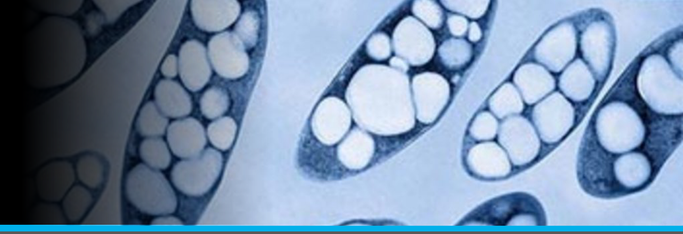
Green Chemical Production by Bacterial CO_2 Reduction



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Introduction

Objectives:

1. Determine essential growth parameters using simple microbiology experiments
2. Apply chemical engineering principles, cell growth model, substrate utilisation and product formation model to simulate biomanufacturing process
3. Establish fed-batch model to describe cell growth in the bioreactor, using parameters from microbiology experiments
4. Apply the optimal parameters to the bioreactor and examine the performance in terms of yield of final product

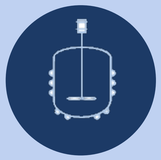
Motivation:

The motivation for this project is the need for low carbon alternatives for chemical production. Fed-Batch biosynthesis provides an alternative approach to ethanol production method such as ethene hydration and fermentation. The advantage of using genetically engineered bacteria for ethanol production over the other methods are:

1. Much lower carbon footprint than ethanol produced by ethene hydration, which is the most used method for industry applications since ethene is a bioproduct of the oil industry
2. Offers the opportunity for valorisation of waste streams, such as organic waste and CO_2 emissions from high carbon industries into value added products
3. The compatibility with renewable energy sources enabling chemicals to be produced with a low to negative carbon footprint
4. Ability to implement fed-batch and continuous processes

Ralstonia Eutropha

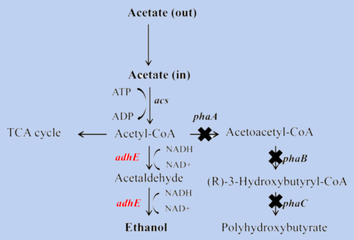
This project focuses on the bioconversion of acetate to ethanol in the bioreactor:



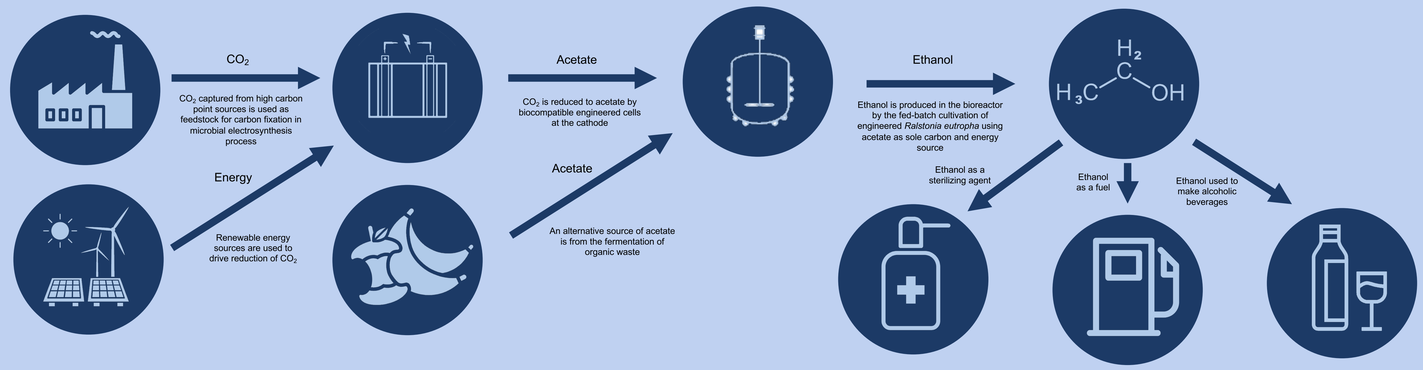
R. eutropha is a non-pathogenic bacteria commonly found in soil and fresh water. The properties this bacteria which make it a suitable candidate for biotechnology application are:

1. Ability to adapt to continuously changing environments
2. Ability to shift between autotrophic and heterotrophic growth
3. Ability to feed on a range of different carbon sources

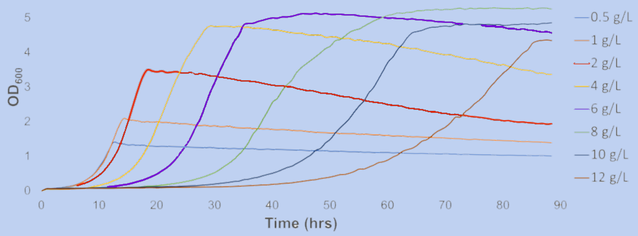
R. eutropha is known for its ability to store up to 80% its cell dry weight as PHB, which is a prerequisite to bioplastic production. In the engineered strain for ethanol production, the gene coding for bioplastic deleted and an *adhE gene* encoding an alcohol dehydrogenase from *Escherichia coli* was overexpressed for the conversion of acetate to ethanol



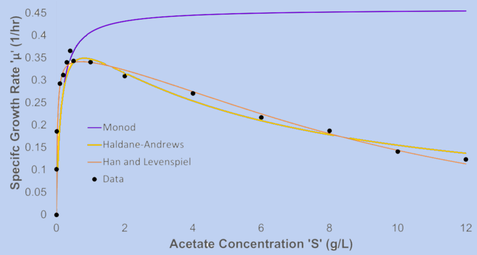
Process Outline



R. eutropha Growth on Acetate



- This shows bacteria growth on acetate at different initial concentrations, with OD_{600} as a measure of cell density
- Max cell density increased with increasing initial concentration up to 8 g/L , above which acetate was growth inhibiting



- The growth rate was found for the exponential growth phase for each initial concentration and fitted with two substrate inhibition models

This showed that high substrate concentrations have significant negative impact on the growth rate in the growth phase

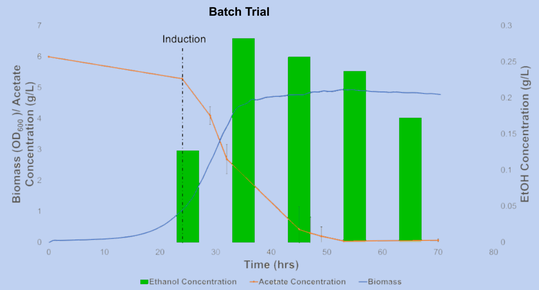
Haldane-Andrews:

$$\mu = \frac{\mu_m S}{S + K_S + \frac{S^2}{K_I}}$$

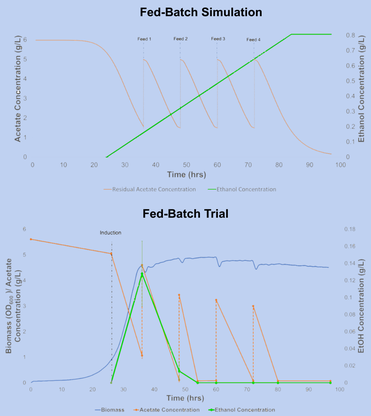
Han and Levenspiel:

$$\mu = \frac{\mu_m S[1-(\frac{S}{S_m})]^n}{K_s + S - [1-(\frac{S}{S_m})]^m}$$

Batch and Fed-Batch Ethanol Production



Shows the batch cultivation of *R. eutropha*. As the cells grow, the carbon source is consumed. After induction of the ethanol synthesis pathway, ethanol accumulates in the medium with max ethanol production occurring in the growth phase since ethanol is a primary metabolite. At end of batch process, cells have run out of carbon and begin to break down stored energy.



The goal of implementing a fed-batch process was to prevent the breakdown of ethanol at the end of the batch process by periodically supplying fresh medium to ensure there was always available carbon source for the cells to use. The trial was unsuccessful at preventing the assimilation of ethanol product despite cells consuming the additional acetate supplied

References

[Lee et al., 2016] Lee, H.M., Jeon, B.Y., and Oh, M.K. (2016). Microbial Production of Ethanol from Acetate by Engineered. *Biotechnology and Bioprocess Engineering*, page 6.