Evaluation of different cleaning agents for the removal of membrane biofilms

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Project Objectives

1. Inhibit and control biofilm on whey reverse osmosis (RO) membranes

Description of Work Performed

Provide a brief description of the work performed and their results.

Dairy concentration membranes are prone to biofouling, which leads to their reduced performance and also poses quality and food safety issues. In the previous studies, we demonstrated the role of bacterial biofilms in RO whey concentration membrane biofouling process. Culture and microscopic techniques provided clear evidence of mixed species biofilm formation on the membranes obtained from an industrial operation at the intervals of 2 months for a total duration of 14 months.

The presence of *Bacillus, Escherichia coli*, *Klebsiella oxytoca, Enterococcus, Streptococcus, Staphylococcus, Micrococcus, Aeromonas, Corynebacterium*, and *Pseudomonas* species were frequently encountered in biofilm matrices. Many of these organisms were also found to produce extrapolysaccharides (EPS) that helped in their firm adhesion to the membrane. The CIP protocol tested against the planktonic and 24h old biofilms of above microflora included five treatment steps based on; alkali, surfactant, acid, enzyme, a second surfactant, and a weekly sanitizer application.

The results confirmed the resistance of isolates in both planktonic and embedded states against most of the five treatment steps. The acid based treatment was found to be quite effective, which resulted in 4.54 to 7.64 logs reduction in case of planktonic cells, and 2.09 to 4.58 logs reduction against embedded cells in 24h old biofilms formed under lab conditions. The sanitizer treatment step also showed similar results against planktonic cells and caused a reduction of 4.93 to 8.32 logs. On the other hand, it was much less effective against the embedded cells in biofilms, and resulted in a reduction of only about 1.19 log counts. *Bacillus* sp. showed highest resistance in planktonic cell, as well as, embedded cell state.

On the basis of these results, it can be concluded that the biofilms formed on the RO whey concentration membranes were resistant to the CIP protocol being used.

Results of Technology or Process Assessed

The existing CIP protocol from a whey concentration plant was tested against the biofilm consortia isolates obtained from used whey RO membranes (2 to 14 months old). All the isolates of the entire constitutive microflora were compared for their resistance to chemicals as an individual step for both as planktonic cells and biofilm embedded state. The six steps CIP process was based on; alkali, surfactant, acid, enzyme, a second surfactant, and a weekly sanitizer application. Of these, the sanitizer treatment was found to be most effective in killing the biofilm isolates in the planktonic cell state resulting in 4.93 to 8.32 log reduction. The other effective step was the acid treatment, which resulted in about 4.54 to 7.64 log reduction. Other treatment steps always resulted in a lower reduction in the range of 1 to 5 logs. The individual isolates showed a great variability in their sensitivity towards different chemical treatments. In general, the *Bacillus sp.* showed greater resistance as compared to the other genera such as *Enterococcus, Escherichia,*

Streptococcus, Staphylococcus, Klebsiella, etc. Acid treatment was most effective against the biofilm embedded cells resulting in a reduction of 2.09 to 4.58 log counts, whereas, the sanitizer treatment resulted in an average reduction of about 1.19 logs only.

All six cleaning steps of the existing CIP protocol were individually tested against single and mixed species biofilms developed under dynamic conditions of growth using a CDC biofilm reactor.

Biofilms (24 h old) of the most resistant *Bacillus* isolate were developed and analyzed. The acid treatment step of the CIP protocol was the most effective cleaning agent followed by the sanitizer treatment. Our previous studies, conducted under static conditions, showed greater reduction levels for the two steps respectively, against the same organism.

In further studies, 24h old mixed species biofilms were developed under the dynamic system by using the constitutive microflora (*Bacillus* sp., *Staphylococcus* sp., and *Streptococcus* sp.) of a 10 month old membrane biofilm, and tested against individual cleaning steps of the CIP protocol. In comparison to the above results on single species biofilms, the acid treatment and sanitizer steps resulted in lower reduction in case of the mixed species biofilms

Benefit to Minnesota Economic Development

Present investigation provided useful information related to the potential modifications of the existing CIP protocol for removing resistant biofilms on membranes. This would not only help in improving the efficiency of membrane processing of whey, but will also help improve the microbial quality safety of the final product.

Conclusions

The studies conducted so far established the resistance of bacterial isolates in both planktonic and embedded state. Acid treatment was observed to be most effective against planktonic, as well as, embedded cells. Sanitizer treatment was more effective against planktonic cells as compared to the biofilm embedded cells. Based on survival pattern of single species *in vitro* biofilms, *Bacillus* isolates were observed to be the most resistant organisms. Differences were noticed in the resistance pattern of biofilm
isolates as the membrane aged. The results obtained from application of the sequential CIP protocol, using steps 1 through 6, against mixed consortium biofilms, revealed the resistance of biofilm embedded cells against the existing CIP protocol. Enzyme β- galactosidase showed promise by degrading the biofilm matrix of *Bacillus* biofilms to a higher extent. It may be able to replace the enzyme step of the existing protocol for better cleaning efficiency.