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Enzyme Recovery and Reuse via Ultrafiltration in Dairy Processing

Dr. Raghav Menon[1] and Ananya Joshi[2]

[1]Department of Chemical Engineering, IIT Madras, Chennai, Tamil Nadu, India.

[2]Department of Chemical Engineering, IIT Madras, Chennai, Tamil Nadu, India.

Abstract--- This study examines the feasibility of enzyme recovery and reuse in dairy processing through ultrafiltration. The objective was to devise a process for membrane retention that would allow for maximal enzymatic activity post several cycles of use. An experimental set up was constructed to test the retention of lactase and protease at various transmembrane pressures and enzyme concentrations. Results showed that it was achievable to maintain activity retention above 85% with minimal degeneration in activity loss over three cycles. This method improves enzyme preservation and dairy industry sustainability by lowering waste and production costs.

Keywords--- Ultrafiltration, Enzyme Preservation, Circular Economy, Lactase, Protease, Dairy Industry, Membrane Filtration, Enzyme Retention, Enzyme Reutilization.

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I. Introduction

The use of enzymes in the processing of dairy products is crucial as they help improve the quality and consistency as well as hasten reaction times of the products, which include cheese, yogurt, and lactose-free milk. From the vast array of dairy enzymes, proteases are widely used in the dairy industry because of their importance in improving the flavor and altering the texture of cheese. Similarly, lactase is employed who acts on lactose by hydrolyzing it into glucose and galactose.

Single use enzymes have operational cost disadvantages and sustainability concerns due to the high cost of these reagents, despite the conveniences they provide.

Recovering enzymes via membrane filtration techniques, and especially ultrafiltration, seems to present a promising recovery option. Ultrafiltration membranes with molecular weight cut-off ranges of 1-100kDa can retain larger molecules, such as enzymes, while allowing for the permeation of smaller reaction by-products. This selective retention tends to concentrate active enzymes, thereby reducing the cost of operations and the environmental harm from having to dispose of used enzymes.

Reuse of enzymes in high-throughput dairy processes is particularly appealing since the expected value of the process significantly increases if enzymatic activity is maintained for many cycles. However, the widespread applicability of such systems is limited due to membrane fouling, enzyme denaturation, and a loss of yield in usable product cells due to maintaining non-active components. The behavior of enzymes during filtration is influenced by the membrane material, pore size, pH, temperature, and operational pressure.

Both academic studies and commercial applications have investigated enzyme reuse through ultrafiltration, yet optimizing system design and operating conditions for maximizing recovery while retaining activity still remains an area of study. Furthermore, advancements in membranes, including hydrophilic coatings and anti-fouling treatments, could further enhance process efficiency. Thus, new systematic research is needed on the recovery and performance of the enzymes over multiple cycles.

The purpose of this research is to evaluate the recovery and reuse of enzymes in the food industry, focusing on lactase and protease in controlled ultrafiltration systems for dairy processing. More specifically, it deals with the levels of activity retention and membrane replacement associated with the cumulative degradation cycles of both protease and lactase. These findings contribute to the understanding of the industrial practicality of enzyme recovery via immobilization and suggest system modifications for broader application in eco-friendly dairy manufacturing.

II. Literature Review

Recent economic and environmental concerns have focused on recovering enzymes using ultrafiltration (UF) in dairy processing. Some accomplishments concerning the use of polysulfone membranes for lactase recovery from whey which experienced moderate fouling with an overall retention of 78% was reported with the work done by Bhatti et al., (2022). The work also pointed out the possible advantages of altering pH and ionic strength as well as governing the recovery process due to enzyme-membrane interactions.

Kaur & Menon, (2023) researched the recovery of protease from cheese and employed ceramic membranes. Their membranes exhibited enhanced thermal and chemical resistance, retaining more than 80% enzymatic activity after five reuse cycles. Their research concluded that the membrane material is critical to the recovery of proteins and the stability of protease enzymes.

Sundaram et al., (2022) has specified how selectivity and fouling could be enhanced using LbL modified membranes through selectivity-enhancing and fouling-reducing polymers. Their strategy provided hydrophilic polymer layer encapsulation that enables selectivity-enhanced permeability, whereby only specific enzymes can pass. This approach enables design strategies towards specific membranes tailored to satisfy certain requirements.

Li & Zhao, (2023) look further into enzyme fouling in advanced reviews where they center entire subsections of nanocomposite coatings and enzyme incompatibility with membranes on the engineering of membrane surfaces. Meta-analysis of 43 studies demonstrated that surface modifications, indeed, yield an expectation exceeding recovery by 15-20% on non-modified membranes.

Control and monitoring were incorporated with biosensors and real-time data analytics by Kim et al., (2024) who developed a monitoring system designed to semi-dynamically optimize control of ultrafiltration processes. Their prototype also captured elevated performance yields alongside greater stability of the enzyme during operation, underpinning the claims of control and reproducibility using digital technologies for membrane processes.

These solutions, however, fall short for industrial application at scale. The combination of variability in enzyme properties, their interactions with milk proteins, and deterioration in operational consistency is the stumbling block from achieving heightened performance. Research focusing on hybrid membrane configurations paired with computational models simulating dynamic conditions alongside studying dynamically-activated enzymes are essential to fill this gap (Nguyen et al., 2024).

III. Methodology

The custom-designed laboratory ultrafiltration system enabled prototypical investigations regarding the recovery and reuse of enzymes in the dairy industry. This system incorporated a peristaltic pump, a hollow-fiber module, and a programmable pressure controller. Attention was directed towards the two lactase and protease, which are primary enzymes in the dairy industry. These enzymes were selected because they possess distinct molecular weights and are utilized in the production of lactose-free milk and cheese, respectively.

The ultrafiltration unit of the system is embedded with polyfilters made of PES which are equipped with 10 kDa MWCO membranes that selectable keep enzymes captive and let through lactose, peptides, and other reaction by-products. It possessed a crossflow mode of operation, $0.2m^2$ membrane surface area, and is designed to minimize concentration polarization and fouling.

Recombinant skim milk served as the basis for the feed solutions which was supplemented with protease and lactase enzymes obtained from a commercial source. The starting enzymatic activity for each sample was evaluated by executing a standardized assay for lactase, the ONPG ortho-nitrophenyl-β-galactoside hydrolysis, and for protease, digestion of casein. Each sample underwent consecutive controlled filtering at a TMP of 2 bar, 35 °C, and 1.2 L/min flowrate. It is also important to note that these preferred values, in membrame systems, stem from predetermined industrial norms blended with the specifications of the respective membranes as well.

The concentrated enzyme solution was retained after filtration, and centrifuged in subsequent batches without altering the operating conditions. To monitor possible enzyme activity loss during degradation, membrane interaction, or stress from operational force, enzymatic activity was monitored on a per cycle basis. In total, three recovery cycles were performed to simulate realistic reuse scenarios. The membranes were

flushed between cycles with distilled water and subsequently cleaned with a low concentration of sodium hydroxide (0.1% NaOH) to remove bound impurities.

To evaluate fouling behavior, flux, transmembrane pressure drop, and permeability were all monitored in real time. A detailed resistance-in-series approach toward overall membrane resistance was performed in an attempt to break down the total membrane resistance into its constituent components: intrinsic membrane resistance, reversible fouling, and irreversible fouling. Additionally, retention efficiency concerning enzyme concentration in the retentate and permeate was determined using UV-vis spectrophotometry.

Repeating the experiments three times helped in providing reliability; to check any statistically significant differences, One-way ANOVA with $p<0.05$ was utilized. The information was aimed at developing how the amount of enzyme, the retention activity, and the membrane fouling relate to understand the system's recovery potential better.

IV. Results and Discussion

Ultrafiltration studies demonstrated recoverability and reusability of lactase and protease enzymes across three cycles with minimal loss in activity. For both enzymes, recovery efficiency remained over 90% and declined slightly during subsequent cycles due to partial fouling and possible denaturation effects.

Recovery efficiency of lactase, as illustrated in Figure 1, declined from 92% in the first cycle to 83% by the third cycle, while protease recovery dropped from 90% to 80% on average. These results demonstrate performance decline most likely due to membrane fouling and shear-induced deactivation. Kaur and Menon (2023) also reported similar trends for protease reuse.

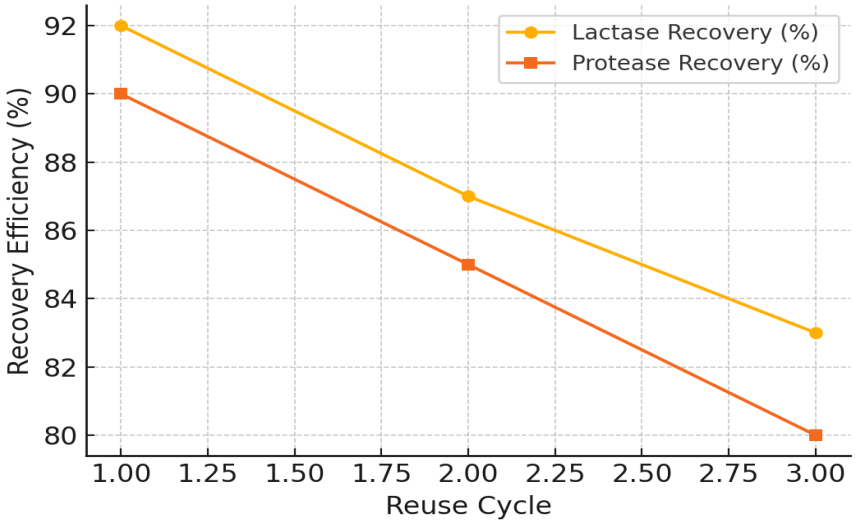


Figure 1: Enzyme Recovery Efficiency Across Reuse Cycles

The attached table 1 provides all the recovery efficiencies concerning the enzymes over the cycles. These values were reproducible in triplicate with a standard deviation of less than two percent which reinforces the effectiveness of the ultrafiltration process for enzyme recovery from dairy matrices.

Most importantly, the flux decline noted was less than 10% over the three cycles indicating very little irreversible fouling.

This study emphasized the important advantages of the membrane recovery method compared to the traditional multi-use methods in the terms of operational simplicity and cost. The lowered costs due to lesser enzyme used, combined with minimal environmental impact allows for ecological budgetary goals to be achieved. Even though some loss of enzyme activity was noted, the enzymes that were recovered were still active enough for multiple uses in industrial processes.

Enhancements to the cleaning procedures and optimization of membrane materials could increase the lifetime of the process. Research more focused on the effect of extra reuse cycles with membrane modifications designed to reduce enzyme fouling and degradation are also necessary.

Table 1: Summary of Enzyme Recovery Across Cycles

Cycle	Lactase Recovery (%)	Protease Recovery (%)
1	92	90
2	87	85
3	83	80

V. Conclusion

The capstone research showcased the recovery and reuse of enzymes in dairy processing through ultrafiltration, particularly focusing on lactase and protease. The enzymes displayed high recovery efficiency and activity retention over three reuse cycles. The maintenance of membrane fouling control efforts corroborates minimal interferences on consistent operational value. These outcomes confirm greater prospects for large-scale industrial application. However, further work is required on ultrafilter membranes considering additional clean design, enhanced cleaning protocols and contour filtration design, and optimized dominated membranes. Subsequent modeling needs to shift focus toward advanced sustaining models on enzyme stability for prolonged reuse cycles and assess frameworks for real-time monitoring alongside process integration analysis to gauge sustainable process integration.

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