic flooded column</td>
<td>24</td>
<td>Woodchips, crude glycerol</td>
<td>1.2 g/L (NH₄)₂SO₄, 0.4 g/L H₃PO₄</td>
<td>4.5</td>
<td>9</td>
<td>SA</td>
<td>7.05</td>
<td>-399</td>
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<tr>
<td>R3</td>
<td>CSTR</td>
<td>30</td>
<td>Woodchips, crude glycerol</td>
<td>1.2 g/L (NH₄)₂SO₄, 0.4 g/L H₃PO₄</td>
<td>4.5</td>
<td>4</td>
<td>SA</td>
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<td>-396</td>
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<tr>
<td>R4</td>
<td>UASB</td>
<td>21</td>
<td>Manure, lactate</td>
<td>None</td>
<td>1.1</td>
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<td>FIN</td>
<td>7.40</td>
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<td>R5</td>
<td>UASB</td>
<td>21</td>
<td>Ethanol</td>
<td>0.06 g/L KH₂PO₄, 0.14 g/L (NH₄)₂HPO₄</td>
<td>8.5</td>
<td>7,2</td>
<td>FIN</td>
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<td>-395</td>
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<td>R6</td>
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<td>21</td>
<td>Ethanol</td>
<td>0.06 g/L KH₂PO₄, 0.14 g/L (NH₄)₂HPO₄</td>
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<td>R7</td>
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<td>Ethanol</td>
<td>0.06 g/L KH₂PO₄, 0.14 g/L (NH₄)₂HPO₄</td>
<td>8.5</td>
<td>7,2</td>
<td>FIN</td>
<td>7,4</td>
<td>-382</td>
</tr>
</tbody>
</table>
Sulphate removal

Woodchips
Hay+manure/ Glycerol
Manure, lactate
Ethanol

Woodchips
Hay+manure/ Glycerol
Manure, lactate
Ethanol

\( g \text{ SO}_4 / \text{ m}^3 \cdot \text{d} \)
The sulphate reducing community

- Only max 10% of the bacteria were sulphate reducers
- Mostly deltaproteobacteria
  - *Desulfomicrobium*
    - Incomplete oxidizers
  - *Desulfovibrio*
    - Incomplete oxidizers
- *Desulfobacteraceae genera*
  - Complete oxidizers
- *Desulfuromonas*
  - Reduces sulfur, but not sulphate…
Shared orders between the 6 best performing reactors

All reactors
1. Anaerolineales
2. Bacteroidales
3. Campylobacterales
4. Clostridiales
5. Desulfovibrionales
6. Desulfuromonadales
7. Methanosarcinales
8. OPB95
9. Pseudomonadales
10. Sphingomonadales
11. Synergistales
12. Xanthomonadales

Woodchip reactors
- No unique taxa

Ethanol reactors
- No unique taxa

Manure reactors
- No unique taxa
Canonical correspondence analysis

- Coordination analysis based on the microbial community composition, abiotic factors and performance
- Red – ethanol reactors
- Brown – manure reactors
- Green – glycerol reactors
Example – ethanol reactors

- pH 6 – most efficient sulphate removal from the minbe water
- ORP -300 - -400 mV
Concluding thoughts

- Optimal pH and redox for optimal results

BUT;

- The microbial communities affect the pH and redox
- Used substrates affect the microbial communities
- The concentration of sulphate (and other components) of the feed affect the microbial communities
- Temperature affect the $\text{SO}_4$ reduction rate
- Hydraulic retention time (HRT)
- Carrier material
Acknowledgements

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- Lerato Seepeि
TECHNOLOGY FOR BUSINESS