



Design and development of a milking machine pilot : monitoring fouling and cleaning under real conditions

A. Hubert, N. Rossi, S. Zouaghi, V. Michel, M. Guilbaud, J-M Herry, A. Hanin, G. Jard, H. Tormo, R. Perion, A. Morazin, J-L Poulet, N. Oulahal, C. Laithier

FCFP 2022, Lille

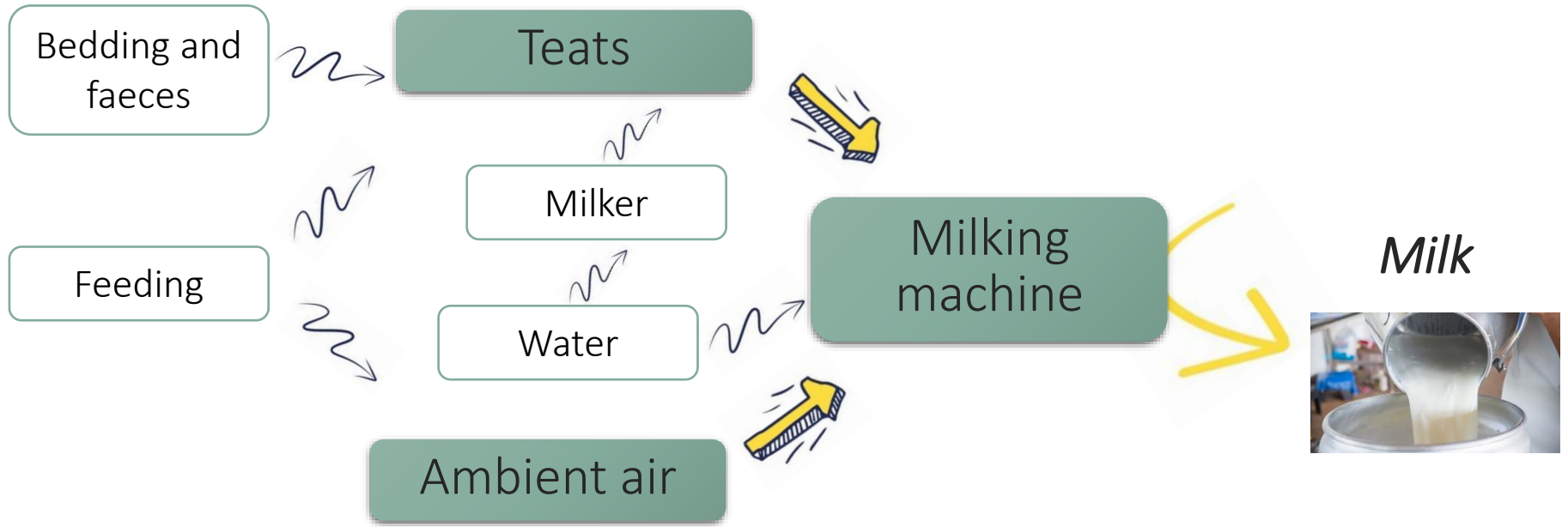


Funded by :



Context and presentation of Pilotraite

Milking machine and microbiological quality



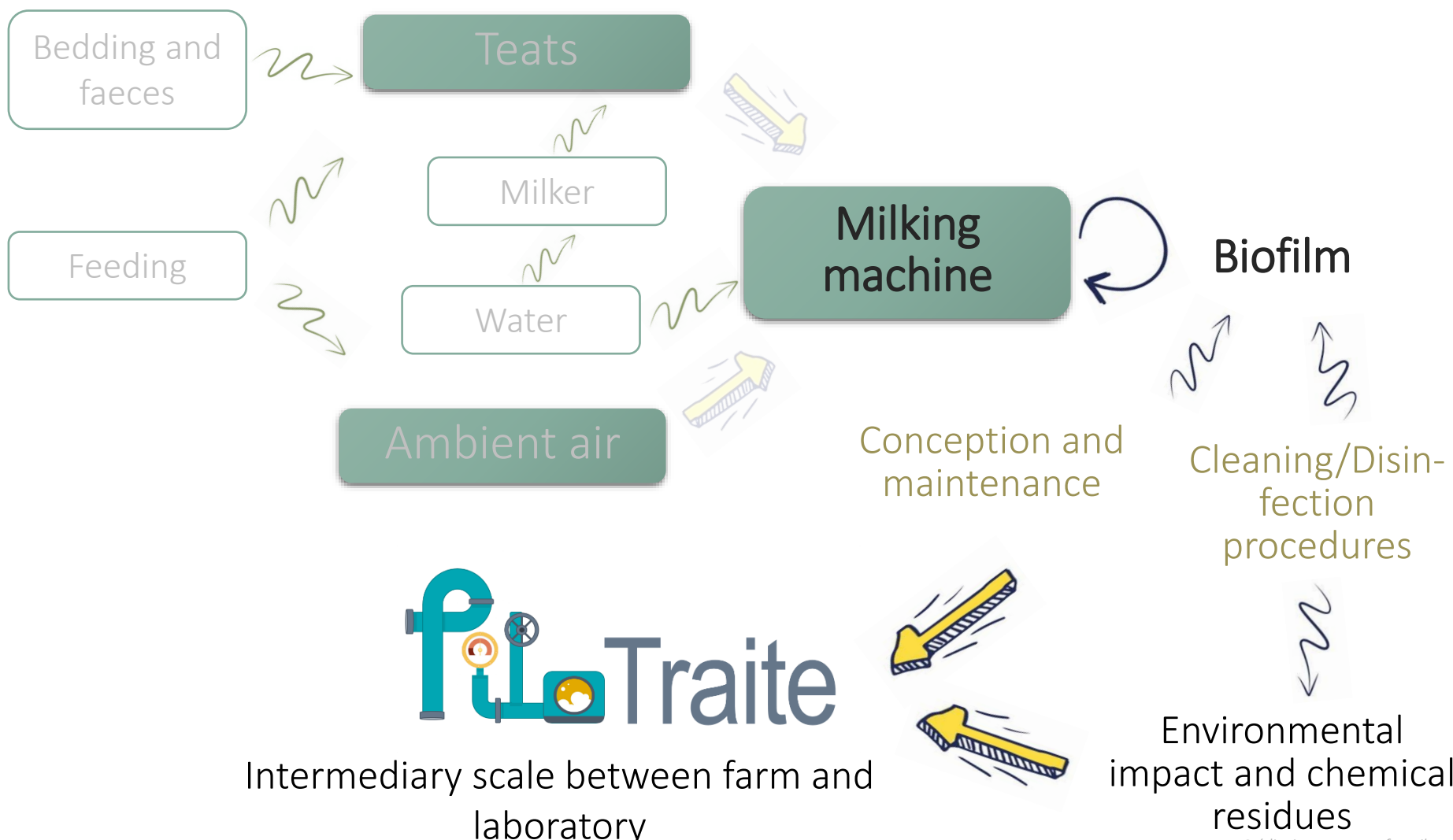
Milking machine : essential on microbiological quality of milk

Sanitary
Quality



Technological and
sensorial Quality

Biofilms in milking machine : inoculation of milk



PiloTraite

Pulsator circuit

Artificial udders

Milking circuit

Cleaning circuit



Device carrying removable stainless steel coupons to study biofilms

Conditions to study biofilms in the pilot

To eliminate biofilms between each experrimentation



Cleaning/disinfection procedures

To remove biofilms in order to characterize them



Methods to remove biofims (not presented here)

To implement complex biofilms in Pilotraite

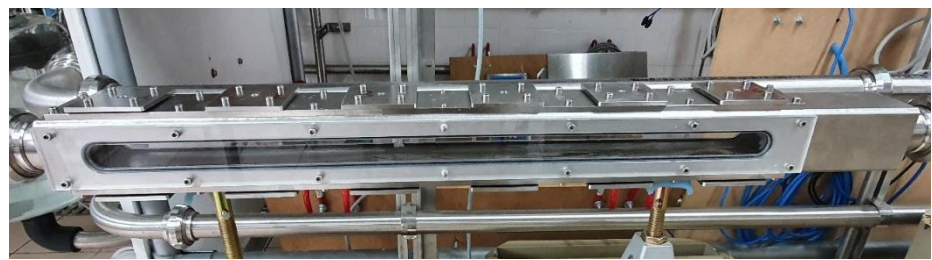
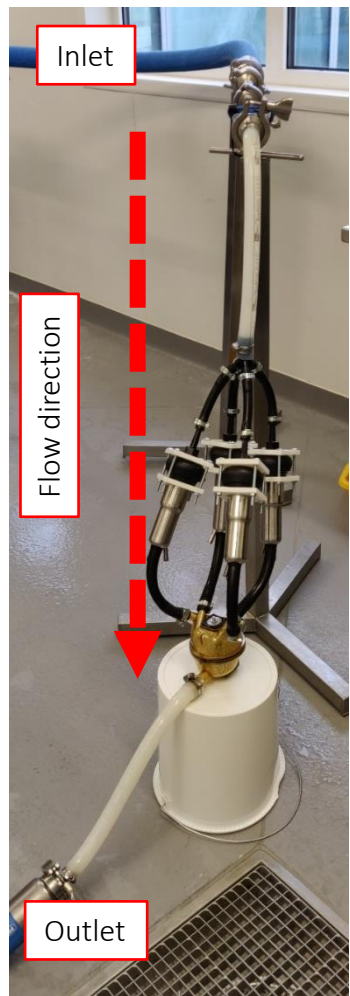


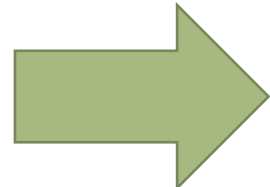
Implementation procedures

Focus on cleaning/disinfection procedures

Focus on cleaning/disinfection procedures

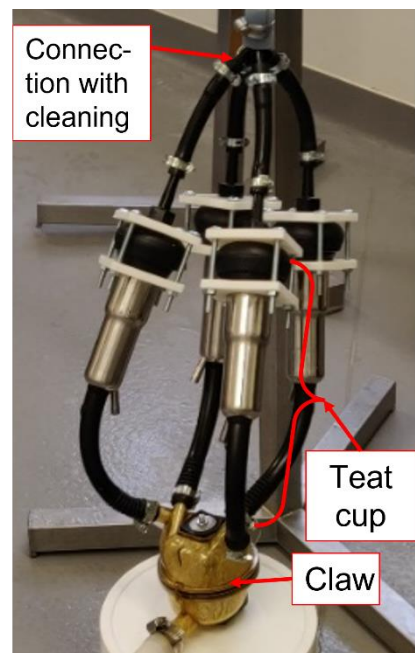
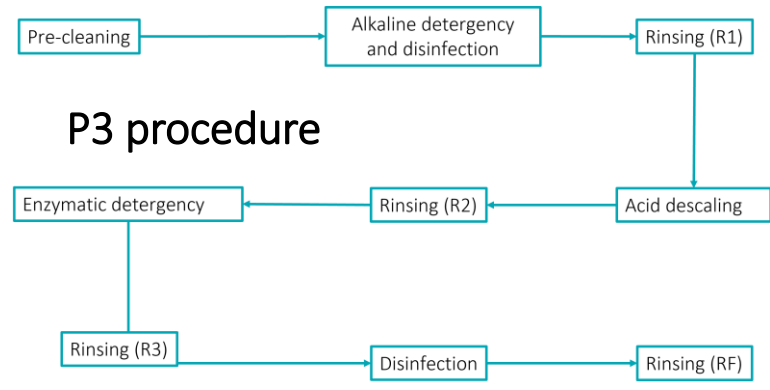
Studies in the ACTALIA's lab :



 Evaluation of cleaning ability thanks EHEDG guidelines (doc.2)



Focus on cleaning/disinfection procedures



Not fully cleaned :

- Connection with cleaning
- Claw



*increase in the dose of disinfectant
2 others disinfection steps*

Persistence of contamination on the claw

Disassembling and manual cleaning of risk areas : identified after an audit by ACTALIA



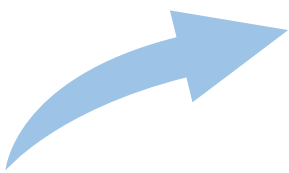
P3 procedure

Absence of contamination

P1 Procedure (alkaline/acid)

To be careful on the joint's position

Focus on implementation of biofilms








Evaluation of the resident biofilm after :

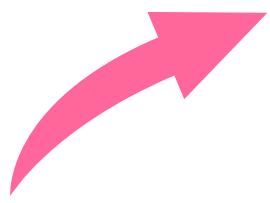
1/ NED Procedure

- Biofilm résident characterization
- Simple Biofilm implementation
- Complex biofilm implementation - milk – 2 days
- Complex biofilm implementation – milk – 1 day
- Complex biofilm implentation – tronçon

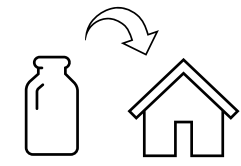
February			March			April			Mai			June			July							
		1	1	2		3		1	1			2	1	2	3	2	3			3		

Evaluation of biofilm in the pilot after :

-  Biofilm résident characterization
-  Simple Biofilm implementation
-  Complex biofilm implementation - milk – 2 days
-  Complex biofilm implementation – milk – 1 day
-  Complex biofilm implentation – tronçon








1/ NED procedure
 2/ **4 circulations of UHT milk inoculated with lactococci** (2 days, 2 times a day followed by simple rinsing)



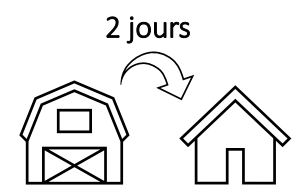
February			March			April			Mai			June			July							
		1	1	2		3		1	1			2	1	2	3	2	3			3		

Evaluation of biofilm present in the pilot after :

-  Biofilm résident characterization
-  Simple Biofilm implementation
-  Complex biofilm implementation - milk – 2 days
-  Complex biofilm implementation – milk – 1 day
-  Complex biofilm implentation – tronçon

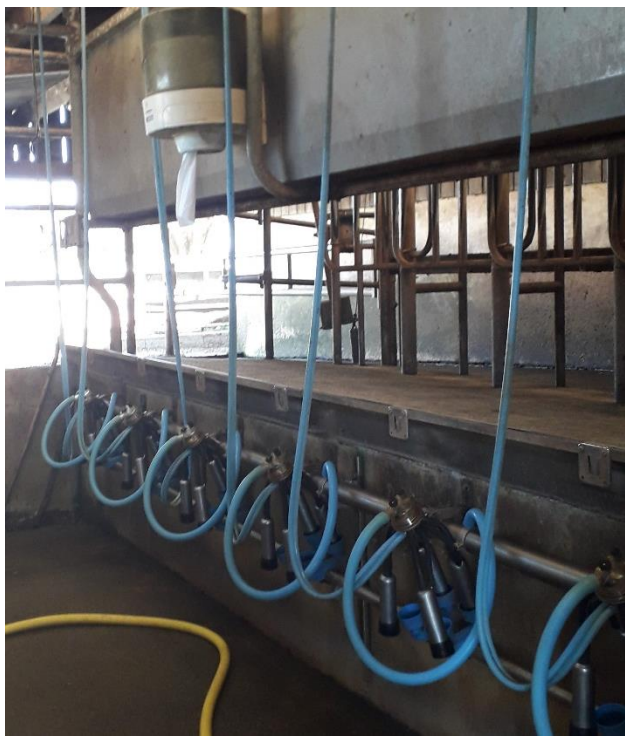


1/ NED procédure
 2/ **4 circulations of UHT milk inoculated thanks circulation in a real milking machine** (2 days, 2 times a day, followed by a simple rinsing)



Sampling of biofilms in the neighbouring farm

Microflora present in the milking machine are removed and collected thanks circulation in the milking machine with UHT milk(just before milking in the evening)



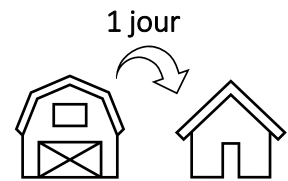
February			March			April			Mai			June			July							
		1	1	2		3		1	1			2	1	2	3	2	3			3		

Evaluation of biofilm after :

- Biofilm résident characterization
- Simple Biofilm implementation
- Complex biofilm implementation - milk – 2 days
- Complex biofilm implementation – milk – 1 day
- Complex biofilm implentation – tronçon








1/ NED procedure
 2/ 2 circulations of UHT milk inoculated thanks circulation in a real milking machine (1 day, 1 time a day, followed by a simple rinsing)



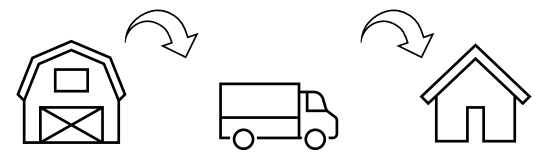
February			March			April			Mai			June			July							
		1	1	2		3		1	1			2	1	2	3	2	3			3		

Evaluation of biofilm present in the pilot after :

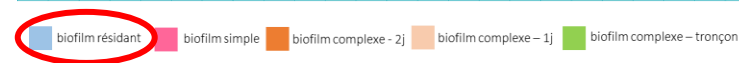
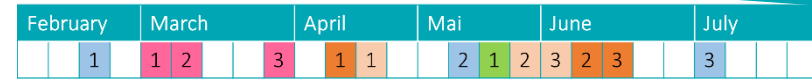
-  Biofilm résident characterization
-  Simple Biofilm implementation
-  Complex biofilm implementation - milk – 2 days
-  Complex biofilm implementation – milk – 1 day
-  Complex biofilm implentation – tronçon



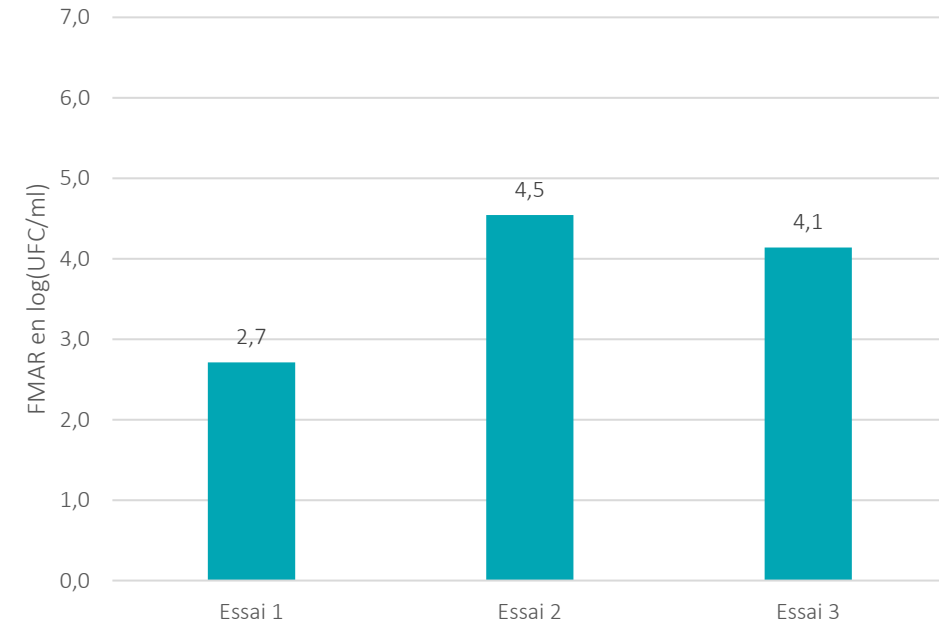
1/ NED procedure
 2/ **2 circulations of UHT milk** (2 days, 2 times a day, followed by a simple rinsing), Biofim of experimental farm brought by **a peace of the milking pipeline** (results not detailed, needed to be confirmed)



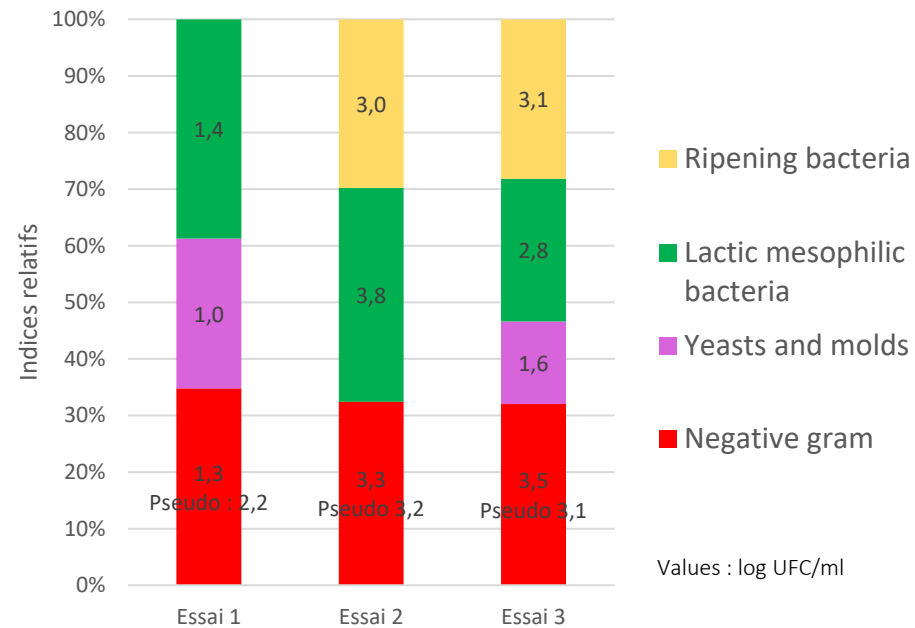
Resident biofilm in the pilot



Total bacteria count of microflora



Relative presence (%) of microflora in the pilot



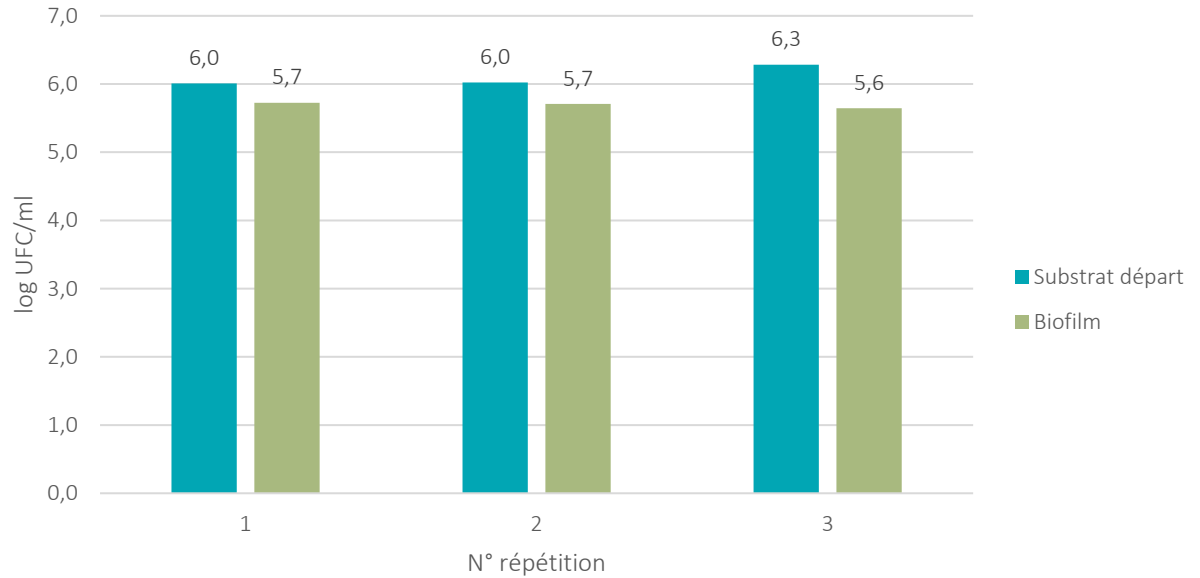
- ❖ No pathogenic microflora detected (*Listeria Monocytogenes*, staphylocoques à coag +, *E. Coli*) during all the experimentation
- ❖ NED not sufficient to eliminate biofilm in the pilot : microflora present before experimentation and increasing during the experimentation
- ❖ Emergence of ripening microflora during experimentation
- ❖ Important presence of spoilage microflora, especially *Pseudomonas spp.*

Implementation of lactococci in the pilot



■ biofilm résiduaire
 ■ **biofilm simple**
 ■ biofilm complexe - 2j
 ■ biofilm complexe - 1j
 ■ biofilm complexe - tronçon

Comparaison between quantity of lactococci in the pilot and in the substrate (n=1)



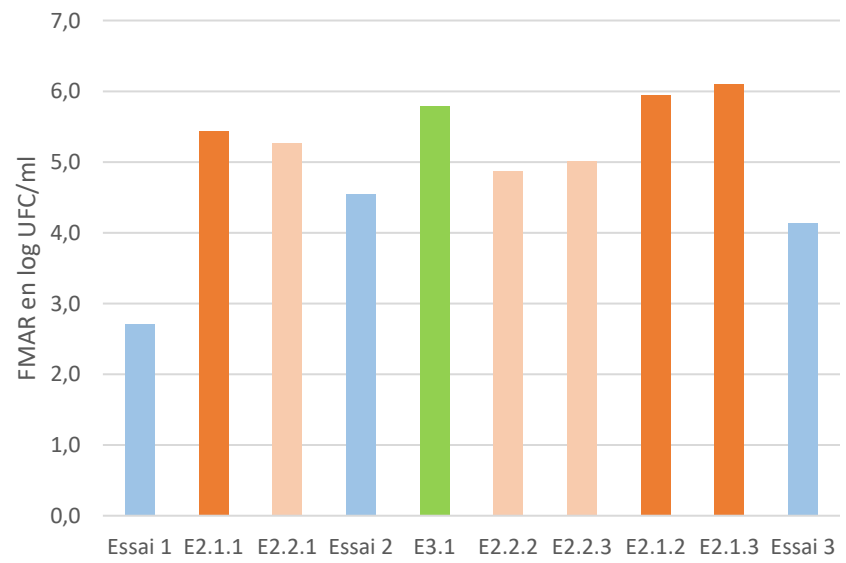
Enrichment of microflora in the pilot with lactococci (evolution of microbiological groups not measured)

Evolution of microflora in the pilot during the experimentation

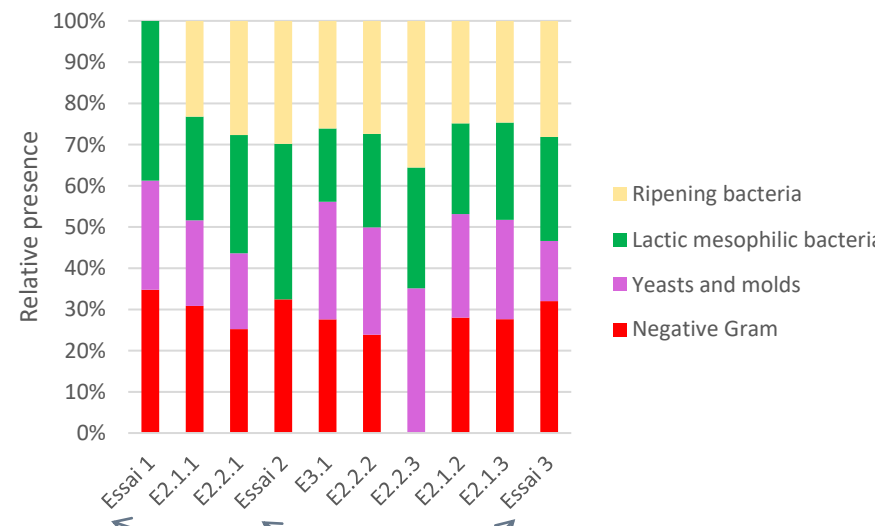
February		March		April		Mai		June			July					
	1	1	2		3	1	1		2	1	2	3	2	3		3

■ Biofilm résident
 ■ Biofilm simple
 ■ Biofilm complexe – 2j
 ■ Biofilm complexe – 1j
 ■ Biofilm complexe – tronçon

Evolution of total bacteria count of UHT milk after circulation in the pilot (n=1)



Evolution of relative importance of microbiological groups in the UHT milk after circulation in the pilot (n=1)



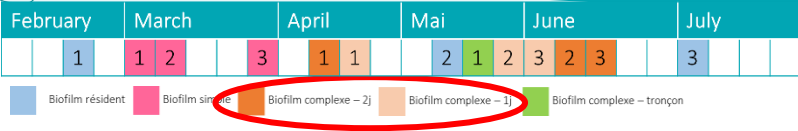
Graphic legend :

- Resident biofilm characterization
- Complex biofilm implementation - milk – 2 days
- Complex biofilm implementation– milk – 1 day
- Complex biofilm implementation– peace of milk pipeline

No implementation procedure

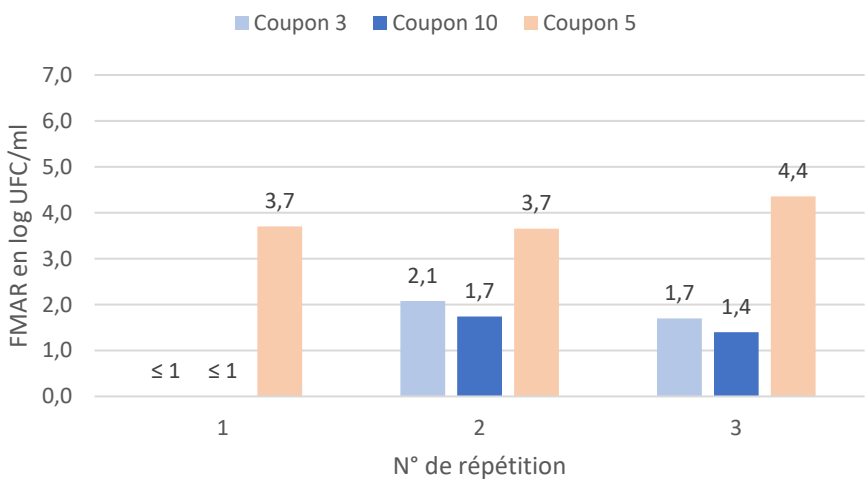


Implementation of complex biofilm on coupons

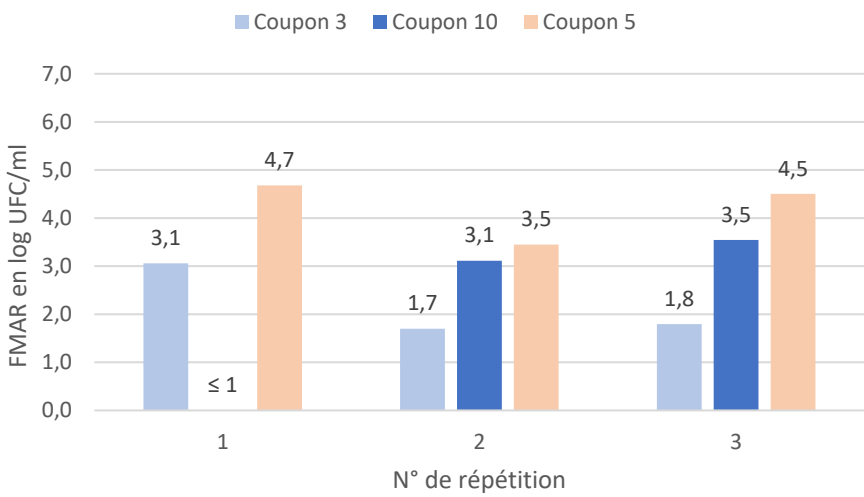


Total bacteria count on stainless steel coupons

Implementation in 1 day

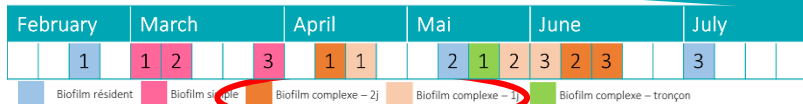


Implementation in 2 days

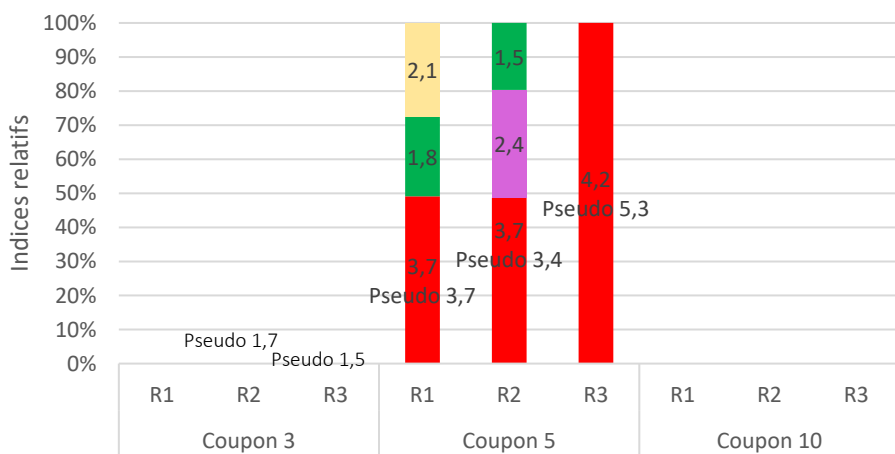


- ❖ Implementation of microflora on the coupons : variable in the time and depending on the coupon
- ❖ More important implementation with 2 days

Implementation of complex biofilm on coupons

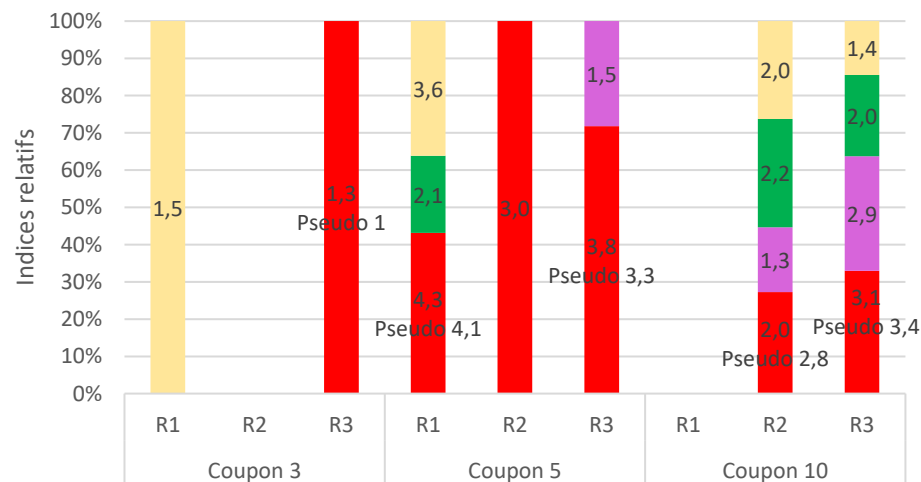


Implementation in a day (n=1)



Values : log UFC/swab

Implementation in 2 days (n=1)



■ Negative Gram ■ Yeasts and molds
■ Lactic mesophilic bacteria ■ Ripening bacteria

- ❖ Spoilage bacteria implant themselves better on coupons (1 day/2 days) and usefull microflora is more present with 2 days to implement biofilms
- ❖ Not homogeneous implementation

Pilot Traité

Conclusions and perspectives

- ❖ Resident biofilm even if drastic cleaning/disinfection procedure before experimentation
 - ❖ Usefull and spoilage microflora : representative of real milking machine
 - ❖ Resident biofilm modified with important addition of some microflora
- ❖ Microbiological groups : stable during experimentation but better analysis is needed (metabarcoding in progress)
- ❖ Longer implementation procedures foster the development of biofilm in the pilot
- ❖ Focus on coupons : spoilage bacteria grow better and implementation with 2 days is more favourable to have a complex biofilm on coupons
- ❖ Other results not presented : evolution of physical surface of coupons



- ❖ A pilot available to study impact of cleaning/disinfection procedures on biofilms in milking machine
- ❖ Resident biofilm : necessary to be controlled before each experimentation
- ❖ Preliminary tests to determine the protocol