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 cosmeceutical/active and 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): pAPln-ADH-16BVM03 C302L
- 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
- Alternatives 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.[2]
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.

Alternative Solutions
The cell broth has various rheological properties as each batch contains different colonies and showed partial purification as about 99% of Oleic ester was retrieved. However, the resin chromatography successfully separated cells from the product containing cell broth which produce plasticizer (polymer), pharmaceuticals (lubricant), chemical (chemical intermediates, adhesives, and solvents) and cosmeceutical/active and ingredients. For a true lab-scale operation, the resin chromatography is shown to be effective and is expected to be adopted by industries towards incorporating new eco-friendly processes.

Figure 1: Process diagram of continuous biotransformation of oleic acid

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. [3] Hidden costs include indirect costs. All cost estimates are calculated on an industrial scale of production.

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.[4] 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

Table 1: Estimated production cost per cycle of each biotransformation unit operation.

<table>
<thead>
<tr>
<th>Cost Type</th>
<th>Cost Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentation</td>
<td>$300 / L</td>
</tr>
<tr>
<td>Resin Column Chromatography</td>
<td>$200 / L</td>
</tr>
<tr>
<td>Sterilization</td>
<td>$0.30 / kg</td>
</tr>
<tr>
<td>Drying</td>
<td>$20 / kg</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Cost Type</th>
<th>Cost Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Capital Investment</td>
<td>$80,000</td>
</tr>
<tr>
<td>Purchased Equipment</td>
<td>$10,761.44</td>
</tr>
<tr>
<td>Installation</td>
<td>$20,333.33</td>
</tr>
<tr>
<td>Total TCI</td>
<td>$121,724.77</td>
</tr>
</tbody>
</table>

Figure 2: Result of resin columns chromatography absorption (left) and desorption with Ethyl Acetate (right)

Figure 3: Experimental industrial layout.