From Beer to Biofuel

Grain Waste Powering Eucalyptol Production

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Abstract

We have designed a system consisting of three strains of *Escherichia coli* (*E. coli*) which will convert hemicellulose into eucalyptol. The first two strains overexpress and secrete enzymes to degrade hemicellulose into xylose. The third strain of *E. coli* converts the xylose into eucalyptol via the mevalonate pathway. A scale-up model was also developed to assess industrial feasibility. The cost of eucalyptol production would drop two-thirds compared to the current market cost, which would make industrial uses plausible. Future studies will be transforming our *E. coli* with the genes of interest and testing for production of eucalyptol.

Introduction

Biological synthesis of commonly used industrial substrates has been shown to lower production costs when properly optimized. The aim of this project was to repurpose an organic waste product in the biological synthesis of eucalyptol. Spent grains from beer production is a common waste product in Milwaukee and contains adequate polysaccharides which can be used as substrate for bacterial growth and synthesis. A specific emphasis in this project was to design a system which could be optimized to industrial scale synthesis of eucalyptol.

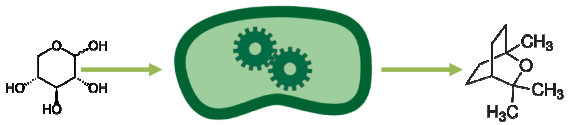

Figure 1: Cartoon representation of our process to transform xylose into eucalyptol.

Input	Output
Problem: Hemicellulose is too large to enter the cell	Purpose: Synthesize and excrete eucalyptol
Purpose: Secrete enzymes for the break-down of hemicellulose to D-xylose	Mechanism: Utilize D-xylose as a sugar source to exploit the mevalonate pathway (6 genes introduced from *E. faecalis, S. pneumonia, A. thaliana*, and biobrick BBa_K849000)
Mechanism: Enzymes xynC-A and xyloA are tagged and secreted	Optimization: Mevalonate pathway was chosen to synthesize isopentenyl-5-pyrophosphate (IPP) for its existing high expression level in *E. coli*. TPS-CIN modifies IPP to produce eucalyptol
Collaboration: Secretion system from University of Washington iGEM	

Table 1: Input and output strain purpose and genetic engineering explanations.

Design

The three *E. coli* system can be divided into two subsystems, input and output, each responsible for completing a separate task. The input subsystem is responsible for breaking down the large polysaccharide hemicellulose from the spent grains into simpler subunits, specifically xylose. Because *E. coli* can naturally metabolize xylose, the output subsystem can then use the xylose produced from the input subsystem to fuel the synthesis of eucalyptol.

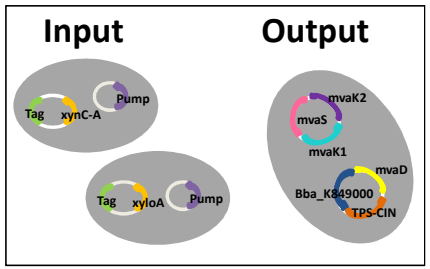

Figure 2: Cartoon of gene inserts for the designed input and output subsystems. While the input genes xynC-A and xyloA, excreted from the cell by the pump, are responsible for the enzymatic break down of hemicellulose, the output genes mvaK1, mvaS, mvaK2, mvaD, Bba_K8490000 and TPS-CIN complete the mevalonate pathway synthesizing and excreting eucalyptol.

Mevalonate Pathway

- The mevalonate pathway is a eukaryotic pathway which contains 8 genes leading to the synthesis of gernayl pyrophosphate.
  - 3 genes naturally exist in *E. coli*
  - 5 genes from other sources were incorporated complete the pathway
- 1,8-cineole synthase was added to the end of the pathway for final eucalyptol synthesis.
- System was attached to a strong T7 promoter and transformed into the BL21 strain of *E. coli* to encourage strong expression.

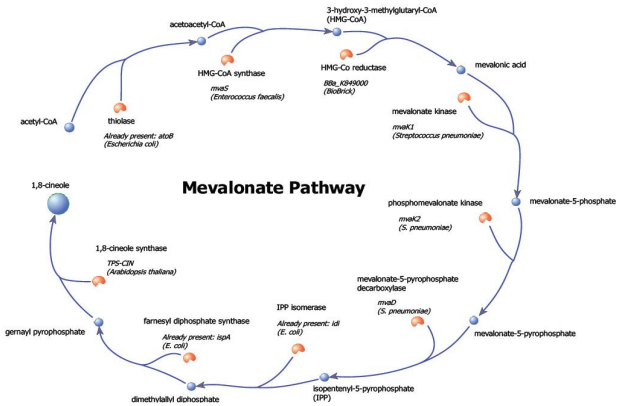

Figure 3: Cartoon depiction of the Mevalonate Pathway.

Feasibility

A large aspect of our project was to assess the feasibility of industrial use. The first portion was to determine the theoretical enzyme activity and total turnover rate. Afterwards, a bioreactor was designed and governing equations were used to calculate the final concentrations for a batch process.

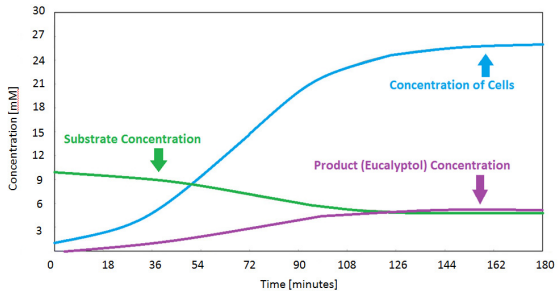

Figure 4: Predicted product concentration produced from a bioreactor inoculated with a small amount of *E. coli* cells with a saturated substrate solution. Graph uses monod growth for *E. coli*.

After the total turnover rate was calculated, product yield was 538mM and the next step was to design a bioreactor to carry out the reaction. A batch reactor was selected over a continuous reactor to best suit the bacterial life cycle. The governing equations of a bioreactor were used to calculate the concentrations of the substrate, cells and product. The inhibitory effect of eucalyptol on cell growth was found to be negligible with respect to the bioreactor concentration. Table 2 shows our preliminary cost assessment.

Reactor Size	Operating Cost	Product Cost	Current Market Cost	Profit at $10/kg	Payoff time
10,000L	$5 million/yr	$5.51/kg	$64/kg	$4.6 million/yr	4 years

Table 2: Preliminary assessment for scaling scale up. All calculations were done using SuperPro Designer ©

Future Direction

Currently delaying the instatement of alternative green fuels as primary fuel for vehicles is the obstacle of creating compatible car engines. An 8:1 eucalyptol-to-diesel ratio has been found to be effective for contemporary diesel engines. This supplemented diesel would require only small modifications to current engine designs, allowing for a smoother and faster transition from imported, nonrenewable, oil-based diesel to sustainable biofuels.

We are hoping to continue our project next year, in which we will try to produce Eucalyptol in vitro through our three E. coli system and work with our SuperMilage team to determine if the Eucalyptol/Gasoline mixture properly works in various combustion engines. We were also hoping to get our upcoming iGEM team ready through journal readings/idea storming and to outreach to nearby high schools to possibly recruit them into iGEM HS and possible future directions in synthetic biology.

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