Metabolite Production of *Thermoanaerobacterium saccharolyticum*

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Thesis: This experiment was part of a larger project. A batch of a gene-deletion mutant strain of *Thermoanaerobacterium saccharolyticum* was grown in a bioreactor to test cell growth and production under certain conditions.

Background

• Many microorganisms reproduce quickly, and as a result adapt to environmental conditions quickly

• In a bioreactor, cells (particularly those with recombinant DNA) are not in optimal conditions and will evolve. This limits how long a cell culture remains useful in a bioreactor

• **This experiment aims to study changes in metabolism of a *Thermoanaerobacterium saccharolyticum* culture over time**
- *Thermoanaerobacterium saccharolyticum* is an anaerobic bacterium used for ethanol production from glucose and xylose.
- In this experiment, a strain genetically modified with gene knockouts was grown using xylose as the carbon source.

• The experimental results will be compared to a computer model prediction to test its efficacy
- An organism's overall metabolism can be described as a collection of basic reaction pathways
- These pathways are called elementary modes, and analysis using them is called elementary mode analysis
- Elementary mode analysis can be used to predict how a substance is metabolized by the organism
- Quantitative analysis of probability of each of the pathways can be used to predict the most favorable pathways, and thus predict eventual evolution of the cells

Experimental Methods

Growing the gene deletion mutant strain of *T sacch* grown in bioreactor:
•**1 liter MTC media** with 20 g/L xylose carbon source
• **Nitrogen sparge** to keep anaerobic conditions
• At 24 hours, lag phase continued so 5% CO_2 gas was added at to the sparge to add carbonic acid
• CO_2 addition ended lag phase
• Bioreactor used base to maintain pH of 6.0 despite CO_2 in sparge and acid production by cells

Experimental samples analysis:
• **Samples taken at 4.5, 24.25, 29, 33.5, 47, 71.5, and 95 hours**, stored in cool environment to inhibit cell growth
•**HPLC identified major components (xylene, ethanol, lactic acid)** consumed or produced, measured concentrations with refractive index detector
• **Cell density measured** by spectrophotometer for optical density at ~260nm, which is linearly proportional to cell density

Figure 1

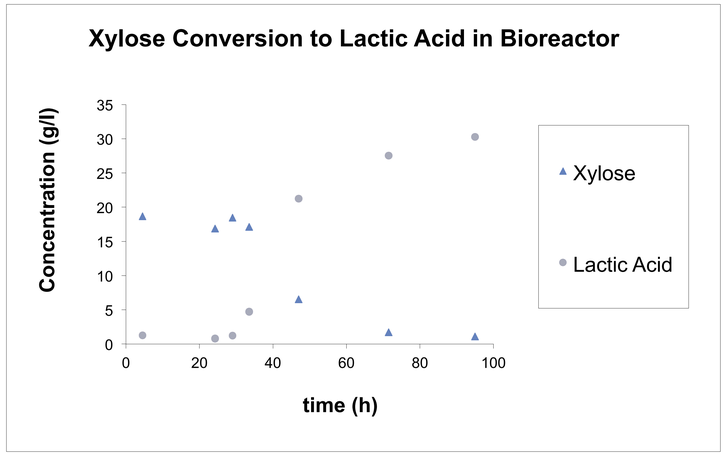
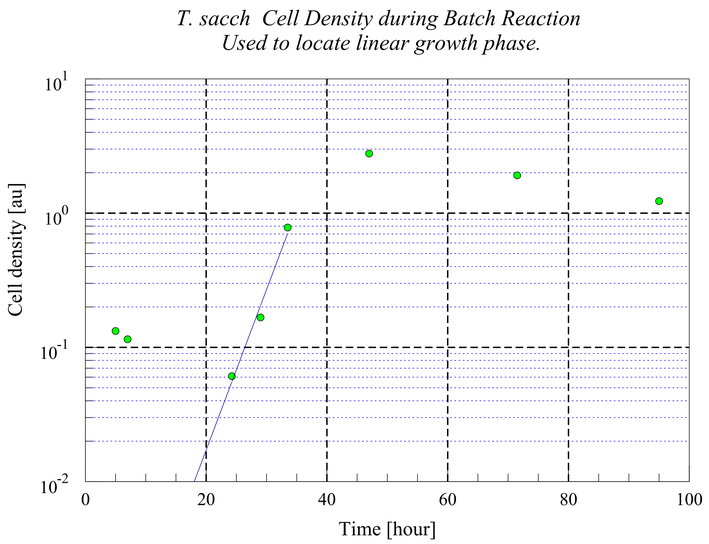


Figure 2



Application

•**These experiments are the first step** in a bioreactor project

• The **goal of this experiment is to test and improve a model** based on elementary mode analysis to predict the evolution of cells in a bioreactor

•Eventual **goal of the project is to create an automated bioreactor** system to automatically take samples, with bioreactor used to continuously add feedstock and reduce cell density to encourage cell growth

- Samples will be further analyzed for cell growth and media composition as generations of *T. sacch* adapt to the environment of the bioreactor
- The experimental results can be compared to a computer model predicting the evolution of the cells' metabolism
- The computer model provides quantitative analysis of metabolic pathways using elementary mode analysis
- The cells are predicted to evolve to the most favorable metabolism, model predicts most favorable metabolism, so it should be able to predict evolution of the cells

Conclusions

Ethanol was expected major product
•Very low yield
• Maximum yield less than 0.18 g/L at 47 hours
• Concentration was below detection limit by 71.5 hours
• Possibly evaporated in sparge or consumed by cells

Lactic acid was produced
• Yield higher than original xylose content
• Yield concentrations exceeded calibration curve, so final result is uncertain

Bioreactor ran successfully
• MTC media and pH level provided environment conductive to cell growth
• Carbon dioxide in the sparge necessary for cell growth

References

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