Undergraduate Thesis Prospectus

Leveraging Enzyme Excretion in Cell-Free Synthesis of Lactic Acid

(technical research project in Chemical Engineering)

Publish or Perish: Pressures on Academia in Technology Development

(sociotechnical research project)

by

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.
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General Research Problem

How may access to quality, cost-effective materials be improved?

Industrial advances have been interlocked with academic discovery for almost 100 years (Satell, 2016). Academia provides the research and development for fundamental science that is translated into market changing products. Ensuring these products are quality and cost-effective requires thorough and purposeful academic discovery, scalable for application in an industrial setting. However, there is a culture in academia that threatens the publishing of meaningful and innovative science, termed publish or perish (Bello et al., 2023). Focusing energy on pushing papers out rather than creating scalable science impedes the translation of fundamental science into consumer products. Industry also must seek out novel technology for industrial application (Satell, 2016). Understanding how to take academic research and create an industrial process utilizing that technology is key to providing access to quality, cost-effective materials. One of these technologies is cell free synthesis, which can be applied to typically fermented products to increase yield, like lactic acid (Xie et al., 2018; You & Percival Zhang, 2017).

Leveraging Enzyme Excretion in Cell-Free Synthesis of Lactic Acid

How can large-scale manufacturing of lactic be optimized using cell-free enzyme technology?

Introduction

Many industries are dependent on large quantities of biocommodities to continuously run their biochemical processes. Biocommodities, the cheap raw materials essential for almost every chemical and biochemical process, are inexpensive compared to high value products. The cost is heavily reliant on the feedstock cost which accounts for 30%-70% of production expenses (Zhang, 2010). One of the most versatile biocommodities in the current market is lactic acid which has applications in the pharmaceutical, cosmetic, food and beverage, and biodegradable

plastics industries (Datta & Henry, 2006). All these industries are vital to standard products in American life. This already sophisticated market is expected to grow at a rapid rate. Lactic Acid production was a 3.46 billion dollar industry last year and is projected to double by 2031 allowing for a well-designed, cheap production process to crack into the market (Datta & Henry, 2006).

One of the cutting edge methods to cheaply produce biocommodities is cell-free fermentation. In 2010, cell-free synthetic (enzymatic) pathway biotransformations (SyPaB) were shown to increase product yield, improve process flexibility, and hasten reaction rate which will decrease the time required to produce commodities like lactic acid (Zhang, 2010). These enzymes are also recyclable without the downside of cell glucose consumption (Wee & Ryu, 2009). Results from anaerobic cell catalysis experimentation find that 10% of the feedstock is lost from the feed stream with more unconverted feedstock being consumed in recycle streams (Zhang, 2010). By removing cell consumption of feedstock in both the initial and recycled streams, the cost is decreased as the efficiency increases making cell-free catalysis a viable alternative to cell fermentation. Cell-free biotransformation along SyPaB also decreases the amount of waste products because other enzymes within the cell can be removed before reactions are performed if enzyme selection is effectively performed (Zhang, 2010).

The production of lactic acid still faces several constraints, chief among them is waste production (Alves de Oliveira et al., 2018). Cell-free fermentations have less data than traditional cell fermentations (Zhang, 2010). Many iterations of trial and error will need to be conducted to develop an efficient process that mitigates the side products and waste accumulating in lactic acid production. Waste production is accompanied by environmental restrictions that severely reduce allowable production and increase the cost compared to less sustainable and traditional

alternatives (Alves de Oliveira et al., 2018). Cell-free fermentation should reduce these lactic acid side product concerns, but complete elimination is not a reasonable expectation.

Methods

Various unit operations will be employed to create lactic acid from a cell-free reactor and the process is outlined in Figure 1. First, we will ferment *bacillus subtilis* in a retentostat using LB broth as a growth media (Cruz Ramos et al., 2000). This microbe is capable of secreting enzymes which will help increase the purity of the system from the beginning (Abedi & Hashemi, 2020). However, the specifics of the genetic engineering required to produce such a cell line are out of the scope of this project. Next, a disk stack centrifuge will be used to remove any cellular debris and to separate the cells from the secreted enzymes (Phanthumchinda et al., 2018). The supernatant containing our target enzymes and other small secreted molecules will then undergo ion exchange chromatography to isolate our target enzymes based on engineered peptide tags that will be selected in the column (Sullivan et al., 2016). The enzymes of interest are GDH, KDGA, ALDH, DHAD, and L-LDH. The purified target proteins will be transferred to a holding tank until they are needed for the reactor, concluding the batch portion of the process. Enzymes and glucose from food waste will be fed into a continuous stirred tank reactor (CSTR) where the cell-free synthesis of lactic acid will occur (Hodgman & Jewett, 2012). The output will be various small molecule intermediates mixed in with the lactic acid product which will then be purified via microfiltration, with enzymes being recycled back into the reactor (Phanthumchinda et al., 2018). The small molecules and lactic acid remaining will undergo liquid-liquid extraction with butanol and sunflower oil as solvents (Kumar & Thakur, 2019). The remaining output stream will be purified lactic acid which will then be packaged and sold in a liquid solution.

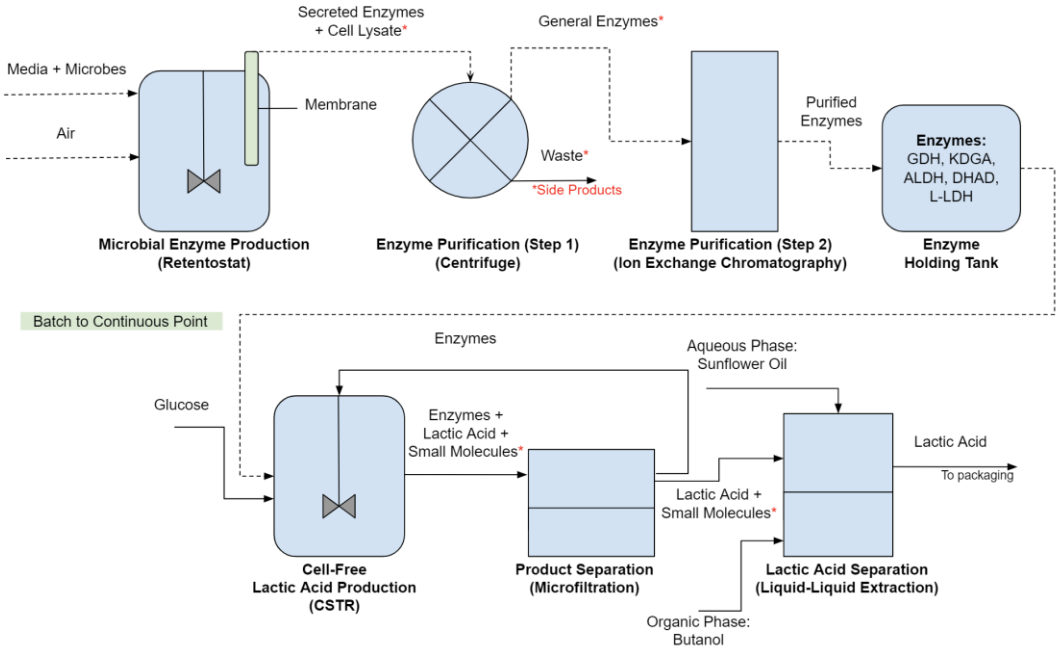

Figure 1: Block flow diagram for cell free synthesis of lactic acid (author).

Logistics

This project will be done by a group of four people (Gavin, Clare, Collin, and Ethan). The initial design will be created in the Fall semester for CHE 4474 and the project will be finished in the Spring semester for CHE 4476. The work will be split amongst the group as follows: Gavin and Clare will be designing the lactic acid reactor and downstream processes needed to purify the lactic acid, Collin will be designing the bioreactor used to produce the necessary enzymes and purification of the enzyme product stream, and Ethan will be researching and analyzing the economics behind the entire process. Every week, the team will meet up to discuss findings and report progress. All of the data needed for the material balances, operating conditions, and economic costs will be obtained from a literature review. Aspen Plus V14 will be used to model the process and simulate its conditions. Matlab will be used to calculate individual material balance equations on each reactor.

Publish or Perish: Pressures on Academia in Technology Development

How are academic researchers, biotechnology companies, and professional societies competing to protect or reform the status quo in academic biotechnology research, in which career incentives pressure researchers to publish frequently and to attract sponsored research?

Academia faces pressure from funding sources, tenure tracks, and their peers to produce science quickly. With recent retractions of peer-reviewed journal articles studying COVID-19 and Alzheimer's disease, understanding academic "publish or perish (POP)" culture impacts on its scientific contributions is crucial.

Involved parties reinforce and reinvent the status quo in academia, with groups often contributing to each practice. Academic institutions provide pressure to its faculty by requiring the "beginning of a national reputation in the candidate's field" which can be achieved primarily through publications (*PROV-017: Promotion and Tenure*, 2011). Academic participants include those committed to ensuring quality research and those who engage in unethical practices (Rawat & Meena, 2014). Publishers are also responsible for peer review of research and defending against fraudulent work (Piller, 2022). Biotechnology companies convert research to consumer products, thus deciding whether they engage in unethical practices (*Securities and Exchange Commission vs. Elizabeth Holmes and Theranos, Inc.*, 2018). Finally, professional organizations like Retraction Watch and Inside Higher Education, which function outside academia, act as a check and balance system on research quality (*Inside Higher Ed*, n.d.; Oransky, 2021).

Researchers explore the interactions between these groups and their agendas. Bello et al. (2023) found POP has a worldwide history in academia and is widely regarded as boosting publication productivity, though these publications may not contribute to scientific innovation. Becker and Lukka (2023) focus rather on gathering empirical, perspective-based evidence of

POP culture, finding instrumentalism to drive academia's beliefs. Bowman (2023) observed the power relations in academia revealed that these relations motivate practices of measuring researchers based on their publications. Connolly (2020) reflects on the impact of COVID-19 on tenure tracked faculty, sharing the experience of one faculty member who was pressured to publish an aged book rather than pursuing innovative teaching methods during the pandemic. Gallup and Svare (2016) also note the shift in higher education institutions towards only encouraging research that brings in external funding, further pressuring faculty to redirect their efforts to be the most profitable.

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