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Paper entitled:

**Removal of *Bacillus* spores from stainless steel pipes by flow foam: effect of the foam quality and velocity**

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## 1        **1.    Introduction**

2        In agro-food industrial environments, surfaces have been reported to be contaminated  
3        by a range of microorganisms, including pathogenic and spoilage bacteria (Srey et al., 2013).  
4        Once introduced, if environmental conditions are suitable, many bacteria are able to persist  
5        on the contaminated surfaces or even to form biofilms. Indeed, despite cleaning and  
6        disinfection procedures, some bacteria are still commonly found on the surfaces of food  
7        processing lines, mostly in the form of adherent spores, e.g. *Bacillus* spores in closed  
8        equipment (Peng et al., 2002) or in the form of biofilms, e.g. *Pseudomonas spp.* (Dogan and  
9        Boor, 2003).

10        Cleaning in place (CIP) leading to residue removal from inner surfaces of processing lines  
11        without disassembling, has been a crucial factor in guaranteeing the safety and quality of  
12        food. If not done properly, consequences can be devastating, especially in the case of  
13        pathogen surface contamination (Pietrysiak et al., 2019; Ribeiro et al., 2019). In order to  
14        clean rapidly, CIP aims to combine the advantages of the high temperature, detergent and  
15        the mechanical action generated by the turbulent flow (or the impact of the spray)  
16        (Moerman et al., 2014). The mechanical effect is created by the flow rate and it is generally  
17        admitted that high flow rates result in high removal rates because of the high shear forces  
18        on the deposit layer. However some works have detailed the role of hydrodynamics and in  
19        particular of the mean wall shear stress and the major role played by its fluctuations (Blel  
20        et al., 2013, 2010). This was very recently judged to be mandatory for any CIP improvement  
21        (Li et al., 2019).

22        The addition of foaming surfactants or even gas-stabilized foam means the cleaning  
23        solution can be applied as a foam, which can increase the retention time, e.g. on vertical  
24        surfaces. Foam is widely used in static conditions throughout the food industry for the  
25        cleaning of large open surfaces (floor, conveyors, workshops and equipment). To clean open  
26        surfaces, foam requires specific qualities, namely density, foamability, stability and void  
27        fraction-based quality. However, foam cleaning agents could also be used for cleaning some  
28        closed equipment such as filtration modules (Gahleitner et al., 2013). Despite its  
29        widespread use, very little work has been carried out on the elimination of surface deposits  
30        using flowing foam, essentially gas-liquid two-phase flows in capillaries (Kondjoyan et al.,  
31        2009) and to our knowledge none have dealt with the elimination of microorganisms.

32 Almost nothing is known about the potential of foam flow to conduct cleaning operations  
33 using much less energy (very low velocity) and much less water. Aqueous foams are non-  
34 Newtonian complex fluids consisting of concentrated dispersions of gas bubbles in a soapy  
35 liquid. Depending on the amount of water they contain, they can be either wet or dry. The  
36 air fraction defines the so-called foam  $\beta$  quality (Tisné et al., 2004). Foams have original  
37 mechanical properties which rely on their low density and high surface area combined with  
38 their ability to elastically respond to low stresses and to flow like a viscous liquid with large  
39 distortions.

40 Foams admit an unexpected and nonlinear rheological behavior (shear thinning and  
41 yielding), where the properties of the liquid and gas, that compose it, have an influence on  
42 it. Their rheological behavior can be compared with some non-Newtonian models such as,  
43 power law, Bingham, and Herschel–Bulkley. Recently it was demonstrated that the foam  
44 rheological behavior can be better described by Herschel-Bulkley model (Dallagi et al., 2019,  
45 2018).

46 Among the consequences of foam flow, the mechanical action exerted on the contact-  
47 surface depends on the velocity, foam composition (air/liquid) and the ability of the system  
48 (foam, geometry and surface properties of the equipment to be cleaned) to maintain a thin  
49 liquid film between the solid surface (wall) and the foam flow.

50 Wall shear stress, especially due to this thin liquid film located between the wall and the  
51 foam flow, plays an important role in the characterization of the rheological properties of  
52 this foam, depending mainly on the bubble size and particularly on the volume fraction of  
53 the liquid (Chovet, 2015; Chovet et al., 2014). These properties can be used to understand  
54 the microorganism detachment phenomena, such as spores from solid surfaces. Therefore,  
55 foam flows would constitute a true novelty in surface hygiene, as low water load and high  
56 mechanical actions under moderate temperatures would permit highly cleaning, which can  
57 easily be combined with disinfection.

58 This study investigates the removal kinetics of *B. amyloliquefaciens* 98/7 and *B. cereus*  
59 98/4 spores using foam flow. The respective roles of the foam quality (importance of  
60 air/water balance), and of the flow rate were analyzed by means removal kinetics modeling.  
61 Results were then compared to spore removal under mild cleaning in place conditions. We  
62 then investigated some foam properties (the flow regime and the mean foam flow velocity,

63 bubble-size distribution, bubble-passage frequency, foam quality and mean wall shear  
64 stress).

65

## 66 **2. Materials and Methods**

### 67 **2.1. Bacterial strains and solid surfaces**

68 In this study, two bacterial strains isolated from dairy processing lines forming spores of  
69 **very different surface energies one hydrophobic and the second hydrophilic** (Faille et  
70 al., 2019, 2016, 2010) were used: *B. cereus* CUETM 98/4 (BC-98/4) and *B.*  
71 *amylolichofaciens* CUETM 98/7 (formerly known as *B. subtilis* 98/7). *Bacillus* spores were  
72 produced as previously described (Faille et al., 2019). Before any experiment, two  
73 further washes were performed and spores were subjected to a 2.5-min ultra-sonication  
74 step in an ultrasonic cleaner (Bransonic 2510E-MT, 42 kHz, 100 W, Branson Ultrasonics  
75 Corporation, USA) to limit the presence of aggregates. In order to evaluate the  
76 hydrophobic character of spores, Microbial Affinity to Hydrocarbons tests (MATH) were  
77 performed as previously described (Faille et al., 2019b).

78 The material, used in the form of rectangular coupons (45 mm x 15 mm), was AISI 316  
79 stainless steel with pickled (2B) finish (kindly provided by APERAM, Isbergues, France).  
80 Prior to each experiment, coupons were cleaned and disinfected using a standard  
81 protocol used at UMET. Coupons was first cleaned using pure alkaline detergent (RBS  
82 T105, Traitements Chimiques des Surfaces, France). They were then subjected to a 10  
83 min immersion in a 5% RBS T105 at 60 °C, followed by thorough rinsing with tap water,  
84 then with softened (reverse osmosis) water for 5 min each. 24 h before the experiments,  
85 stainless steel coupons were treated in a dry heat oven at 180°C for 1 h.

86

### 87 **2.2. Surface soiling and cleaning**

88 The soiling suspensions were prepared with ultra-purified sterilized water and a spore  
89 concentration of around  $10^6$  CFU/ml. The coupons were vertically immersed in a Beaker  
90 containing 250 ml of the soiling solution, then kept at room temperature for 4 hrs.

91 Coupons were then inserted into a  $23 \times 10^{-2}$  m long stainless steel test duct with a  $1.5 \times 1$   
92  $\times 10^{-2}$  m rectangular cross-section. The geometry of the rectangular test ducts used for the

93 experiments was previously described (Cunault et al., 2015). The three central coupons out  
94 of the five installed in the ducts were soiled and further analyzed after cleaning.

95 The production foam prototype was built according to previous work (Chovet and Aloui,  
96 2016; Tisné et al., 2003a). The experimental set-up, with an open foam flow circuit, was  
97 designed to allow the foam flow to develop within horizontally-placed square ducts, with  
98 the coupons to be cleaned at the top. The test ducts were situated after a transparent  
99 Plexiglas rectangular duct, of identical inner size, to visualize the foam flow. To allow steady  
100 state flow conditions, the test ducts were placed at intervals exceeding 80 times the  
101 hydraulic diameter of the vein inlet (i.e. 1.5 m).

102 The prototype is presented in Figure 1. The rig includes a mother tank (capacity: 100 L)  
103 filled with Sodium Dodecyl Sulfate (Sigma-Aldrich ReagentPlus®, over 98.5% purity)  
104 dissolved in osmosed water (0.15% ww). The SDS solution is pumped into the feeding tank  
105 (50 L) located at a height of 3 m using a positive displacement pump (VARMECA 21TL055,  
106 Leroy-Somer). This set-up creates a constant flow rate in the foam generators due to gravity.

107 Three foam generators were designed as previously described (Tisné et al., 2003a).  
108 The foam is generated by injection of pressurized air through a porous medium (DURAN®,  
109 pore sizes ranging from 1 to 1.6  $\mu\text{m}$ , Dislab, Lens, France), inside cylindrical containers filled  
110 with the SDS solution. The foam quality describing the air/water content of the foam was  
111 calculated as follows (Equation 1) according to (Chovet and Aloui, 2016) where  $Q_g$  and  $Q_l$   
112 are respectively the gas and liquid flow rates:

113 .

114

$$115 \quad \beta = \frac{Q_g}{Q_g + Q_l} \quad (1)$$

116

117 The three independent parallel generators allowed us to increase the bulk velocity  
118 without affecting the foam structure. Three foam qualities were chosen for the cleaning  
119 experiments 50%, 60% and 70%. The mean velocity was calculated taking into account the  
120 global flow rate ( $Q_l + Q_g$ ) divided by the cross-section area  $S$  of the test duct. The Reynolds  
121 number was calculated according to Equations (2) and (3), taking into account the density  
122 of both gas and liquid phases. Foam viscosity was calculated using the relationship based  
123 on a heuristic model of concentrated emulsions (Equation 4).

124

125 
$$Re = \frac{\rho_f \cdot \bar{v} \cdot d_h}{\mu_f} \quad (2)$$

126

127 
$$\rho_f = (1 - \beta) \cdot \rho_l + \beta \cdot \rho_g \quad (3)$$

128

129 
$$\mu_f = \frac{\mu_l}{1 - \mu_l^{1/3}} \quad (4)$$

130

131 Three liquid/air flowmeters enabled the adjustment of the flow rate from 0 to 35 l h<sup>-1</sup>  
132 and 0 to 70 l h<sup>-1</sup> respectively.

133 Flowing from the generators, the foam passes through a transparent Plexiglas pipe of 1.1  
134 m length. The transparent pipe enables the visualization of the foam texture, bubble size  
135 and foam velocity measurement. Two pressure outlets allow the connection of 2 manifold  
136 tubes placed over a scaled plate that measures on a length L of 1m the pressure drop  $\Delta P$  to  
137 calculate the mean wall shear stress  $\bar{\tau}$  ( $d_h \Delta P / 4 L$ ). For each cleaning experiment, only one  
138 test duct containing the soiled coupons was clamped to the transparent pipe.

139 The different test ducts were thus cleaned with three foam qualities at 20°C, at foam  
140 mean velocities ranging from 2.1 to 6.7 m s<sup>-1</sup>, for 15 and 35 s, 1, 3, 5, 10 and 20 min and  
141 other experiments were carried out to mimic CIP conditions. The test ducts were connected  
142 to a CIP pilot rig (Jullien et al., 2008) and a simple CIP procedure was then carried out under  
143 the same conditions as those used for the foam tests, i.e. SDS concentration, temperature,  
144 cleaning times. The flow rate was selected to generate a mean wall shear stress of 5 Pa,  
145 falling within the range of the mean wall shear stress conditions induced by the flowing  
146 foam as described in the Results Section. After the cleaning process, the coupons were  
147 removed from the test tubes and rinsed by dipping in a beaker containing one liter of sterile  
148 ultrapure water. The residual spore contamination was then analyzed as follows.

149 To determine the number of adhering spores before (N0) or after the (Nresid) cleaning  
150 procedure, coupons were subjected to an ultrasonication step in 10 ml of 2% Tween 80 (v/v)  
151 in peptone water without indole 0.015 g/L (Biokar), diluted to 1L with ultra-purified  
152 sterilized water (5 min, Ultrasonic bath, Branson 2510, 40 Hz). This treatment has been  
153 previously shown, in our laboratory, to remove more than 99% of the adherent spores  
154 (Tauveron et al., 2006). The detached spores were enumerated on nutrient agar composed

155 of 1.3% w/v nutrient broth (Biorad, France) and 1.5% w/v bacteriological agar type E (Biokar  
156 Diagnostics, France) after 48 h at 30°C. The percentage of residual spores after cleaning was  
157 then calculated  $[(N_{\text{resid}}/N_0) * 100]$ .

158 For microstructure examination, some rinsed coupons were first dried at 20 °C for at  
159 least 1 hour to prevent spore detachment during the staining procedure. The coupons were  
160 then stained with orange acridine (0.01%) for 15 min at 20°C, gently rinsed with softened  
161 water and allowed to dry before observation. Finally, the surface contamination  
162 organization was observed using an epifluorescence microscope (Zeiss Axioskop 2 Plus,  
163 Oberkochen, Germany) at magnification 1000X.

164

### 165 **2.3. Foam flow visualisation**

166 The method is based on the observation of the displacement of moving bubbles at the  
167 walls of the pipe, for a given interval. In situ measurements were carried out at the last part  
168 of the pipe where the foam flow could be considered as established. The velocity of the  
169 bubbles was measured by marking the Plexiglas pipe wall by two thin marks spaced at a  
170 known distance. For both the lateral and the top walls of the pipe, three locations were  
171 chosen: two at 1 mm from the edges and one in the middle of the observed wall. The time  
172 taken for a bubble to pass between the two marks was recorded to calculate the its velocity.  
173 The smallest easily-visible bubbles (0.3 mm) were considered for tracking and 10 successive  
174 measurements were carried out. Mean values were then calculated. These could be  
175 considered as representative of the local velocity at the wall whatever the bubble size (Tisné  
176 et al., 2003). This method gives an approximation of the bubbles' speed. A selection of  
177 photos of the foam flow (camera Panasonic LUMIX DMC-FZ62, High speed video [HS], at a  
178 speed of up to 200 frames / second) were analyzed using Piximètre 5.1 R1540 image analysis  
179 software. The clearest two images in terms for each flow condition induced by the  
180 generators and for the three foam qualities were filtered to better observe the borders of  
181 the bubbles. It was thus possible to evaluate the bubble size distribution in all the cases  
182 studied.

183 An optical probe (© RBI instrumentation, Meylan, France) based on the discrete variation  
184 of the refractive indicator optics between the two-phase flow (air/liquid) was used to  
185 evaluate the void fraction and the air bubbles' passage frequency at the wall top (at 0.5 mm  
186 from the top). Data were analysed using the ISO software provided by RBI.



187

## 188 **2.4. Kinetics modelling**

189 A two-phase kinetics model was used to fit the data as previously proposed for the  
190 detachment kinetics of biofilms during CIP (Benezech and Faille, 2018). The fitting was  
191 performed using GlnaFIT (Geeraerd et al., 2005) **using a biphasic model composed of two**  
192 **first order kinetics** (Dallagi et al., 2018b, 2019).

193

## 194 **2.5. Statistical analysis**

195 At least 3 repetitions were carried out for the quantitative analysis of the residual  
196 contamination after foam cleaning. Data were analysed by general linear model procedures  
197 using SAS V8.0 software (SAS Institute, Gary, NC, USA). Variance analysis was performed to  
198 determine how the bacteria removal described by the kinetic parameters (residual  
199 contamination at different cleaning times and model parameters were affected by the  
200 cleaning conditions tested .

201

## 202 **3. Results**

203

### 204 **3.1. Foam flow organization and mechanical action induced by the foam**

205 Three foam qualities were prepared with a concentration of SDS of 0.15 % w/w in order  
206 to exceed the Critical Micelle Concentration (CMC). **The SDS as an anionic surfactant is a**  
207 **good representative of the “sulphate” surfactants largely used in formulated detergents.**  
208 **The SDS is known to be highly soluble and easy to rinse and is largely used in academic**  
209 **studies (Mai et al., 2016). Anionic surfactants are recognized for their cleaning, foaming and**  
210 **emulsifying properties.** The foam generated was found to be very stable (no changes were  
211 observed in terms of foam drainage and bubble size over one hour – data not shown). We  
212 also checked that the use of one, two or three generators in parallel failed to modify the  
213 foam structure significantly, despite the differences in the foam velocity. Thanks to the  
214 transparent Plexiglas tube, placed upstream of the test duct with the soiled coupons  
215 subjected to the cleaning procedure, it was possible to visualise the foam flow through the  
216 rig and to take images or videos. Observations were made from one side and from through  
217 the top.

218 The bubble velocity was first measured as shown in Figure 2 in three locations on each  
219 selected duct wall (top and lateral). As shown in Figure 2, depending on the experimental  
220 conditions (number of generators, foam quality), the velocity profiles were quite different.  
221 As the mean velocity increased, a difference in the flow velocity of the bubbles appeared  
222 depending on their position in the duct. Indeed, when a single generator was used, bubble  
223 velocity was generally constant and the foam flow therefore behaved like a plug flow. The  
224 increase induced by two generators showed no change at the top of the duct, except for  
225 the foam quality of 70%. Conversely, the bubble velocities increased from the top to the  
226 bottom of the duct as shown in Figure 2 B. This is due especially to the underlying liquid  
227 film, which pulls the foam in contact because its velocity increases. At the top wall, bubble  
228 velocities were highest at the centre of the side, and thus decreased as the flow approached  
229 the duct edges. All conditions used are summarized in Table 1.

230 The foams' flow conditions varied from 2.0 to 8.6 cm s<sup>-1</sup>, whilst for the CIP conditions the  
231 velocity was significantly higher at 120 cm s<sup>-1</sup> and the flow regime was turbulent ( $Re >$   
232 14500). The mean wall shear stress (WSS) condition for the CIP was chosen to fall within  
233 those induced by the foam, i.e. ranging from 2.2 to 6.4 Pa, allowing comparison between  
234 the CIP mechanical action and the use of foam flow. One can note that the plug flow regime  
235 with constant foam velocity profile corresponding to 1 generator flow (all foam qualities)  
236 related to a Reynolds number maximum of 67. At over 100, the foam flow velocity profile  
237 at the top wall could not be considered as constant (Figure 2A).

238 In Figure 3, an example of photos of the foam flow arrangement at the top surface of the  
239 transparent duct is shown.

240 In order to identify the distribution of bubble size within the foam under different  
241 conditions, photos were taken at the top wall of the Plexiglas duct (Figure 3). The size  
242 distribution appeared to be affected by both velocity and foam quality. For example, the  
243 greater the velocity at the top wall, the smaller the bubbles.

244 The bubble sizes were then measured and the data are given in Figure 4. When only one  
245 generator was used, the bubble sizes were more heterogeneous than those obtained with  
246 two or three generators, whatever the foam quality. Moreover, a significant number of big  
247 bubbles (between 1 mm and 10 mm in size) was also observed. The increase in the velocity  
248 was thus more conducive to smaller bubbles. In accordance with Figure 3, some larger

249 bubbles (size > 1mm) could still be measured with 2 and 3 generators for the foam flow  
250 where  $\beta = 50\%$  and with 2 generators for the foam flow where  $\beta = 60\%$ .

251 The mean frequencies of the bubbles' passage observed by the optical probe (Figure 4,  
252 D) near the top wall increased with the number of generators. However, this increase could  
253 not be explained solely by the mean velocity, but is apparently also linked to the reduction  
254 in the bubble sizes e.g. for the foam 50%, the doubling or the tripling of the mean velocity  
255 induced an increase in the frequency by factors of respectively 2.9 and 7.1.

256

### 257 **3.2. Spores' detachment under the different flowing conditions**

258 Spore adhesion to the stainless steel coupons was  $5.6 \pm 0.4$  log CFU cm<sup>-2</sup> for *B.*  
259 *amyloliquefaciens* and  $5.4 \pm 0.3$  log CFU cm<sup>-2</sup> for *B. cereus*. Before any detachment  
260 experiments, we checked that spore incubation in SDS 0.15% did not result in any significant  
261 viability loss (data not shown). The detachment of *B. amyloliquefaciens* spores was  
262 investigated under all the flow conditions with each of the three foam qualities. In Figure 5,  
263 only the mean values of the remaining contamination at the different kinetic times were  
264 presented. In all cases, the detachment curves clearly showed two distinct phases.

265 Both phases appeared to be exponential and therefore were quite accurately described  
266 by the biphasic model, with R<sup>2</sup> ranging from 0.62 to 0.98 and mostly over 0.80.

267 During the first detachment phase (less than 1 min), the spore detachment was very fast,  
268 with a 0.6 to 1.8 log decrease in the population of surface-attached spores. Large  
269 differences were observed according to the number of generators used with the 50%, foam  
270 quality whereas the number of generators had little effect on the detachment of the other  
271 two foams (60% and 70%). Taking into account all the conditions used, it appears that spore  
272 detachment during this first phase was much more efficient with the 50% foam quality  
273 when 1 or 2 generators were used. After this first step, the detachment continued for at  
274 least 20 minutes, i.e. the duration of the cleaning procedure, though more slowly. Here  
275 again, the spore detachment rate seemed dependent on the experimental conditions  
276 (number of generators, foam quality). The cleaning kinetics with foam were compared to a  
277 CIP using the SDS 0.15% and a mean wall shear stress of 5 Pa (Figure 5D). The first  
278 detachment step was close to the most efficient one with foam, i.e. allowing the  
279 detachment of over 1.5 log CFU, close to the one observed when 1 or 2 generators were

280 used with the  $\beta = 50\%$  foam. Conversely, no further detachment occurred after this first  
281 phase, indicating that a plateau value had been reached.

282 The decimal reduction at 20 min, i.e. the end of the second phase of the cleaning kinetics,  
283 was statistically analysed to compare the role of the flow rates conditions induced by the  
284 generators, the different foam qualities and by the CIP conditions. At 20 min, cleaning  
285 efficiencies observed were comparable between CIP and the flow rates induced by one and  
286 two generators (letter A, Tukey's grouping) as shown in Figure 6. In addition, the cleaning  
287 efficiency induced by the 50% and 60% foam qualities appeared significantly better than  
288 the 70% foam (different letters according to the Tukey's grouping).

289 Focusing on the cleaning conditions with foam, the variance analysis confirmed that  
290 the variability observed on the three kinetics parameters ( $f$ ,  $k_{max1}$ , and  $k_{max2}$ ), was  
291 significantly related to the flow rate induced by the foam generators. However, some  
292 discrepancies should be noted (see Figure 7). Considering the potential combined effects of  
293 the foam quality and the flow rate (one, two and three generators) on the parameter  $f$  ( $f$  is  
294 the poorly adherent fraction of the population and/or less resistant to detachment), the  
295 variance analysis gave a  $p$  value of 0.027. The Tukey's grouping as shown in Figure 7,  
296 highlighted a slight effect of the flow rate:  $f$  being higher under the lowest flow rate  
297 conditions and higher with the foam where  $\beta=50\%$ , compared to the foam with  $\beta=70\%$  (no  
298 common letters, Tukey's grouping). More visible was the role of the flow rate on the  
299 constant rate  $K_{max1}$  ( $p=0.001$ ), the lowest flow rate clearly being the most efficient  
300 condition for spore removal under this first phase:  $K_{max1}$  was multiplied by a factor up to  
301 300. Foam quality appeared to play a role as the Tukey's grouping highlighted that  $K_{max1}$   
302 values for the  $\beta=70\%$  foam were very low compared to 50% and 60% foams. While taking  
303 into account data obtained with the CIP conditions, as also shown in Figure 7, flow  
304 conditions were still highly significant ( $P=0.0012$ ) and three classes were defined by Tukey's  
305 grouping (A, AB and B). In this case, CIP conditions gave an intermediate mean value of 55  
306 for  $K_{max1}$  compared to 87 with one generator and 6.7 or 1.3 respectively for two or

307 The effect of cleaning using foam flow, was tested with another *Bacillus* species. In Figure  
308 8 the two kinetics appeared very similar with a quick detachment in less than one min  
309 followed by a second phase, with about 0.5 log removal in both cases. Such a cleaning  
310 condition was chosen as the most efficient, according to the results described above. The  
311 main difference lied in the  $K_{max1}$  values, *B. cereus* spores being more difficult to remove

312 than *B. amyloliquefaciens* ones at the initial phase of the kinetics. Conversely, the removal  
313 during the second phase of the kinetics appeared very similar and this was confirmed by  
314 close values of the detachment rate  $K_{max2}$  for the two bacteria.

315 The microscopic observations showed the spores distribution on the coupons before and  
316 at different cleaning times. Only times 0 (fouling), 15 s, 3 minutes and 20 minutes were  
317 considered for comparison between foam cleanings (0.5 and 1 generator), one of the most  
318 effective foam cleanings observed and CIP. Microscopic observation showed that *B.*  
319 *amyloliquefaciens* 98/7 spores formed some clusters as shown in Figure 8, but these spores  
320 were mainly evenly distributed on the steel surface after the 4 hours soiling. Clusters were  
321 limited by the sonication of the spore suspensions prior to the soiling step and these were  
322 rapidly removed after only 15 s by both CIP and foam flow. Yet, according to Figure 9,  
323 removal was visibly greater with foam flow than CIP. The difference observed here (almost  
324 one log) is less than the one given by the removal kinetics (Figure 5B; 0.5 log difference),  
325 which considers viable and cultivable bacteria. However, the variability (up to 0.5 log)  
326 between trials could easily explain this discrepancy.

327

#### 328 **4. Discussion**

329 Foam is a two-phase gas-liquid fluid, in which gas is the dispersed phase and liquid is the  
330 continuous phase, where the volume of gas greater than that of liquid. In this work, only  
331 wet foam was used, meaning that foam is formed only of spherical bubbles, as observed at  
332 the top wall as previously described (Chovet and Aloui, 2016; Tisné et al., 2004) . Each mean  
333 velocity induced by one, two or three generators engendered a different flow regime.  
334 Indeed, for the lowest mean velocity, the axial component was uniform over the entire  
335 cross-section, thereby corresponding to the mono-dimensional flow regime or plug foam  
336 flow regime. For the mean velocity of  $4 \text{ cm s}^{-1}$ , the flow appeared partially sheared with a  
337 sliding at the walls, the axial velocity component no longer being uniform, depending on  
338 the ordinate and corresponding to the two-dimensional (2D) foam flow regime. One can  
339 notice that the top wall velocity remained constant (foam at  $\beta=50\%$  and  $\beta=60\%$ ) or was only  
340 slightly modified at the center of the top wall (foam at  $\beta=70\%$ ) under our experimental  
341 conditions. For the highest mean velocities, the foam flow was completely sheared with a  
342 sliding at the walls and could therefore be considered as three-dimensional (3D), with the  
343 underlying liquid film at the bottom of the duct flowing at a higher velocity, pulling the foam

344 flow above and therefore inducing a significant increase in the bubbles' velocity directly in  
345 contact with this thick liquid film. **This phenomenon is accompanied by a rearrangement of**  
346 **bubble sizes, with the largest bubbles being mostly moved up the pipe.** Such a phenomenon  
347 was already described by (Tisné et al., 2003). In this work, the cleaning of surfaces by the  
348 foam was evaluated at the top wall as a first evaluation of the role of flowing foam in the  
349 removal of surface contaminations (bacteria spores). In parallel, even if the experimental  
350 conditions were supposed to maintain the foam structure with the increase in the mean  
351 velocity, it appeared that the bubble size repartition at the top wall varied with the foam  
352 quality tested, meaning that bubble rearrangement had occurred: the increase in the mean  
353 velocity induced a reduction in the bubble size at the top wall.

354 Furthermore, it has been demonstrated that the variation at the top wall of the thin  
355 liquid film between the bubbles and the wall is directly affected by the bubbles passage and  
356 depends on their size (Tisné et al., 2004), which could have an effect on the effectiveness  
357 of adherent bacteria removal. The thickness fluctuations thus induced under their  
358 experimental 1D flow conditions varied between 5  $\mu\text{m}$  and 35  $\mu\text{m}$  with a foam quality of  
359 70%. Under 1D flow conditions for a foam quality of 55%, conditions close to our  
360 experimental conditions Chovet and Aloui, 2016, observed fluctuations at the top of the  
361 channel liquid film varying from 2  $\mu\text{m}$  to 40  $\mu\text{m}$ . In addition, this amplitude decreased with  
362 the increase in the foam quality, probably due to a change in the bubble size arrangement  
363 at the wall.

364 Microscopic scale studies (Tisné et al., 2004), reveal that it is possible to rely on studies  
365 of bubble flows inside circular capillaries, which will help in understanding the underlying  
366 phenomena (Bretherton, 1961). Assuming that there was no tangential shear stress at the  
367 fluid–fluid interface, he predicted that the film thickness was dependent on four  
368 parameters: the tube radius  $r$ , the liquid viscosity  $\mu_L$ , the surface tension  $\gamma$  and the bubbles'  
369 velocity  $V_b$ . The film thickness is as follows:

$$370 \quad e = 1.337 r Ca^{2/3} \quad (5)$$

371 where  $Ca$  represents the capillary number defined as:

$$372 \quad Ca = \mu_L V_b / \gamma \quad (6)$$

373 In relation to Bretherton's approach,  $r$  was assimilated to the radius of the bubble. Tisné  
374 et al., 2004 representing the evolution of the measured contact film thickness versus  $Ca^{2/3}$   
375 observed a close agreement with the Bretherton law (Bretherton, 1961), the bubble size

376 considered being 0.5 mm. Our experiments are in a capillary number range of  $11 \cdot 10^{-4} < Ca$   
377  $< 44 \cdot 10^{-4}$  ( $0.011 < Ca^{2/3} < 0.026$ ) falling within the range proposed by these authors,  $3 \cdot 10^{-4}$   
378  $< Ca < 28 \cdot 10^{-4}$  ( $0.005 < Ca^{2/3} < 0.02$ ). Given the agreement observed with the Bretherton law,  
379 the contact liquid film thickness in our experimental conditions would have ranged from 7  
380 to 18  $\mu\text{m}$  given a mean bubble radius of 0.5 mm, the thinnest liquid films being observed at  
381 the lowest velocities. Such a range of variation is in agreement with previous works (Chovet  
382 and Aloui, 2016; Tisné et al., 2004).

383 In parallel, it was shown (Tisné et al., 2003) that the wall shear stress was lower in the  
384 liquid film between each bubble and the wall. The wall friction was especially concentrated  
385 at the two ends of the bubbles; the wall shear stress fluctuations' amplitude being linked to  
386 the bubble size. When compared with the spore detachment kinetics, the greatest  
387 detachment efficiency appeared to be obtained with larger bubble size, notably when their  
388 diameter exceeded 0.1 mm, as clearly observed with the foam qualities of 0.5 (1D and 2D  
389 foam flow conditions) and 60% (1D foam flow condition) during the first step. Under CIP  
390 conditions, the detachment rate appeared to be comparable to the best foam cleaning  
391 conditions tested for comparable mean WSS conditions. In both cases the cleaning agent  
392 was the SDS under cold conditions (20°C). Previous work (Faille et al., 2018), highlighted the  
393 significant role of the fluctuation in the local wall shear stress on the cleaning efficiency  
394 under CIP conditions. One can draw a parallel here with these previous observations  
395 (Chovet and Aloui, 2016; Tisné et al., 2003), as the presence of the WSS fluctuations induced  
396 by the foam at the top wall appeared to play a role in the detachment mechanism and was  
397 clearly visibly under the 1D flow regime. However, for the 70%, foam quality, larger sized  
398 bubbles were observed at the top wall, which failed to ensure cleaning efficiency.  
399 Conversely, the increase in foam velocity meant a re-arrangement of the bubble sizes at the  
400 top wall (smaller bubbles). This phenomenon appeared to be unfavorable for efficient  
401 cleaning, as despite an increase in mean WSS, fluctuation amplitude decreased. **Local wall**  
402 **shear stress decreases dramatically while bubble passes and increases to a maximum**  
403 **between bubbles** (Tisné et al., 2003). **Therefore, the frequency of fluctuation of the local**  
404 **wall shear stress with large bubbles is less than the fluctuation with small bubbles but the**  
405 **amplitude is higher and would explain the differences in the spores' removal.**

406 The kinetics of bacteria spore detachment in the different flow and foam quality  
407 conditions were investigated and modelled according to previous work (Benezech and

408 Faille, 2018) on biofilm removal under CIP conditions. An identical simple two-phase model  
409 was found to be suitable for describing biofilm removal kinetics. The first bacterial removal  
410 phase corresponded to a quick removal of biofilm matrix with embedded cells, while the  
411 second phase accounted for the removal of cells directly attached to the steel surface. For  
412 bacterial spores removed by foam flow, the mechanisms appeared to be totally different,  
413 as the bacteria were evenly distributed on the stainless steel surfaces with very few clusters.  
414 This is unlikely to explain the quick and strong removal at the very beginning of the cleaning  
415 (less than 1 min). The parameter  $f$  corresponding to the part of the spore's population easily  
416 affected by the foam flow appeared to be significantly higher at the lowest velocities and  
417 for the wettest foam ( $\beta=50\%$ ). This also corresponded to the highest values of the  $K_{max1}$   
418 constant rate, i.e. the first phase of the removal kinetics. However, the second kinetic phase  
419 did not significantly improve the cleaning efficiency as a whole, whatever the conditions.  
420 For biofilms, it was observed that the chemical action contrarily to the mechanical action  
421 induced by the foam flow, was only involved in the first removal kinetic phase. The addition  
422 of chemicals such as NaOH during CIP conditions would largely improve this initial kinetics  
423 removal phase (Benezech and Faille, 2018). The difficulty in removing the remaining spores  
424 during the second phase of the kinetics was probably due to the stainless steel surface finish  
425 2B used, which was proven to be less hygienic than other finishes, such as bright annealed  
426 2R, as it presents boundary grains where spores can accumulate. The fluctuations in the  
427 liquid film thickness and/or of the wall shear stress appeared to impact the detachment  
428 phenomenon to a lesser extent.

429 A comparison with previous work on particles detachment by bubbles moving in a  
430 capillary duct, will allow the potential role of the capillary forces in the bacterial detachment  
431 to be taken into account. Two types of particles in terms of surface energy (hydrophobic  
432 and hydrophilic) of a size comparable to the Bacillus spores were used (Kondjoyan et al.,  
433 2009b), the entire air-liquid interface was modelled and the time-variation of the capillary  
434 force during transit of the bubble at the surface was determined. The particle detachment  
435 curve was thus predicted from near zero velocity to the highest velocity value, at which  
436 capillary force was supposed to vanish.

437 The approach was validated using latex particles  $2\mu\text{m}$  in diameter. The bell-shaped  
438 detachment curves experimentally obtained showed a width dependent on the value of the  
439 contact angle of the particles, the curve being narrower for hydrophilic particles than for



440 hydrophobic ones. The effective contact angle values of the particles could thus be deduced  
441 directly from the width of the detachment curves. *Bacillus amyloliquefaciens* 98/7 spores  
442 according to previous work were highly hydrophilic (Faille et al., 2010) with a contact angle  
443 to water of 20.5° (data not shown). For hydrophilic particles (Kondjoyan et al., 2009b), the  
444 detachment occurred at bubble velocities of around 3 cm s<sup>-1</sup> and dramatically decreased at  
445 5 cm s<sup>-1</sup>. As far as a direct comparison is conceivable, such a velocity range corresponded  
446 to the variation range (2.2 – 5 cm s<sup>-1</sup>), where the greatest detachment rate was observed,  
447 as illustrated by high K<sub>max1</sub> constant rate values under 1D flow conditions. For  
448 hydrophobic particles, the bell-shaped detachment rate was wider and detachment started  
449 at greater bubble velocities, starting at 3 cm s<sup>-1</sup> and peaking at around 7 cm s<sup>-1</sup>. This could  
450 partly explain the very low cleaning efficiency of surfaces soiled by the *Bacillus cereus* 98/4  
451 spores by the best foam cleaning conditions observed for *B. amyloliquefaciens*. *B. cereus*  
452 spores presented a high contact angle value (111.1°) as described recently (Faille et al.,  
453 2019a), largely over the value of 59° for the hydrophobic particles deduced from the bell-  
454 shaped curve (Kondjoyan et al., 2009b). With *B. cereus* spores, greater foam velocities  
455 should be tested, while conserving the bubble pattern obtained in this work under 1D flow  
456 conditions.

457 Time-variations relating to the capillary forces as an inlet condition in a modified  
458 adhesion and dynamic model were suggested as a way of predict the nano- and micro-  
459 movements of particles during their detachment from a surface (Kondjoyan et al., 2009).  
460 These movements are probably emphasized by the shear force fluctuations in our  
461 experimental conditions, which differ greatly to capillary flow conditions.

## 462 **Conclusions and perspectives**

463 **This work constitutes a cornerstone for future work on the implementation of foam flow**  
464 **cleaning in the industry. This requires** further activities on foam flow characterisation in  
465 order to be able to design a new efficient cleaning foam structure **e.g. less drainage**  
466 **phenomenon and increase of the wall shear stress at the bottom of the ducts**, which would  
467 take into account the surfactant used **(more profitable and usable industrially) than the SDS**  
468 and the temperature of the foam. The decrease of the temperatures seemed to play a  
469 significant role in its cohesion strengths (data not shown) **potentially corresponding to food**  
470 **processing sectors working under positive cold conditions e.g. fresh-cut or frozen vegetable**

471 **and fruit industries.** The novelty of this concept is to clean complex equipment while using  
472 far less potable water, at a lower energy consumption level.

473

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478

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Figure 1. Diagram of foam cleaning in place prototype

Figure 2: Bubble velocities measured at the top and lateral walls of the transparent duct measured at three positions (in red) in relation to the number of generators (one: square, two: diamond, three: triangle) and with the foam qualities (white: 50%, grey: 60% and black: 70%)

Figure 3. Foam visualization at the top wall of the transparent Plexiglas duct just upstream of the test ducts for the three foam qualities and foam flow conditions induced by the generators

Figure 4. Bubble size (mm) pattern; A: one generator, B: 2 generators, C: 3 generators and frequency of bubbles' passage (D) in front of the optical probe at the top wall for the three foam qualities: 0.5 (dark blue), 0.6 (light blue), 07 (yellow)

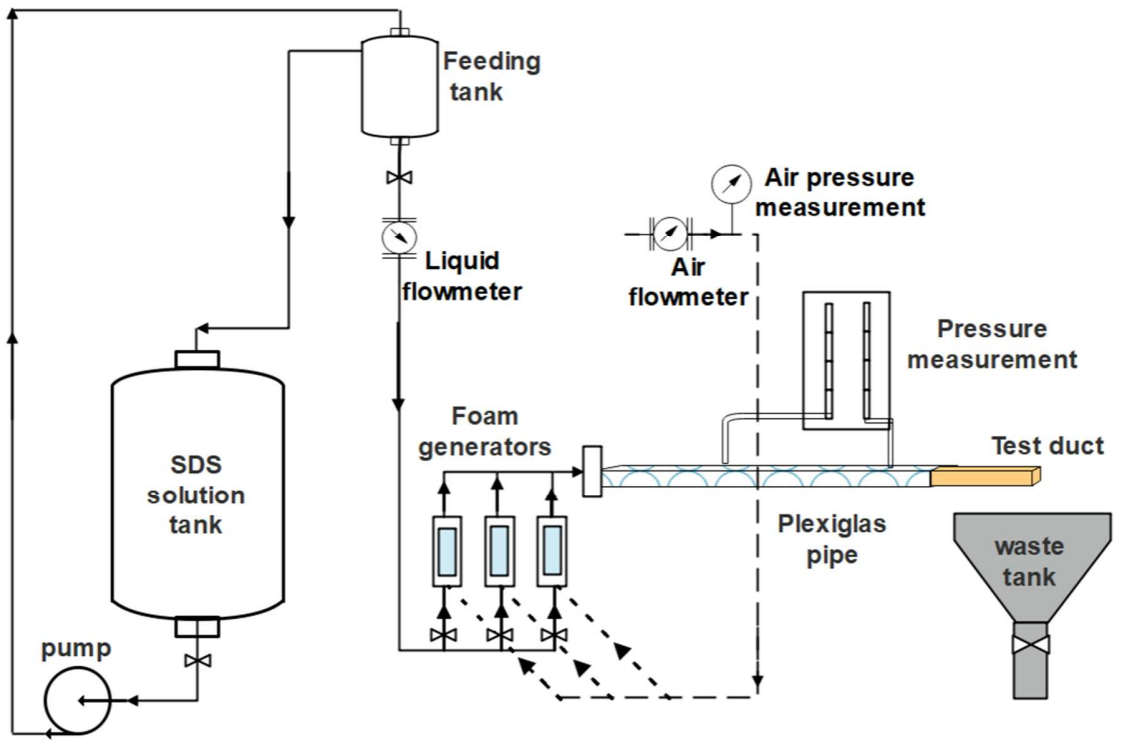
Figure 5. Removal kinetics of *B. amyloliquefaciens* spores under different flow conditions (mean values): 1 generator (square), 2 generators (diamond), 3 generators (triangle) for the foam qualities of 50% (A), 60% (B) and 70% (C); Removal kinetics with the foam quality of 50%, one generator compared to CIP ("foam quality" being equal to zero in that case) (D)

Figure 6. Decimal reduction of the *Bacillus amyloliquefaciens* spores induced by different flow conditions at 20 min cleaning time: comparison of the combined effects of the flow conditions and the foam quality including CIP conditions

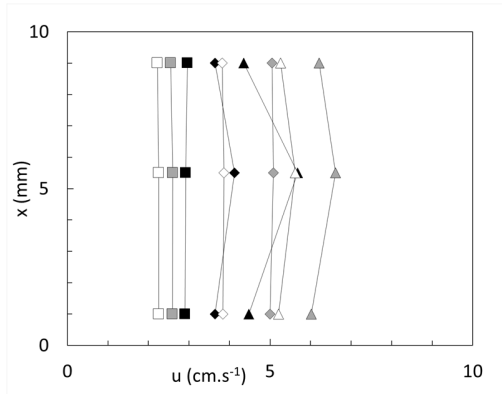
Figure 7. Variations induced by the combination of the foam quality (including CIP conditions for the last two graphs) and the flow rate induced by one, two or three generators on the kinetics parameters  $f$ ,  $K_{max1}$  and  $K_{max2}$ . According to the Tukey grouping, letters were indicated with potentially three classes A, AB and B; common letters meaning no significant differences

Figure 8. Comparison between the removal of *Bacillus amyloliquefaciens* and *Bacillus cereus* spores: cleaning with foam of  $\beta=50\%$  and one generator

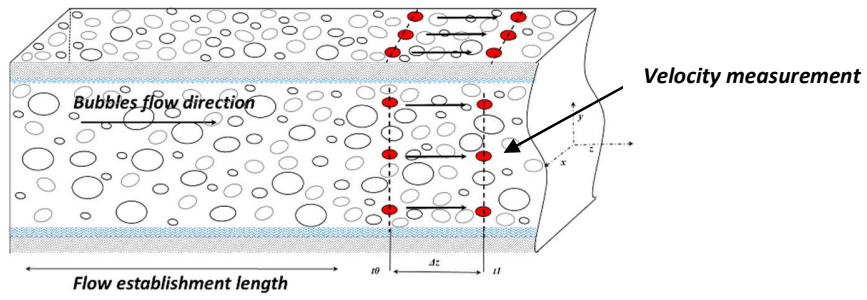
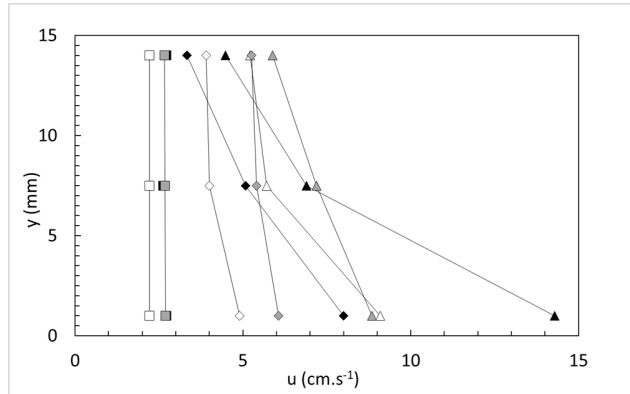
Figure 9. Observations of the stainless steel coupon contamination before cleaning and after 15 s, 3 min and 20 min with a foam quality of  $\beta=50\%$  using one generator or with CIP



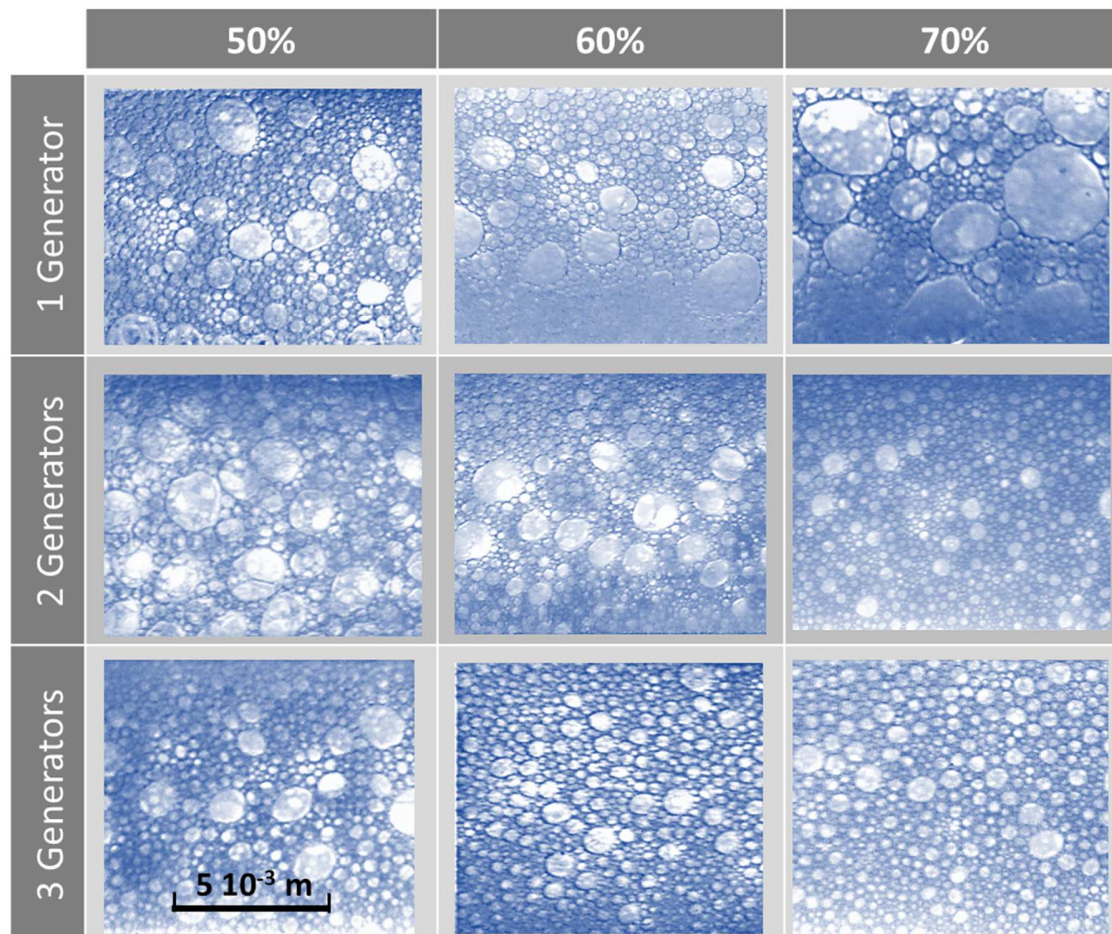
**Top wall**

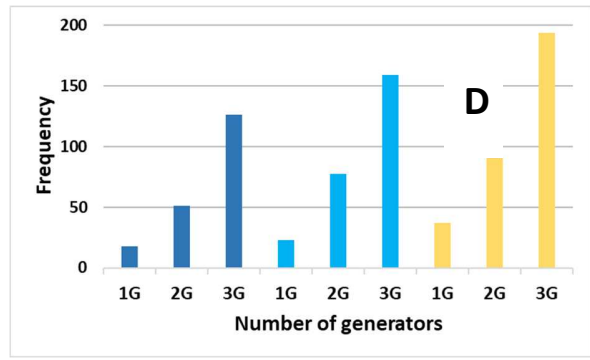
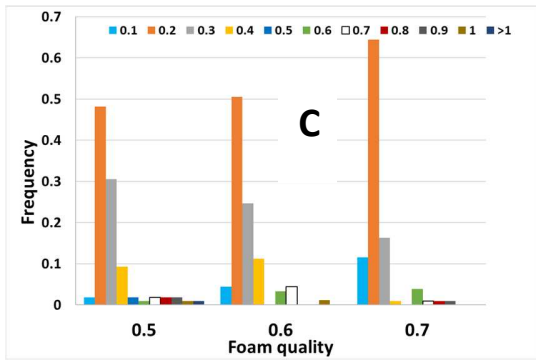
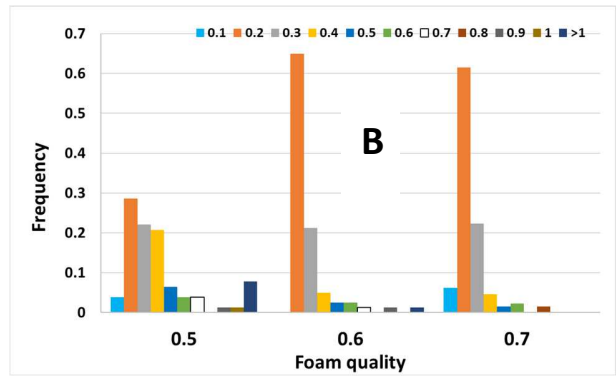
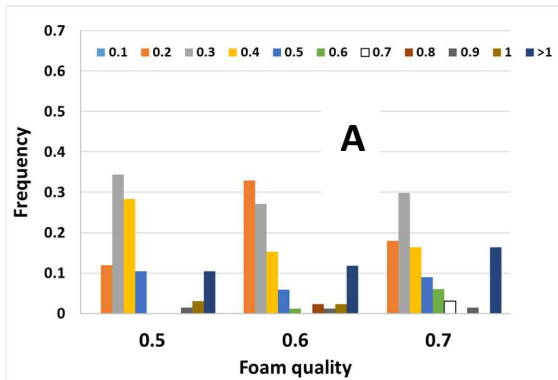


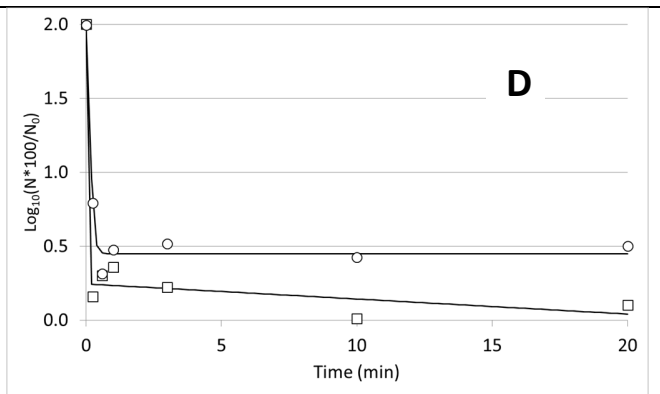
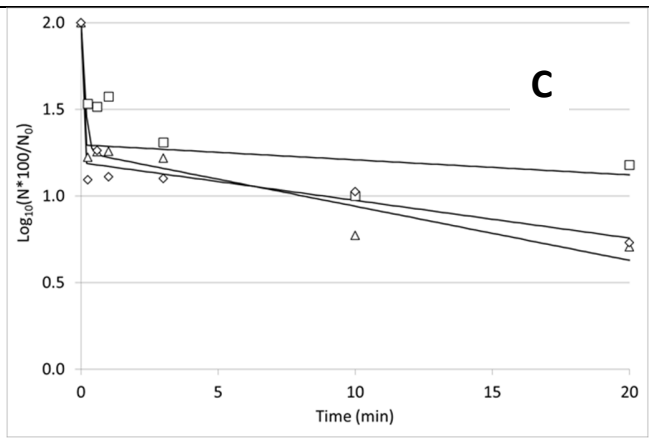
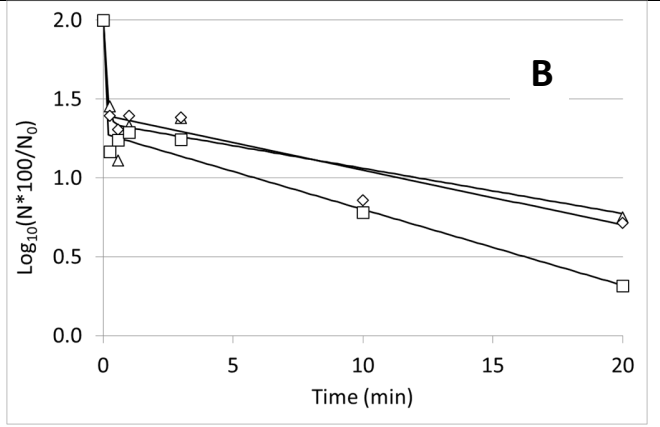
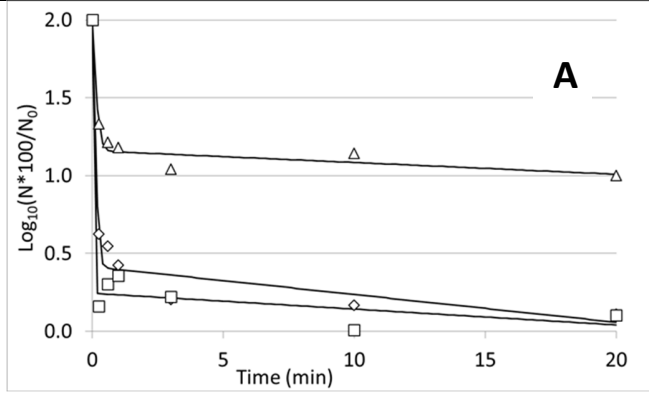
**Lateral wall**

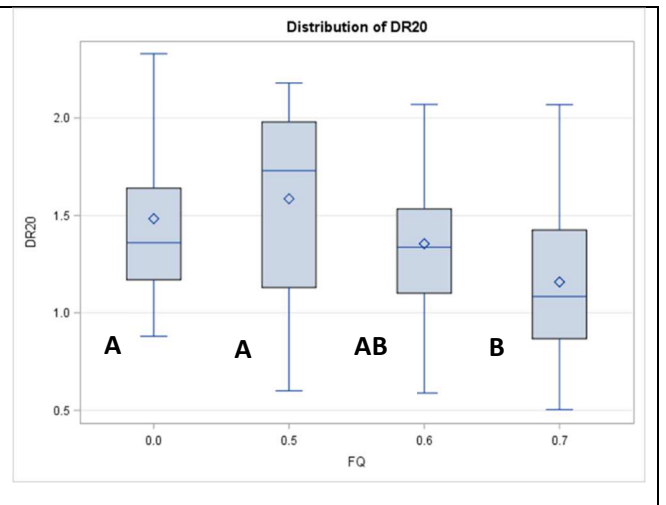
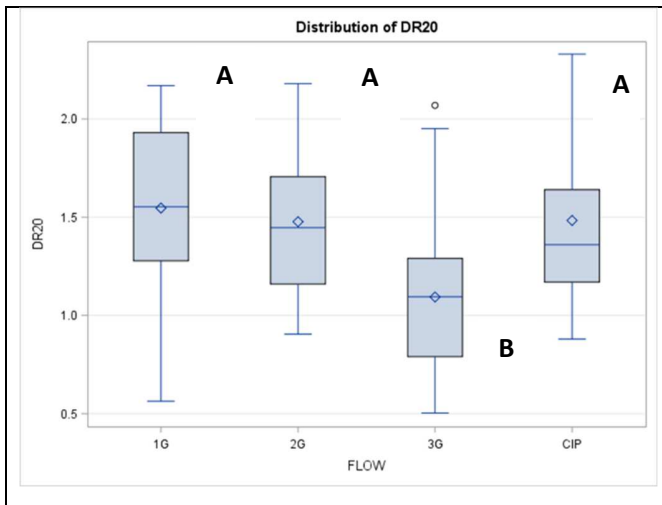


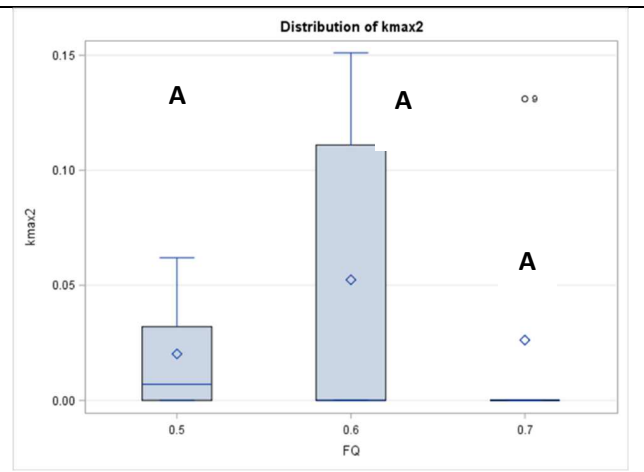
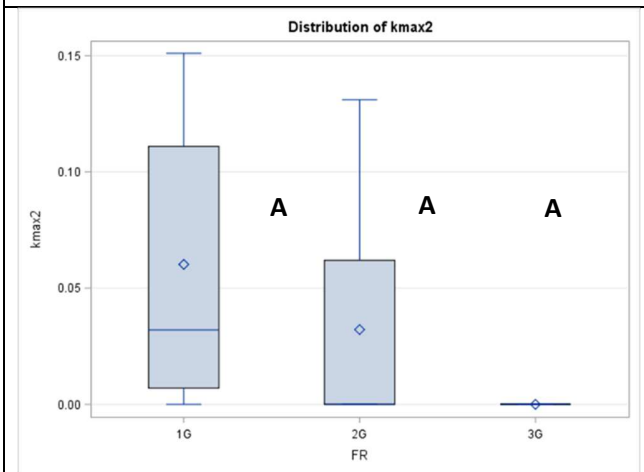
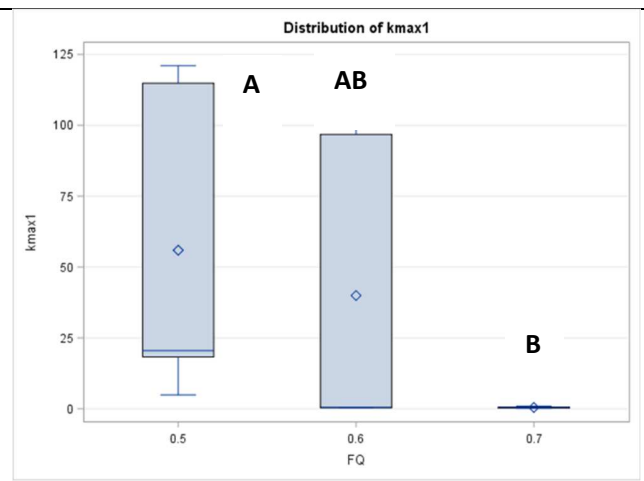
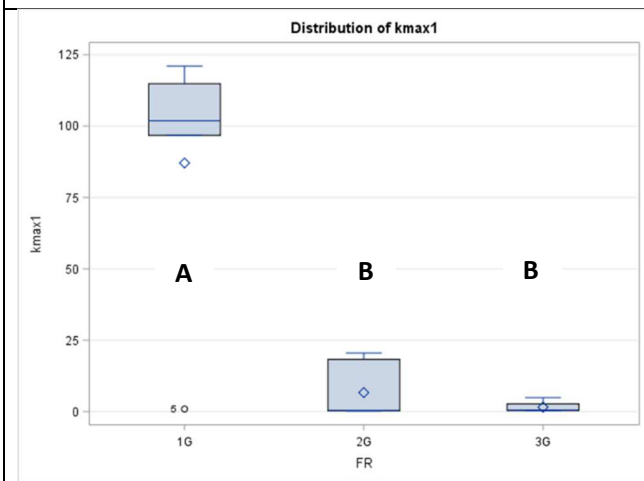
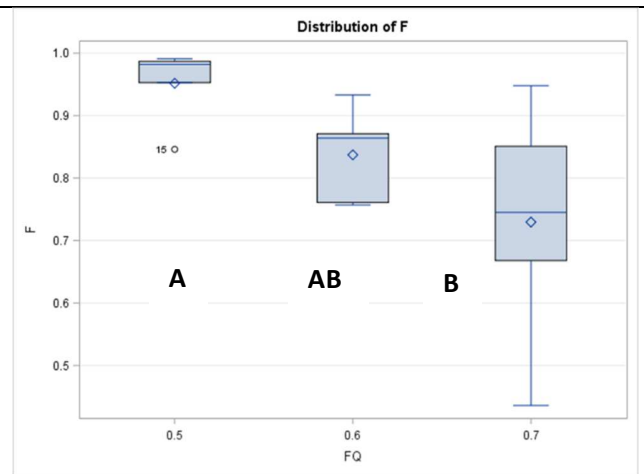
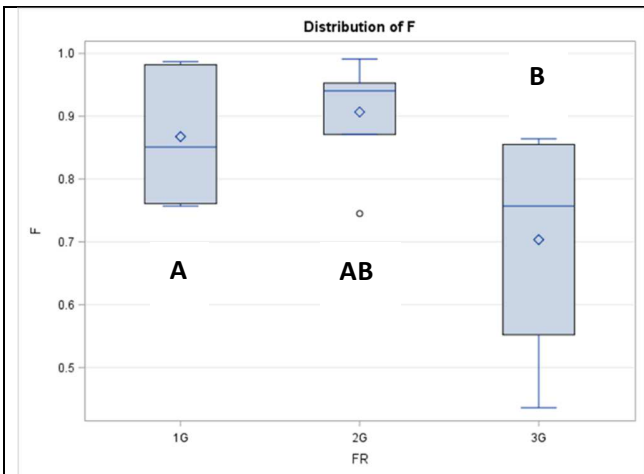


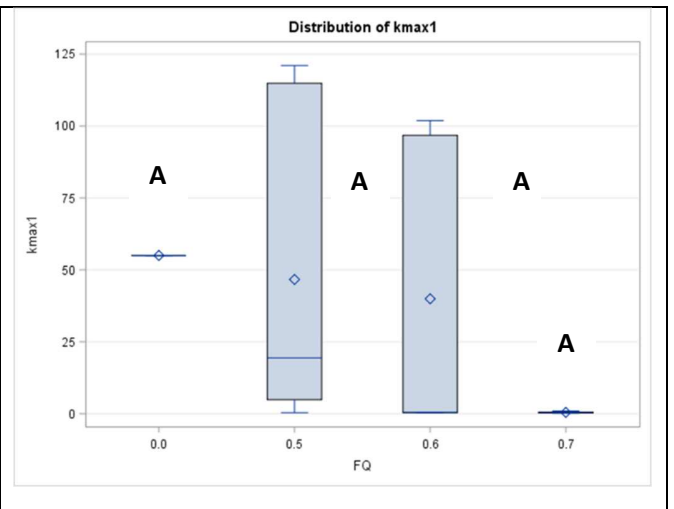
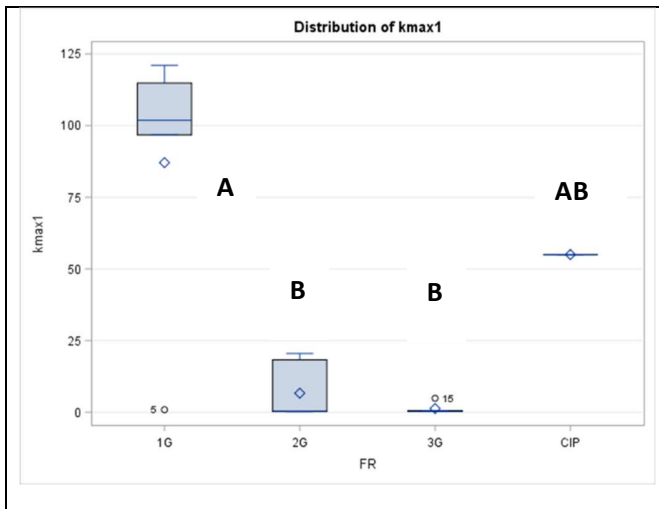


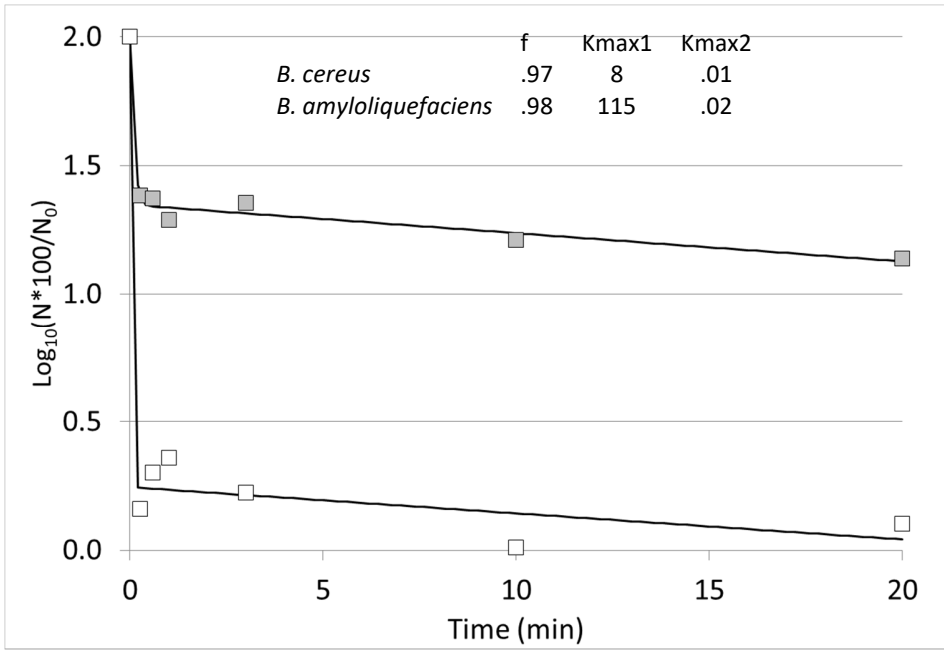


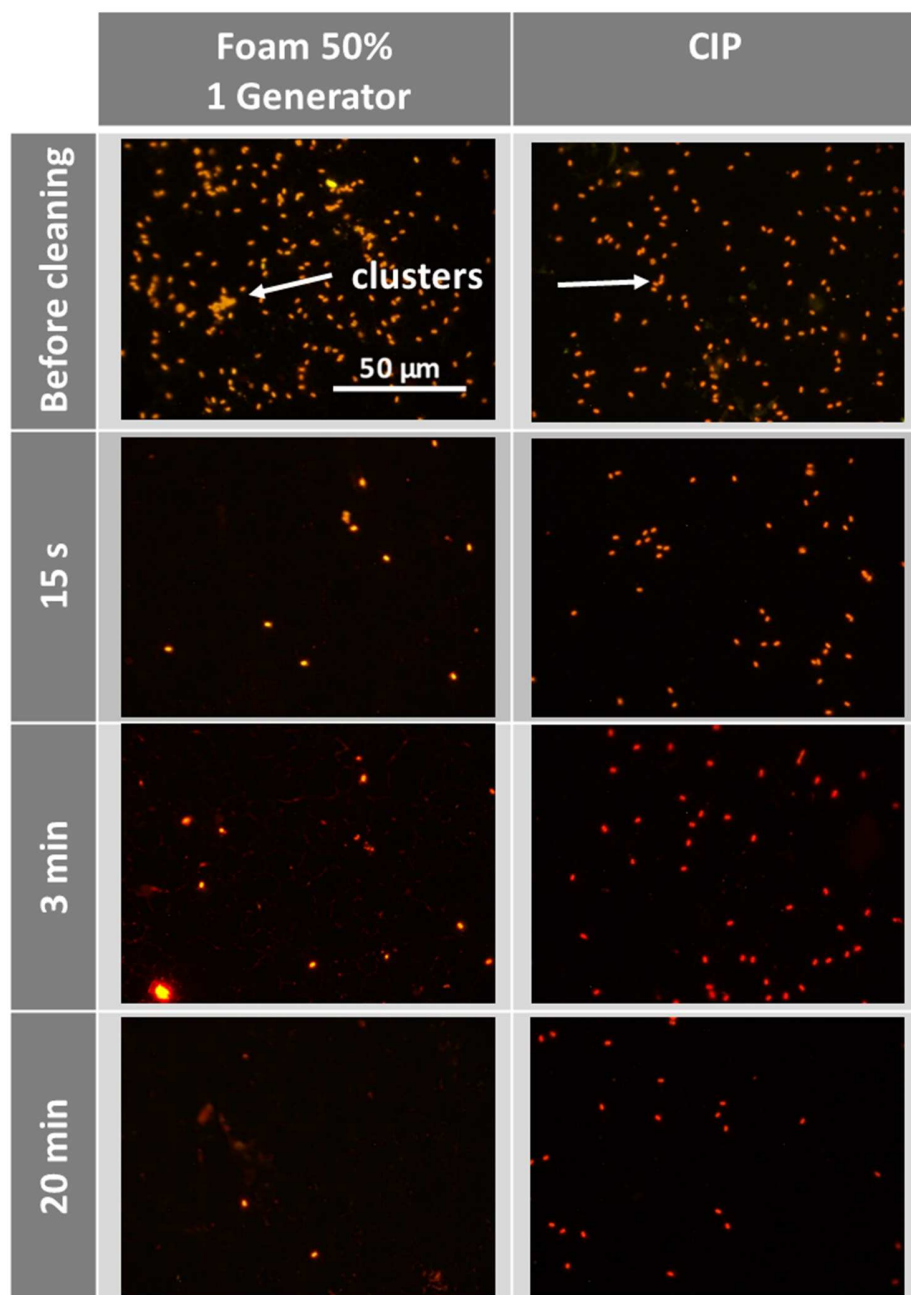














Liquid flow rate (l.h <sup>-1</sup> )	Air flow rate (l.h <sup>-1</sup> )	Foam quality $\beta$	Mean velocity (cm.s <sup>-1</sup> )	$\bar{\tau}_w$ (Pa)	Re
6	6		2.0	2.2	43
9	9	50%	4.0	4.2	87
13.5	13.5		6.1	5.9	130
4.2	6.3		2.4	2.2	51
8.4	12.6	60%	4.9	4.4	101
12.6	18.9		7.3	6.0	151
4.2	9.8		2.9	2.4	67
8.4	19.6	70%	5.7	5.1	135
12.6	29.4		8.6	6.4	202
650	-	0 (no foam)	120	5.1	14500

Table 1. Flow conditions for the foam flow and the CIP (foam quality = 0)