Short communication: A comparison of biofilm development on stainless steel and modified-surface plate heat exchangers during a 17-h milk pasteurization run

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ABSTRACT

Flow of milk through the plate heat exchanger (PHE) results in denaturation of proteins, resulting in fouling. This also accelerates bacterial adhesion on the PHE surface, eventually leading to the development of biofilms. During prolonged processing, these biofilms result in shedding of bacteria and cross-contaminate the milk being processed, thereby limiting the duration of production runs. Altering the surface properties of PHE, such as surface energy and hydrophobicity, could be an effective approach to reduce biofouling. This study was conducted to compare the extent of biofouling on native stainless steel (SS) and modified-surface [Ni-P-polytetrafluoroethylene (PTFE)] PHE during the pasteurization of raw milk for an uninterrupted processing run of 17 h. For microbial studies, raw and pasteurized milk samples were aseptically collected from inlets and outlets of both PHE at various time intervals to examine shedding of bacteria in the milk. At the end of the run, 3M quick swabs (3M, St. Paul, MN) and ATP swabs (Charm Sciences Inc., Lawrence, MA) were used to sample plates from different sections of the pasteurizers (regeneration, heating, and cooling) for biofilm screening and to estimate the efficiency of cleaning in place, respectively. The data were tested for ANOVA, and means were compared. Modified PHE experienced lower mesophilic and thermophilic bacterial attachment and biofilm formation (average log 1.0 and 0.99 cfu/cm^2, respectively) in the regenerative section of the pasteurizer compared with SS PHE (average log 1.49 and 1.47, respectively). Similarly, higher relative light units were observed for SS PHE compared with the modified PHE, illustrating the presence of more organic matter on the surface of SS PHE at the end of the run. In addition, at h 17, milk collected from the outlet of SS PHE showed plate counts of 5.44 cfu/cm^2, which were significantly higher than those for pasteurized milk collected from modified PHE (4.12 log cfu/cm^2). This provided further evidence in favor of the modified PHE achieving better microbial quality of pasteurized milk in long process runs. Moreover, because cleaning SS PHE involves an acid treatment step, whereas an alkali treatment step is sufficient for the modified-surface PHE, use of the latter is both cost and time effective, making it a better surface for thermal processing of milk and other fluid dairy products.
Key words: biofilm, stainless steel, plate heat exchanger, pasteurization, cleaning

Short Communication

Milk is considered an ideal medium for growth of many pathogenic and spoilage-causing bacteria because it contains nutritional components essential for their growth (Degeest and De Vuyst, 1999; Cherif-Antar et al., 2016). Thermal processes, such as pasteurization of milk, are used to reduce spoilage bacteria as well as pathogens in order to provide protection from illness (Visser and Jeurnink, 1997). Plate heat exchangers (**PHE**), which have a stainless steel (**SS**; SS-316) surface, are used for pasteurizing milk and other products in the dairy industry (Shah et al., 1988). As milk flows through PHE, milk proteins denature as a result of heating of milk and stick to the equipment surface (Rosmaninho and Melo, 2006). In an earlier study, Bouman et al. (1982) observed the fouling of PHE to be greater in the regeneration section of the pasteurizer. The composition of the deposit with a temperature reaching 57°C after 12 h of processing was found to be 30 mg/plate for phosphorus, 51 mg/plate for calcium, and 52 mg/plate for protein. However, the composition changed significantly in the heating section where a temperature of 70°C was maintained and was found to be 36 mg/plate for phosphorus, 95 mg/plate for calcium, and 133 mg/plate for protein. Further, the fouling due to protein and mineral deposits on the

surface of PHE during pasteurization also accelerates bacterial adhesion to the surfaces, leading to biofilm development. Thermophiles were reported by Hinton et al. (2002) at a level of 10^5 cfu/cm^2 on fouled surfaces, whereas no bacterial activity was detected on the clean surface. An important source of microbial cross-contamination in the dairy industry is the formation of microbial biofilm during milk storage and processing due to the adherence of bacterial contaminants on SS surfaces (Flint et al., 1997). Development of biofilms in milk processing environments leads to increased opportunity for microbes to cross-contaminate the processed dairy products. Moreover, spoilage and pathogenic bacteria trapped in biofilms are protected from sanitizers due to multispecies cooperation and the presence of extracellular polymeric substances, which result in their survival (Watnick and Kolter, 2000). Rapid fouling of PHE is thus undesirable for both economic and technical reasons. According to a study conducted by Van Asselt et al. (2005), 80% of the total production cost in dairy processing industries is attributed to fouling and cleaning. Further, the production run times are limited due to both shedding of bacteria in the product, leading to microbiological concerns, and restoring the heat transfer characteristics of PHE (Sandu and Singh, 1991). It also has been reported that metal surfaces get corroded (Bryers, 1987; Gupta and Anand, 2017) due to metabolic activity of the microorganisms present in the biofilm.

The initial establishment of biofilm highly depends on the properties of the surface material, such as surface energy and hydrophobicity (Flint et al., 1997). Many researchers have reported that bacterial adhesion is reduced with the lowering of surface energy and enhanced surface hydrophobicity (Fletcher and Loeb, 1979; Pringle and Fletcher, 1983; Tsibouklis et al., 2000; Liu et al., 2017). Hence, it is of high relevance to modify the SS surface and to use antimicrobial coatings to have less deposition, bacterial adhesion, and establishment of biofilms. One of our recent studies (Jindal et al., 2016) using modified coupons revealed that an SS-316 surface modified with an Ni-P-polytetrafluoroethylene (**PTFE**) blend supports lower bacterial adherence, consequently resulting in minimal biofilm formation. A native SS surface with the highest value of surface energy (42.94 ± 0.67 mN/m) recorded a maximum bacterial attachment of log 5.11 ± 0.03, whereas an Ni-P-PTFE modified surface with the least surface energy (15.96 ± 1.21 mN/m) exhibited lower bacterial attachment (3.15 ± 0.04). Results from this study were promising; however, the experiments were conducted on coupons in a laboratory-scale static environment. As a follow-up, the purpose of this study was to investigate the extent of biofouling on native (SS) and modified

(Ni-P-PTFE) PHE under dynamic conditions for an extended run time of 17 h.

The 2 pasteurizers used for this study included a native (SS) and an Ni-P-PTFE modified-surface PHE (AGC Heat Transfer, Portland, OR). Milk was allowed to flow continuously through both pasteurizers for 17 h to simulate the conditions encountered in a typical dairy plant. Prior to the pasteurization step, the incoming raw milk was split into 2 balance tanks. At the end of pasteurization, both PHE were flushed with water and were dismantled for biofilm sampling. Plates were reassembled in both PHE for cleaning in place (**CIP**) and were then dismantled again to check the efficiency of CIP. Two-stage CIP treatment followed, which involved the use of sodium hydroxide and an acid wash of nitric acid for native PHE. The Ni-P-PTFE modified-surface PHE, being sensitive to the acid treatment, was treated with only alkali during the CIP cycle, as recommended by the manufacturer.

To study the microbial changes, milk samples were collected from both the balance tanks (raw milk sample) and outlets (pasteurized milk sample) of both pasteurizers at the start of the run (h 0) and at hourly intervals from h 11 until the completion of the run (h 17). Raw and pasteurized milk samples were maintained at 4°C from collection to completion of analysis. Appropriate serial dilutions were made aseptically and plated on plate count agar, followed by incubation at 32°C for 48 h. Colony-forming units were calculated, and the counts were reported as log colony-forming units per milliliter (Wehr and Frank, 2004).

At the end of the run, when PHE were flushed with water, 3M quick swabs (3M, St. Paul, MN) were used to recover biofilms from the regeneration section. Standard protocols were followed to enumerate mesophiles and thermophiles embedded in the 17-h-old biofilms. To validate the surface cleanliness of each section of both PHE, Charm's ATP swabs (Charm Sciences Inc., Lawrence, MA) were used. A bioluminescence luminometer (Charm Sciences Inc.) was used to measure the relative light units (**RLU**). Matrix-assisted laser desorption/ionization time of flight (**MALDI-TOF**) was used to identify the selected isolates from raw milk, pasteurized milk, and the surface of both PHE. It is a reliable method for identification of bacteria and examines ribosomal proteins based on mass spectrophotometry. The bacterial counts were analyzed using ANOVA, and means were compared.

The results obtained in this study are presented in Figures 1 to 5. We observed that the SPC started to increase in the pasteurized milk samples after h 11. The SPC at h 11 for the pasteurized milk of native PHE (log 2.54 cfu/cm^2) was far higher compared with that for the modified-surface PHE (<1.0 log); however, the rate