Controlling Biofilm Development on Ultrafiltration and Reverse Osmosis Membranes Used in Dairy Plants

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ABSTRACT

This study aimed to develop improved cleaning strategies for controlling biofilms on the surfaces of membranes used in dairy ultrafiltration (UF) and reverse osmosis (RO) plants.

Eleven UF / RO membrane modules from 7 different New Zealand dairy membrane processing plants were received after typical cleaning-in-place (CIP) procedures. Microorganisms were isolated from both the retentate and permeate sides of these membrane surfaces and from the liquids collected from a UF membrane plant. Also some foulants scraped from a RO membrane were tested. The routine CIP currently used in the dairy plants was not adequate to completely remove organic material, including microbial cells, proteins and carbohydrates from the membrane surfaces. These residues may influence the surface characteristics and interactions between microorganisms and membranes and thus affect biofilm formation. Thirteen isolates including both bacteria and yeast were identified using biochemical techniques. *Klebsiella oxytoca* were isolated from 3 different membrane plant sites. This is, so far as we know, the first report of *K. oxytoca* being isolated from dairy membrane surfaces. The ability of the 13 strains to attach to negatively charged polystyrene surfaces was tested using a microtitre plate assay. Three *K. oxytoca* strains demonstrated higher ability to adhere than the other strains, suggesting that these strains might play an important role in developing biofilms on dairy membrane surfaces. Two *K. oxytoca* strains (*K.* B006 from plant A, UF and *K.* TR002 from plant C, RO) that performed best in the microtitre screening assay with respect to attachment capabilities were chosen for the remainder of the study.

The cell surface hydrophobicity of all isolates was determined using the microbial adhesion to hydrocarbon assay (MATH) and the cell surface charge was determined by measuring the surface zeta potential. These two characteristics did not show a clear relationship with the adherence of the isolated strains. However, it was found that bacterial attachment was enhanced in the presence of whey or mixed strains.

A commercial biofilm reactor CBR 90 was modified for developing biofilms on membranes and investigating strategies for biofilm removal. Biofilms of single and dual *K. oxytoca* strains were developed under a continuous flow of whey. The saturated biofilm was approximately 8 $\log_{10}$ CFU cm^{-2}. The results of our study suggested that the whey protein concentration, membrane type including membrane material (polyethersulfone (PES) and polyvinylidene fluoride (PVDF)), membrane age (used and new), bacterial strain and the interactions between different microorganisms are all significant factors for biofilm development on membrane surfaces.

Three enzymatic cleaners and four sanitisers, including sodium hypochlorite (pH 6.5, 200 ppm free available chlorine (FAC)), Perform® (peracetic acid/hydrogen peroxide, 2% v/v), ozonated water (pH 7.0, 0.5 ppm free available ozone (FAO)) and anolyte of MIOX® electrolysed water (EW) (pH 6.8, 120 ppm FAC) were tested for their efficacies in killing culturable cells from biofilms formed by single or dual *K. oxytoca* strains on used PES membrane surfaces. With no sanitation applied, two of three enzymatic cleaners performed better than sodium hypochlorite (pH 10.8-11, 200 ppm FAC) commonly used for CIP of UF membranes in the dairy industry. The four sanitisers were used to treat the membranes after a CIP wash regime. The results indicated that if a dairy processor were to use a standard CIP on membrane systems, then a further flush with MIOX® EW anolyte would reduce residual attached microbial populations further. In addition, using protease followed by a sanitation (sodium hypochlorite, Perform® or anolyte of MIOX® EW) produced the best clean based on a greater than 2 log reduction in residual cells and left no culturable and viable cells at a detection limit of 0.1 $\log_{10}$ CFU cm^{-2}.

Keywords**:** biofilm, dairy, ultrafiltration, reverse osmosis, membrane, *Klebsiella*, attachment, surface hydrophobicity, surface charge, CIP, electrolysed water, enzymatic cleaner

LIST OF PUBLICATIONS

This work has been published in part in the following papers:

1. Tang, X., Flint, S.H., Brooks, J.D., & Bennett, R.J. (2010). The efficacy of different cleaners and sanitisers in cleaning biofilms on UF membranes used in the dairy industry. *Journal of Membrane Science, 352*(1-2), 71-75.

2. Tang, X., Flint, S.H., Brooks, J.D., Bennett, R.J., & Morton, R.H. (2009). Biofilm growth of individual and dual strains of *Klebsiella oxytoca* from the dairy industry on ultrafiltration membranes. *Journal of Industrial Microbiology and Biotechnology, 36*(12), 1491-1497.

3. Tang, X., Flint, S.H., Brooks, J.D., & Bennett, R.J. (2009). Factors affecting the attachment of micro-organisms isolated from ultrafiltration and reverse osmosis membranes in dairy processing plants. *Journal of Applied Microbiology, 107*(2), 443-451.

LIST OF PRESENTATIONS

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4. Tang X., Flint S. H., & Brooks J. D., (2007). Controlling biofilm development in membrane plants. *New Zealand Institute of Food Science and Technology (NZIFST) 2007 Conference* (pp. 53), Wellington, New Zealand

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LIST OF CONTENTS

ABBREVIATIONS

Atomic Force Microscopy	AFM
Attenuated Total Reflection – Fourier Transform Infrared spectroscopy	ATR-FTIR
Autoinducer-2	AI-2
Bovine Albumin	BA
Bovine Serum Ablumin	BSA
Cellulose Acetate	CA
Cholerae Autoinducer 1	CAI-1
Clean-in place	CIP
Commercial Biofilm Reactor	CBR
Confocal Laser Scanning Microscopy	CLSM
3, 5-dinitrosalicylic acid	DNS
Electrolysed Water	EW
Extracellular Polymeric Substances	EPS
Free Available Chlorine	FAC
Free Available Ozone	FAO
Glycomacropeptides	GMP
Hydrophobic Interaction Chromotography	HIC
Microbial Adhesion to Hydrocarbon Assay	MATH
Microfiltration	MF
Milk Permeate	MP
Milk Protein Concentrate	MPC
Molecular Weight Cut-Off	MWCO
N-acylhomoserine Lactones	AHLs

Nanofiltration	NF
N-nonanoyl-cyclopentylamide	C_9-CPA
New Zealand's Biotech	NZBio
NEW Zealand Institute of Food Science and Technology	NZIFST
New Zealand Microbiological Society	NZMS
New Zealand Society for Biochemistry and Molecular Biology	NZSBMB
Optical Density	OD
Peracetic Acid	PAA
Phosphate Buffer Saline	PBS
Polyamide	PA
Polyethersulfone	PES
Polymerase Chain Reaction	PCR
Polysulphone	PS
Polyvinylidence Fluoride	PVDF
Reverse Osmosis	RO
Scanning Electron Microscopy	SEM
Skim Milk Agar	SMA
Standard Deviation	SD
Standard Plate Count Agar	SPCA
Thin Film Composites	TFC
Trans-membrane Pressure	TMP
Tryptocase Soy Broth	TSB
Ultrafiltration	UF
Whey Permeate	WP
Whey Protein Concentrate	WPC

LIST OF FIGURES

LIST OF TABLES