PURDUE UNIVERSITY

CAPSTONE/SENIOR DESIGN EXPERIENCE 2018

Agricultural & Biological ENGINEERING

Continuous Fermentation of Fatty Acids

Hyunwoo Chung, Prasanna Janakiraman, Shrishti Jaganmohan, Priya Raju (BS BE)

PROBLEM STATEMENT

Production of medium length carbon chain esters containing fatty acid molecules are high value molecules which are conventionally acquired from petroleum derivatives. These molecules are demanded by industries which produce plasticizer (polymer), pharmaceuticals (lubricant), chemical (chemical intermediates, adhesives, and solvents) and cosmetics(conservatives and active ingredients). However, the conventional methods of producing medium length (C9~C12) fatty acids from petroleum derivatives require to undergo harsh processes, such as high temperature and pressure operation, ozonolysis, and toxic and dangerous chemicals, such as sulfuric acid or nitric acid.[1]

Overall Goal

Provide laboratory scale result, which could substitute conventional production processes with eco-friendly and cost-effective biotransformation .

Design Goal

Lab-scale design of continuous process that connects batch fermentation to resin column chromatography.
Lab-scale design of ion-exchange resin packed column chromatography.

This Design Includes:

- Biotransformation of Oleic acid using E.Coli BL21(DE3)::pAPTm-ADH-E6BVMO$_{(opt)}$C302L$_{mutant}$
- Continuous batch fermentation process
- Cell/liquid separation and liquid/liquid separation via Ion-Exchange resin chromatography

Background

- Purpose of whole-cell biotransformation is to **produce functional ingredients** for cosmetics, pharmaceuticals, and chemicals
- Recombinant E. coli is **fermented**, substrate is **injected** into the cell broth to **enable biotransformation**
- Whole-cell biotransformation **targets** renewable fatty acids and plants oils as substrate.
- Stearic acid is **beneficial** for pharmaceutical use - normalizing blood pressure
- Advantages of recycling E.coli
  - Suitability for **vector design** in catalytic enzymes
  - Cost Reduction

Market Analysis:

Purpose: Identify the trend in the plastics and pharma industries towards incorporating new eco-friendly biotransformation processes to replace traditional ones.

Methods: Studying news sources and/or scientific publications

Findings: The use of biotransformations has been shown to be effective and is expected to be adopted by many industries.[3]

Conclusion: There is a need to develop and test new biotransformation unit operations in a lab setting so that successful projects can be scaled up. This will be economically sound and beneficial to the environment.

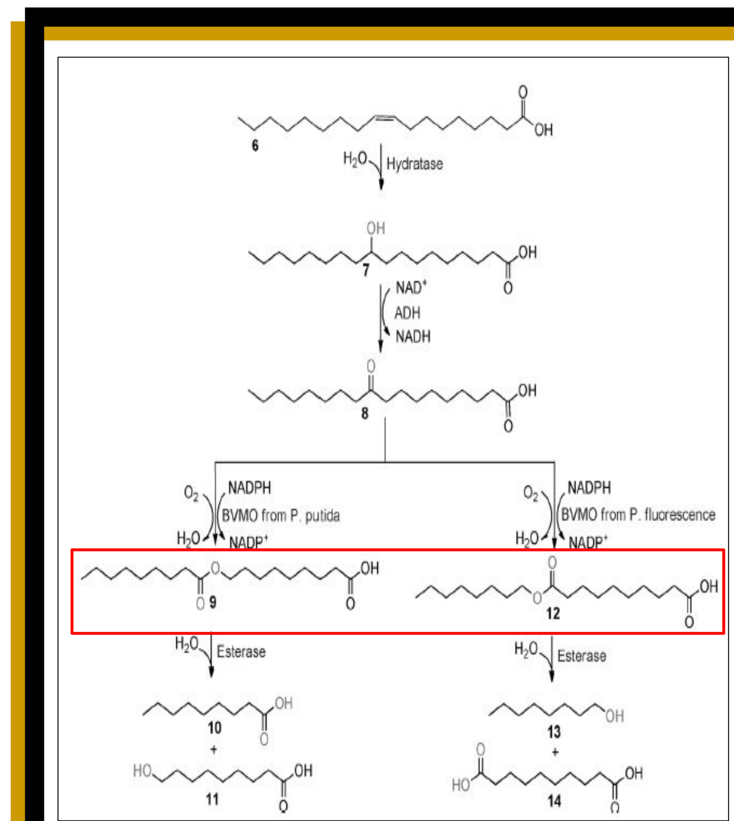

Figure 1: Multi-step enzymatic reactions in order to convert oleic acid (**6**) to oleic esters (**9,12**) [2].

Experimental Design

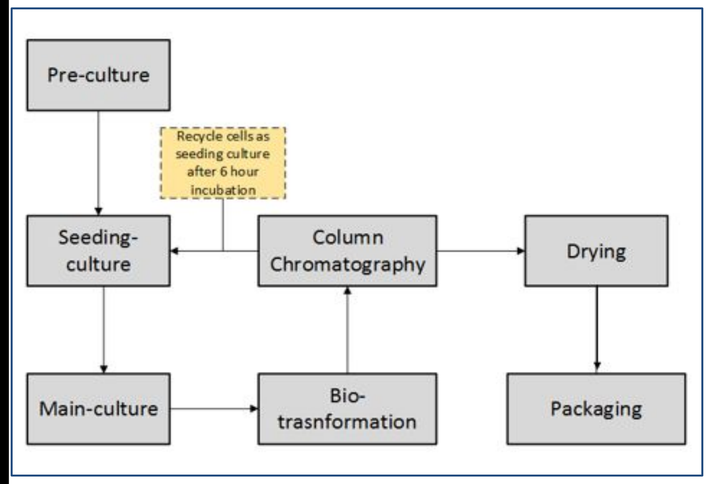

Figure 2: Process diagram of continuous biotransformation of oleic acid

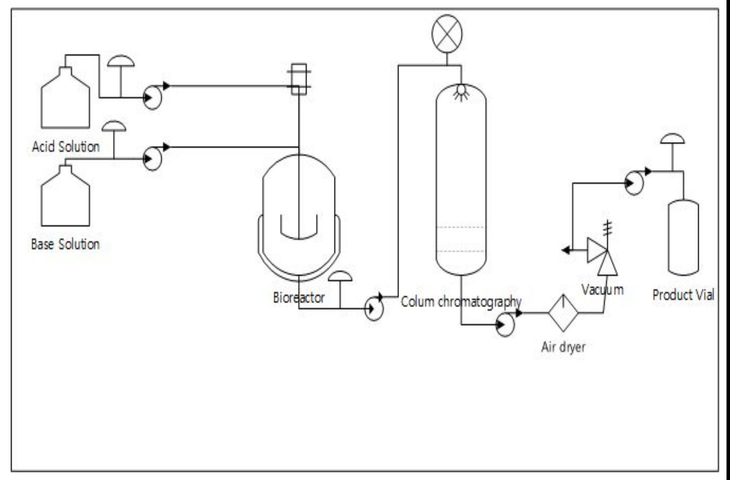

Figure 3: Expected industrial equipment layout

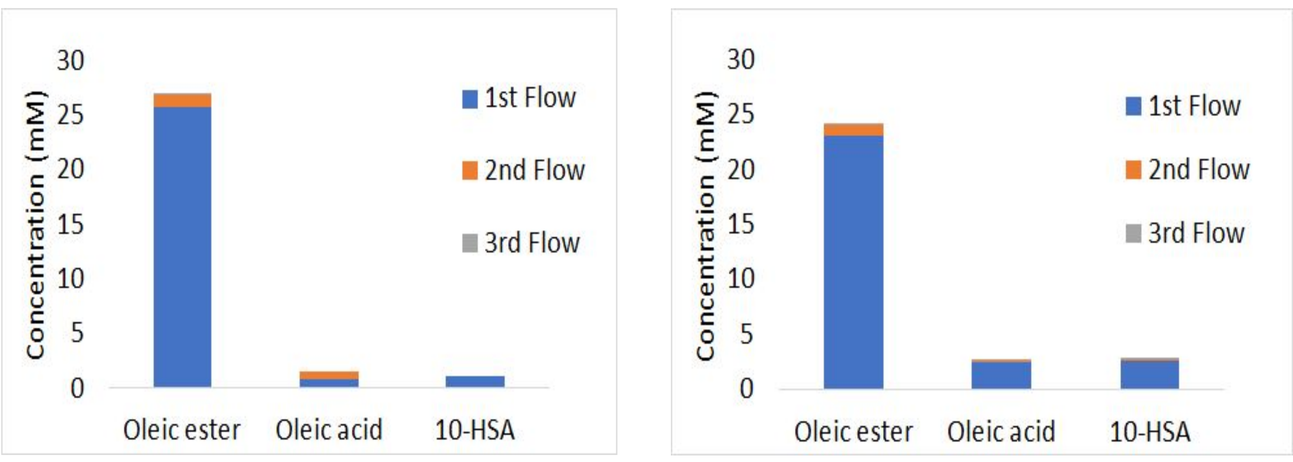

Figure 4:Result of resin column chromatography absorption (left) and desorption with Ethyl Acetate (right)

The Oleic acid (30 mM) reaction (OD at 600nm = 30) was converted to 28.06 mM of Oleic ester, 1.71 mM of Oleic acid, and 0.14 mM of 10-Hydroxystearic acid (10-HSA) using E.coli as the biocatalysis. The absorption of cell containing broth consisted of three flows rates: 800 mL (flow volume) / 900 seconds, 800 mL / 1980 seconds (33 min), and 800 mL / 180 seconds (vacuum). The reacted cell broth was tested three times.

400 mL of R1 media (containing 4 g of $(NH_4)2HPO_4$, 13.5 g of KH_2PO_4, 1.7 g of citric acid, and 10 mL of TMS / 800 mL of R1 media) was added to wash down the remaining cells within the column. 800 mL of Ethyl acetate was flown at a rate of 800 mL / 1800 sec (30 min) three times in order to desorb the product that was absorbed by the resin.

From the experiment, 98.7% of Oleic ester was absorbed while of 100% was desorped by Ethyl acetate. 100% of Oleic acid was absorbed; 78% is desorbed. 71.4% of 10-HSA was absorbed while 61.7% was desorbed. The resin chromatography successfully separated cells from the product containing cell broth and showed partial purification as about 99% of Oleic ester was retrieved.

Alternative Solutions

The cell broth has various rheological properties as each batch contains different colonies of the cell. The following alternative solutions incorporate the cell's effect on the process.

1. Centrifugation separation of cells prior to the Ion-exchange resin column chromatography
   Pros: Cells do not block the spaces between resin bids and secure minum flux.
   Cons: Requires an additional process (centrifugation) causing an increase of production cost.
2. *In-situ* product recovery by mixing resin beads with cells within the bioreactor.
   Pros: The resin beads are in free movement within the bioreactor and the bids are large enough to separate from the cell and cell broth.
   Cons: *In-situ* recovery is still under developmentary phase in research, which requires further studies on the process itself and selection of resin.

Economic Analysis

Our economic analysis assumes costs per cycle batches for fermentation, resin chromatography, sterilization, and drying processes. Our process should be able to be added onto the existing processes. We are able to sell our product for around $80 per gram.[4] US production is in a large scale of around 100 million grams , but our small scale production will produce 1,000 grams of final product.[4] The total capital investment costs are based on direct costs, since the purchased and installation equipment.[5] Hidden costs include indirect costs. All cost estimates are calculated on an industrial scale of production.

Total Revenue		$80,000
Cost of Goods	Luria-Bertani Broth	$24.95
	Urea Solution	$48
	Biotin Solution	$24
	Oleic Acid	$9.99
	TWEEN 80	$1,171.38
Yearly Profit		$78,722

Table 1: Yearly profit costs for the final product.

	Direct Costs	**Estimated Costs**
Total Capital Investment	Purchased Equipment	$160 701.44
	Installation	$20,333.33
	Total TCI	$181.034.77
Fixed Costs	15% of TCI	$27,155.22

Table 2: Total capital investment and fixed costs estimates of final product.

Total Cost Per Cycle (Cost Summary)	
Fermentation	$300 / L
Resin Column Chromatography	$200 / L
Sterilization	$0.36 / kg
Drying	$20 / kg

Table 3: Estimated production cost per cycle of each biotransformation unit operation.

Global / Social Impact:

Biotransformation processes such as what is being tested in our experiment prove to be less harmful to the environment and less dangerous to plant workers as they do not involve extreme temperatures or harsh reagents.[1] Reducing environmental impact is important for sustainability. Pharma & Plastics industries are found all over the world, so it is expected that improvements in this unit operation will have a global impact.

References

[1]a) Y. Fujita, H. Matsuoka, K. Hirooka, Mol. Microbiol. 2007, 66,829; b) T. G. Liu, H. Vora, C. Khosla, Metab. Eng. 2010, 12, 378; c) B. Bhler, J. B. Park, L. M. Blank, A. Schmid, Appl. Environ. Microbiol. 2008, 74, 1436; d) J. B. Johnston, H. Ouellet, L. M. Podust, P. R. O. de Montellano, Arch. Biochem. Biophys. 2011,507, 86.

[2]Song, Ji-Won, Jeon, Eun-Yeong, Song, Da-Hyun, Jang, Hyun-Young, Bornscheuer, Uwe T., Oh, Deok-Kun, & Park, Jin-Byung. (2013). Multistep Enzymatic Synthesis of Long-Chain α,ω-Dicarboxylic and ω-Hydroxycarboxylic Acids from Renewable Fatty Acids and Plant Oils. *Angewandte Chemie International Edition, 52*(9), 2534-2537.

[3]Speight, J. G., & El-Gendy, N. S. (2018). Introduction to petroleum biotechnology. Cambridge, MA: Gulf Professional Publishing.

[4] Oleic Acid Propyl Ester | CAS 111-59-1. from https://www.scbt.com/scbt/product/oleic-acid-propyl-ester-111-59-1

[5] Peters, M. S., Timmerhaus, K. D., & West, R. E. (2003). *Plant design and economics for chemical engineers*. New York: McGraw-Hill.

Special Thanks to:
Dr. Martin Okos, Technical Advisor and Instructor
Dr. Park, Jin Byung, Professor of Ewha Womans University's Dept. of Food Science and Engineering (Granting permission to use Hyunwoo Chung's collaboration)

Acknowledgements:
Troy Tonner, Alyssa Christoffer, Carol Weaver

PURDUE AGRICULTURE PURDUE UNIVERSITY

PURDUE ENGINEERING Think impact.