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Modeling Butanol Production by *Clostridium beijerinckii*

Although fossil fuels are currently the most economical source of energy, many other alternative energy sources are being explored as replacements for fossil fuels. Currently, millions of dollars are being spent on ethanol research. However, ethanol's energy content is only two-thirds that of gasoline. Butanol, another alternative biofuel, has similar energy content when compared to gasoline and has been gaining attention. The class *Clostridia* is known to contain species that carry out butanol fermentation from a variety of 5 and 6 carbon sugars. *Clostridium beijerinckii* is well known for its ability to grow in easily facilitated, inexpensive media and its ability to produce butanol well into the stationary phase of growth. However, butanol is known to be toxic to the bacteria in larger amounts, inhibiting growth and often lysing the cell membrane. Although butanol is miscible up to roughly 70g/L with water, standard batch fermentations only yield around 25g/L.

Experiments were conducted using the bacterium *C. beijerinckii* in 1-L screw-top shaker flasks filled with 500mL of xylose media. Samples were taken at 24, 48, and 72 hours during the experiment. Turbidity measurements were taken at each time interval. The supernatant was separated from the pellet in each sample and was analyzed to measure the substrate and solvent concentrations using high performance liquid chromatography. Cell pellets were stored at -15 degrees Celsius and were examined by 2DGE to determine differential protein expression. The data shows declining sugar concentrations and increasing production of acetic and butyric acid in the early part of the growth cycle and butanol production in the later part of the growth cycle.

The fermentation process is modeled by a system of differential equations based on metabolic reactions using Michaelis Menten enzyme kinetics. The equations, built from those of previously published models, are analyzed and numerically solved to explore the efficient conversion of glucose and xylose into butanol by these bacteria. The mathematical model predicts the concentrations of intermediaries and products formed throughout the course of each experimental run, and results are compared to experimental data. Each reaction equation represents the result of enzymes binding to a substrate or an intermediary to create another intermediary or one of the final products. Parameters and initial conditions are altered to best fit experimental data, and numerical simulations are represented visually in concentration versus time graphs. Results from the model are analyzed and compare favorably to experimental data. The goal of this analysis and experimentation was to find the necessary conditions for optimum yields of butanol production.