Assessing bactericidal properties of materials: the case of metallic surfaces in contact with air

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Abstract

A new method for assessing bactericidal properties of metallic materials, soiled by aerosol, was developed and applied to stainless steel in conditions close to reality. The airborne bacteria survival on different stainless steel grades and massive copper is presented here. The investigating bacterium was *Enterococcus faecalis*, which is a well-known contaminant strain in the indoor environments. It was observed that the bacterial aerosol lethality increased proportionally with the relative humidity (RH) of the environment. A significant difference in survival rate was measured depending on the tested supports, the greatest lethality being observed on clean massive copper. Moreover, the addition of nutrients on metallic surfaces, even in small quantities, was enough to ensure the revival of quiescent microorganisms. © 2002 Elsevier Science B.V. All rights reserved.

1. Introduction

Numerous airborne biological particles and their components, bacterial endotoxin and mycotoxin are found in the air of buildings or food industry shops. The survival and development of these biocontaminants in the air are partly linked to the colonisation of material surfaces (Brown, 1953; Finch et al., 1978; Robine et al., 2000).

Bactericidal materials for hygienic applications are subjects of recent development, as for instance, stainless steel. Consequently, these new products highlight the importance of pertinent tests to assess the hygienic properties of materials or surfaces.

Hygiene uses all means available: tools, chemical products, designed to guarantee the health and safety of the persons. In a hygienic environment, the microbial population needs to be controlled and reduced. However, very few tests assessing the bactericidal property of materials in contact with air have been proposed. However, many studies dealing with the fouling and cleaning aspects of materials in contact with foodstuffs have been carried out (Visser, 1997, 1998).

The traditional method employed for assessing bacterial viability is cultivation. A criterion of non-cultivation could only be connected to a bacteriostatic property of the material but not to its bactericidal property. In order to test the bactericidal activity of such materials, it is necessary to use discriminating methods describing the bacterial physiology. Recent studies dealing with bacterial stress have demonstrated

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that after adhesion or after an external stress such as osmotic shock, thermal shock or disinfection, a loss in the cultivation criterion is observed (Caro et al., 1999).

Bacteria can lie dormant, but their physiological state is reversible and the pathogenic microorganisms can conserve their virulence (Pommeruy et al., 1996). Dormant bacteria cannot be detected by cultivation, but they still represent a danger. Therefore, it is necessary to develop new methods of microorganism detection using fluorochromic coloration (Heldal et al., 1996; Kildes and Nielsen, 1997).

The objective of this work is to perfect a method for assessing bactericidal properties of materials. This is done by soiling stainless steel surfaces by an aerosol and controlling microorganisms. In this test, a ubiquitous strain *Enterococcus faecalis* contaminated the steel surfaces after spraying. *E. faecalis* is an opportunist agent very often implicated in nosocomial infectious outbreaks. Gram positive cocci are very common in indoor environments and could represent 85% to 90% of the species recovered from airborne bacteria (Etkins, 1994). The materials studied are austenitic cold-rolled stainless steel AISI 304, the most common steel used in buildings and health applications as well as copper. An experimental copper-rich stainless steel is also tested since the bactericidal activity of pure copper is well-known (Gillet, 1999).

2. Materials and methods

2.1. Tested materials

2.1.1. Selection

The metallic surfaces selected in this study are massive copper (Cu, Goodfellow, UK) with a purity higher than 99.99% and different stainless steel grades (Ugine, France) including copper-rich stainless steel (AISI 211 + 3Cu) and a standard cold-rolled stainless steel (AISI 304, bright annealed or 2R finish). The elementary composition of tested materials is summarised in Table 1.

The AISI 304 stainless steel selected for this study is mainly composed of chromium (18%) and nickel (9%) and widely employed in hygienic applications (Bavay, 1993). In addition, a new austenitic grade composed of 3% copper (AISI 211 + 3Cu) is also added, and in this case, nickel is replaced by manga-

Table 1
Elementary composition of materials (wt.%)

	AISI 304	AISI 211 + 3Cu
Fe	balance	balance
Cr	17.09	16.75
Ni	8.14	1.60
Si	0.432	0.774
Mn	1.31	7.58
Mo	0.121	0.039
Cu	0.12	3.02
C	0.040	0.050
N	0.044	0.20
Ti (ppm)	3	16

nese. As a rule, the maximal copper content accepted in a stainless steel composition cannot exceed 5% (wt). Above these values, some liquid phases can form into the metallic structure at a temperature higher than 1100 °C, making impossible the hot-rolling process (Raghavan, 1992).

2.1.2. Surface treatment of copper-rich stainless steel

A final heating treatment is applied to copper-rich stainless steel in order to form copper precipitates in the metallic phase (Raghavan, 1992). The sheets are placed in a furnace at 700 °C during 100 h and slowly air-cooled afterwards. Then the surfaces are polished under water to eliminate the oxide layer formed during the heating treatment.

2.1.3. Surface cleaning

Prior to the air contamination, the materials are dipped in ethanol (95%) for 10 min and dried on adsorbent paper (Joseph paper). As the copper surface is oxidised by such treatment, it is subsequently cleaned using HNO_3 0.5 M.

2.1.4. Metal releasing

In contact with water, metallic ions like copper, which could have bactericidal properties, can be released from stainless steel. Prior to the air contamination test, the Cu-releasing is measured in water by immersing new samples tested in triplicate for 24 h in synthetic potable water ($MgSO_4$ 4×10^{-3} M, NaCl 5.6×10^{-3} M and K_2SO_4 5.5×10^{-4} M). The total surface in contact with water is 0.03 dm^2. After 24 h, a volume of 50 ml is taken and acidified by HNO_3 5%. Iron, chromium, nickel, manganese and copper are analysed by Flame Spectroscopy Atomic Absorption.

The metallic concentration (C) is expressed in mol/dm^2. A blank (B) is also carried out in potable water. Under our experimental conditions, the metallic releasing from materials (R, in mol/dm^2) is expressed by $R = (C - B)V/S$, where $V = 0.05$ dm^3 and $S = 0.03$ dm^2 and $R = 1.67(C - B)$. The metallic releasing determined for all materials selected in this study is presented in Table 2.

2.2. Microorganisms

2.2.1. Cultivation conditions

The tests are carried out with a Pasteur Institute Collection strain *E. faecalis* 10.30.15 stored at 4 °C on inclined gelose (tryptic soy Sanofi Pasteur). In order to establish the effect of culture conditions on the viability of the aerosolised cells, the strains undergo three subcultures under gelose before each use. The colonies are obtained after 24 h at 37 °C. We checked that this mode of conservation and culture had no effect on the aerosol survival (Robine, 1999). The bacterial colonies are then put in suspension in 20 ml of filtered deionised water. The final concentration is measured by epifluorescence microscope count.

2.2.2. Determination of the minimum inhibitive concentration of copper (MIC)

The antimicrobial activity is measured by determining the smallest quantity of Cu^{2+} necessary to inhibit the growth [MIC] of a strain of *E. faecalis*. A series of cultures in agar medium is prepared; each test contained different concentrations of $CuSO_4$ (corresponding to Cu^{2+} concentrations ranging from 0 to 0.025 mol/ml of gel medium). All the tests are carried out using an inoculum with a final concentration of 10^2 cells/test, in order to count the cells that can be cultivated in agar medium. Each concentration of copper-enriched agar medium is tested in triplicate. After incubation, the MIC is determined by the initial dilution series where no bacterial growth is detected.

2.3. Characteristics of the experiment's surface aerobiocontamination system

As shown in Fig. 1, the aerobiocontamination assembly is made up of a Collison 3-jet atomiser (1) with pressure (0.5 and 3 bars) controlled by a manometer. For a pressure of 1 bar, the air flow is 7.9 dm^3/min with an air speed of 1.34 m/s and a water flow of 10.6 ml/h. The compressed air is previously purified by a filtration line (10) made up with coalescing filter, a carbon filter and a High-Efficiency Particulate Air (HEPA) filter (Robine et al., 1998).

The humid aerosol is diluted and dried in an evaporator with a controlled dry airflow (Q_2) prior to entering a homogenisation sphere (3). In this section, the temperature and humidity are controlled and measured (6). The concentration of cultivable and metabolically active bacteria as well as the particles concentration are evaluated at the end of the production line. An automated contamination cell (4) (Robine, 1999; Robine et al., 1998) is used to contaminate the surface of materials. The aerocontamination is performed on new materials. An optical counter (5) (Laser-X, Instrumat) measures the optical diameter (0.12 to 3 μm) and the number of aerosolised biological particles. The sampling flow rate of the counter is 10 cm^3/s. The complete assembly operated dynamically and the flow rates are balanced in order to obtain isokinetic sampling. Finally, as a safety measure, the complete assembly is placed under a laminar flow extractor hood (Scupcrisis 1127, ESI Flufrance).

2.4. Control of environmental conditions

2.4.1. Relative humidity and temperature

The samples are prepared before and during the test within humidity and temperature-controlled enclosures according to ASTM Standard (E-104-85, 1991). Relative humidity is provided by saturated saline solutions (Table 3).

Table 2
Metallic releasing after 24 h in potable water (mol/dm^2) (<D.L. under the Detection Limit)

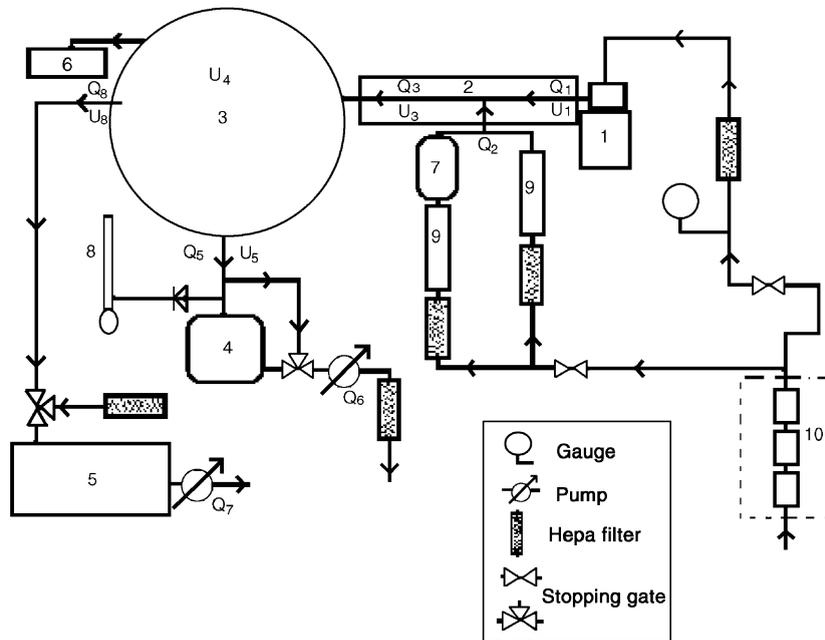| Elements	Fe	Cr	Ni	Mn	Cu
Stainless steel (AISI 304)	5.7×10^{-7}	5.8×10^{-9}	8.5×10^{-9}	<D.L.	<D.L.
Stainless steel (AISI 211 + 3Cu)	1.2×10^{-5}	1.6×10^{-6}	5.3×10^{-7}	2.9×10^{-7}	2.6×10^{-6}
Massive copper (Cu)	<D.L.	<D.L.	<D.L.	<D.L.	6.4×10^{-6}

Fig. 1. Aerocontamination assembly diagram.

These solutions are placed at the bottom of leak-tight dryers (inside diameter 100 mm, Fisher), introduced in an oven (INCUCELL 110, Bioblock) providing a constant temperature (25 ± 0.5 °C). The effect of relative humidity on bioaerosol viability is performed on AISI 304 stainless steel at three relative humidities: 0%, 31% and 85%. On the other hand, the effects of material and fouling on the bioaerosol survival are realised at extreme relative humidities such as 0% and 100%.

2.4.2. Surface fouling

The bacteria are placed in a solution with 1 g glucose and 0.1 g asparagine corresponding to the optimal C/N ratio for bacterial survival under our test conditions. This solution containing both bacteria and nutrients is then aerosolised on the three underlying materials. This procedure ensures a uniform distribution of the fouling and of the bacterial contaminants with a satisfactory reproducibility.

2.5. Evaluation of bacterial survival

The evaluation of the bacterial survival is based on fluorochromic staining and epifluorescence micro-

scopy numeration. Two types of staining are used on inert and biocide-based materials.

2.5.1. Epifluorescence microscopy

The labelled bacterial samples are observed using an epifluorescence microscope (Axioscop Zeiss) equipped with a 50 W mercury lamp. Two filters are used: N∘2 (excitation 365 nm, emission 420 nm) and N∘9 (excitation 450–490 nm, emission 520 nm). The observations are made with a CP Achroplan lens ($100 \times /1.25$ immersed, $40 \times$ and 10 when dry) (Zeiss). The bacterial cells are counted automatically by an image analysis system (Microvision) on 10 epifluorescence microscope fields, which correspond to 4.7×10^{-3} mm^2.

Table 3
Saturated salts and associated relative humidities

Composition of saturated salts	Relative humidities at 25 °C (%)
SiO$_2$ with saturation indicator	≈ 0
MgCl$_2$	31
KCl	85
H$_2$O	> 99

Table 4
Active bacterial population after the contamination of the surfaces

	E. faecalis
Survival %	86.7
Standard deviation	5.7
Confidence interval	1.36
Number of samples	120

2.5.2. Evaluation of the membrane integrity

This type of coloration is performed on various materials including copper as this marker does not interfere with the copper surface reactivity. The *Enterococcus* cell's cytoplasm membrane integrity is evaluated by passively passing two fluorescent markers (Backlight kit, stains A and B by Molecular Probe, Leiden, The Netherlands), with a spectrum band of 510–540 nm (stain A, fluoresces green) and 620–650 nm (stain B, fluoresces red). Green staining of the bacteria demonstrates cytoplasm membrane integrity, whereas red fluorescence is a sign of alteration (Robine, 1999). The numeration was realised at 0, 24 and 96 h after the initial contamination on clean or fouled underlying materials exposed to various relative humidities (0% or 100%). The rate of survival bacteria (%) is given by the number of active bacteria (green-stained bacteria) divided by the number of total flore (obtained by red and green-stained bacteria).

3. Results

3.1. Determination of the MIC

The minimum inhibitory concentration determined in agar medium for *E. faecalis* is 0.013 M corresponding to the agar medium in which no bacterial growth is observed after incubation.

3.2. Checking the initial aerobiocontamination

The purpose of these preliminary tests is to check the viability of the bacterial cells contaminating the surface at the initial time (Table 4).

The survival percentage of microorganisms on surfaces immediately after aerosolising (T0) is approximately 80%. The results of the tests comply with the standards with relation to the initial survival rate (brought to 100%).

3.3. Effect of relative humidity on the aerosol survival

The evolution of the survival percentage of *E. faecalis* after aerobiocontamination on standard stainless steel at different humidities is represented in Fig. 2.

It is observed that the bacterial cells tested live longer in dry media. Low mortality is observed in
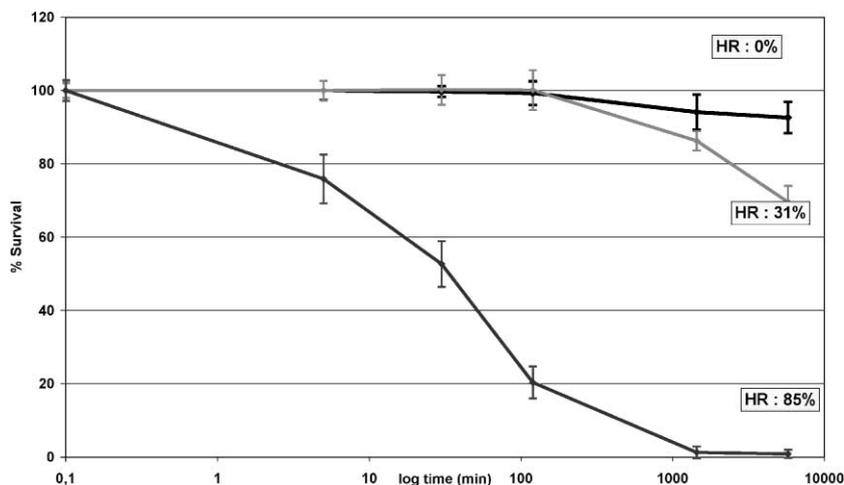

Fig. 2. Survival kinetics of an aerosol of *E. faecalis* fixed on AISI 304 stainless steel according to the relative humidity of the environment (the bars correspond to the standard deviation).

populations of *Enterococcus* incubated at 0% and 31% relative humidity (RH) during 48 h. On the other hand, at 85% RH, the strains are completely inactivated, respectively, after 24 h according to an exponentially decreasing curve.

3.4. Effect of the underlying material on the aerosol survival

The survival of *E. faecalis* aerosol is studied over 96 h for two extreme relative humidities (0% and 100% RH at 25 ∘C) on three surfaces: stainless steel (steel AISI 304), copper-enriched steel (steel 211 + 3Cu) and copper (Fig. 3a and b).
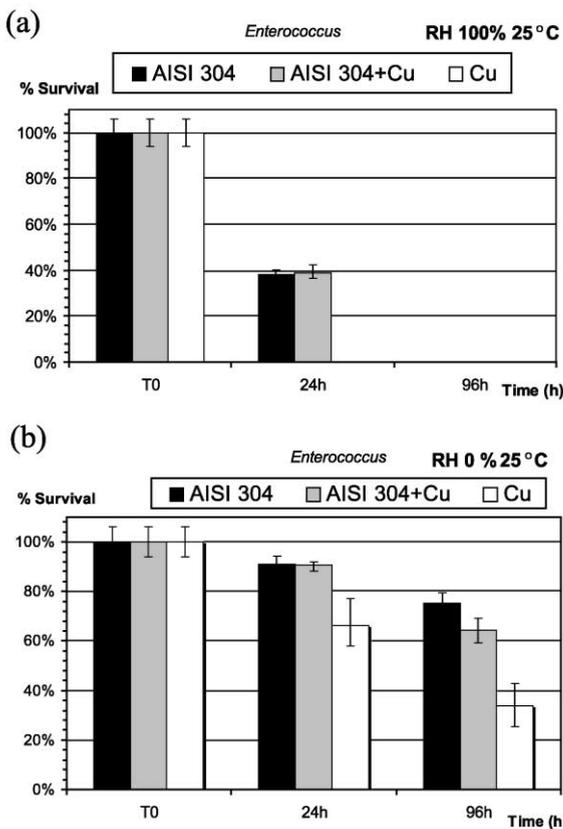

Fig. 3. (a) Measurement of the survival of a bacterial aerosol, fixed to three underlying materials (stainless steel AISI 304, stainless steel AISI 211 + 3Cu and copper) at a relative humidity of 100% at 25 ∘C (the bars correspond to the standard deviation). (b) Measurement of the survival of a bacterial aerosol, fixed to three underlying materials (stainless steel 304, stainless steel AISI 211 + 3Cu and copper) at a relative humidity of 0% at 25 °C (the bars correspond to the standard deviation).
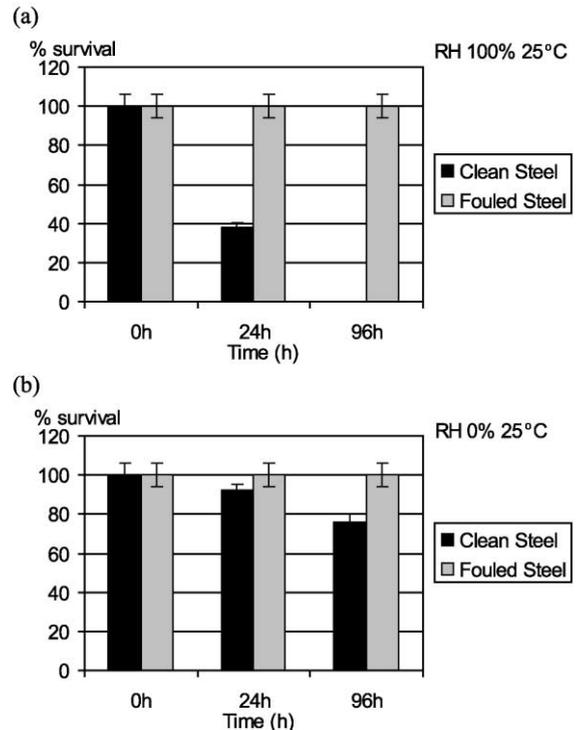

Fig. 4. (a) Measurement of the survival of a bacterial aerosol, fixed to clean control steel and fouled at a relative humidity of 100% at 25 °C. (b) Measurement of the survival of a bacterial aerosol, fixed to clean control steel and fouled at a relative humidity of 0% at 25 °C.

In a humid medium, no significant difference is observed between the standard stainless steel 304 and the steel with copper-enrichment surface treatment (steel AISI 211 + 3Cu). The lethal effect noted is essentially linked to the humidity of the medium. Under the same operating conditions, copper has a strong bactericidal activity. It should be noted that all bacterial cells are inactivated after a contact of 24 h at 100% RH.

In a dry environment, no significant difference is observed between the control steel 304 and the copper-enriched steel (steel AISI 211 + 3Cu). Only the solid copper surface has a significant depressive effect on the fixed biomass with a 24% difference with the control steel after 24 h of contact.

3.5. Effect of fouling on aerosol survival

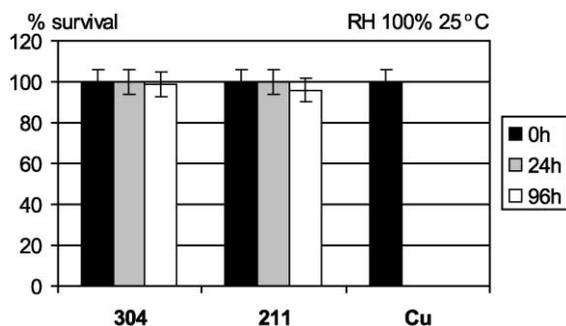The survival of bacterial cells is determined after the impact on stainless steel AISI 304, new or fouled

Fig. 5. Measurement of the survival of an *Enteroccocus* aerosol, fixed to three underlying materials (stainless steel AISI 304, stainless steel AISI 211 + 3Cu and copper) and fouled at a relative humidity of 100% at 25 °C (the bars correspond to the standard deviation).

by nutrients for two RH of 100% (Fig. 4a) and 0% (Fig. 4b) at 25 °C. In a humid medium and on fouled steels, no significant reduction of the bacterial survival is noted over 96 h, since nutrients are available and used for the growth of microorganisms (Fig. 4a). In a dry environment, the survival of the bacterial aerosol on the same fouled steel also remains at its maximal level during 96 h (Fig. 4b).

The survival of *E. faecalis* is also determined over 96 h on three surfaces (control stainless steel, copper-rich stainless steel and solid copper) and fouled by nutriments at two relative humidity of 100% and 0% at a temperature of 25 °C.

At 100% RH, no reduction in the active biomass is noted on control steel and on copper-enriched steel. On the contrary, solid copper shows a strong lethal effect with inactivation of all fixed biomass after 24 h of contact (Fig. 5). In such environment, nutrients are available and used by microorganisms for their growth explaining the maximal viability observed on the two steels and the strong toxic effect of the released copper supposed to be assimilated by the microorganisms.

In a dry environment, no significant difference in the survival rate is demonstrated between the three underlying materials tested, since nutriments are not available for microorganisms. These compounds would seem to protect aerosolised bacterial cells from the lethal effect of the oxidising compounds present in the air and on the underlying materials.

4. Discussion

4.1. Choice of the aerosol methodology

As far as we know, there is no experimental device for the purpose of controlled contamination of surfaces by bacterial aerosols in order to assess materials. At present, very few experiments are done on bioaerosols, and those techniques are used only by some teams to study the efficiency of samplers or filtration systems (Decosemo and Griffiths, 1992; Griffiths and Decosemo, 1994).

Most of the time, the surface contamination tests are liquid tests: a bacterial suspension with a known concentration is put into contact with a given surface. It is also the easiest technique to implement. However, it is hard to follow and above all to reproduce the layout of inoculums over surfaces from one experiment to the next. Moreover, the liquid medium put into contact with the receiving surface to be tested does have an effect on its physico-chemical properties. Choosing to aerosolise viable cells ensures that experimental conditions can be more easily controlled and reproduced (Herbert, 1990; Chang et al., 1995). Such a technique also makes possible to introduce stress parameters, like dehydration and direct exposure of cells to the abiotic factors of the natural environment (temperature, various radiation, etc.). Under such stress, bacterial cells are brought to a specific physiological state, which governs their adhesion and survival properties on surfaces. Since it is necessary to control the microbial level in the environment for many human activities, and as we know that air is one of the most important microbial contamination vectors, this experiment is most likely to be applicable to various fields.

4.2. Influence of the relative humidity on aerosol survival to inert materials

After aerobiocontamination on stainless steel 304, the bacterial survival of *Enterococcus* increases as relative humidity decreases, this is in agreement with the previous papers (Robine et al., 1998, 2000). Because of their proteinic, polysaccharidic and lipidic nature, bacteria can be considered as hygroscopic bodies with a desiccation rate changing according to relative humidity (Cox, 1989).

Once fixed on supports in a humid environment, cells rapidly fill up again with water. That rehydration leads to a conformational change in structures greatly reducing their activity.

A relevant influence of relative humidity on inert materials such as stainless steel AISI 304 is attested by our results. This is in accordance with a recent study on bacterial survival after aerosol soiling on glass and polymeric surfaces (Robine et al., 1998).

As we used the same experimental conditions than in this previous study (Robine et al., 1998), we can compare the observed results. A noticeable reduction is observed for glass in comparison with stainless steel. These differences could be attributed to materials surface properties. The glass surface presents polar characteristics, as a water layer is highly associated with the surface (Vernhet and Bellon-Fontaine, 1995), whereas stainless steel is a more apolar surface (Boulangé-Petermann et al., 1993). Consequently, the water layer associated with the glass surface can play a direct role for a faster rehydration of the cellular structure inducing higher mortality (Dunklin and Puck, 1948; Bateman et al., 1962). As mentioned in a recent paper, the mortality measured on PVC surfaces was undoubtedly the result of this polymer's high reactivity, implying the presence of oxidising sites, the release of hydrochloric acid (HCl) and the presence of additives (Robine et al., 2000).

4.3. Influence of biocide-based metallic materials on aerosol survival

The effect of relative humidity on lethality is also examined on biocide-based metallic materials. At high relative humidity, only massive copper displays a bactericidal effect synergetic with that of relative humidity. In contact with water, copper-rich stainless steel or massive copper releases some copper ions. Nevertheless, the total quantity of copper released in water after 24 h is four times higher for massive copper than for copper-enriched stainless steel. A tentative hypothesis can be proposed: in high relative humidity media, the bacterial surface is more hydrated and copper could be more easily carried to the cell wall, which finally facilitates its absorption. If the copper is absorbed in a sufficient quantity, it induces irreversible damage to the enzymatic system, leading to cellular death. In dry environments, the bacterial surface is poorly hydrated and copper is less easily transported to the cell wall, thus explaining the high survival of around 30% after 96-h adhesion on solid copper. In dry environments, where water cannot directly play a role in copper transport, biocide action of copper can be explained by the oxidation of surface in contact with air. It could be concluded that copper displays an anti-bacterial property even in a dry environment. These initial findings need to be confirmed by further experiments.

4.4. Influence of material fouling on aerosol survival

Surface fouling is widespread in the environment or in food industry plants (Visser, 1997, 1998). Surface steels mechanisms are now well described when fouling is due to biological fluids (Boulangé-Petermann, 1996). More researches on surface fouling have been conducted in connection with the creation of a conditioning film on solid surfaces immersed in liquids inducing changes in adhesion processes (Boulangé-Petermann et al., 1997; Faille et al., 1999). However, no studies have been devoted so far to the effect of fouling on bacterial survival in aerial environment.

In this study, we show that whatever the material and the relative humidity considered, bacterial survival is enhanced on fouled surfaces. In our experimental conditions, we conclude that fouling by saccharidic and amino compounds plays a protective role in the survival.

This protective effect could be explained by two hypotheses. First, metallic surfaces are covered with a layer composed of nutriments initially deposited by the aerosol. Consequently, the initial metallic surface is masked with nutriments and the release of copper is limited. In the second hypothesis, copper is released from the metallic surface but immediately trapped by the nutriments adsorbed on the surface.

Therefore, the addition of nutriments on metallic surfaces, even in small quantities, is enough to ensure the revival of quiescent microorganisms.

4.5. Determination of minimal inhibitory concentration

The antimicrobial activity of copper is measured by determining the smallest amount of copper needed to inhibit the growth of Enterococccus. The MIC in agar

medium is 0.013 M. It is possible to correlate this MIC to the loss in cultivation criterion. However, this measurement is not sufficient to draw conclusions about an efficient and durable antimicrobial activity of a material. In an agar medium, antimicrobial activity is determined only for cultivated bacteria but not for a bacterial aerosol in contact with a metallic surface.

It is possible to estimate the MIC obtained in our experimental conditions. The antimicrobial properties of copper are explained by a release of copper. When immersed in water for 24 h, it is possible to determine the total quantity of copper released from metallic surfaces: 2.6×10^{-6} mol Cu^{2+}/dm^2 stainless steel containing copper and 6.4×10^{-6} mol Cu^{2+}/dm^2 massive copper. In order to express the MIC in a concentration, it is necessary to assume that in environments with high relative humidity, the surface is covered with a 1-μm water layer, roughly the bacteria size. Then, the concentration of copper released from stainless steel (0.26 M) and for pure copper (0.64 M) can be estimated. As attested by our results, in environments with high relative humidity, bacterial survival is higher on copper-rich stainless steel (around 40%) than on pure copper (0%). Consequently, the MIC can be estimated to 0.64 M. This MIC value determined in these experimental conditions is directly connected to a loss in structural integrity. This value is 50 times higher than the value determined for the loss in the cultivation criterion. In conclusion, the MIC is not an absolute constant for a given bacterium, but it is possible to compare different biocides and their activities for rigorously standardised conditions.

Globally, our results confirmed that most bacteria can enter the viable but non-cultivable (VBNC) state in response to adverse environmental conditions (Caro et al., 1999; Pommeruy et al., 1996; Whitesides and Oliver, 1997). However, there are some uncertainties regarding the health risk from such VBNC forms. These uncertainties call into question the use of traditional microbiological media to monitor the quality of surface.

Concerning the development of copper-rich stainless steel, to our current knowledge, the poor interest in hygiene is demonstrated here as these materials display poor or limited hygienic efficiency. Moreover, very little work has been done concerning the long-term effect of biocide-based metallic materials on microbial ecology.

5. Conclusions

The experiments conducted as part of this study have highlighted the importance of environmental factors and test conditions when assessing hygienic properties of materials. It is imperative that the assessment techniques of a material be adapted to its final application.

It is important to stress that very few studies have so far been carried out on microbial survival after aerosolisation compared to the research conducted on biofilm development in liquid media. The mechanisms at work during microbial contamination in liquid medium are different from bacterial aerosol phenomena, so we should not venture to extrapolate.

One of the ways being currently developed to fight biological pollutants in the air is the use of chemicals (biocides). It is rightly to be feared that they could bring about negative effects on health and the environment and on resistance qualities to chemical molecules in the species studied.

From a practical standpoint, the hygienic control of media could be obtained by choosing materials less ''receptive'' to microorganisms, and by initially taking account of their surface physico-chemical properties and the way they are maintained, if they can be subjected to cleaning and disinfection operations. In the case of the equipment, which can be cleaned on site, it is necessary to have easy-to-clean materials. More generally, our results show that it is important to control fouling conditions in hygienic applications to avoid bacterial survival.

References

Bateman, J.B., Stevens, C.L., Mercer, W.B., Carstensen, E.L., 1962. Relative humidity and the killing of bacteria: the variation of cellular water content with external relative humidity or osmolality. J. Gen. Microbiol. 29, 207–219.

Bavay, J.C., 1993. Austenitic stainless steels in stainless steel. In: Lacombe, P., Baroux, B., Beranger, G. (Eds.), Les Editions de Physique. Les Ulis, France, pp. 549–591.

Boulangé-Petermann, L., 1996. Process of bioadhesion on stainless steel surfaces and cleanability: a review with special reference to the food industry. Biofouling 10, 275–300.

Boulangé-Petermann, L., Baroux, B., Bellon-Fontaine, M.N., 1993. The influence of metallic surface wettability on bacterial adhesion. J. Adhes. Sci. Technol. 10, 275–300.

Boulangé-Petermann, L., Rault, J., Bellon-Fontaine, M.N., 1997. Adhesion of *Streptococcus thermophilus* to stainless steel with

different surface topography and roughness. Biofouling 11, 201–216.

Brown, A.D., 1953. The survival of airborne microorganisms. Aust. J. Biol. Sci. 6, 470–485.

Caro, A., Got, P., Lesne, J., Binard, S., Baleux, B., 1999. Viability and virulence of experimentally stressed non-culturable *Salmonella typhimurium*. Appl. Environ. Microbiol. 65, 3229–3232.

Chang, C.W., Grisphun, S.A., Willeke, K., Macher, J.M., Donnely, J., Clark, S., Juozaitis, A., 1995. Factors affecting microbiological colony accuracy for bioaerosol sampling and analysis. Am. Ind. Hyg. Assoc. 56, 979–986.

Cox, C.S., 1989. Airborne bacteria and viruses. Sci. Prog., Oxford 73, 469–499.

Decosemo, G.A.L., Griffiths, W.D., 1992. Problems associated with the assessment of airborne microorganisms. J. Aerosol Sci. 23, 655–658.

Dunklin, E.W., Puck, T.T., 1948. The lethal effect of relative humidity on airborne bacteria. J. Exp. Med. 87, 87–101.

Etkins, D.S., 1994. Biocontaminants in indoor environments. Indoor Air Qual. Update, 11–37.

Faille, C., Dennin, L., Bellon-Fontaine, M.N., Benezech, T., 1999. Cleanability of stainless steel surfaces soiled by *Bacillus thuringiensis* spores under various flow conditions. Biofouling 14, 143–151.

Finch, J.E., Prince, J., Hawksworth, M., 1978. A bacteriological survey of the domestic environment. J. Appl. Bacteriol. 45, 357–364.

Gillet, A., 1999. Mécanisme d'action des désinfectants et de leurs formulations. In: Leveau, J.Y., Bouix, M. (Eds.), Nettoyage, désinfection et hygiène dans les bio-industries. Editions Tec&Doc, Paris, pp. 237–255.

Griffiths, W.D., Decosemo, G.A.L., 1994. The assessment of bioaerosols: a critical review. J. Aerosol Sci. 25, 1425–1458.

Heldal, K., Skogstad, A., Eduard, W., 1996. Improvements in the quantification of airborne microorganisms in the farm environment by epifluorescence microscopy. Ann. Occup. Hyg. 40, 437–447.

Herbert, R.A., 1990. Methods for enumerating microorganisms and determining biomass in natural environments. Methods Microbiol. 22, 8.

Kildes, J., Nielsen, B.H., 1997. Exposure assessment of airborne microorganisms by fluorescence microscopy and image processing. Am. Occup. Hyg. 41, 201–206.

Pommeruy, M., Butin, M., Derrien, A., Gourmelon, M., Colwell, R.R., Cormier, M., 1996. Retention of enteropathogenicity by viable but non-culturable *Escherichia coli* exposed to seawater and sunlight. Appl. Environ. Microbiol. 62, 4612–4626.

Raghavan, V., 1992. Phase diagrams of ternary iron alloys. In: Raghavan, V. (Ed.), Monograph Series on Alloy Phase Diagram. Indian Institute of Metals, Calcutta, India, pp. 687–688.

Robine, E., 1999. Fixation et survie des aérosols bactériens sur les surfaces. PhD-Thesis, University of Paris XII.

Robine, E., Attoui, M., Dérangère, D., Moreau, R., 1998. Aerocontamination testing procedure for the evaluation of building materials and surfaces hygienic properties. J. Aerosol Sci. 29, 551–553.

Robine, E., Dérangère, D., Robin, D., 2000. Survival of *Pseudomonas fluorescens* and *Enterococcus faecalis* aerosol on inert surfaces. Int. J. Food Microbiol. 55, 229–234.

Vernhet, A., Bellon-Fontaine, M.N., 1995. Role of bentonites in the prevention of *Saccharomyces cerevisiae* adhesion to solid surfaces. Coll. Surf., B 3, 255–262.

Visser, H., 1997. Fouling of heat exchangers in the diary industry. Exp. Fluid Sci. 14, 407–424.

Visser, H., 1998. The role of surfaces forces in fouling of stainless steel in the dairy industry. J. Dispersion Sci. Technol. 19, 1127–1150.

Whitesides, M.D., Oliver, J.D., 1997. Resuscitation of *Vibrio vulnificus* from viable but non-culturable state. Appl. Environ. Microbiol. 63, 1002–1005.