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# Modifications in the CIP protocols for removing biofilms on whey RO membranes



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## **Project Objectives**

Evaluation of Modified CIP in biofilm control on whey RO membranes.

## **Description of Work Performed**

The present investigation was undertaken to evaluate a typical CIP protocol against membrane biofilm isolates in planktonic and embedded states. The CIP protocol tested against the constitutive microflora of membrane biofilms included six treatment steps based on; alkali, surfactant, acid, enzyme, a second surfactant, and a weekly sanitizer application. Single and mixed species biofilms developed under *in vitro* static and dynamic conditions were treated with individual steps of existing CIP protocol.

The results obtained from the chemical treatments against 24h old biofilms confirmed the higher effectiveness of acid treatment against biofilm embedded cells amongst all the CIP chemicals. *Bacillus* isolates showed the highest resistance in planktonic, as well as, biofilm embedded states. Studies based on the application of sequential CIP protocol against all the consortia indicated survivors even after the complete cleaning process including the sanitizer treatment step. This study thus helped to conclude that the existing CIP protocol was not effective to completely remove biofilms developed on the membrane surface.

The study aimed to remove membrane biofilms by developing improved cleaning strategies, especially the enzyme cleaning step.  $\beta$ -galactosidase enzyme was observed to be the most effective under static and dynamic conditions. In addition, a Protease and a Lipase were also observed to be effective against biofilms developed using the most resistant *Bacillus* isolate. Studies related to the CIP modifications using  $\beta$ -galactosidase enzyme alone (CIP-1) revealed lower efficacy as compared to the existing CIP protocol against single and mixed species biofilms. Similarly, the surfactant replacement trials using Span 85, and Tween 85 also did not result in any improvements in the existing CIP protocol. The second approach (CIP-2) by modifying the cleaning conditions (pH and time) resulted in better biofilm removal as compared to the existing CIP protocol. Similarly, combination of protease, lipase, and  $\beta$ -galactosidase enzymes, and the modified cleaning conditions used as the third approach. (CIP-3) indicated higher log reductions against single and mixed species biofilms as compared to the CIP-1 and CIP-2 treatments.

## **Results of Technology or Process Assessed**

The modifications in the cleaning protocol based on replacement of enzyme cleaning step and the other modified cleaning conditions resulted in better cleaning of biofilms as compared to the existing protocol.

## **Benefit to Minnesota Economic Development**

The project findings will benefit the dairy industry by producing better microbial quality whey products with eventual benefit to the industry. The better cleaning protocol will also save expenses incurred in premature replacement of membranes due to membrane biofouling.

**Conclusions**

The existing CIP protocol is inadequate to control membrane biofilms, and modifications such as enzyme cleaning step can help in improving its efficacy.

**Future Needs/Plans**

Further work is necessary to study combinations of various enzymes and surfactants as modifications to the existing CIP protocol to make it more effective in controlling biofilms in membrane processing operations.