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Hygienic design of food processing lines to mitigate the risk of bacterial food contamination with respect to environmental concerns

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ABSTRACT

Public authorities, chain stakeholders and consumers are all concerned by microbial food safety. Microbiological hazards are one of the most common causes of food poisoning that has been considered for many years but still on the track nowadays considering the recent foodborne disease outbreaks largely reported by the media. Microbial contamination origins are diverse from the field to the plate e.g. soil, air, equipment surfaces, packaging material and staff.

Firstly, this article highlights the ways in which the choice of materials plays a major role in surface hygiene. Hydrodynamic conditions directly linked to the equipment geometry are focused on, as is the role played by surfaces in contact with air in surface drying. Surface environmental conditions during processing or cleaning are discussed and new proposals described. Better knowledge of surface contamination and cleaning mechanisms would positively impact hygienic design principles, thereby mitigating any environmental impact of the cleaning operations in the food and beverage industries: new strategies are therefore proposed.

Industrial relevance:

Hygienic design of food processing equipment is nowadays considered to be mandatory in the reduction of the risk microbial food contamination. The presentation of potential roles of materials on the remaining bacterial soil, after soiling and cleaning, provide new insights when envisaging any hygienic improvements. Equipment design plays a major role in

contamination trapping or in the ease of removal via the flow arrangements, notably during cleaning and rinsing operations. More detailed knowledge of the flow mechanical actions combined with the unavoidable chemical action of the detergents would allow industries to go further in enhancing certain “good” phenomena, such as pulsed flow conditions. Last but not least are those possibilities offered by a hygienically designed processing line, in the quest for environmental impact mitigation of cleaning operations i.e. drinking water and energy reductions, or the use of new “green” mild detergents.

Key-words: Surface hygiene, cleanability, bacterial food contaminants, biofilms, drying

Highlights

- Promising novel approaches to improve hygiene of equipment surfaces cleaned in place
- Mitigation of environmental impacts of cleaning operations
- Major role of drying phenomena in the resistance to cleaning of adherent bacteria

1. Introduction

Foodborne diseases are considered an emergent public health concern throughout the world. Food contact surfaces in processing equipment are considered to be major factors in the risk of food contamination in the food and beverage industries. Joint responsibility of equipment manufacturers and food producers could be suggested. Hence surface hygiene concerns could be tackled both by looking at the machinery design (geometry and material) and at the cleaning and disinfection operation conditions and frequencies.

Over recent years, apart from numerous studies of surface disinfections, great interest has been shown in both the mechanisms of (i) surface contamination, including the potential roles of materials and environmental conditions and of (ii) surface cleaning, largely governed by the machinery design impacting the mechanical action of the detergent flow under cleaning-in-place (CIP) conditions.

Another constraint for the food industries today, is the reduction of environmental impacts, all the more so, as cleaning operations are largely concerned in this issue.

The understanding of all the mechanisms involved in soiling and cleaning operations would provide combined strategies for providing cost effective results, without causing any adverse effect on human health or the environment. The purpose of this paper is to gather together all of the relevant insights from most of the recent studies to better decipher and hopefully, solve such issues.

2. Substrata: Role of material properties on bacterial contamination and detachment

Even when hygiene procedures are applied, adherent micro-organisms can be observed on every surface of food-industry plants, including stainless steel surfaces, gaskets, floors, conveyors belts or equipment. The ability of micro-organisms to contaminate these surfaces varies greatly according to the substratum composition. Indeed, differences are frequently observed in the ease with which bacteria not only adhere to materials (Faille et al., 2002), but also form biofilms (Somers & Wong, 2004). One reason given is that some of the substratum components, such as the nickel in some stainless steel alloys (Lopes, Morin, Oliveira, & Melo, 2005), or the elastomer NBR (Storgards, Simola, Sjoberg, & Wirtanen, 1999) would impact the physiology (growth or viability) of adherent bacteria. Furthermore,

the nature of the materials would also clearly affect the ease of removal of adherent bacteria and biofilms and consequently the hygienic properties of the substrata. Reported works have highlighted that bacterial detachment was easier from stainless steel than from other substrata such as PEHD, Teflon®, PVC… (Midelet & Carpentier, 2002; Somers & Wong, 2004), but differences have also been identified between polymers (Sénéchal, Carrigan, & Tabrizian, 2004). Along with their chemical composition, surface properties of materials used in the food industry also vary greatly. Some of them, e.g. physico-chemistry and topography, have been assumed to affect interactions between micro-organisms and materials, in terms of surface contamination and/or further ease of cleaning. It is worth noting that these surface properties may change over time, due to surface conditioning and/or of surface ageing.

2.1. Physicochemistry

Contrarily to what happens between cells and plant or animal tissues, the interactions between materials and micro-organisms are driven by physicochemical interactions, e.g. acid-base, electrostatic or hydrophobic interactions. Two major theories involving these physico-chemical interactions have been proposed for predicting the level of bacterial attachment to inert surfaces. The Derjaguin-Landau-Verwey-Overbeek (DLVO) theory describes the total Gibbs energy of interaction between a microorganism and a surface as a function of the separation distance (Hermansson, 1999). The thermodynamic theory is based on the comparison of the interfacial surface free energies for bacteria in suspension and bacteria in an adhered state (van Oss, 2006).

First of all, it is noteworthy that in most environmental conditions (neutral pH), bacteria are negatively charged, as are most of the substrata (stainless steel, glass, polymers). Consequently, repulsive interaction generally occurs, lowering bacterial adhesion, as demonstrated in a few works, by using positively- and negatively-charged materials (Mi, Bernards, Cheng, Yu, & Jiang, 2010; Terada et al., 2005), or by suspending media of low and high ionic strength (Li & Logan, 2004). Conversely, the subsequent colonisation could be inhibited on positively-charged surfaces because of strong interactions between bacteria and substratum which would impair cell division (Gottenbos, 2001). Along with these interactions, hydrophobic interactions are also supposed to play a major role, due to the unusually strong attraction of hydrophobic surfaces in water (Meyer, Rosenberg, & Israelachvili, 2006). According to van Oss (Van Oss, 1997), hydrophobic interactions would even be the strongest long-range non-covalent interactions in biological systems. However, even if much has

been written on the role of substratum hydrophobicity in the attachment of bacteria and in the formation of biofilms, there are a number of inherent contradictions. The bacterial adhesion, interaction strength (Xiaoxia, Peng, & Olavi, 2006) and ease of removal of adherent bacteria (Boulangé-Petermann, Gabet, & Baroux, 2006; Sénéchal et al., 2004) has often been reported to be impaired on hydrophobic substrata, but other reports suggest that hydrophobic substrata would promote bacterial adhesion, given the greater extent of bacterial adhesion (Cerca, Pier, Vilanova, Oliveira, & Azeredo, 2005), or the higher resistance to cleaning procedures of *Bacillus* spores, whatever the spore wettability (Faille et al., 2002). These discrepancies could be, at least in part, relevant to bacterial properties, e.g. the presence of fimbriae (Otto et al., 1999), or of a mucous layer (Faille et al., 2014). Conversely, only a few works have suggested that biofilm development and structure are affected by the substratum hydrophobicity (Patel, Ebert, Ward, & Anderson, 2007; Ploux, Beckendorff, Nardin, & Neunlist, 2007). Concerning surface free energies, little hard evidence has been published stating that substratum surface energy is correlated to bacterial adhesion (Hüsmark & Rönner, 1993; Ong, Razatos, Georgiou, & Sharma, 1999; Vadillo-Rodríguez, Busscher, Van Der Mei, De Vries, & Norde, 2005). This lack of correlation would be the result of a non-linear relationship, with a lowest adhesion for intermediate values of the surface free energy (20–30 mJ m^{-2}), as shown in reported works (Pereni, Zhao, Liu, & Abel, 2006; Tang et al., 2009; Zhao, Wang, Liu, & Wang, 2007).

The difficulty in attributing a specific role to each of the physicochemical interactions might be related to the coupling of several interactions in bacterial adhesion and biofilm formation (Sheng, Ting, & Pehkonen, 2007). One could also hypothesize that the discrepancies observed between models and real phenomena would reflect non-homogeneous surface free energies, that would provide localized sites favourable for bacterial attachment or retention, for example on stainless steel grain boundaries (Geesey et al., 1996). Indeed, micro- or nano-scale heterogeneities in substratum physicochemistry have been proven to affect bacterial adhesion (Bos, van der Mei, Gold, & Busscher, 2000; Kalasin, Dabkowski, Nusslein, & Santore, 2010). At PIHM, the central role of surface micro-heterogeneities on *Bacillus* spore adhesion was displayed (Galopin et al., 2010). Indeed, an accumulation of spores was clearly observed at the borders of the superhydrophilic/superhydrophobic patterns (Figure 1). However, these aspects have been currently poorly investigated, despite their possible consequences in terms of bacterial contamination of heterogeneous substrata, or of border areas between different materials in contact.

To add further complexity and to render the comprehension of interfacial phenomena even more challenging, the substratum is continually subjected to surface conditioning

resulting from the rapid adsorption of materials from the liquids in contact (proteins, fat, minerals, detergents, surfactants, disinfectants, etc.). The formation of the so-called conditioning film may strongly affect not only the surface physico-chemistry (such as the basic polar component) of materials, but also their hygienic properties. In order to investigate the influence of this conditioning film on substratum properties, Jullien *et al.* (Jullien et al., 2008) subjected stainless steel surfaces to up to 25 successive soiling (with milk or meat) and cleaning (with detergent) runs in a pilot rig. They observed a significant decrease in the hygienic status of the stainless steel surfaces for both conditioning procedures (milk or meat), but the decrease was more marked when milk was used. Other reported works have also demonstrated that conditioning conditions strongly affected further bacterial surface contamination, yet that the modifications resulting from this conditioning film were bacteria-dependent (Palmer, Flint, & Brooks, 2007).

To conclude, the composition/structure of the conditioning film is closely dependant on environmental conditions, e.g. nature of foods and detergents in contact, fluid temperature… In other words, the physicochemistry and consequently the surface hygiene of materials found in food environments may change radically, depending on the food chain and the processes in place.

2.2. Topography

In addition to the physicochemical properties, substratum topography has been recognized to largely influence bacterial contamination. Indeed, in industrial plants it is common to find materials with an irregular surface, some of which are poorly accessible and difficult to clean, such as gaskets or complex pieces of equipment (Lemos, Mergulhão, Melo, & Simões, 2015) and these areas are often associated with a persistence of bacterial contamination.

Intuitively, one can assume that surface areas available for bacterial attachment or growth are greater on rougher surfaces and that topographic features provide protection to adhered bacteria from shear forces, as for instance during cleaning procedures. However, despite the volume of results available in the literature, the mechanisms by which surface topography modulates bacterial contamination remain largely unclear. The inconsistencies in the published results might be primarily due to the fact that, in most reported works, the

surface topography was solely described by the parameter Ra (average roughness, arithmetical mean deviation of the absolute ordinate values within a sampling length). For example, some results suggested that bacterial adhesion/colonisation of stainless steel surfaces (Ra ranging from 0.06 to 0.89 µm) decreases with Ra, until a threshold average roughness of 0.16 µm, below which no further reduction could be expected (Medilanski, Kaufmann, Wick, Wanner, & Harms, 2002), while others (Hilbert et al., 2003) failed to observe any effect on the adhesion and removal by a cleaning procedure (Ra ranging from 0.01 to 0.9 µm). This is doubtlessly partially linked to the fact that Ra is unable to account for the shape of the surface irregularities, or even the presence of some rare irregularities despite their role in surface hygiene. For example, in a work published by Faille *et al.* (Faille et al, 2000), two stainless steel surfaces characterized by different topographies (periodic roughness profile with regular waves, vs irregular profile without any organisation but with many pits and valleys) exhibited similar Ra values (+/- 1.15 µm), but quite different hygienic statuses. Furthermore, adherent bacteria have been often associated with surface features similar in size to, or slightly larger than the cells or spores involved, as observed in the bottom of crevices (Díaz, Schilardi, Salvarezza, & De Mele, 2007), or in the depth of the grain boundaries and crevices (personal communication, Figure 2). We may assume that surfaces with features of microbial sizes may retain bacteria in greater numbers than smoother or rougher surfaces by increasing the exchange surface with adherent bacteria and providing a better protection from shear forces.

The relevance of other roughness parameters than Ra, such as those derived from the Abbot-Firestone curve was investigated. These parameters such as the reduced valley depth (Rvk), the reduced peak height (Rpk described in the standard ISO 13565-2 (1997), or the levelling depth (Rp) described in the standard DIN 4762 (1989) better take into account the presence and shape of peaks and valleys on the substratum surface and have been proven to be better linked to the hygienic status of the materials (Faille et al., 2000; Jullien, Bénézech, Carpentier, Lebret, & Faille, 2003).

It is important to consider that the above studies often involved different material types with a wide range of shapes and sizes of surface features, which are spread more or less randomly over the whole surface. In order to gain the best possible understanding of the role of specific features in bacterial adhesion, surfaces with regular, well-defined topographical patterns were produced and investigated for their hygienic status. Works

reported by Whitehead and Verran were among the first involving these kind of patterned surfaces. Using microbial retention assays with a panel of differently sized micro-organisms, they determined that the sizes of both surface defects and bacterial cells significantly affected the resistance to removal of adherent bacteria (Whitehead, Colligon, & Verran, 2005). More recently, Perera-Costa *et al.* (Perera-Costa et al., 2014) showed that spatially organised micro-topographic surface patterns reduced bacterial adhesion (30−45%) relative to the smooth control samples, whatever the bacterial shape or surface energy. Unfortunately, despite the lack of data available in the literature, it is highly likely that the presence of micro- and nano-sized topographical features may increase the retention of organic matter which in turn would affect interaction between bacteria and substrata. Therefore, further study needs to be carried out to validate the effectiveness of patterned materials after an accumulation either of complex food residues or polymeric substances produced by biofilms.

3. Geometry: flow dynamic roles from surface contamination to surface cleaning

3.1 Surface contamination

Hydrodynamic conditions are known to impact biofilm dynamics at any stage, from the adhesion step to the appearance of fully developed biofilms. Much literature is available on this topic, mainly in environmental sciences. Very recently, Lemos *et al*. (2016) and Araujo *et al*. (2015) have shown the effect of the hydrodynamic conditions under which biofilms were formed on selected macromolecular characteristics, demonstrating that higher flow velocities can give rise to more complex and denser biofilms. More than 20 years before, Vieira *et al*. (1993) had already shown the impact of turbulences respectively on the biofilm macro-structures and microstructures. Under too high wall shear stress conditions (Tsai, 2005) observed a dramatic reduction in the surface microbial load at the stationary phase of the biofilm growth. All of these activities were done at the laboratory scale. Indeed, few works have looked into what happens in complex food equipment or processing lines. Quite recently Blel *et al.* (2010) worked on the soiling of an industrial two-way valve by a suspension of *Bacillus cereus* spores in water. The authors reaffirmed that in turbulent flow conditions, the transport process of particles is mainly governed by convection and diffusion.

However, when viscous forces near the wall are high enough, the transport mechanism is mainly carried out by diffusion throughout the boundary layer. The adhesion process occurs due to attraction forces (van der Walls and electrostatic forces) between particles and the wall. Moreover, for bacterial spores of approximately 1 μm width and 2 μm long, adhesion forces are complex due to their surface energies and the potential presence of specific 3D surface structures. Indeed, in addition to the hydrophobicity and the relative charge of the surface which induce high attraction forces, *B. cereus* spores are surrounded by an exosporium thereby increasing contact surfaces, as its appendages are also in contact with the steel surface. In addition Blel *et al.* (2010) demonstrated the highly significant role of confined zones in terms of hydrodynamics, in constituting huge deposit areas at Reynolds numbers as low as 3300. They interpreted the surface load observed as a result of two phenomena, (i) spore convection and adhesion when the applied wall shear stress is moderate, but sufficient to ensure the transport of spores to the wall and (ii) spore deposition in confined zones characterized by weak mixing and thus low shear stress forces, when the residence time for spores is sufficient.

Very recently, Cunault *et al.* (2015) working with a set-up composed of a series of mock-ups mimicking the design of washing tanks widely used in fresh-cut food industries, have studied the growth dynamics of biofilms developed on internal surfaces under turbulent flow conditions. These vats were filled with water containing *Pseudomonas fluorescens* cells and agitated by a Rushton impeller inducing a turbulent flow regime. The flow pattern inside the vats was highly modified by the design of the vats (corners, folds or flat surfaces). The authors demonstrated a strong link between the flow pattern induced by the design and the biofilm development dynamics. Using CFD, ranking of the different zones was based on the contamination load. The differences observed were compared to the flow arrangement inside the vats and to the CFD evaluation of the wall shear stress. Recirculation areas impact on the contamination load was emphasized by low wall shear stress conditions, such as in corners inducing high levels of attached bacteria over 10^7 CFU cm^{-2} after 72h. Conversely for flat and vertical walls affected by the highest mean wall shear stress values, 10 times more, the observed surface contamination load was lower at less than 10^2 CFU cm^{-2} after 72h. As in Blel *et al.* (2010), the wall shear stress parameter appeared to be insufficient to explain this difference. PIV (Particle image velocimetry) measurements have shown that fluctuations in the local flow velocity occurred directly due to the impeller blade rotation.

Such fluctuations over time, which has been shown to be a key factor to understand the surface contamination removal (Blel *et al.*, 2007), would probably deeply affect the initial contamination and the further biofilm growth dynamics as demonstrated in the recent study by Araujo et al. (2016).

3.2. Surface cleaning

In closed processing lines, the microbial safety of the products must be ensured by efficient hygienic procedures using Cleaning In Place (CIP) processes. However, the literature shows that some bacterial contamination does remain on equipment surfaces after standard CIP procedures (Elevers et al., 1999). It is now commonplace to consider the definite role of the wall shear stress on the cleaning efficiency. Models were proposed to describe the microbial removal integrating this parameter in the past (Lelièvre et al., 2002). However, Jensen et al. (2005) and Lelièvre et al. (2003) demonstrated that wall shear stress alone is not sufficient to explain the level of removal of bacterial contamination from a surface. Indeed, in turbulent flow regimes, the fluctuation of this parameter would explain some of the discrepancies observed, such as high cleanability levels combined with low mean wall shear stress values. Blel et al (2007) working on the cleaning efficiency of specific pipe features widely encountered in food processing lines arrangements, such as sudden or gradual contraction or expansion pipes and bends with various curved edges, have measured the local wall shear stress in parallel to the velocity profile to better understand how the flow arrangement could affect the bacterial removal through cleaning. They confirmed previous observations and highlighted the significant role of the flow dynamics induced by the geometry e.g. recirculation areas, high wall shear stress fluctuations, and how such flow perturbations could be transmitted along the line to deeply change the cleaning efficiency downstream.

Despite the number of studies dealing with turbulence generation and transport near the wall, few authors have been interested in adhesion and detachment mechanisms of bacterial cells in the confined area near wall zones. Braaten *et al*. (1988) however, mentioned detachment under a turbulent flow regime of *Lycopodium* spores with an average diameter 10 times larger than the bacteria. Generally, the size and the density of particles are considered as major parameters, which control the kind of interaction with

coherent structures as equipment walls. According to Ziskind et al. (2000), particles high enough to reach the external region of the boundary layer, are subjected to the effect of the large scale motions in the external region contrarily to small particle dimensions probably located deeper in the viscous sub-layer, where the instantaneous velocity distribution is linear, as in the case of bacteria spores or initial biofilm stages composed of few cells directly attached to the surface or in small, flat clusters. In this zone, the effect of the large scale motion on particle re-suspension is excluded. Nevertheless, Corino and Brodkey (1969) showed that ejections and sweeps of coherent structures contribute to the increase in the wall shear stress. Ziskind *et al.* (2000) reported that horseshoe or hairpin vortices are the most important flow patterns inducing the detachment of small particles from the wall due to their contribution to the shear stress forces generated. Thus, whatever the size of the particles, eddies generated near the wall induce fluctuating shear forces. When these hydrodynamic forces are greater than the adhesion ones, re-suspension occurs. In Blel *et al.* (2007) *B. cereus* spore transport, adhesion and detachment mechanisms from a stainless steel wall during cleaning were discussed. Some fundamental concepts, such as the key characteristics of a turbulent flow near the wall and the nature of interaction between turbulence and an individual bacteria spore under simplified conditions, are used in order to explain residual levels of contamination of surface equipment after a CIP procedure. Briefly, the comparison between turbulent eddies' size and repartition and the dimension of *B. cereus* spores suggest the possible re-deposition phenomenon at zones characterized by high dissipation of the turbulent energy. This eventuality is also closely related to spore morphology and to the relative density between the fluid and the suspended spores. The work of Le Gentil *et al.* (2010) demonstrated this deposition phenomenon of contaminants even during CIP procedures. Experiments were focused on the surface contamination during the CIP procedure of pipes and of a two-way valve (cleaned and disinfected before the test) coming from soiled pipes (around 5 10^6 CFU cm^{-2}) inserted in a clean loop. The authors observed that re-adhesion was controlled by the flow pattern, the valve being significantly contaminated after CIP. It is clear that equipment design plays a significant role in contamination trapping (Blel *et al.,* 2010), but as shown here by Le Gentil *et al.* (2010), potential new cleaning in place strategies should take this phenomenon into account.

More recently, Faille *et al.* (2013) have evaluated the respective roles of mechanical and chemical effects on the removal of *Bacillus* spores during cleaning-in-place. The authors

demonstrated that the mechanical action alone, such as the one delivered during standard industrial CIP conditions is not sufficient to remove any *Bacillus* strains. Hence, a CIP at 4 Pa (mean wall shear stress) in pipes appeared to be as efficient as a rinsing at 500 Pa. Spores from most of the species tested were shown by electron microscopy to be slightly affected by the chemical and temperature CIP conditions. The detachment efficacy during CIP is probably largely influenced by the spore's outer layer's susceptibility to chemicals, which may lead to the weakening with time of the interaction strength between spores and substrata. Hence along with the cleaning time, the removal kinetics of bacterial surface contamination has to be established in order to highlight such phenomena. Removal kinetics of *B. cereus* biofilms (Sylla *et al.*, 2011) were modelled by a simple two-phase model accounting for two-"species" removal, with each of the "species" removal being described by first order kinetics. The phenomenon was described as a removal of two differing adherent bacteria sub-populations with constant rates in relation to their poorly or highly adherent properties. Chemical action or mechanical actions alone appeared to be far less effective that a combination of the two. The poorly adherent population removal constant rate was found to be doubled when comparing CIP and rinsing conditions, the flow rate being the same. More interesting was the very low value of the removal constant rate of the "strongly attached sub-population", being up to 100 times lower than the removal constant rate of the "poorly attached population". Consequently, a 30 min cleaning appeared to be insufficient to remove all of the surface contamination. An example of the removal kinetics thus observed is presented in Figure 3.

4. Role of areas in contact with air: interface air-liquid-material, drying of contaminated surfaces

A number of other conditions found in food environments could influence the bacterial surface contamination or further cleanability. Such is notably the case regarding the presence of interfaces between substratum, liquid and air. Indeed, several reports on the bacterial contamination of partially immersed surfaces have indicated that the interface between air and liquid would be favourable to bacterial adhesion, as well as to the formation and/or the persistence of biofilms (Giaouris & Nychas, 2006; Liu et al., 2015; Wijman, de Leeuw, Moezelaar, Zwietering, & Abee, 2007). One can easily imagine that the

oxygen availability at the air/liquid interface would contribute to this preferential location of biofilms. As an example, *Salmonella enteritidis* produced between 2 and 3 log more biofilm on half-immersed stainless steel coupons, as compared to wholly-immersed coupons (Giaouris & Nychas, 2006). Similar observations were made on *B. cereus* strains (Wijman et al., 2007), which clearly form biofilms at the interface between the liquid and air in wells of microtiter plate assays, but also on partially-immersed stainless steel coupons. The differences between wholly- and half-immersed coupons was of the same order as above, which means that the local bacterial concentration at the air-liquid interface biofilm will be even higher, notably up to 10^8 cells cm^{-2}. Along with the oxygen availability, it was suggested that motility is a key element of biofilm formation at the air-liquid interface (Majed, Faille, Kallassy, & Gohar, 2016). In conditions close to those encountered in fresh-cut food processing equipment, the air/liquid/wall interface was also shown to be particularly favourable to surface contamination (Cunault et al., 2015). More interestingly, the structure of the biofilm formed by *P. fluorescens* greatly differed in both environments as observed by SEM (Figure 4): Huge, thick patches were easily observed at the interface between air and liquid, while the potential for biofilm growth was limited on submerged areas. As a consequence, industrial storage and piping systems, which are partly filled during process or where residual liquid has remained after a production cycle (mainly if inadequately cleaned), are conducive to biofilm formation (Wijman, de Leeuw, Moezelaar, Zwietering, & Abee, 2007) and would contribute to recurrent contamination of food during further production cycles.

Furthermore, many bacterial soils undergo a drying step in food environments, which can occur during the production phase or between the production phase and the hygiene procedure. That is the case, for instance, on open surfaces such as conveyor belts, cutting boards, or once again at the air-liquid interface on surfaces of partly-filled equipment, including tanks, pumps, or piping systems. Unfortunately, many bacteria are able to withstand these periods of desiccation, whether in the form of biofilms (Halavi & Hansen, 2013) or of adherent cells (Kusumaningrum, Riboldi, Hazeleger, & Beumer, 2003). Moreover, exposure of a *B. cereus* biofilm to air was recently shown to accelerate spore formation (Hayrapetyan, Abee, & Groot, 2016), a bacterial form known to be strongly adherent and able to survive pasteurisation. These areas may represent a vastly underestimated risk for food contamination. Indeed, in a recent work (Faille, Bihi, Ronse, Ronse, Baudoin, &

Zoueshtiagh, 2016), the cleanability of adherent *Bacillus* spores was proven to be significantly lowered when subjected to a drying step, both time and temperature playing a significant role in the resistance to detachment (Figure 5). Several hypotheses have been put forward to explain this phenomenon. Among these, the shape of the liquid bridge between the sphere and the substratum, could play a major role in the interaction force between bacteria and substrata. The authors also demonstrated that the shape of the liquid bridge, which determines the pressure inside the liquid, and therefore the type of interaction between the particle and the substratum (attraction vs repulsion), was greatly influenced by the hydrophilic/hydrophobic characters of the two surfaces. The physico-chemistry of the substratum would therefore play a role in the various phenomena occurring at the interfaces between bacteria and materials. The areas where drying process occur must therefore be of concern in the hygiene management in the food industries.

5. Sustainability of cleaning operations in the food and beverage industries

It is generally admitted that the cleaning process efficiency generally depends on four energy factors: mechanical energy due to hydrodynamics for soil detachment, chemical energy breaking down soil and rendering them easier to remove, thermal energy which increases the effects of the two first factors and the cleaning time. It is commonplace to state that a restriction in one energy source may be compensated for, by increasing energy from the other factors. Nevertheless, a better understanding of cleaning mechanisms and of their dynamics is still needed not only to improve hygiene, but to take into account the impacts of such operations on the sustainability of food process industries. This would drive research toward a complete renewal of cleaning condition proposals.

This has prompted investigations into pulsed flow, where a significant change in velocity is imposed on a steady flow either intermittently or continuously.

Based on the major role of the flow perturbation in the cleaning efficiency, the effects of pulsating turbulent flow conditions in the cleaning efficiency were investigated by Gillham et al. (2000) showing the increase in both mass transfer and wall shear stresses in pipe flows. More recently, Bode et al (2007) showed the effect of the waviness ratio between the amplitude of velocity pulse and the baseline flow velocity on the cleaning time of milk-type deposits. Blel et al. (2009a) using the non-intrusive electrochemical method in straight pipes,

showed that the pulsating flows induced an increase in the local velocity gradient at the wall pipe. This result was explained by the periodic renewal of the boundary layers. Spectral analysis showed strongly increasing rates of the fluctuation energy for the different pulsating conditions in comparison with a steady flow. The test conditions involving a recirculation flow inducing a modification in the energy dissipation cascade, which can be explained by the redistribution of eddy size near the wall. In Blel et al. (2009b), experimental data on the removal of bacteria spores from pipe surfaces showed the contribution of the different pulsation parameters in the removal of adhered bacterial spores, in addition to the effect of the mean velocity of the flow. A high cleaning rate level is observed despite the reduction in the magnitude of the mean velocity. The role of the variation of the wall shear stress due to pulse conditions on bacteria removal efficiency should be investigated as a significant improvement observed in both spore removal rate and in the time necessary to reach a complete removal.

More recently Blel et al (2013) working on a highly complex equipment, namely a scraped-surface heat exchanger soiled by biofilms of *P. fluorescens*, demonstrated again the interest of pulsed flow during CIP procedures in tough conditions. The study focused on the entry bowl of the exchanger, where three critical areas in terms of ease of cleaning were observed. This phenomenon was explained by the low mean wall shear stresses and low fluctuations measured under standard CIP flow conditions. The use of a pulsating flow completely modified the flow and thereby significantly reduced the residual contamination in those areas. Recently Foeste et al. (2013) has developed a CFD model based on the assumption of a mass transfer controlled cleaning process. A validation of the cleaning mechanism, using experimental data of local cleaning times in several complex geometries with varied static and transient flow velocities, was carried out. The authors stated that based on the new CFD model presented the local cleaning efficiency can be predicted, including any particular specificities of the pulsed flow conditions.

Pulsed flow increases instantaneous rates of heat, mass and momentum transfer, equivalent to the use of much larger steady flow velocities and larger volumes of liquid. Less liquid can be used, cleaning carried out more quickly and more reliably, as a result of relatively cheap hydraulic energy input and pulsing equipment. Further research is still needed in this area for further industrial implementations. Srey *et al.* (2013) in a quite recent review of biofilms in food industries, mentioned ultrasonication, a well-known technique, as

an efficient biofilm removal method. Today, such a technique cannot replace current cleaning operations, but could be of help in reducing the criticality of some areas in processing lines, allowing milder cleaning conditions for the whole processing line cleaned-in-place. It has been documented that even though lower-frequency in sonication is remarkably more efficient than higher frequency for reducing biofilm cells' viability, bacteria in food industries cannot be eliminated solely using the present ultrasonic technologies, thus ultrasound techniques are combined with other treatment techniques (ethylene-diamine-tetra-acetic acid, enzymes or ozonation) have been recommended (Baumann et al. 2009). The sustainability of these combined techniques has yet to be proven, but should be considered in a global strategy.

Cleaning operations in food industries require great quantities of drinking water, inducing environmental impacts today considered to be inacceptable. The dairy industry is for instance, consumes large quantities of water for food processing. Depending on the final product (milk, cheese, yogurt or milk powder), water consumption can go from 0.6L to as high as 6L per litre of raw milk treated (EDA, 2002). These figures make the dairy sector the highest water-consuming process per unit of raw material in the food sector. Apart from the production of the raw product (cattle breeding for milk production), most of the water consumption is imputable to food processing, which significant quantities required for cleaning operations.

One strategy to reduce the amount of water used, could be to clean with diphasic flows e.g. air and water. Too few works deal with this topic and to our knowledge, with the removal of microorganisms. However, Kondjoyan et al. (2009) demonstrated the interest of bubbles circulating in water generating a capillary force strong enough to detach micron-sized particles from solid surfaces. Such micron particles could be considered as "model bacteria". Another option would be the use of foam. Hence, although foam cleaning, followed by rinsing operations, is widely used for open surfaces in the food sector, it has rarely been used for closed system cleaning. Indeed, foam flows are known to generate parietal shear stresses at least 100 Pa higher than in the case of liquid flow under identical flow rates. A recent study by Chovet (2015) demonstrated the potency of horizontal foam flow in pipes in the generation of high wall shear rate conditions using small amounts of surfactants and of water (void fraction over 80%). Preliminary data have recently been

proposed showing the interest of foam flow for the removal of *Bacillus subtilis* spores (Figure 6).

In addition, the soil generated by cleaning in place solutions induces large economic and environmental costs. Hence, recycling such solutions appears as an attractive alternative to reduce these costs. Blel et al. (2015) have proposed a cleaning solution regeneration technique using Na-bentonite and combining adsorption/coagulation and flocculation. The cleanability of stainless steel surfaces showed that regenerated solutions allow better surface wettability, thereby explaining the observed improvement in the cleaning of stainless steel surfaces including microbial soiling by *B. subtilis* spores.

6. Conclusion

In surface contamination, material surface properties roles, mechanisms induced by environmental conditions during soiling or cleaning lead to new proposals for the future as described above. It can be also mentioned that novel surface coatings and alternative chemicals for cleaning are being actively researched at an academic scale. A recent study by Moreira *et al.* (2016) proposed the use of surface alteration of stainless steel. They observed that after performing a cleaning protocol with chlorine, a reduction of bacterial counts was much higher on a stainless steel modified by the incorporation of silicon (a-C:H:Si or SICAN) compared to stainless steel. However, the interest of these new surfaces demonstrated at the laboratory level could be jeopardized by the surface conditioning after several soiling and cleaning cycles, which may potentially radically change their surface properties. Any improvement should be balanced with research approaches integrating industrial condition particularities. A way of attenuating this obstacle would be to favour milder cleaning conditions. This could be envisaged using more hygienically-designed production systems in combination with less aggressive cleaning substances. For instance, Lequette *et al.* (2010) demonstrated the interest of enzymes in the efficient removal of biofilms working with a panel of bacteria isolated from different food industry sectors. However, the longevity of surface coatings and the traceability of enzymes from closed systems remain to be fully demonstrated.

While maintaining high hygiene standards any option proposed to lower energy, chemicals or water used are currently much sought-after. As described in this paper, new

proposals for materials and equipment design combined with mild alternate cleaning conditions, minimizing water load and all environmental impacts of cleaning operations will become mandatory, if food industries are able to meet future challenges while preserving our environment.

Figure captions

Figure 1: Observation by scanning electron microscopy of a patterned silicon nanowire surface (50 x 50 µm^2 superhydrophilic squares on a superhydrophobic surface) after incubation in a *Bacillus* spore suspension (Galopin et al., 2010).

Figure 2: Observation by scanning electron microscopy of a biofilm formed by *Kocuria varians* and mainly located in surface defaults (PIHM)

Figure 3. Examples of removal kinetics of *Bacillus cereus* biofilms under CIP (empty dots: 60°C, NaOH 0.5%, and a mean wall shear stress of 2 Pa), under static conditions (empty triangles: 60°C, NaOH 0.5%) and rinsing conditions (empty diamonds: mean wall shear stress of 2 Pa)

Figure 4: Observation by scanning electron microscopy of a biofilm formed by *P. fluorescens* in a fresh-cut food processing equipment on fully-immersed areas [WET] and at the interface between air and liquid [DRY] (Cunault et al., 2015)

Figure 5: Stainless steel coupons contaminated with *B. subtilis* 98/7 spores were allowed to dry for 3 h at 25°C or 50°C. Coupons were inserted into a parallel-plate flow chamber with a rectangular flow channel (PIHM). Observation of spore detachment was performed under an optical microscope (Axio-scop 2 plus, Zeiss) at a magnification of ×400. Images were recorded by camera (Olympus, DP21).

Figure 6. Examples of removal kinetics of *Bacillus subtilis* spores at a mean wall shear stress of 5 Pa, SDS 0.3 % w/w under flowing foam cleaning conditions at 3 l h^{-1} (diamond captions) or liquid flow conditions at 300 l h^{-1} (rectangle captions)

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Fig 1.

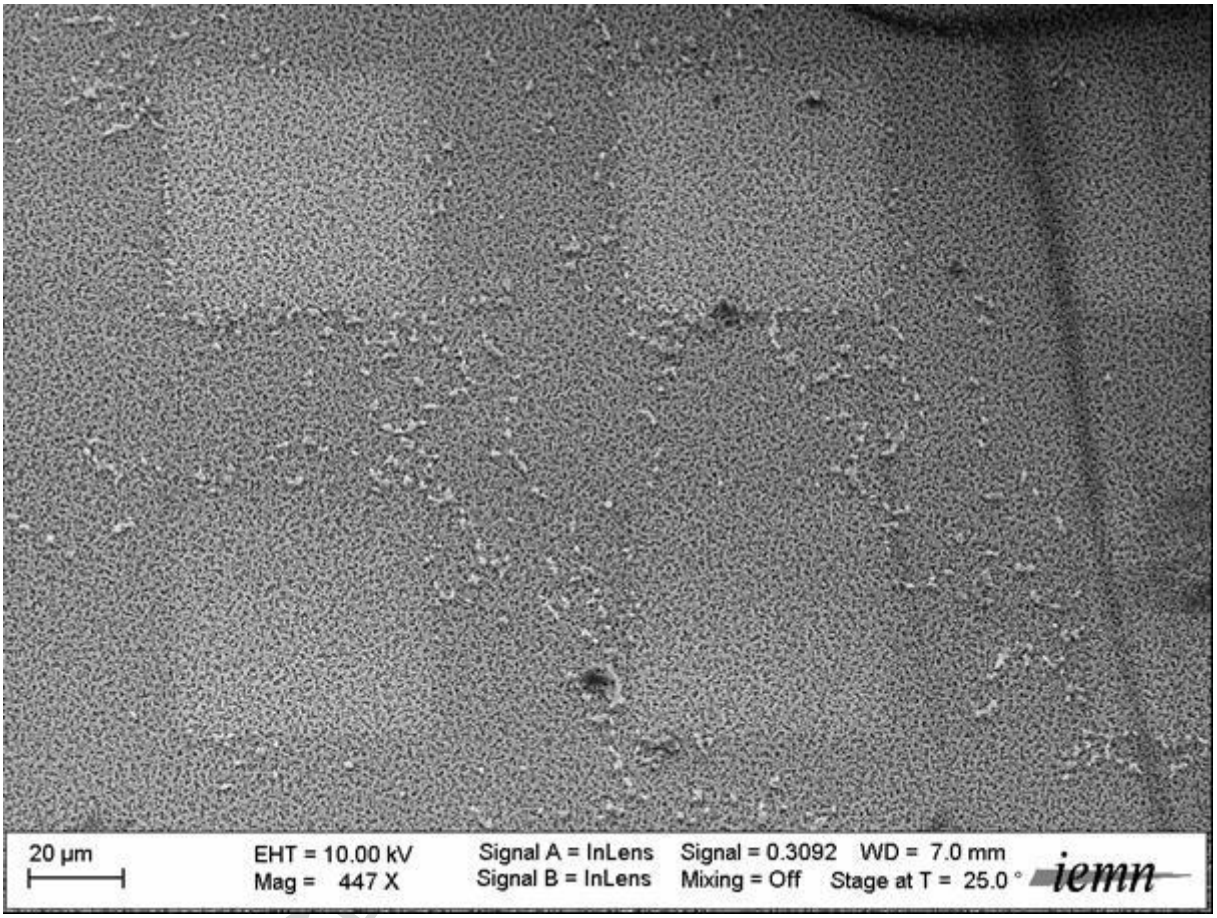


Fig. 2.

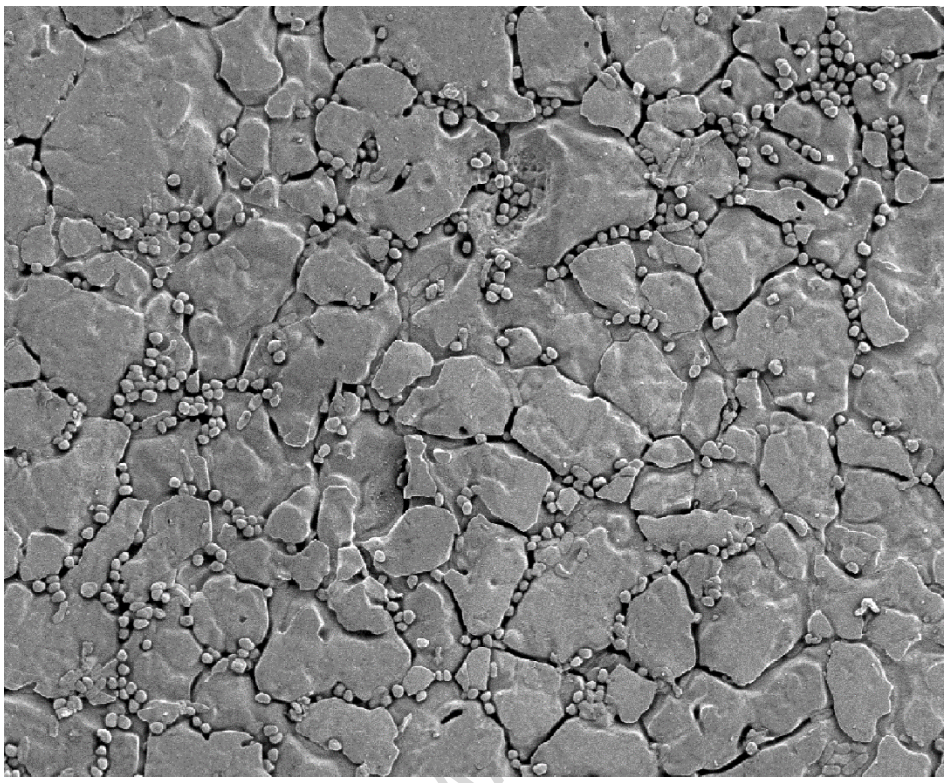
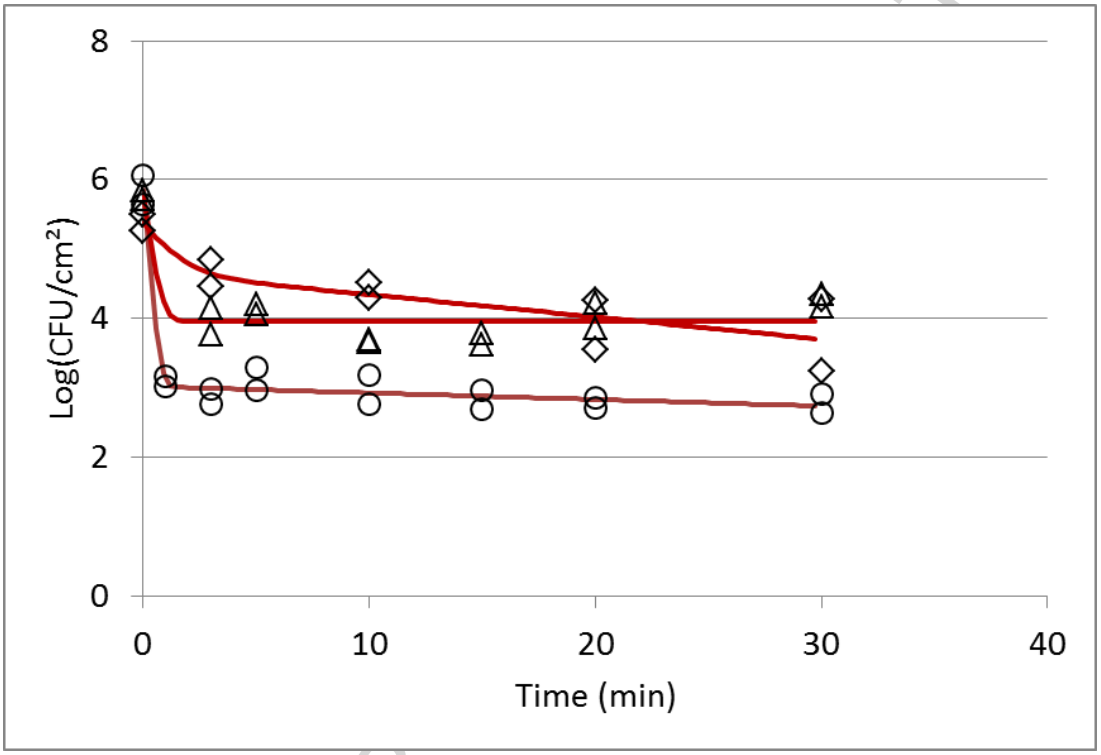


Fig. 3.



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Faille, C., Cunault, C., Dubois, T., Bénézech, T. (Auteur de correspondance) (2018). Hygienic design of food processing lines to mitigate the risk of bacterial food contamination with respect to environmental concerns. Innovative Food Science and Emerging Technologies, 46 (SI), 65-73. . DOI : 10.1016/j.ifset.2017.10.002

Fig. 4.

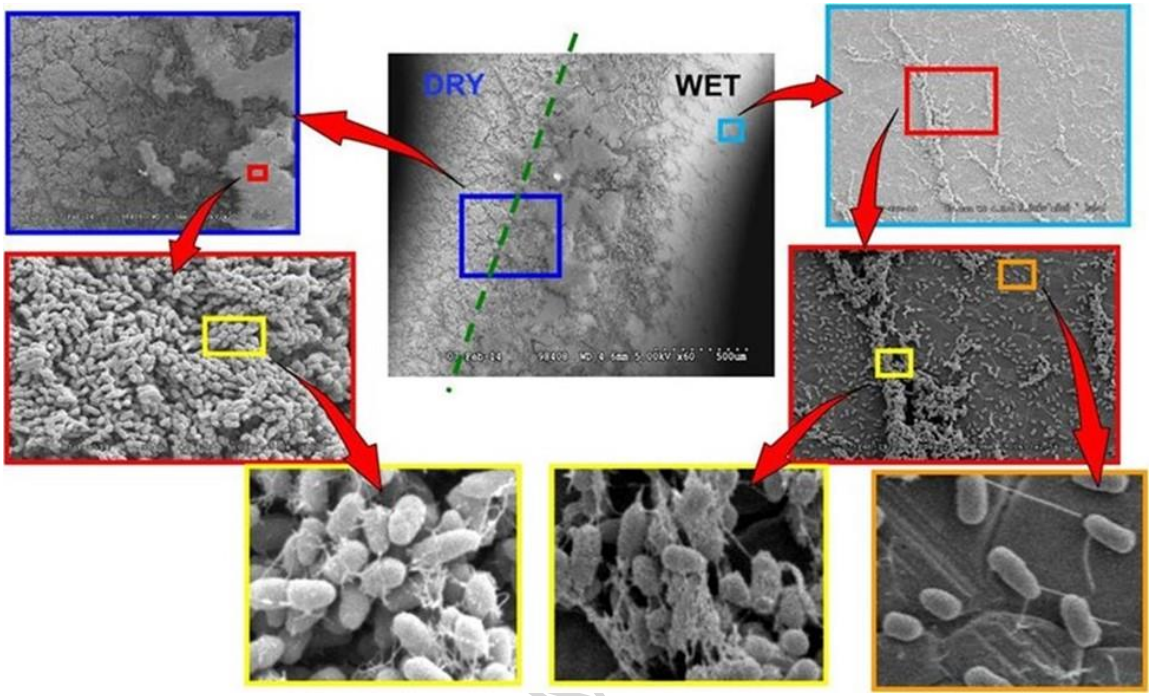

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Fig. 5.

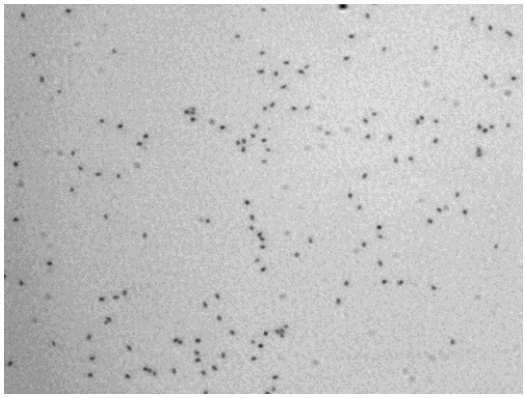
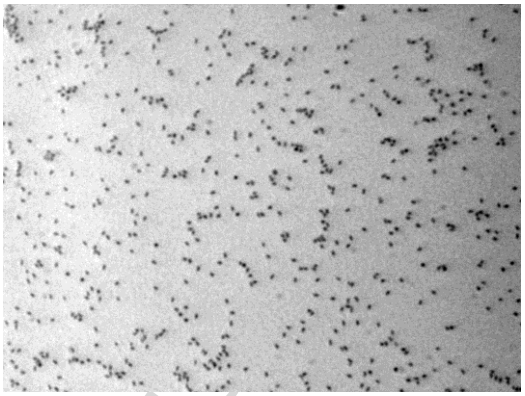
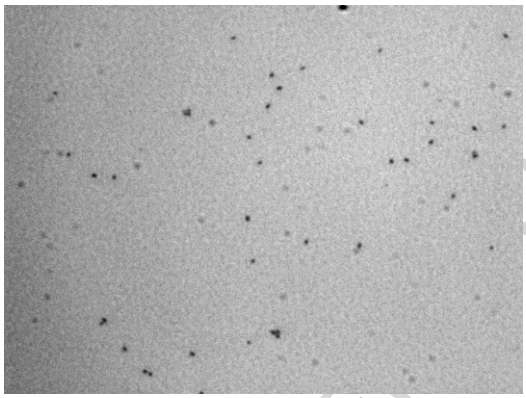
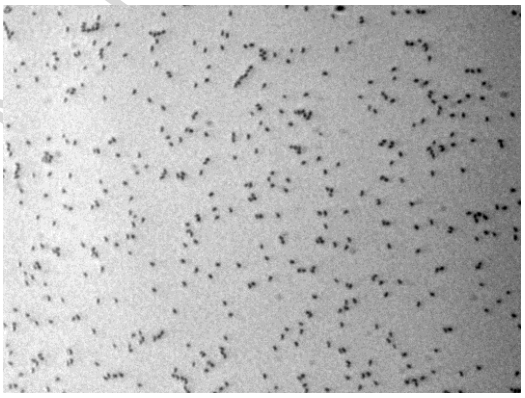
Coupons dried for 3 h at 25°C	Coupons dried for 3 h at 50°C
 Initial contamination	 Initial contamination
 Residual contamination (447 Pa)	 Residual contamination (447 Pa)

Fig. 6.