Modelling Biofilm Formation in a Dairy Wastewater Irrigation System

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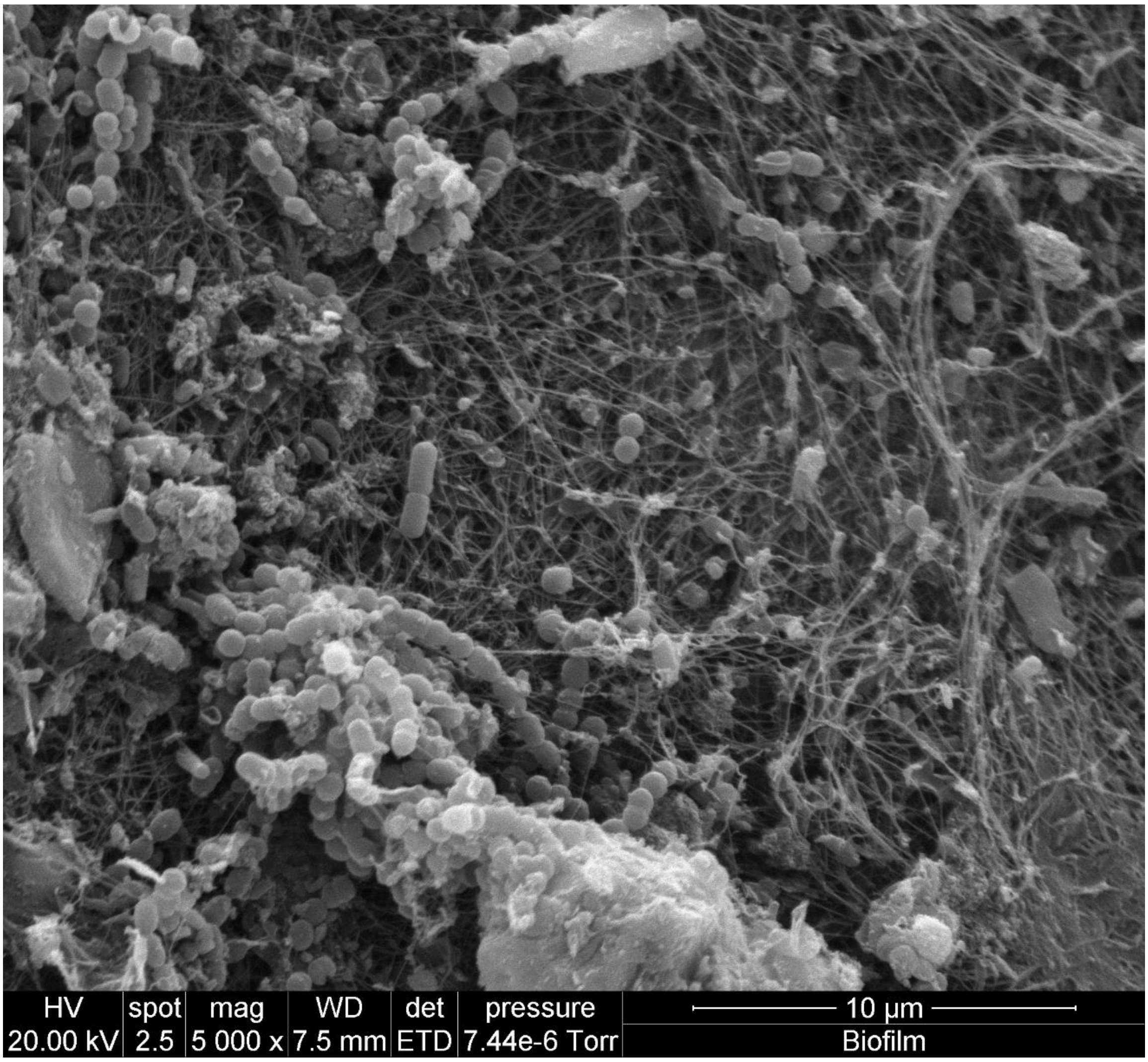
Frontispiece

SEM picture of the extreme biofilm isolated from a dairy wastewater irrigation system.

Credit: Manawatū Microscopy imaging centre.



Abstract

The increase in international demand for milk products has resulted in a corresponding increase in dairy wastewater from dairy manufacturing plants that requires treatment. Every stage of the manufacturing process generates wastewater with up to 10 L produced for every litre of milk processed.

This thesis focuses upon a case study from a New Zealand milk powder plant, which experienced an extreme biofilm formation that blocked the irrigator nozzles of a primary treated dairy wastewater irrigation system. The total microbial population entering the wastewater system and the biofilm formation of the culturable fraction were determined in an attempt to understand the cause of the extreme biofilm formation. Next Generation Sequencing (NGS), showed Gram-negative dominated the microbial population, which was reflected in the culturable isolates from the extreme biofilm and wastewater samples taken after the extreme biofilm event. 16s rRNA sequencing identified 23 isolates: 10 *Citrobacter* (43.5%), six *Klebsiella* (26%), two *Pseudomonas* (8.7%), three *Enterobacter* (13%), one *Raoultella* (4.4%) and one *Bacillus cereus* (4.4%). The *Raoultella* spp was considered unique as it was only cultured from the extreme biofilm, however, this genus was also detected in the wastewater using NGS.

Four isolates from the extreme biofilm where assessed for their responses (biofilm formation, growth rates, yield, and saturation constants) to varying environmental conditions. Nutrient level, temperature and Ca^{2+} significantly affected the biofilm formation individually while Na^{+} and Mg^{2+} had interactions with other effects. Growth rates were dependent on the nutrient level and ion content, however, growth in aerobic and anaerobic environments was found to be statistically ($P < 0.05$) indistinguishable. Bacteria exhibited slowest growth in the presence of Ca^{2+}, however, Ca^{2+} significantly increased the yields over other ions in three of the four bacteria. These different effects on the growth rates, yields, saturation constants and biofilm forming ability suggest that more than one mechanism is involved in the use of these ions. These ions could influence the excretion and production of extracellular polymeric substances, metabolic pathways, or divalent cation bridging (DCB).

The developed model predicted the planktonic and biofilm populations of the four isolates taken from the extreme biofilm. Two trials were performed to test the capabilities, a high nutrient (optimal levels as found in laboratory) 20% TSB and a low nutrient (levels at average wastewater conditions) 4% TSB. It was found that the model accurately predicted the biofilm population level in the low nutrient environment while over predicting the observed levels by 0.5 log CFU/m^2 in the high nutrient environment. Planktonic predictions in both environments were approximately 1 log CFU/m^3 below the observed levels. It was also noted that predicted steady state levels in the planktonic populations were reached approximately 7 hours after those observed. This is most likely due to either the measured bio-transfer rate being slightly different in the reactor trials or death of bacteria in the system. However, the two trials show the model providing good predictions of the biofilm levels with varying nutrient contents. Therefore, this will allow for the quick assessment of the biofilm levels in the dairy wastewater irrigation system with changing conditions.

Limitation of the study

1. The high through put microtiter plate assay did not replicate the flow conditions of the dairy wastewater irrigation system

2. The lab scale reactor did not match the turbulent flow in the dairy industry as this was impractical

3. This is only applicable to the site in question that the model was developed for

4. The model was developed using Monod kinetics

5. This was performed on the observation that the ions Ca^{2+}, Mg^{2+}, and Na^{+} were limiting microbial growth

List of Publications

Dixon, M. Flint, S. Teh, K. H. & Mellow, K. (2015) Biofilm issues in dairy waste effluents In: Teh, K.H. Flint, S. Brooks, J. & Knight, G. Biofilms in the dairy industry. Chichester. Wiley, pp. 189-202

Dixon M. Flint S. Palmer J. Love R. Biggs P. Beuger A. (2017) Analysis of culturable and non-culturable bacteria and their potential to form biofilms in a primary treated dairy wastewater system. Environmental Technology. 1-8. doi.org/10.1080/09593330.2017.1352034

Dixon M J L. Flint S H. Palmer J S. Love R. Chabas C. Beuger A L. (2018) The effect of calcium on biofilm formation in dairy wastewater. Water Practice & Technology. 13:2, 1-10. doi: 10.2166/wpt.2018.050

List of Presentations

Dixon, M., Beuger, A., Love, R., Palmer, J. & Flint, S. H. (2014) Case Study of bacteria in dairy wastewater biofilms. In NZ Microbiological Society Conference, Wellington

Dixon, M. (2014) Update Fonterra Environment Technical group PhD progress

Dixon, M. Beuger, A., Love, R., Palmer, J. & Flint, S. H. (2014) Biofilm Formation in dairy wastewater Systems. Research Group PhD seminar day

Dixon, M. (2015) Update Fonterra Environment Technical group PhD progress

Dixon, M., Beuger, A., Love, R., Palmer, J. & Flint, S. H. (2015, October 24). The effect of varying nutrients, pH and ion content on the biofilm formation of bacteria from dairy plant wastewater streams. In 7th ASM Biofilm Conference.

Dixon, M., Beuger, A., Love, R. Palmer, J. & Flint, S. H. (2015, November 2). The effect of varying nutrients, pH and ion content on the biofilm formation of bacteria from dairy plant wastewater streams. In NZ Microbiological Society Conference, Rotorua

Michael Dixon

Dixon, M., Palmer, J., Love, R., Beuger, B., Bamford, T. (2016) Biofilm formation in dairy wastewater treatment systems. Research Group PhD seminar day

Dixon, M., Palmer, J., Love, R., Beuger, B., Bamford, T. (2016) Modelling biofilm formation in dairy wastewater treatment systems. In NZ Microbiological Society Conference, Christchurch

Dixon, M. (2016) Update Fonterra Environment Technical group PhD progress

Dixon, M. Beuger, A., Love, R., Palmer, J. & Flint, S. H. (2017) Modelling Biofilm Formation in dairy wastewater Systems: An Overview. Research Group PhD seminar day

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