Introduction

Why Biological Hydrogen Production?
Defined as hydrogen produced by a biological process. Viable alternative fuel source due to:
• Global warming and rapid depletion of fossil fuel reserves
• Less energy intensive hydrogen source
• Allows simultaneous treatment of industrial wastewater if used as a growth substrate
• No competition with food crops

Clostridium beijerinckii
• A species of anaerobic bacteria (grows only in the absence of oxygen)
• Produces high yields of hydrogen through anaerobic fermentation
• Able to metabolize a broad range of substrates, therefore suitable with industrial wastewater

Objective
To determine the minimum nutrient concentrations required for hydrogen production by C. beijerinckii in order to minimize costs.

Synthetic Wastewater
For controlled experimental conditions, defined media must be used. Therefore, synthetic wastewater is produced for this project. Its composition is fixed and based on previous work involving Clostridia bacteria, and consists mostly of trace amounts of various salts and nutrients.

Of these nutrients, biotin, MgSO₄·7H₂O and glutamic acid are among the most costly. In order to minimize the cost of fortifying industrial wastewater, their minimum required concentrations must be determined. The following nutrient concentrations have thus been tested:

Table 2: Experimental nutrient concentrations

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Concentration/minimum concentration in bold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>mg/L: 0, 0.05, 1, 3</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>g/L: 0.00, 0.02, 0.03, 0.07, 0.1, 0.2</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>g/L: 0.00, 0.01, 0.05, 0.1, 0.42</td>
</tr>
</tbody>
</table>

Methodology

C. beijerinckii is first cultivated in the recommended nutrient broth, with subsequent inoculations of synthetic wastewater occurring towards the end of the exponential growth phase.

Maintenance of Anaerobic Conditions
• The synthetic wastewater is boiled for 20 minutes to drive off oxygen
• Nitrogen gas is bubbled through both the synthetic wastewater and nutrient broth, with the media bottles then closed tightly with rubber stoppers and aluminium crimps.

• Prior to inoculation through a hypodermic needle, a reducing agent, Na₂S·9H₂O, is added to the media bottles.

Results

The figures below show the effects of the three different nutrient concentrations on biological hydrogen production. The error bars represent the 95% confidence interval on the results, and an overlap indicates no significant difference in hydrogen production at α=0.05.

Control concentrations are marked in green. 12 replicates were executed for the control condition, to ensure consistency in methodology and measurements prior to experimentation.

Effect of Biotin Concentration (4 replicates)

- Biotin concentration does not have a significant effect on hydrogen yield.
- No difference in lag time or production rate observed

Effect of Glutamic Acid Concentration (4 replicates)

- Glutamic acid concentration does not have a significant effect on hydrogen yield.
- No difference in lag time or production rate observed

Effect of MgSO₄·7H₂O Concentration (3 replicates)

- The absence of MgSO₄·7H₂O leads to no hydrogen production.
- However, further increases in its concentration have no significant effect on total yield.
- Lower concentrations of MgSO₄·7H₂O were associated with longer lag times for measurable hydrogen production: 42h at 0.01g/L, 22h at 0.02g/L and 12-18h for all other concentrations

Conclusion

- Biotin and glutamic acid are not required for biological hydrogen production.
- MgSO₄·7H₂O is a required nutrient, but hydrogen production is still viable and timely at concentrations significantly lower than the control.
- Significant savings can be made when fortifying industrial wastewater by eliminating or reducing these three nutrients.
- Future work should focus on minimizing the lag time in biological hydrogen production, by varying nutrient or buffer concentrations. Other metabolic by-products should also be identified to better understand the pathways for hydrogen production, and potentially increase its yield.

References

Skomronczyk, M.T., Biological hydrogen production from industrial wastewater with Clostridium beijerinckii. Submitted to McGill University towards completion of the degree of Master of Engineering (Thesis Option), 2008.

Acknowledgements

Special thanks to Prof. Viviane Yargeau and to Dominic Aebi for their guidance and support. Thanks to the McGill University Faculty of Engineering for their financial support.