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Influence of feed temperature to biofouling of ultrafiltration membrane during skim milk processing

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ABSTRACT

The temperature of the feed is known to be an important factor affecting the formation and the growth rates of bacterial communities on dairy filtration membranes. However, decades after the integration of filtration processes in the dairy industry, there is still questioning whether filtration should be performed at cold (< 15 °C) or hot (> 48 °C) temperature. A biofilm reactor designed to mimic a filtration system was used to provide answers to this question. Bacteria adhering and growing on ultrafiltration membranes in contact with pasteurised skim milk were characterised at 15 °C and 50 °C through a metabarcoding approach. Our results suggested that the processing time should be limited to 10 h at 50 °C to avoid the exponential growth of thermophilic spore-former bacteria, while the use of 15 °C combined with daily cleaning procedures appeared the best way to retard the formation of biofilms on membranes.

1. Introduction

Biofilm formation is generally undesirable in the food industry, because it represents a potential source of microbial contamination and spoilage enzymes affecting food quality (Teh et al., 2014), and affect processing efficiency (Seale, Bremer, Flint, Brooks, & Palmer, 2015a). In the dairy industry, processing equipment such as filtration systems are used in a continuous mode for extended operation times (> 20 h). These conditions are particularly susceptible to biofilm formation (Anand, Singh, Avadhanula, & Marka, 2014). Indeed, the filtration environment is steady (which is essential for biofilm formation, even in the presence of turbulence), offers a constant supply of nutrients (proteins, sugars and minerals) and is at a temperature suitable for microbial growth (between 10 °C and 50 °C) (Tang, Flint, Bennett, Brooks, & Zain, 2015). Filtration membranes are further susceptible to biofilm formation since mechanical cleaning is not possible in this closed environment and the constant convective flow through the membrane, due to transmembrane pressure, may facilitate bacterial adhesion to the membrane surface (Choi, Zhang, Dionysiou, Oerther, & Sorial, 2005; Ridgway et al., 1999; Simões, Simões, & Vieira, 2010).

The main strategy used to control biofouling in the dairy industry is to clean membranes daily with a clean-in-place (CIP) system, circulating acid and alkaline solutions at the membrane surface following a filtration time of up to 24 h (Anand et al., 2014; Berg et al., 2014). This cleaning process restores the membrane permeation flux (Trägårdh, 1989). However, traditional CIP may not remove or kill bacteria cells adhered on stainless steel or filtration membrane surfaces (Bénézech & Faille, 2018; İpek & Zorba, 2018; Marka & Anand, 2017), even with optimised CIP protocols (Kumari & Sarkar, 2014). At the laboratory scale, innovative biofilm removal or control

strategies dedicated to the membrane separation industry have been suggested: the use of ozonised water (Henderson et al., 2016), surfactants (Hijnen et al., 2012) or specific enzymes (Khan et al., 2014; Tang, Flint, Bennett, & Brooks, 2010), quorum quenchers (Yeon et al., 2009), quorum quenching bacteria (Kampouris et al., 2018; Oh et al., 2017) or bioengineered biofilms (Wood et al., 2016). The use of specific enzymes is actually recommended in the industry to control short-term issues related to biofouling, but are still inefficient in a long-term purposes (Simões et al., 2010).

The complete removal of biofilms from filtration membranes may not be possible (Bucs et al., 2018; Simões et al., 2010). Instead of eradicating them from filtration systems, it appears more realistic to delay biofilm formation (Bucs et al., 2018), or to reduce their negative impact through the selection of operational parameters that limit the growth of problematic bacteria such as those that are thermoresistant. Recently, the chemistry of the membrane material was found to affect the persistence of a problematic spore-former (*Bacillus* sp.) on filtration membranes following whey ultrafiltration (UF) (Chamberland et al., 2017a). Lowering the temperature of the feed also appears a good strategy since it reduced the incidence of biofouling on reverse osmosis membranes in the desalination industry (Farhat, Vrouwenvelder, Van Loosdrecht, Bucs, & Staal, 2016), and the adhesion of *Streptococcus* sp. on PES membranes during milk or whey UF (Chamberland, Lessard, Doyen, Labrie, & Pouliot, 2017b).

Two common feed temperatures are used during UF of dairy fluids. Although higher feed temperatures (i.e., 50 °C) provide higher permeation fluxes (Méthot-Hains et al., 2016; St-Gelais, Haché, & Gros-Louis, 1992; Yan, Hill, & Amundson, 1979), better microbial quality of the retentate is expected when filtration is performed at a colder temperature (< 20 °C) due to a lower number of bacteria in it (Kapsimalis & Zall,

1981; Maubois & Mocquot, 1975; Pompei, Resmini, & Peri, 1973). However, when filtering fluids with fat or concentrating milk at a high concentration factor, a higher feed temperature is required to reduce the viscosity of the feed, even if the growth of thermophilic bacteria can occur (Maubois & Mocquot, 1975; Seale et al., 2015b). Consequently, this study was needed to document the composition of bacterial community forming on dairy filtration membranes at different time points and temperatures. It permitted to assess if the formation of biofilm on membranes is possible in a single processing day at cold temperature (15 °C), and to determine a safe processing time at hot temperature (50 °C) to avoid the exponential growth phase of bacteria adhering on membranes in contact with pasteurised milk. Bacteria were characterised by a metabarcoding approach and quantified by real-time PCR (qPCR), from membranes sampled in a biofilm reactor designed to mimic a filtration system.

2. Material and methods

2.1. Milk source

A different batch lot of raw milk (temperature < 4 °C, pH between 6.65–6.75) was obtained from a local dairy prior each experiment. Milk was skimmed (< 0.01% fat) and pasteurised (75 °C for 16 s) as previously described (Chamberland et al., 2017b). The pasteurised milk was directly collected in a sterilised 20 L feed tank (Cole-Parmer, Montreal, QC, Canada) called the milk tank, and was stored at 4 °C. The time between the pasteurisation and the beginning of each experiment was 12 h or less.

115 *2.2. Biofilm reactor assembly and operation*

116

117 A biofilm reactor (CBR 90, BioSurfaces technologies corporation, Bozeman,
118 MN, USA) that mimics the filtration environment was used to study the attachment of
119 bacteria on 10 kDa polyethersulfone (PES) UF membranes (ST membrane, Synder,
120 Vacaville, CA, USA) (Fig. 1). Membrane coupons measuring 6 cm^2 were fixed on eight
121 membrane holders, between the holders and the restraining frames specifically designed
122 for the biofilm reactor. The eight holders are placed in such a way in the bioreactor that
123 the flow condition is identical for each membrane (Fig. 1B). The milk tank was always
124 maintained at 4 °C in an incubator (MIR-153, Sanyo, Osaka, Japan) throughout the
125 biofilm development experiments (Fig. 1). A peristaltic pump (Masterflex, Model
126 7518-00, Barnant Company, Barrington, IL, USA) continuously transferred milk from
127 the milk tank to the biofilm reactor through silicone tubing (Tubing M-Flex L/S 16,
128 Cole-Parmer, Montreal, Canada) at a constant flow rate of 0.25 mL s^{-1} (flow rate value
129 between those of Bremer, Fillery, and McQuillan (2006) and Dufour, Simmonds, and
130 Bremer (2004)). The stirring rate inside the reactor was 180 rpm, as described by Tang
131 et al. (2009), to generate a flow regime considered as turbulent according to
132 Buckingham-Meyer, Goeres, and Hamilton (2007).

133 The biofilm formation experiments were performed at 15 °C and 50 °C. The
134 reactor was maintained at 15 ± 1 °C by a cold water bath or at 50 ± 1 °C by a stirring
135 hot plate (Troemner, LLC; Thorofare, NJ, USA) connected with a sterile temperature
136 sensor inside the biofilm reactor. Experiments were performed in triplicate (n = 3) for
137 each temperature condition.

138 Efforts were made to mimic as much as possible the filtration system
139 environment by operating the biofilm reactor in the continuous mode, at representative

operating temperatures, with the highest level of turbulence possible, and with pasteurised milk having its natural microflora (thermoresistant bacteria and environmental contaminants). However, the turbulent flow regime and shear stress at membrane's surface in a filtration system could not be reproduced using the bioreactor.

2.3. Bioreactor preparation

Prior to each experiment, the biofilm reactor and its different parts (tanks and tubing) were autoclaved at 121 °C for 15 min. Membrane coupons were then fixed aseptically on the membrane holders and a conventional CIP of the system (conditioning step) was performed as recommended by the membrane manufacturer. Briefly, the CIP procedure consisted of alkaline (pH 10.5), acid (pH 2.0) and chlorinated alkaline (pH 10.5, 150 ppm of free chlorine) cleaning steps, executed consecutively. Cleaning solutions were prepared with commercial chemicals: Membra-base 210 (Sani-Marc, Victoriaville, QC, Canada), Ultrasil 75 (Ecolab, Saint Paul, MN, USA) and Chloreco (Sani-Marc). Each cleaning step was performed for 30 min at 50 °C. A rinsing step with deionised water at 50 °C preceded and followed each individual step of the CIP. The stirring rate of 180 rpm was maintained in the biofilm reactor during the cleaning procedure. A cleaning step under the same conditions was also performed at the end of each experiment.

2.4. Membrane and milk sampling

During experiments performed at 15 °C, membranes were collected with a sterile scalpel after the membrane conditioning step (0 h), after 20 h, 24 h, 28 h, 32 h,

36 h, 48 h and after the final cleaning step at the end of the experiment. Considering the shorter development time of bacteria at warmer temperatures, the membranes were collected in shorter intervals of time at 50 °C: after the membrane conditioning step, after 7.5 h, 10 h, 12.5 h, 15 h, 20 h and after the last cleaning step. Prior to being stored at –80 °C until DNA extraction, membranes were gently rinsed in a sterile phosphate buffered saline (PBS) solution (pH 7.4) to remove planktonic bacteria from membranes (Anand, Hassan, & Avadhanula, 2012).

Milk inside the biofilm reactor was sampled at the same time points as the membranes. For each sampling time, three samples of 2 mL were pelleted and stored at –80 °C until DNA extraction.

2.5. Targeted genomic analysis of the bioreactor microbiome

2.5.1. Genomic DNA extraction

For both temperature conditions, genomic DNA, which contains DNA from dead and viable cells, was extracted in duplicate from the milk and the membrane samples as described previously (Chamberland, Lessard, Doyen, Labrie, & Pouliot, 2017c), except that sodium acetate (pH 5.2) at a final concentration of 0.3 M was used to precipitate the DNA (Sambrook & Russell, 2001).

2.5.2. High-throughput sequencing and bioinformatics

Amplicon sequencing targeting the V6-V8 region of the 16S ribosomal RNA (rRNA) gene was performed on a Miseq sequencer at the Institut de Biologie Intégrative et des Systèmes (Université Laval, Québec, QC, Canada). Two replicates for each temperature condition were used for the sequencing step. Raw demultiplexed paired-end

reads of each replicate were deposited in the GenBank database under the accession number SRP140914 (first replicate, 3,285,298 raw demultiplexed reads) and SRP150623 (second replicate, 3,169,154 raw demultiplexed reads). The computations of the reads of each replicate were processed separately, as previously described (Chamberland et al., 2017a), with the modifications described next. Computations were done with the pipeline FROGS from the Galaxy portal of the INRA MIGALE Bioinformatics platform (Jouy-en-Josas Cedex, France; Escudié et al., 2017; Goecks et al., 2010). Following reads preprocessing (quality filter, read trimming and read assembly), contiged reads were clustered with the Swarm clustering method using the denoising clustering step and the suggested aggregation distance of 3 (Mahé, Rognes, Quince, de Vargas, & Dunthorn, 2014). A first chimera filter was performed with VSEARCH (Rognes, Flouri, Nichols, Quince, & Mahé, 2016). Operational taxonomic units (OTU) with abundance lower than 0.005% were removed (Bokulich et al., 2013). The SILVA database (release 132, December 13, 2017) was used to perform the OTU affiliations. Each OTU was manually inspected a second time to remove undetected chimeras. The final OTU tables obtained for each replicate were finally merged by taking the sum of the absolute abundance of each taxon. Ecological metrics (alpha- and beta-diversity) were computed with the Mothur pipeline (v1.35.1) (Schloss et al., 2009), as previously described (Chamberland et al., 2017c).

2.5.3. Quantification of bacterial growth

The number of 16S rRNA gene copies found on membranes and in milk at each time point were quantified by qPCR as described previously (Chamberland et al., 2017a).

2.6. Statistical analysis

As mentioned, the experimental design was repeated three times for each temperature. A one-way analysis of variance (ANOVA) of the number of 16S rRNA gene copies variable was performed with RStudio (v.1.0.136) using the package agricolae (v1.2-8). The analysis was performed separately for membrane and milk samples. The number of gene copies among samples were considered significantly different with a *p*-value smaller than 0.05 (Fisher's least significant difference test).

3. Results

3.1. Quantification of the number of 16S rRNA gene copies on membranes

Changes in bacterial communities in milk and on membranes were studied as function of time for two temperatures, 15 °C and 50 °C (Fig. 2). The bacterial communities observed on membranes were significantly different depending on whether the membranes were used at 15 °C or 50 °C (Fig. 2A). At 50 °C, a significant increase in the number of 16S rRNA gene copies was observed throughout the experiment ($P < 0.05$, Fig. 2A). The number of gene copies per cm^2 increased significantly from 3.21 ± 0.12 $\log_{10}$ after the first cleaning to 5.40 ± 0.97 $\log_{10}$ gene copies per cm^2 ($P < 0.05$) after 7.5 h, finally reaching a maximum of 8.83 ± 1.58 $\log_{10}$ gene copies per cm^2 after 15 h ($P < 0.05$, Fig. 2.A). Following the cleaning step performed after 20 h of operation, the number of gene copies (5.57 ± 3.38 $\log_{10}$ gene copies per cm^2) was still significantly higher than at the beginning of the process (3.21 ± 0.12 $\log_{10}$ gene copies per cm^2, $P < 0.05$, Fig. 2.A).

240 At 15 °C, the number of 16S rRNA gene copies did not increase significantly on
241 the membranes throughout the 48 h experiment. It varied from 3.31 ± 0.24 $\log_{10}$ gene
242 copies per cm^2 after the first cleaning to 3.86 ± 0.58 $\log_{10}$ gene copies per cm^2 after 48 h
243 ($P > 0.05$, Fig. 2A). The initial count was not significantly different from the final count
244 measured after the last cleaning (3.48 ± 0.58, $P > 0.05$, Fig. 2A).
245
246 *3.2. Composition of bacterial communities formed on membranes*
247
248 The temperature of the feed also affected the diversity and richness of bacteria
249 observed on membranes. Globally, communities formed at 15 °C had more diversity
250 and richness (**Error! Reference source not found.**). For example, the diversity index
251 (Inverse Simpson) was between 1.00 and 6.57 on membranes operated at 50 °C while it
252 was between 8.35 and 20.58 at 15 °C (Table 1). In the same vein, membranes operated
253 at 50 °C had a lower richness, estimated between 7.84 to 67.83 species, compared with
254 between 63.51 and 180.70 at 15 °C (Chao index, Table 1).
255 Communities formed at 50 °C were dominated by the Bacilli class (more than
256 60.15% from 7.5 h, Supplementary material Table S1). In contrast, those formed at the
257 colder temperature were composed of Actinobacteria (13.46% to 26.26%), Bacteroidia
258 (12.53% to 27.00%), Bacilli (8.01% to 20.69%), α- and γ-Proteobacteria (2.77% to
259 23.94% and 21.80% to 35.27%, respectively) classes (Supplementary material Table
260 S2). Low proportions of the Clostridia class were also observed (0.04 to 4.32%) at
261 15 °C (Supplementary material Table S2), while this class was not present at 50 °C, or
262 in trace concentrations (0.67% after 7.5 h, Supplementary material Table S1).
263 Of the bacterial genera observed on membranes, the *Lactococcus* genus was the
264 most abundant during the first part of the experiment performed at 50 °C, representing

265 37.18% of the community on the membrane sampled after 7.5 h (Fig. 2A). However, at
266 this temperature, exponential growth of *Bacillus* spp. occurred, presumably primarily
267 *B. licheniformis* based on a local BLAST alignment, from 10 h to 15 h of operation. The
268 number of 16S gene copies affiliated to the *Bacillus* genus increased from 1.66 to
269 6.14 $\log_{10}$ gene copies per cm^2 between these sampling times (Fig. 2A). The *Bacillus*
270 genus reached stationary phase at 15 h of operation and persisted on membranes, even
271 after cleaning.

272 At 15 °C, psychrotrophic (cold-tolerant) bacterial genera were observed on
273 membranes, such as *Corynebacterium* (ratios between 3.51 to 12.28%), *Halomonas*
274 (ratios between 1.10% and 7.67%), *Pseudomonas* (ratios between 0.09 to 11.65%) or
275 *Psychrobacter* (ratios between 10.26% to 26.75%) (Fig. 2A and Table S2).
276 *Psychrobacter* was the most abundant genus, however, no one genus was dominant, and
277 no exponential growth was observed throughout the 48 h-experiments (Fig. 2A). The
278 *Bacillus* genus was only found in low ratios (0.19% to 3.92%) at this temperature (Fig.
279 2A).

280

281 *3.3. Changes in bacterial diversity in milk inside the bioreactor*

282

283 As on the membranes, there were few variations in the proportions of bacterial
284 OTU in milk circulating in the bioreactor at 15 °C. A decrease in the number of 16S
285 rRNA gene copies was suspected, from 6.08 ± 1.41 $\log_{10}$ gene copies per mL at the
286 beginning of the experiment to 4.52 ± 0.12 $\log_{10}$ gene copies per mL after 48 h of
287 operation, but the difference was not significant ($P > 0.05$, Fig. 2B). At 50 °C, an
288 increase in *Bacillus* spp. was observed in milk, similar to the finding with the
289 membranes, but an increase in the number of 16S gene copies was only significant after

290 15 h of operation, where the number of copies reached 8.49 ± 0.67 $\log_{10}$ gene copies
291 per mL ($P < 0.05$, Fig. 2B).
292
293 **4. Discussion**
294
295 *4.1. Operating at 50 °C, a race against spore-former bacteria*
296
297 The most important predictable issues resulting from UF of dairy fluid at 50 °C
298 is the formation of biofilms composed of thermophilic bacteria on UF membranes
299 during the process (Anand et al., 2014; Burgess, Lindsay, & Flint, 2010; Pompei et al.,
300 1973), and the subsequent contamination of the feed, as seen in milk circulating inside
301 the bioreactor (Fig. 2B). These bacteria, including the commonly found
302 *Anoxybacillus flavithermus*, *Geobacillus* spp. and *Bacillus* spp. are generally not
303 pathogenic, but their biofilm-forming and spore-forming abilities make them extremely
304 difficult to remove or kill using conventional heat treatments, and their heat-stable
305 enzymes may also affect the quality of dairy products (Burgess et al., 2010; Cho et al.,
306 2018; Sadiq et al., 2017). Complete CIP may be sufficient to kill them on stainless steel,
307 which support high temperature cleaning (Parkar, Flint, & Brooks, 2004). However, on
308 weaker materials such as polymeric filtration membranes, it was observed that biofilms
309 of *Bacillus* spp. are one of the most resistant to CIP solutions (Anand & Singh, 2013).
310 This study determined that a 15 h run at 50 °C (time needed to reach a stationary
311 phase on membranes) allows mature and saturated biofilm composed of spore-former
312 bacteria (*Bacillus* spp.) to form on a cleaned membrane during its first use. The *Bacillus*
313 genus entered exponential growth phase after approximately 10 h and grew significantly
314 in a short period of time, as found in other studies with doubling times corresponding to

0.25 h and to 0.52 h at this temperature (Burgess et al., 2010; Dufour et al., 2004; Gauvry et al., 2017). In comparison, *Streptococcus thermophilus* biofilms may be formed in 6 h in the regeneration section of a pasteuriser, at a temperature between 30 °C and 40 °C (Bouman, Lund, Driessen, & Schmidt, 1982; Knight, Nicol, & McMeekin, 2004), while the contamination of thermophilic spore-forming bacteria appears from 9 h during an evaporation process (Murphy, Lynch, & Kelly, 1999; Scott, Brooks, Rakonjac, Walker, & Flint, 2007).

Bacillus spp. have been shown to persist on reverse osmosis membranes in contact with whey for 24 h following every step of a CIP (Anand & Singh, 2013). According to the findings of Keren et al. (2004), the number of persistent cells of *Bacillus* would have increased in this study since the biofilm reached stationary phase. The high ratio of *Bacillus* spp. observed on the cleaned membrane following the 50 °C experiment (Fig. 2A) may thus represent viable bacteria not removed by the CIP. However, since the genomic approach selected for this study also detects DNA from dead cells, additional work complementary to those of Anand and Singh (2013) is needed to confirm the metabolic state of bacteria on membranes following a CIP. This work will permit to determine if bacteria detected on membranes are alive and contribute actively to the formation of biofilms during the following process, or if they are dead, and rather contribute to the formation of biofilms by enhancing further bacterial adhesion on membranes.

4.2. Cold temperature as a key parameter to delay biofilm formation

As mentioned previously, there is a trend in the dairy industry to perform continuous unit operations such as UF at colder temperatures (Tang, Flint, Brooks, &

340 Bennett, 2009) to provide dairy products with a better microbial quality, even if this
341 reduces membrane performance because of higher milk viscosity (Kapsimalis & Zall,
342 1981; Yan et al., 1979).

343 As mentioned by Yuan, Burmølle, Sadiq, Wang, and He (2018), few studies
344 have looked at biofilm formation assays at cold temperatures and whether some
345 psychrotrophic bacteria found in raw milk, such as *Pseudomonas fluorescens*, have
346 increased biofilm-forming abilities at colder temperatures (Aswathanarayan & Vittal,
347 2014). Indeed, biofilms have already been found on dairy industrial filtration
348 membranes operated around 15 °C with pasteurised milk (Chamberland et al., 2017c;
349 Tang et al., 2009).

350 In this study, membranes operated at cold temperature were colonised with
351 bacteria such as *Pseudomonas*, *Lactococcus* and *Sediminibacterium*, as observed
352 previously following 5 h UF at 10 °C in a model cross-flow filtration system
353 (Chamberland et al., 2017b). However, this 48 h experiment did not provide sufficient
354 evidence for biofilm formation or for growth on the membranes since the numbers of
355 16S rRNA gene copies on membranes did not differ significantly across the times
356 sampled ($P > 0.05$, Fig. 2A). At 15 °C, both psychrotrophic and mesophilic bacteria can
357 grow in the dairy environment (Seale et al., 2015a). However, mesophilic bacteria such
358 as lactic acid bacteria have a slow growth rate at this temperature (Adamberg, Kask,
359 Laht, & Paalme, 2003) and psychrotrophic bacteria from raw milk that survive
360 pasteurisation are scarce (Coghill, 1982; Quigley et al., 2013a). Even if it was
361 demonstrated that a psychrotrophic genus such as *Pseudomonas* was more resistant to
362 heat treatment than previously stated (Quigley et al., 2013a), most of the psychrotrophic
363 bacteria found in pasteurised milk originate through post-contamination from the water
364 source or the plant environment (Chamberland et al., 2017b; Coghill, 1982; Quigley et

al., 2013b).

Since water used to perform the cleaning cycle of the pasteuriser and the membranes in this study was demineralised and of high purity, the number of metabolically active bacteria in the pasteurised milk and adhering to membranes was probably very low. The biofilm reactor and its components were also autoclaved prior to each experiment, which limited the possibility of environmental contamination of milk. Habimana, Heffernan, and Casey (2017) formed *Pseudomonas fluorescens* biofilms on nanofiltration membranes in less than 48 h at 20 °C, but their inoculation rate was higher. Extended membrane usage time is required to determine the time needed for psychrotrophic biofilms to be formed.

In this study, communities formed at 15 °C did not have a detectable exponential growth phase. Their tolerance to the CIP cycle was possibly lower than communities formed at 50 °C, as revealed by a similar number of 16S rRNA gene copies at the beginning and at the end of the 48 h experiment (Fig. 2A) (Anand & Singh, 2013; Keren et al., 2004). Since the membrane cleaning is generally performed at 50 °C, it would be interesting to determine if the higher thermal stress imposed by the CIP on communities formed at 15 °C could be a long-term advantage to reduce the biofilm formation rate on membranes. The destabilisation of bacteria through temperature changes permitted control of *S. thermophilus* biofilms in the regenerative section of pasteurisers (Knight et al., 2004). This stress, imposed with a multi-temperature filtration process, may contribute to reduce biofilm formation on filtration membranes.

4.3. The representativeness of the biofilm reactor

Necessarily, results obtained from a biofilm reactor in this study did not reflect

perfectly the reality of industrial filtration systems. Indeed, according to observations made in the desalination industry (reverse osmosis membranes), a higher flow velocity in filtration system or the presence of a concentration polarisation at the membrane surface may affect the time needed to form biofilms on membranes (Suwarno et al., 2014). On the other hand, the use of a biofilm reactor helped to generate membrane samples at different time points without affecting the retentate flow, and to determine more precisely the impact of the temperature in a controlled environment. In comparison with other studies made previously, the ratio of the bacteria among communities forming on membranes in model filtration system or in the biofilm reactor were different (Chamberland et al., 2017a,b). However, common dairy bacterial genus such as *Bacillus* spp., *Lactococcus* spp., *Pseudomonas* spp. or *Streptococcus* spp. were observed in both systems (Chamberland et al., 2017a,b).

5. Conclusions

The temperature of the feed, as shown in this study, is a crucial parameter to consider for preventing biofilm formation on membranes. Indeed, at the industrial scale, the conclusions presented here cannot be taken as an argument to perform cold milk UF over 48 h. However, this study revealed that in a system wherein bacterial contaminants are well controlled (system perfectly cleaned and cleaned with good quality water), the biofilm formation rate on membranes is considerably slower at 15 °C than at 50 °C. Indeed, in a system were the daily cleaning procedures are rigorously performed, biofouling may not be an issue at 15 °C. However, as noted, additional work is needed to determine how psychrotrophic biofilms persist on filtration membranes and affect cleaning efficiency in real filtration systems. Industrially, if the feed viscosity does not

permit operation at cold temperatures, or if industrial installations are not available, filtration at 50 °C may be mandatory. Then, the operating time should be limited to 10 h to avoid the exponential growth of thermoresistant bacteria such as *Bacillus* sp. on membranes.

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Figure legends

Fig. 1. Biofilm reactor (A) consisting of a feed tank (1) maintained at 4 °C in an incubator (2), bacterial air vents (3), a peristaltic pump (4), a flow break (5), a CDC biofilm reactor (6), a temperature sensor (7), eight membrane holder rods (8) and eight membranes (9), a stir bar (10), a stirring/hot plate (11) and a waste collector (12), and (B) biofilm reactor seen from the top.

Fig. 2. Portrait of bacterial communities formed on membranes (A) and bacteria found in milk (B) inside the bioreactor at 15 °C or 50 °C. Bars with the same letter do not have a significantly different gene copy number (Fischer's least significant difference test, $P > 0.05$, n =3). The percentages indicate the proportion of each genus.

Table 1

Ecological metrics of communities formed on membranes at 15 °C and at 50 °C. [a]

Temperature (°C)	Time (h)	S_{obs}	Diversity index (Inverse Simpson)	Richness index (Chao)
15	0	149	13.69	162.42
	20	125	20.58	132.18
	24	142	19.44	149.99
	28	88	12.78	94.49
	32	60	8.35	63.51
	36	98	11.69	109.44
	48	148	20.11	172.40
	After cleaning	172	12.70	180.70
50	0	18	6.57	18.00
	7.5	62	5.35	67.83
	10	61	5.41	65.34
	12.5	50	1.27	57.87
	15	15	1.01	25.41
	20	5	1.00	7.84
	After cleaning	25	1.14	27.61

[a] Abbreviation: S_{obs}, number of OTU observed. For diversity and richness indices, each sample had sequencing coverage of greater than 99%; analyses were performed on rarefied samples containing 16,536 sequence reads.