

## Food and Beverage

# Securing Microbial Quality Of Cheese Brine With Microfiltration

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#### Introduction

Salting by immersion in brine is widely used for many varieties of cheese worldwide.

Open brine pools or raceways systems are entry points for multiple sources of microbial contamination, such as the water used to prepare the brine, the wet environment (splashes, condensate drops from walls and ceilings) and potentially contaminated cheeses. Fat, curd particles, plus the accumulation of proteins and other components from the cheese build up a nutrient-rich environment for the salt-resistant microorganisms. Reused brine may then become a reservoir of unwanted microorganisms, such as gas- or pigment-producing bacteria, yeast and mold, or salt-resistant pathogens, crosscontaminating the cheese and impacting its quality.

Reducing operating costs, minimizing water and environmental footprint is driving the need for extending the brine life for reuse. The control of brine quality has become essential to ensure consistent daily production.

Brine plant configuration and treatment systems, can vary significantly to fit different performance targets related to cheese type, end markets, plant and process environment. While UV lights and pasteurization equipment claim microbial deactivation, filtration can provide clarification and microbial reduction or removal at the same time. Different systems and media from coarse filtration down to final membrane filtration are available.

Pall Microflow XL Brine microfiltration systems (Figure 1) efficiently combine constant turbidity reduction and reliable control of microbial contamination levels over long automated operation cycles. They also demonstrate cost-effective operation versus non filtration methods, reductions of up to 50% in water usage and 30% in cleaning time and chemicals compared to other membrane systems, and satisfy the major drivers of the dairy industry: brand protection, constant quality and cost reduction.

#### **Microbial Challenge Tests in Brine**

While microbial removal efficiency values are commonly found to characterize final cartridge filters, based on standard microorganisms in model fluids, there is limited data established with typical spoilage microorganisms in the respective application fluids and even less data for crossflow hollow fiber filters.

The purpose of this study is to establish removal efficiency data for Pall Microflow microfiltration membranes based on microbial challenge tests specially designed for brine operating conditions and typical contamination organisms. Challenge tests with *Brevundimonas diminuta*, a standard microorganism commonly used for filter validation, were also included.

The study was composed of the following steps:

- Selecting microorganisms present in brine
- Defining growth conditions and characterizing challenge microorganisms in brine
- Challenge tests under brine typical conditions with lab scale hollow fiber modules
- Characterizing the removal efficiency by Log Reduction Values (LRV) for 6 different microorganisms

#### Selecting Microorganisms for the Challenge Tests

Various types of cheese microbial contamination are reported in the literature, which involve bacteria and yeast species. While some studies focus on the presence of pathogenic bacteria linked to product recall, such as *Listeria* and its survival in brine<sup>(1)</sup>, most of them relate to bacteria or yeast

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isolated from brine and involved in cheese downgrades<sup>(2,3,4)</sup>, such as late blowing defects in semi-hard cheese, color change in mozzarella and flavor deviation related to unbalanced presence of bacteria and yeasts.

From microorganisms reported in cheese brine, *Pseudomonas fluorescens*, a water contaminant involved in mozzarella downgrades, *Lactobacillus casei, Debaryomyces hansenii, Kluyveromyces marxianus*, typical cheese microflora, also involved in some downgrade cases were selected. *Listeria monocytogenes*, one of the most dangerous pathogen, was included as a worst case scenario organism. *Brevundimonas diminuta*, which is not present in cheese processing, but commonly used to qualify 0.2 µm filters under standard conditions (modified ASTM 838-05) was added to the program as a reference microorganism<sup>(5)</sup>.

#### Defining Growth Conditions and Characterizing Challenge Microorganisms

The first part of the study was designed to provide growth curves, establishing the optimal harvest point for each microorganism and to evaluate their viability in brine.

Growing the selected microorganisms in appropriate nutrient media and under defined conditions is a key factor to establish reliable data, as high cell counts are needed for challenge tests in order to reach a defined specific level in CFU/cm<sup>2</sup> of effective filter area. Furthermore the cells should be robust enough to survive when inoculated into the 20% salt brine model, to avoid that cellular stress generates a false response to the challenge with an underestimated contamination level. Morphology and dimensions of the cells were then measured by microscopy.

The growth curves (Figure 2), obtained from at least 3 main cultures for each microorganism, show the typical 3 stages called lag, log and stationary stage. The colony count was determined by dilution series and plating onto suitable nutrient agars and the optical density measured over a time period of minimum 20 hours up to 50 hours. Colony counts, of up to 4.10<sup>9</sup> CFU/ml were reached corresponding to optical densities (measured at 546 nm) between 2 and 5 depending on the microorganism.

For challenge testing, the microorganisms were harvested in the late log- or early stationary stage, marked by the red arrow in Figure 2, in order to avoid a high number of dead cells challenging the filter without being counted by the determination of the viable count on agar plates.

Viability of each test microorganism was tested over three hours after inoculation into brine, to

evaluate the impact of brine exposure during the microbial challenge test. None of the test organisms were influenced by the brine solution during the test, as shown in Figure 3.

The impact of inoculation into brine was also evaluated on cell dimensions, which is an important parameter when challenging a filter. Reduction of the length, width or both for all microorganisms, from 1/6 up to 1/3 was observed for all test organisms during the first hour in brine. Cell size after 1 hour, shown in Figure 4, remained stable. Starting from this result, microorganisms were inoculated in brine one hour prior to the challenge, enabling cells to adapt to the brine and get stable size during the 1-hour filtration run.

## Microbial Challenge Test Under Brine Typical Conditions

The PVDF hollow fiber USP143 modules evaluated in this study are small versions of the modules used in the Pall Microflow XL Brine microfiltration systems.

The challenge tests were performed in a specifically designed test set-up (Figure 5) enabling realization of disinfecting, rinsing and filtration steps (Figure 6), with brine characteristics close to an industrial operation.

Challenge flowrate was set at the level of 37 L/m<sup>2</sup>h, an average flux obtained in cheese plants operating Pall Microflow XL Brine systems. The filtration temperature for the contaminated brine was chosen to a realistic value of 10°C. Direct filtration mode was selected in order to control the microbial concentration level in the feed stream for a precisely defined challenge test. A specific challenge level in CFU/cm<sup>2</sup> could thus be determined, which is not possible in crossflow mode. Table 2 summarizes the test conditions.

# Characterizing removal efficiency by Log Reduction Values (LRV)<sup>6</sup>

Microbiological performance of filters is typically evaluated under defined laboratory conditions. This is done by challenging the filter under reproducible conditions with certain microorganisms suspended in a defined volume, at a defined concentration with a suitable flow of the suspension through the filter. The specific conditions for the brine challenge tests were described in the above paragraph.

The relationship between upstream and downstream concentration of microorganisms is measured and used to calculate the titer reduction. For convenience these usually very high numbers are expressed by indicating the Logarithmic Reduction Value (LRV), representing the common logarithm of the titer reduction. Log Reduction Values efficiently express microbial performance of filters.



 $\label{eq:LRV} LRV = log \; \frac{\mbox{Total number of microorganisms influent to the filter}}{\mbox{Number of colonies recorded on the downstream analysis disc}}$ 

Log Reduction Values are linked to the concentration in the feed stream, or challenge level. If the challenge level is too low, the small number of microorganisms downstream of the filter could limit the precise calculation of titer reduction. In this case, sterile effluent may be incorrectly interpreted as high performance. Alternatively, if the calculation is performed correctly with the theoretical limit of the method a false low performance may be the result.

To provide a clear interpretation of the filter performance, LRV is expressed together with the specific challenge level per effective filter area in CFU/cm<sup>2</sup> and with the total colony count or absence of microorganism in the total filtrate.

With each microorganism type, three hollow fiber modules type Pall US<sup>-143</sup> were tested out of 3 different lots. In a first attempt, specific challenge level was set to 10<sup>5</sup> CFU/cm<sup>2</sup> for the smallest test organisms *B.diminuta* and *P.fluorescens*. Since these resulted in sterile filtrate, i.e. no colony count downstream the filter, the challenge level was raised to 10<sup>7</sup> CFU/cm<sup>2</sup> for all other test runs and organisms.

#### High Performing Hollow Fiber Microfiltration

In this microbial performance study, Pall USP 143 hollow fiber modules were tested with 5 typical cheese brine contaminating microorganisms, under realistic dairy process conditions.

#### All tests showed that microorganisms were efficiently removed at a challenge level of 10<sup>7</sup> CFU/cm<sup>2</sup> filtration surface area with Log Reduction Values >10.4.

Although the microbial removal performance of hollow fibers shown in this study cannot be directly compared to sterilizing grade filter cartridges operated in direct mode, the removal efficiency of which is correlated to integrity test values as a result of the filter validation process, it is a good indicator of the security level which can be brought into the brining process by Pall Microflow hollow fiber systems.

## Benefits of Pall Microflow XL Brine Systems in Cheese Plants

The Pall USP 143 modules evaluated in this study are small versions of the XL modules used in the Microflow XL Brine microfiltration systems.

Typical performance of Pall Microflow XL Brine systems is filtrate turbidity below 0.8 NTU, combined with high microbial reduction efficiency, when operated in crossflow mode. On site measurements at soft cheese plants, showed high removal performance, with typical microbial level in brine of  $10^2$  to  $10^5$  CFU/mL and log reduction value (LRV) up to >5 on total microflora. Typical turbidity of raw brine, filtrate and concentrate is shown in Figure 7.

The Pall Microflow technology is easy to integrate into brining systems, with operating modes designed to match the various dairy requirements related to cheese type and plant organization. For example, the system can operate in a "kidney loop" mode, continuously filtering 5 to 20 % of the total brine volume during cheese brining, thus continuously reducing suspended solids and microbial load, or in batch mode e.g. filtering batch volumes overnight, for the highest microbial control level, such as requested for soft and semi-soft cheese types.

Key features of the Pall Microflow XL Brine system include:

- PVDF membranes with high mechanical strength for longer service life
- Backflush capability of the Microza\* hollow fiber, maintaining a higher flux over a longer operating cycle
- Hollow fiber membrane with a 1.4 mm open channel for optimized cleaning
- On board cleaning / concentration tanks
- Automated chemical dosing for operator safety
- Water filtration, cleaning temperature control
- All product-wetted components in 316L stainless steel
- Fully automated cycle programming for unattended operation and reduced labor and downtime

The Pall Microflow technology platform is an advanced solution for microbial contamination control, enabling dairies to perform clarification at low operating costs while providing constant and high brine filtrate quality. It provides a reliable environmentally-friendly solution to extend the life of brine and prevent cheese quality downgrades, thus improving the cheese manufacturer's economics.

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# **Figures and Tables**

Figure 1: Pall Microflow XL Brine system



#### Table I: Selected strains and culture media

Strain	DSMZ No.	Other Collection No.	Nutrient broth	Nutrient media	
Pseudomonas fluorescens	4358	NCDO 2085	NB	TSA	
Listeria monocytogenes	15675	NCTC 11994	TSB	TSA	
Lactobacillus casei	20011	—	MRS	MRS	
Debaryomyces hansenii	70590	_	YPB	YPD	
Kluyveromyces marxianus	5419	ATCC 8582	YPB	YPD	
Brevundimonas diminuta	_	ATCC 19146	_	TSA	



Figure 2: Growth curves of selected microorganisms at different temperatures



Figure 3: Microorganisms viability in brine versus buffer

time [h]









180

160









Figure 4: Microorganism cell dimensions in buffer and in brine













L. monocytogenes







in buffer length
in buffer width
in brine length
in brine width



### Table II : Test conditions

Filter	Pall crossflow module USP143		
Effective filtration surface area	0.12 m <sup>2</sup>		
Test flow rate	37 L/m²h in direct filtration mode		
Filtration temperature	10 °C		
Number of tests	3 different filter modules per challenge organism		
	2 to 3 challenge tests per filter module		
Brine model solution	20 % NaCl + 0.1% CaCl2		
	pH adjusted at 4.6 – 4.8 with HCl		
Volume filtered	4.5 L		

Total filtrate sampled by control membranes for analysis

Figure 5: Challenge test set-up - Schematic description



Figure 6: Challenge test - Main Steps



Figure 7: Typical brine turbidity





#### Table III: Microbial challenge test results on Pall USP 143 hollow fiber module

Module no.	Organism	CFU in total challenge	CFU / cm <sup>2</sup>	CFU in total filtrate		LRV
1	P. fluorescens	3.43E+08	2.86E+05	0	>	8.5
2	P. fluorescens	3.80E+08	3.17E+05	0	>	8.6
3	P. fluorescens	2.71E+08	2.26E+05	0	>	8.4
1	P. fluorescens	3.99E+10	3.33E+07	0	>	10.6
2	P. fluorescens	3.76E+10	3.13E+07	0	>	10.6
3	P. fluorescens	3.12E+10	2.60E+07	0	>	10.5
4	L. monocytogenes	8.55E+10	7.13E+07	0	>	10.9
5	L. monocytogenes	3.89E+10	3.24E+07	0	>	10.6
6	L. monocytogenes	8.24E+10	6.86E+07	0	>	10.9
4	L. casei	4.29E+10	3.57E+07	0	>	10.6
5	L. casei	5.36E+10	4.46E+07	0	>	10.7
6	L. casei	4.22E+10	3.51E+07	0	>	10.6
1	K. marxianus	2.62E+10	2.19E+07	0	>	10.4
2	K. marxianus	2.70E+10	2.25E+07	0	>	10.4
3	K. marxianus	2.52E+10	2.10E+07	0	>	10.4
1	D. hansenii	4.50E+10	3.75E+07	0	>	10.7
2	D. hansenii	4.73E+10	3.94E+07	0	>	10.7
3	D. hansenii	4.13E+10	3.44E+07	0	>	10.6
4	B. diminuta	2.12E+08	1.76E+05	0	>	8.3
4	B. diminuta	2.53E+10	2.11E+07	0	>	10.4
5	B. diminuta	3.42E+10	2.85E+07	0	>	10.5
6	B. diminuta	2.92E+10	2.43E+07	0	>	10.5

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