Enzyme-Assisted Pathogen Detection Applied to a Microfiltration System for Food Safety

Jaycey Hardenstein1,2, Alisha Tungare1,4
Seockmu Ku1,2, Tommy Kreke1, Kirk Foster3, Eduardo Ximenes3, Xingya Liu1, Michael Ladisch1,2,3,4
1Laboratory of Renewable Resources Engineering, 2Department of Agricultural and Biological Engineering, 3Weldon School of Biomedical Engineering, 4School of Chemical Engineering, Purdue University, West Lafayette, Indiana, USA

The Problem

With a growing number of consumers in the American market and with food production at an all-time high, food safety is a huge priority for both consumers and corporations everywhere.

Recent progress has slowed in the fight against *Escherichia coli* infections, with the number of infection rates gradually growing.1

56% of *E. coli* outbreaks are a result of contaminated beef.2

5 days are required for detection of *E. coli* according to the enrichment method, which is the standard protocol used by the Food Safety and Inspection Service (USDA).3

Though commercial alternatives are available, detection methods still require 1-2 days before results can be confirmed.4 Thus, there is a critical need for improving the methods of detecting potential contaminants in food.5

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The C3D Technology

The Continuous Cell Concentration Device (C3D) developed by the Laboratory of Renewable Resources Engineering (LORRE) at Purdue University has the potential to reduce the testing time required to detect pathogens in food by concentrating microorganisms and thereby reducing the sample volume.6

Operating Principle of Cross-Flow Microfiltration

The C3D utilizes hollow fiber membranes to concentrate the food sample.

Pretreatment Process

- Enzyme Treatment
  - Enables microfiltration within the C3D
- Pretreatment food solution
  - (Large macromolecules are filtered out to prevent membrane plugging in C3D)

Microfiltration Process (C3D)

- Microfiltration
  - The C3D utilizes hollow fiber membranes to concentrate the food sample
- Concentrated food solution
  - 250 mL Food sample solution

Project Goal

This research investigates the role of enzymes to enable microfiltration and ensure recovery of *E. coli* in ground beef. Experiments were conducted to determine the effect of enzyme hydrolysis, if any, on microbial cell growth and recovery.

Methodology

25 g of ground beef

250 mL Solution

2 mL - 2.3 log CFU/mL E. coli

Homogenate by stomaching

Pre-Filtration

Inoculate to enable enzyme hydrolysis

Cell population (Salmonella, 10¹ CFU/mL) growth rate when inoculated with protease[6]

Addition of protease (Promod™ 298L or Protex™ 7L) to egg white homogenates compared to a control. Population growth rates up to 120 minutes of inoculation time are not significantly different at the 95% confidence level.

Once processed, cell populations are quantified by plating on selective media. Microfiltration rates are obtained throughout the concentration process in the C3D.

Results

The C3D allows for faster microfiltration rates to increase percent cell recovery.

Table 1. Enzyme Loading and Percent Recoveries

<table>
<thead>
<tr>
<th>Enzyme Loading</th>
<th>Trial</th>
<th>% Recovery from C3D</th>
<th>Total % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.40%</td>
<td>1</td>
<td>1%</td>
<td>4.88%</td>
</tr>
<tr>
<td>Average</td>
<td>2</td>
<td>4%</td>
<td>16.67%</td>
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<tr>
<td></td>
<td>3</td>
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<tr>
<td>2%</td>
<td>5</td>
<td>30%</td>
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<tr>
<td>Average</td>
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<td>61%</td>
<td>39.24%</td>
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<tr>
<td></td>
<td>7</td>
<td>55%</td>
<td>36.50%</td>
</tr>
<tr>
<td>4%</td>
<td>Average</td>
<td>59%</td>
<td>37.67%</td>
</tr>
</tbody>
</table>

As seen in Table 1, percent recovery for 0.4% enzyme loading was extremely low, indicating that a higher enzyme loading may be necessary to facilitate *E. coli* recovery. 4% enzyme loading results showed the highest average percent recovery for both the C3D and overall process.

Conclusions

Enzyme Loading on Cell Viability

90 mins The maximum enzyme hydrolysis time up to which differences in cell viability are not significant when compared to non-enzyme treated samples.

>0.40% enzyme loading is viable for treating ground beef solutions. Differences in cell viability are not significant between 0.40%, 2%, and 4% enzyme loading up to 2 hours.

C3D Processing and Cell Recovery

4% Enzyme loaded samples had significantly larger cell recoveries for both the C3D and overall process.

Generally, as the percent enzyme loading increases, percent cell recovery increases.

Future Work

Although these experiments have validated the use of enzymes in enabling microfiltration and ensuring recovery of *E. coli* in ground beef, additional research must be completed to further optimize the concentration process:

- Test additional enzyme ratios to further optimize microfiltration
- Analyze options for various selective enrichment mediums in minimizing background microorganisms and maximizing the *E. coli* recovery
- Determine the optimal enzyme hydrolysis time to minimize cell losses during pre-filtration

Sample microfiltration rate throughout the C3D

The microfiltration rate over the course of a sample run significantly decreases. Optimization of the pretreatment process will enable faster microfiltration rates to decrease the time for pathogen detection.

References


Acknowledgements

This research was funded by Cooperative Agreement ARS 5955-42000-061-020 and the Purdue University Center for Food Safety Engineering, as well as USDA Hatch Project 10677 and 10646. Additional support was provided by the Agricultural and Biological Engineering Department, School of Chemical Engineering, the Honors College, and the College of Agriculture at Purdue University. The authors would also like to thank Dr. Amanda Deering for helpful discussions, Dr. Lisa Mauer for support of undergraduate involvement in CFSE, and Catharine Patrone for coordinating event logistics.