Biofilms and Hygiene on Dairy Farms and in the Dairy Industry: Sanitation Chemical Products and their Effectiveness on Biofilms – a Review

Hana VLKOVÁ[1], Vladimír BABÁK[1], Růžena SEYDLOVÁ[2], Ivo PAVLÍK[1] and Jarmila SCHLEGELOVÁ[1]

[1]Department of Food and Feed Safety, Veterinary Research Institute, Brno, Czech Republic; [2]Dairy Research Institute, Prague, Czech Republic

Abstract

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Microbial biofilms which form on all types of surfaces of technological systems in the dairy industry and on dairy farms adversely affect the quality and safety of final products, i.e. both foodstuffs and raw materials used for their production. The fact that a number of microorganisms are alimentary pathogens, e.g. *Staphylococcus aureus* or *Listeria monocytogenes*, makes a serious problem directly affecting human health. Biofilms are usually formed by various species of microorganism, which protect each other against the effects of biocidal (antibacterial) agents and are resistant to these agents. The colonisation of surfaces of the open and closed piping systems, floors, waste, walls and ceilings of the production halls becomes a major problem in the selection of effective sanitation agents for their control. Based on the existing model studies, practical methods for testing the effectiveness of sanitation procedures should be evaluated, including the selection of biocides and comparison of the physical parameters of the sanitation procedures. Testing the effectiveness of the sanitation agents should be performed with the use of standardised tests, which consider microbial, structural, and chemical characteristics of the living microbial communities on specific contact surfaces in the food-processing industry.

Keywords: microbial communities; food process surfaces; mechanisms of resistance; food safety

BIOFILM AND ITS CHARACTERISATION

Biofilm is not a phenomenon of recent time, having been investigated for more than 20 years in the food processing industry (Costerton *et al.* 1978; Zoltai *et al.* 1981). The knowledge of the microbial communities formed on surfaces was significantly extended, particularly in association with medical studies. Biofilms were detected on medical substitutes and other medical aids (Dankert *et al.* 1986; Arciola *et al.* 2001; De Silva *et al.* 2002); these caused persisting and serious diseases in man (Costerton *et al.* 1999). Accordingly, the ability of some microorganisms such as *Staphylococcus epidermidis* to form biofilms is viewed as a virulence factor (Cafiso *et al.* 2004).

The presence of microbial biofilm on the contact surfaces in the food industry is considered as an

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evident health hazard due to the fact that, besides bacteria insignificant to health, they may contain pathogenic microorganisms. The direct contact with raw materials or foodstuffs can cause secondary contamination due to which the product will become unsafe. Bacterial contamination that causes food decay and decreased quality is technologically important. The presented study, which was supported by project No. QF4048/2004 of the Ministry of Agriculture of the Czech Republic, was focused on the review and presentation of the most important factors associated with the production of milk products under good hygienic conditions from the aspect of sanitation and disinfection of the contact surfaces.

Biofilm and biofouling

Primarily, **biofilm** is a basic term for both positive and adverse effects of microbial adhesion. It is referred to as the aggregation of microbial cells interconnected by extracellular polymeric substances (glycocalyx) that proliferate fast and grow on the surfaces of different materials (Frank 2001). It was found by confocal laser scanning microscopy that the contact between the lower cell layer and the external environment is carried out through channels present in the biomass (Donlan 2001). Microbial biofilm lives as a community with primitive homoeostasis, primitive circulatory system and metabolic cooperation; the response of each attached cell in a community is quite different from the response of the planktonic cells of the same species. Hence, it is a complex differentiated community and the process of its formation can be considered as unique in biology, regarding the coordinated activities of the relatively small genomes of prokaryotes (Dunsmore *et al.* 1981).

Biofouling does not only comprise microorganisms growing in the biofilm structure, but also organic matter that is trapped from the environment and they together form sediments or deposits. This capability of biofilm is utilised in industry, e.g. for sewage purification in rotary biocontactors (Rodgers & Zhan 2003). However, in the food-processing industry, it exerts biological activity that is downright harmful (Zottola & Sasahara 1994).

Biofilm formation

Microbial colonisation of solid surfaces and the formation of biofilm is a process consisting of three successive stages, which can be characterised by definite states of the microbial community:

(1) **Adherence** of free planktonic microbial cells; milk or meat juice proteins, which stuck on the solid contact surfaces in the food-processing industry, usually make preconditions for the sedimentation and attachment of microbial cells to the surface (Kumar & Anand 1998).

(2) **Colonisation** of the preconditioned surfaces; this stage, which is still reversible, can be changed into the irreversible stage of the biofilm development, followed by the biofilm formation itself. By cell redistribution, microcolonies are produced. Channels and pores are formed through which water and nutrients pass into the deeper layers of the cell community (Lawrence *et al.* 1991; Davies *et al.* 1998).

(3) **Release** of microbial cells from the biofilm structures or from the surface (Stoodley *et al.* 2001a); the process of active release is physiologically controlled. Separate cells and small clusters are released more often, but large pieces are also released; the latter constitute a health hazard regarding the infectious dose (Stoodley *et al.* 2001b). The released bacteria or their clusters drif within the liquid environment or are attached to damp semi-finished products and ready-made foodstuffs and can become sources of microbial contamination of other surfaces (Zottola & Sasahara 1994) or the products of both animal and vegetable origins (Rayner *et al.* 2004).

Besides the genetic regulation factors, physical forces present in the fluid environment of biofilm are involved. Physical-chemical properties of the phase interface (hydrophobicity/hydrophilia, speed of liquid stream, osmotic pressure, pH, temperature, microtopography of surfaces) are factors facilitating the biofilm production. Morphology of bacterial cells also plays a role. Morphological shapes that exert less energy in overcoming barriers have a greater chance of biofilm forming (Van Loosdrecht *et al.* 1989; Wirtanen & Salo 2005).

Biofilm characterisation

Biofilms may be formed by one species, but usually more bacterial species are isolated from them. For example on farms and in the dairy industry; these may consist of pathogenic bacteria (*Escherichia coli, Listeria monocytogenes, Yersinia*

enterocolitica, Staphylococcus aureus, Salmonella spp., *Bacillus cereus* etc.). An exopolymeric substance produced by one microorganism may offer an environment for the attachment and growth of another microorganism as documented for the *L. monocytogenes* bacterium attached to the exopolymer produced by *Pseudomonas fragi* (Zottola & Sasahara 1994).

Generally, microorganisms living in biofilms are much more resistant against disinfectants than microorganisms in a planktonic culture. The increased resistance is caused by several factors besides the genetically conditioned processes. Biofilm glycocalyx limits diffusion and can cause deactivation of the disinfectants. The density of bacterial suspension inside the biofilm and the physiological state of cells affecting the production of degrading enzymes are also involved (Vidal *et al.* 1997; Wirthlin *et al.* 2005). Besides that, the adoption of mechanisms causing resistance to biocide agents may occur after the sublethal effect of biocide agents on the biofilm cells (Gilbert *et al.* 2002).

Biofilms formed by different species of microorganism are dangerous because they protect one another during the application of chemical agents (for example alkaline chlorine solutions). This is caused by the different resistance of respective microbial species against the agents used (Vidal *et al.* 1997; Wirthlin *et al.* 2005).

BIOFILMS ON DAIRY FARMS AND IN THE DAIRY INDUSTRY

Location

The surfaces of conveyor belts in the food industry premises, whereupon the products may be in the direct contact with the contaminated surface, are frequently contaminated even after sanitation according to the valid procedures. The contact surface of the conveyor belt in a dairy plant was contaminated with 10^5 to 10^6 CFU/100 cm^2 (CFU = Colony Forming Units) of *Staphylococcus* spp., *Pseudomonas* spp., and other bacteria even after cleaning (Peters 2003).

Bioaerosol may become a source of contamination on these open surfaces, owing to the fact that microorganisms are stuck to the liquid particles of aerosol. In the environment of the premises, bioaerosol is formed during water and air flow and by the release of bacteria from the biofilm present in the waste or on the floors of the production plants with coarsened or otherwise damaged surfaces. Such surfaces may be contaminated with bacteria (up to 10^8 CFU/100 cm^2). Dangerous to health pseudomonades and staphylococci were most often isolated from these places in the meat processing plants and dairies (Mettler & Carpentier 1998); *L. monocytogenes* was also isolated (Suihko *et al.* 2002).

Dangerous biofilms were detected also in closed systems. Pathogenic microorganisms (from genera *Bacillus*, *Streptococcus* and *Staphylococcus*, *Shigella*, *Escherichia*, and *Enterobacter aerogenes*) participated in the biofilm formation on the surfaces of a post-pasteurisation unit in a dairy plant. Moreover, the isolates were often resistant to carbenicillin, cloxacillin, cephaloridin, novobiocin, and vancomycin (Sharma & Anand 2002).The bacteria growing in a biofilm on a stainless steel surface of a heat exchanger in the pasteurisation unit in the dairy contaminated milk in concentration 10^6 CFU/ml after pasteurisation (Flint *et al.* 1997). It was found that the cause of an extensive crisis in Japan in 2000, which affected over 13 000 people, was the production of a thermoresistant toxin by the *S. aureus* bacterium surviving in a conduit tap in the dairy (Asao *et al.* 2003).

Biofilms and materials in the food industry

It follows from the above mentioned facts that biofilms are formed on different materials necessary for different production facilities in the food industry provided favourable conditions for adherence and biofilm formation exist. For example on a farm, phenotypically and genotypically biofilm-positive strain of *S. epidermidis* was isolated from 6 different places in the system for obtaining milk. The contact surfaces in these places were made of glass, rubber, synthetic materials, and of stainless steel (unpublished). Pathogenic microorganisms (*L. monocytogenes*, *S. aureus* etc.) were detected on both the solid contact surfaces made of glass and of stainless steel and on surfaces made of rubber, Teflon, wood, and plastics (Mafu *et al.* 1990; Austin & Bergeron 1995).

The majority of surfaces in the food-processing industry are made of stainless steel that can be easily cleaned and is resistant against chemical agents (Mattila-Sandholm & Wirtanen 1992). However, it was detected by microscopy that even smooth surfaces made from stainless steel can be damaged by mechanical cleaning. Small cracks

and scratches are formed on their surfaces and microorganisms and remnants of raw materials can stick to them (Wirtanen *et al.* 1996). The biofilm structures consisting of several layers with the density of up to 10^8 CFU/cm^3 were detected on the facilities made of stainless steel (Holah & Gibson 1999).

With respect to the clean ability and chemical safety of the equipment for the production of safe foodstuffs, the European Hygienic Engineering & Design Group (EHEDG) provided the guidance for hygienic engineering (Guidance on the hygienic engineering aspects of manufacturing of safe and wholesome food) including the demands on processing (finishing) stainless steel for the food industry engineering systems. However, no great difference was detected in the numbers of the microorganisms attached to the surfaces made of stainless steel, processed to less than is the required degree of 0.8 μm (Hilbert *et al.* 2003; Guðbjörnsdóttir *et al.* 2005). It may be due to the fact that the microbial adherence to the stainless steel surfaces as one of the stages of the biofilm formation does not always occur and is preconditioned by the stainless steel quality (Somers & Wong 2004) and above all by the characteristics of microorganisms (Peng *et al.* 2001).

HYGIENE OF CONTACT SURFACES ON DAIRY FARMS AND IN THE DAIRY INDUSTRY

Sanitation and disinfection, basic requirements

Sanitation of surfaces includes both the process of cleaning off deposits of organic and inorganic matters mixed with microorganisms and the process of devitalisation of microorganisms, which may be the primary cause of the formation of these plaques. The purpose of **disinfection** in the food industry is the reduction of the numbers of live microorganisms to the level which cannot affect the quality and safety of the produced foodstuffs.

Basic requirements for sanitation at the processing facilities are effectiveness, low economic demands, and safety (Salo *et al.* 2001). An effective and safe sanitation regime reaches economic effectiveness if the three following conditions are accomplished:

(1) Efficient sanitation agent, which can be easily washed out; i.e. an agent with the highest efficiency at a low concentration and the minimum time needed for the operation, without leaving dangerous residues.
(2) Low demand on energy and work.
(3) The least damage to the environment and disinfected surfaces.

It follows that the sanitation regime, which meets the basic requirements, must be based on the knowledge of the particular conditions of the sanitised environment:

(1) detected pathogens, colonising the cleaned surface;
(2) knowledge of the aimed effects of the selected biocides against the pathogens;
(3) chemical and physical properties of the surfaces;
(4) hygienic design of technological systems (Salo *et al.* 2001).

Factors and effectiveness of sanitation

The effectiveness of cleaning is preconditioned by four main factors: (1) chemical agent, (2) mechanical power, (3) temperature, and (4) time of the procedure, which together form the Sinner circle (Wirtanen & Salo 2003). Sinner circle described an economically ideal cleaning process using the optimum interactions between these basic characteristics. The reduction of one of them must be compensated by strengthening other factors. However, the compensation cannot be applied without the knowledge of specific causes and the microorganism that forms the biofilm. For example, the combination of the effects of the EDTA chelating agent with ultrasound had an unambiguous synergistic effect on the release of a model biofilm formed by *E. coli.* However, that was not found for the biofilm formed by *S. aureus* (Oulahal *et al.* 2004). In contrast, a strong water stream of up to 17.2 bars showed to be effective for surface cleaning from biofilms formed by *P. aeruginosa* and *S. aureus*.

Besides the above mentioned factors, the following ones also influence the effectiveness of cleaning and disinfection: (1) water hardness (Jones *et al.* 1986), (2) character of contamination, (3) microtopography of surfaces, (4) straightness of the passageways, (5) compatibility of surfaces and agents, (6) agent application method, (7) speed of application and related speed of penetration in to the biofilm structure (Springthorpe 2000). It is necessary to take into account these specific factors, especially in the event of performing sanita-

tion on closed piping systems, which are the most common contact surfaces in the dairy industry.

The effectiveness of cleaning agents is of primary concern in the food industry, because these remove the deposits present on biofilms and protect them from the effect of disinfectants (Gibson *et al.* 1999). During the cleaning stage, up to 99.8% bacteria present on a stainless steel surface can be removed (Dunsmore *et al.* 1981).

Naturally, the selection of the disinfection agent – biocide – is equally important. Before its selection, the following questions should be answered: (1) how effective is it in the pH range of the sanitised environment, (2) how stable is it in a solution, (3) whether it evaporates, (4) whether it is toxic, irritating, or safe, (5) what is the range of its effectiveness, (6) its activity relative to temperature, (7) whether it causes corrosion of the sanitised surfaces, (8) whether it is surface-active, (9) how stable is it in the reactions with organic materials and finally, (10) what is its effectiveness relative to the price (Sequiera *et al.* 1988; Larson & Morton 1991; Troller 1993; Wirtanen 1995).

Besides aqueous solutions, sanitation agents in the form of foam or gel can be used for the cleaning of closed systems (Holah 1992; Wirtanen *et al.* 2000). It must be possible to wash out every agent easily, and this must not affect the characteristics of the surfaces of technological systems (Lelieveld 1985; Holah 1992; Wirtanen & Salo 2003).

These cleaning and disinfection procedures consist of a series of washings, applications of detergents and disinfectants in different combinations of temperature and concentration to be able to ensure their best effect (Czechowski & Banner 1990; Holah 1992; Troller 1993; Wirtanen 1995). The ventilation of the system is essential as the final step of the cleaning procedure, as it allows drying-up the surfaces at the end of the whole process (Holah 1992; Wirtanen *et al.* 2000). It is generally known that a decreased activity of water in the environment leads to the inhibition of the growth or even devitalisation of microorganisms (Troller 1986). The biofilm formation in the food processing industry is associated above all with damp surfaces, whereupon the microorganisms can aggregate more easily (Chmielewski & Frank 2003).

Sanitation of closed systems

The sanitation procedure, comprising both cleaning and disinfection, has been defined as the "Clean In Place" (CIP) for the piping systems in the food processing industry. The procedures for sanitation of the piping systems on dairy farms and in all other areas of the food processing industry can be similarly defined. CIP should be viewed as the in-place sanitation system of closed technological facilities; CIP does not require taking apart the facilities and it ensures the cleaning of all places owing to the fact that these technologies have hygienic designs (EHEDG Document No. 8 1993; EHEDG Document No. 2 2000; EN 1672-2:2005).

The sanitation regime of CIP is set up so as to guarantee (1) elimination of organic and inorganic contaminations (cleaning), (2) disinfection of the cleaned surface from live cells of microorganisms (to 99.9%) and (3) elimination of the residues of the sanitation agents. The purpose of the sanitation regime is to ensure safety of the foodstuffs or raw material produced. The methods for the evaluation of CIP system effectiveness have been described by EHEDG in the Document No. 2 (2000).

CIP sanitation in the dairy industry and on farms has some specific features. It is necessary to remove both the remaining organic contamination (milk in the system) and deposits caused by milk and water combination (milk and water plaque). The standard procedure includes the following steps:

(1) rinsing of the system with clean cold water for 5 to 20 min (ensures washing out the remnants of the produced or processed raw material – milk);
(2) cleaning with an alkaline agent solution (suitable concentration is usually 1.0–1.5% solution of the effective agent, e.g. NaOH) at a temperature 75– 80°C for 6–45 min (removes the deposited organic contaminants – oil, proteins, polysaccharides – from the piping surface);
(3) rinsing with warm water (washes out alkaline environment);
(4) cleaning with an acid agent (suitable concentration of the effective agent is 0.5–2%) for 5–45 min at a temperature between 60°C and 90°C (ensures the elimination of inorganic sediments; it is not necessary to apply it repeatedly during every sanitation; once a week is sufficient – the frequency is preconditioned by water hardness and the degree of inorganic contamination);
(5) rinsing with cold water for 5 to 20 min (Bylund 1995; Tamine & Robinson 1999).

CHEMICAL SANITATION PRODUCTS AND THEIR EFFECT AGAINST BIOFILMS

Explanation of basic terms

Disinfectant or **biocide** is a common term for an agent used for killing, inhibiting, or restricting the growth of harmful organisms in all spheres of human activity. Biocides kill microorganisms by the direct action on the cellular membrane, disruption of the permeability of the cell membrane, and/or through the disruption of fundamental cellular processes, e.g. protein synthesis (Capita *et al.* 2002; Keener *et al*. 2004*)*. The disinfection of surfaces is aimed at the reduction of the counts of live microorganisms, but not at cleaning the surfaces. Practically it means that the disinfection agents do not destroy all live microorganisms in the biofilm structures if these are (after poorly performed cleaning) protected by organic and inorganic deposits from the environment (Carpentier & Cerf 1993).

Biocidally active agents according to the European Chemicals Bureau (ECB) are (1) one or more chemical components with known chemical structure, (2) substances with unknown or variable composition reacting with biological material (UVCB substance – substances with Unknown or Variable composition, Complex reaction products or Biological materials), (3) particular microorganisms (bacteria, fungi, viruses), (4) extracts from oil, plant, or microorganism or (5) products of microorganism fermentation, which chemically or biologically destroys the organisms.

Biocidal product: according to the Directive No. 98/8/EC concerning the placing of biocidal products on the market, it is a product composed of one or more biocidally active agents in a form which serves for the deactivation of any harmful organism in a chemical or biological way. This Directive does not only define the biocidal products (Article 2.1.(a)) but also categorises the products into four main groups: No. 1 Disinfectants and general biocidal products, No. 2 Preservatives, No. 3 Pest control, and No. 4 Other biocidal products.

The first group of products is used on farms and in the dairy industry. This group comprises the veterinary hygiene biocidal products including products used directly for the animals, for disinfection of the ambient environment of animals and the facilities on farms. This group also comprises food and feed area disinfectants. The agents with only cleaning properties and without biocidal effects are not included in this group No. 1.

Sanitation agent is a type of biocidal product which reduces the number of live cells of microorganisms on contact surfaces to an amount which does not pose any risk to food safety (raw materials), and at the same time prevents their future growth (Wirtanen *et al.* 2002). The option of a sanitation agent and its concentration is given above all by the following particular practical conditions: water hardness, contamination level of the system, the size of the contact surface, and the range of bactericidal effects of the agent (Bessems 1998).

In association with the extended knowledge of the problem of secondary contamination of raw materials and foodstuffs, a new aspect opened at the same time of the sanitation procedures and agents applied to the sanitised surfaces in the food-processing industry. The effectiveness of the agents against biofilms is one of the criteria considered. Wirtanen and Salo (2003) described in their review the advantages and disadvantages of some disinfectants used in the process of food production. The effectiveness and way of their activity – penetration, decomposition, damage or release of biofilms or microorganisms – was reported for six of the most commonly used biocidal products. However, their instability or toxicity (agents on chlorine basis), effectiveness in only high concentrations (hydrogen peroxide) or their corrosive effects on surfaces (peracetic acid, ozone or hypochlorites) are the disadvantages of biocidal products.

Testing methods of effectiveness of biocidal products

Development of methods

Most microorganisms, growing under poor environmental conditions deficient in nutrients are usually deposited on the surfaces of materials in the form of mixed biofilm and not in the form of floating plankton. The life conditions in the suspension cultures are quite different from the conditions in the biofilm structures (Wirtanen 1995). Despite these facts, the testing of the disinfection and cleaning agents has been performed on cell suspensions and usually only on one species of microorganisms. Only recently have some commercially manufactured agents been directed at biofilm elimination (Templeton 2005).

The standard tests must ensure repeatable and reproducible results; that would be a problem if biocides were tested on suspensions under practice conditions. It is reasonable that the development of the agents tested in this way should be focused on (1) higher quality of cleaning, (2) increasing economic effect, and (3) reducing adverse effects of the sanitation agents on the contact surfaces in the food processing plants and generally on the environment.

The development of the methods for disinfectants testing is a long-term process associated with the investigation of different factors that can affect the effectiveness of the agents. The effects of the sanitation agents on microbial cultures should be viewed as chemical processes, in which the time of the mutual effect of the reactants is involved. Krönig and Paul (1897) were the first to apply the law of chemical kinetics in the sphere of disinfection. At the same time, they expressed graphically for the first time the correlation between the logarithm of the number of surviving microorganisms and the contact time; this is almost linear. Chick (1908), Watson (1908) and Phelps (1911) continued in this study. They suggested a mathematical model which correlated the concentration of the disinfection agent with the level of the disinfection effect on the tested microorganisms. A long time ago, this model showed the correlation between the disinfection speed and the present chemical reaction type. These studies were neglected, and were recalled again when the European Committee for Standardization tried to develop and verify the suspension tests (CEN-group T216). Analogous models were used in the study by Johnston *et al.* (2000) who tried to explain the variability of the results obtained with bacterial suspension tests.

The need for the evaluation and validation of the disinfection agents produced by the pharmaceutical industry for the use in human and veterinary medicine led to the development of harmonised testing methods in 1989. The standards established by the CEN contained, among others, the "Surface test method". Their effectiveness against the surface-located dried cultures of microorganisms made up of bacteria or fungi was assessed. This was a crucial criterion because microorganisms attached to the surfaces are less sensitive to the disinfectants (Bloomfield *et al.* 1993).

The study of Wirtanen *et al.* (1998) significantly contributed to the development of the test **methods** for the estimation of the **effectiveness of disinfecting agents**. They used an aqueous solution of poloxamer Pluronic F127 that had shown thermo-reversible gelation as a bacterial vehicle for routine efficacy testing of disinfectants on the base of hypochlorite, alcohol, peroxide, and tenside. It was shown that the cell suspensions (10^7 CFU/ml) decreased by more than 5 log CFU within five minutes, while in biofilm aggregations only by 0.4 to 2 log units. The application of a gel as a vehicle for checking and testing the disinfection agents showed to be convenient.

The development of a standard test for the assessment of microorganism resistance in biofilm against disinfection agents was described by Luppens *et al.* (2002). The biofilm was produced by the *S. aureus* bacterium on the glass, stainless steel, and polystyrene surfaces. Nutritive conditions of the test were similar to the standard conditions specified by the European Standard EN 1040. The biofilm formation covered on average 60% of surfaces and the majority of the cells (92%) were viable. They found out that 50 times higher concentration of benzalkonium chloride and up to 600 times higher concentration of sodium hypochlorite caused a comparable decrease by 4 log of *S. aureus* cells when growing in biofilm in comparison with cells in suspension. Similar results were obtained by Møretrø *et al.* (2003). The criterion of the necessary reduction by more than 4 log of cells in biofilm after 5 min in comparison with the initial concentration was used for the first time here; previous studies reported 3 log CFU as a sufficient reduction (Wirtanen 1995). Wirtanen and Salo (2003) viewed the surface tests of effectiveness as much more reliable because they respected both the surface quality and viability of cells that dried on the surface.

The standardised methods used

According to the above-mentioned results, CEN defined the conditions suitable for testing the germicidal, fungicidal, and sporocidal activities of different agents used for various purposes. The criteria for the evaluation of the effectiveness of the agents were defined.

It is very important to test the products on the selected test microorganisms, typical for each application. The microorganisms used in the standard tests are referred to the respective tests:

(1) **Basic suspension test EN 1040** works with microorganisms *Pseudomonas aeruginosa* ATCC 15442 and *S. aureus* ATCC 6538 for bactericidal tests,

Candida albicans ATCC 10231 and *Aspergillus niger* ATCC 16404 for fungicidal tests.

(2) **Quantitative suspension tests** for the agents used in the food industry EN 1276 and for the biocides used in the sphere of veterinary care EN 1656 work with *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 15442, *E. coli* ATCC 10536, *Enterococcus hirae* ATCC 10541 for germicidal tests, *C. albicans* ATCC 10231 and *A. niger* ATCC 16404 for fungicidal tests; the above mentioned tests for the evaluation of bactericidal activity work with two methods: dilution neutralisation method and method of membrane filtration.

(3) **Quantitative surface test** on non-porous surface EN 13697 works with the same microorganisms as the basic suspension test; however, the contact time of the agents with the surface located culture is 5 min for bacteria and 15 min for fungi.

(4) **Test for the evaluation of sporocidal activities** EN 13704 employs *Bacillus subtilis* ATCC 6633 as the test strain.

(5) **The conditions for storage of the control microorganism strains** have been set by EN 12353 test.

The criteria of the effectiveness of the above mentioned tests determine the minimum reduction of 5 log CFU within 60 min for bacteria and the reduction of 4 log for fungi in comparison with the reduction at 200°C. In using the EN 13697 test, the reduction of microorganisms higher than 4 and 3 log for bacteria and fungi, respectively, must be obtained.

Effectiveness of biocides against biofilms in the food industry

After the introduction of the quantitative surface tests, the criteria for the evaluation of biocides became stricter. In these tests, practical conditions of testing are taken into consideration (Morwood 2006). The effectiveness of the cleaning and disinfecting regimes used in the food industry was primarily assessed using the method of swabs for the sample collection from the investigated surfaces and the subsequent analysis of the numbers of viable cells. The comparison was made of the effectiveness of acid and alkaline detergents, applied in the form of aerosols on the surfaces overgrown with biofilm. The testing showed that both acid and alkaline agents significantly ($P < 0.05$) influenced the viability of *S. aureus* and *P. aeruginosa* bacteria growing in biofilm. Further potential spread of contamination was thus decreased to minimum by these agents and it was not necessary to use another disinfectant for sanitation (Gibson *et al.* 1999).

Comparable results were obtained by Bremer *et al.* (2006). The CIP system of sanitation was simulated under laboratory conditions. The model of a flow system in dairy premises was made, operating under the conditions favourable for the biofilm formation in this environment. The effectiveness of acid and alkaline agents on the reduction of viable bacterial cells adhering to the surfaces and forming biofilm was investigated in the system. It was found that the tested alkaline solutions and additives and subsequently acid solutions infused into the system and were able to reduce the numbers of viable bacterial cells on stainless steel surfaces by 3.8 log under the standard conditions. Using a disinfectant in this system, however, did not appear as a positive step in the biofilm elimination.

All the suspension tests showed that the microorganisms used in them were more easily devitalised under model laboratory conditions than in the real conditions in industry or on farms. Aarnisalo *et al.* (2000) reported such a situation using 8 strains of *L. monocytogenes* and testing the effectiveness of 10 disinfectants typical for the food industry. Le Chevallier *et al.* (1988), Wirtanen and Juvonen (2002) and other authors compared the basic tests with the surface tests and gave evidence of the primary importance of the surface tests.

The effect of an disinfectant agent however is not the same against all cells forming the biofilm structures. The range of the reduction of the biocide activity is affected, besides others, by the physiochemical character of the disinfection agent itself. As detected by Ntsama-Essomba *et al.* (1997), the highest reduction in activity was observed with the agents of the lowest hydrophilic-lipophilic balance, such as benzalkonium chloride and hexadecyltrimethylammonium bromide. The activity of the oxidising agents was only partly reduced and the activity of phenol derivatives was only slightly decreased or even unchanged. They also found out that the effectiveness of a biocide is reduced by the presence of milk or exopolymere surrounding the bacterial cells participating in the biofilm formation.

Due to the fact that the agents on the chlorine base are very often used for sanitation in the food

industry, a number of studies were performed testing their effectiveness. In a model study concerning the biofilm formed on stainless steel, sodium hypochlorite was tested. Biofilms from the *E. coli* bacteria isolated from foodstuffs were formed after 6 to 24 h on the inserted smooth or scratched stainless steel cards. Under these conditions, the intactness of the steel had a significant effect, as well as the age of the biofilm, on the effectiveness of its removal. Based on the results of this study, it was recommended to perform the sanitation of the surfaces, on which biofilms are formed by this and similar strains, at intervals shorter than 12 h and to prevent mechanical damage to the steel surface (Lomander *et al.* 2004).

The comparison of hypochlorites (alkaline hypochlorite), free chlorine, and chloramines (chlorosulphamates) has been performed many times. For example, the biofilms formed by *P. aeruginosa* and *K. pneumoniae* were exposed to the effects of these agents and the concentration of active chlorine inside the biofilm was measured in viable cells by using microelectrodes (Stewart *et al.* 2001). It was shown that chloramines penetrated in to the biofilm 6–8 times sooner than hypochlorite; however, the bacteria forming the biofilm were highly resistant to both agents. In an analogous measurement using microelectrodes (Jang *et al.* 2006), ClO_2 (chlorite dioxide) was studied; this is more and more often used in different branches of the milk and food-processing industries. The investigation of ClO_2 penetration into biofilm showed that only the initial concentration above 25 mg ClO_2/l was effective in the biofilm up to a depth of 100 μm. The effectiveness of monochloramine was much higher in comparison with free chlorine (Türetgen 2004).

It follows from the studies performed that the chlorinated agents can penetrate into the biofilm, but fail to devitalise bacteria because the bacteria in the biofilm carry mechanisms of protection against the killing effect of these antimicrobial agents (Stewart *et al.* 2001).

Besides the evaluation of different agents separately, their combinations were also investigated. For the elimination of the biofilm formed by *L. monocytogenes* in the environment with strong organic contamination, sanitation was performed with the combination of peracetic acid and alkaline hypochlorite solution (Somers & Wong 2004).

However, another problem appeared in association with the use of the sanitation agents. The increasing emphasis on health safety of foodstuffs means that the companies processing raw materials and food manufacturers use considerable amounts of chemical disinfection agents. As well as with the therapeutic application of the biocidal substances (antibiotics), the selective pressure contributed to the emergence of microorganisms resistant to the disinfection agents. Microorganisms isolated after the performed sanitation were resistant more than usually (Langsrud *et al.* 2003). The adaptation to one type of disinfection agents may be associated with the cross-reactivity to analogous agents or to therapeutic antimicrobials. For example, a genetic association was detected between the resistance of *Staphylococcus* spp. isolated from the food industry to quarter ammonium salts and β-lactam antibiotics (Sidhu *et al.* 2001; Langsrud *et al.* 2003). The *S. aureus* and coagulase-negative staphylococci isolates from non-pasteurised cow's milk and milk from milk cows affected by mastitis contained the plasmid carrying *smr* gene, which encoded the resistance to this group of disinfection agents (Bjorland *et al.* 2005).

In many cases, it is possible to prevent the formation of bacterial populations resistant to the disinfection agents by the use of effective cleaning and disinfection procedures and thus prevent the development of hazardous environmental conditions in the food industry and on farms (Langsrud *et al.* 2003). Hence it follows that it is necessary to test first the effectiveness of the sanitation agents and sanitation procedures under model laboratory conditions, above all in the dairy industry and on farms.

Legislation instructions for sanitation and production of health-safe foodstuffs

The use of all the sanitation agents – biocides for meeting hygienic standards on farms and in dairies is governed by the valid legislative regulations. The most important regulations are listed below with brief indications of use:

(1) **Directive 98/8/EC** concerning the placing of biocidal products on the market. In the following years, **Directive 98/8/EC** was **supplemented by Commission regulation (EC)** No. 1896/2000 of 7 September 2000, Commission regulation (EC) No. 1687/2002 of 25 September 2002, Commission regulation (EC) No. 2032/2003 of 4 November 2003 and Commission regulation (EC) No. 1048/2005 of 13 June 2005,

amending regulation (EC) No. 2032/2003 on the second phase of the 10-year work programme referred to in Article 16 of Directive 98/8/EC concerning the placing of biocidal products on the market.

Directive 98/8/EC was implemented by the EU member states on 14 May 2000. In Article 2.1.a) The biocides are defined as active substances and preparations containing one or more active substances in the form in which they are placed on the market and are intended to combat harmful organisms; the biocides encompass a wide range of applications including disinfection, preservation, and pest control, prevention or other control activity on any harmful organism by biological or chemical activity. Annex No. V classifies biocidal products into the main groups, of which **group No. 1. is important for us: Disinfection products and universal biocidal products and the subgroups Nos. 3 and 4.**

Products of **subgroup No. 3 are "Biocidal agents for veterinary hygiene"**. The products of this group are used for veterinary hygiene purposes including the products used in places where animals are kept or transported. The products are used for cleaning the teats and udders, for hoof disinfecting baths, for disinfection of stores of feeds and feeding lines, and for sanitation of milking facilities and spaces in milking parlours.

Products of **subgroup No. 4 are "Disinfection products for the sphere of foods and feeds"**. The products are used for disinfection of the facilities, reservoirs, equipments for consumption, surfaces and piping necessary for production, transportation, storage or food, feed and drink consumption (including drinking water) for people and animals.

(2) **The European Parliament (EP) and Council (EC) directives concerning milk and dairy products.** Relative to the investigated problems, it is necessary to mention the group of directives of the European Union: Regulation (EC) No. 852/2004, No. 853/2004 and No. 854/2004. These directives resulted from the European Parliament and Council for simplify European legislation. They follow from EP and Council (EC) Regulation No. 178/2002, which sets down general principles and requirements of food law.

The European Parliament (EP) and Council (EC) Regulation No. 852/2004 of 29 April 2004 on the hygiene of foodstuffs laid down general hygiene rules for the producers of foodstuffs based on the risk assessment analysis and critical check points. The producers must realise structural requirements based on these principles. In Chapter V, Annex II, Items Nos. 1 to 3, the surface material and hygiene demands on technology, which are in contact with foodstuffs, are listed.

The European Parliament (EP) and Council (EC) Regulation No. 853/2004 of 29 April 2004 laid down specific hygiene rules for food of animal origin including milk and dairy products. The requirements concerning the treatment of producing animals and hygiene of producing farms are included in Section IX: Raw milk and dairy products, Chapters I., I.1.d) and II.A.1 to II.A.4. The design and cleanability of the technological facilities, the biocides used for disinfection of animals and mechanical devices are emphasised.

The European Parliament (EP) and Council (EC) Regulation. 854/2004 of 29 April 2004 laid down specific rules for official control on products of animal origin, in Chapter II, Article 4.4 requires good hygiene practice of producers and the resulting necessity of existence of functional sanitation rules.

Within EC, CEN was entrusted with the development of standard procedures for hygienic and safe engineering; afterward, these standards were published in the EHEDG documents. All the standards are published in the form of EN.

CONCLUSIONS

The current knowledge can be summarised as follows:

(1) Microbial biofilms that can be formed on all types of surfaces of technological systems on farms and in the dairy industry adversely affect the quality and safety of the final products (many microorganisms are alimentary pathogens), both processed raw materials and food products.

(2) The biofilms are mostly formed by different species of microorganisms, which mutually protect one another against the effects of biocidal products and are concurrently resistant to these products. The colonisation of surfaces in the closed piping systems, open systems, floors, waste, walls and ceilings in the production halls

by microorganisms represents a problem in the selection of sanitation agents effective for their devitalisation.

(3) Based on the existing and new model studies, practical methods for measuring the effectiveness of the sanitation procedures, including the selection of biocides and comparison of the physical parameters of the sanitation procedures, should be evaluated. The testing the sanitation agents should be performed by standardised tests, which will consider microbial, structural and chemical properties of the viable surface communities of microorganisms on specific contact surfaces in the food processing industry.

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References

Aarnisalo K., Salo S., Miettinen H., Suihko M.L., Wirtanen G., Autio T., Lundén J., Korkeala H., Sjöberg A.M. (2000): Bactericidal efficiencies of commercial disinfectants against *Listeria monocytogenes* on surfaces. Journal of Food Safety, **20**: 237–250.

Arciola C.R., Balsdassarri L., Montanaro L. (2001): Presence of *icaA* and *icaD* genes and slime production in a collection of staphylococcal strains from catheter-associated infections. Journal of Clinical Microbiology, **39**: 2151–2156.

Asao T., Kumeda Y., Kawai T., Shibata T., Oda H., Haruki K., Nakazawa H., Kozaki S. (2003): An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: estimation of enterotoxin A in the incriminated milk and powdered skim milk. Epidemiology and Infection, **130**: 33–40.

Austin J.W., Bergeron G. (1995): Development of bacterial biofilms in dairy processing lines. Journal of Dairy Research, **62**: 509–519.

Bessems E. (1998): The effect of practical conditions on the efficacy of disinfectants. International Biodeterioration and Biodegradation, **41**: 177–183.

Bjorland J., Steinum T., Kvitle B., Waage S., Sunde M., Heir E. (2005): Widespread distribution of disinfectant resistance genes among staphylococci of bovine and caprine origin in Norway. Journal of Clinical Microbiology, **43**: 4363–4368.

Bloomfield S.F., Arthur M., Begun K., Patel H. (1993): Comparative testing of disinfectants using proposed European surface test methods. Letters in Applied Microbiology, **17**: 119–125.

Bremer P.J., Fillery S., McQuillan A.J. (2006): Laboratory scale Clean-In-Place (CIP) studies on the effectiveness of different caustic and acid wash steps on the removal of dairy biofilms. International Journal of Food Microbiology, **106**: 254–262.

Bylund G. (1995): Dairy Processing Handbook. Tetra Pak Processing Systems AB, Lund: 436.

Cafiso V., Bertuccio T., Santagati M., Campanile F., Amicosante G., Perilli M.G., Selan L., Artini M., Nicoletti G., Stefani S. (2004): Presence of the *ica* operon in clinical isolates of *Staphylococcus epidermidis* and its role in biofilm production. Clinical Microbiology and Infection, **10**: 1081–1088.

Capita R., Alonso-Calleja C., Rodriguez-Perez R., Moreno B., Garcia-Fernandez M.C. (2002): Influence of poultry carcass skin sample site on the effectiveness of trisodium phosphate against *Listeria monocytogenes*. Journal of Food Protection, **65**: 853–856.

Carpentier B., Cerf O. (1993): Biofilms and their consequences, with particular reference to hygiene in the food industry. Journal of Applied Bacteriology, **75**: 499–511.

Chick H. (1908): An investigation into the laws of disinfection. Journal of Hygiene, **8**: 92–158.

Chmielewski R., Frank J.F. (2003): Biofilm formation and control in food processing facilities. Comprehensive Reviews in Food Science and Food Safety, **2**: 22–32.

Commission regulation (EC) No 1048/2005 of 13 June 2005 amending Regulation (EC) No 2032/2003 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Official Journal of the European Union, No L 178, 09/07/2005: 1–98.

Commission regulation (EC) No 1687/2002 of 25 September 2002 on an additional period for notification of certain active substances already on the market for biocidal use as established in Article 4(1) of Regulation (EC) No 1896/2000. Official Journal of the European Communities, No L 258, 26/09/2002: 15–16.

Commission regulation (EC) No 1896/2000 of 7 September 2000 on the first phase of the programme referred to in the Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council on biocidal products. Official Journal of the European Communities, No L 228, 08/09/2000: 6–17.

Commission regulation (EC) No 2032/2003 of 4 November 2003 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, and amending Regulation (EC) No 1896/2000.

Official Journal of the European Union, No L 307, 24/11/2003: 1–96.

Costerton J.W., Geesey G.G., Cheng K.J. (1978): How bacteria stick. Scientific American, **238**: 86–95.

Costerton J.W., Stewart P.S., Greenberg E.P. (1999): Bacterial biofilms: a common cause of persistent infections. Science, **284**: 1318–1322.

Czechowski M.H., Banner M. (1992): Control of biofilms in breweries through cleaning and sanitizing. Technical Quarterly Master Brewers Association of the Americas, **29**: 86–88.

Dankert J., Hogt A.H., Feijen J. (1986): Biomedical polymers: bacterial adhesion, colonization, and identification. Critical Reviews in Biocompatibility, **2**: 219–301.

Davies D.G., Parsek M.R., Pearson J.P., Iglewski B.H., Costerton J.W., Greenberg E.P. (1998): The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science, **280**: 295–298.

De Silva G.D.I., Kantzanou M., Justice A., Massey R.C., Wilkinson A.R., Day N.P.J., Peacock S.J. (2002): The *ica* operon and biofilm production in coagulase-negative staphylococci associated with carriage and disease in neonatal intensive care unit. Journal of Clinical Microbiology, **40**: 382–388.

Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market. Official Journal of the European Communities, No L 123, 24/04/1998: 1–63.

Donlan R.M. (2001): Biofilm formation: a clinically relevant microbiological process. Clinical Infectious Diseases, **33**: 1387–1392.

Dunsmore D.G., Twomey A., Whittlestone W.G., Morgan H.W. (1981): Design and performance of systems for cleaning product-contact surfaces of food equipment: a review. Journal of Food Protection, **44**: 220–240.

EHEDG Document No. 8 (1993): Hygienic equipment design criteria. European Hygienic Engineering & Design Group, Frankfurt/Main.

EHEDG Document No. 2 (2000): A method for the assessment of in-place cleanability of food processing equipment. European Hygienic Engineering & Design Group, Frankfurt/Main.

EN 1040:2005: Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics. Test method and requirements (phase 1).

EN 12353:2006: Chemical disinfectants and antiseptics. Preservation of test organisms used for the determination of bactericidal, mycobactericidal, sporicidal and fungicidal activity.

EN 1276:1997: Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas. Test method and requirements (phase 2, step 1).

EN 13697:2001: Chemical disinfectants and antiseptics. Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. Test method and requirements without mechanical action (phase 2, step 2).

EN 13704:2002: Chemical disinfectants. Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. Test method and requirements (phase 2, step 1).

EN 1656:2000: Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary field. Test method and requirements (phase 2, step 1).

EN 1672-2:2005: Food processing machinery. Basic concepts. Part 2: Hygiene requirements.

EN ISO 14159:2004: Safety of machinery. Hygiene requirements for the design of machinery.

Flint S.H., Bremer P.J., Brooks J.D. (1997): Biofilms in dairy manufacturing plant – description, current concerns and methods of control. Biofouling, **11**: 81–97.

Frank J.F. (2001): Microbial attachment to food and food contact surfaces. Advances in Food and Nutrition Research, **43**: 319–370.

Gibson H., Taylor J.H., Hall K.E., Holah J.T. (1999): Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms. Journal of Applied Microbiology, **87**: 41–48.

Gilbert P., Allison D.G., McBain A.J. (2002): Biofilms *in vitro* and *in vivo*: do singular mechanisms imply cross-resistance? Journal of Applied Microbiology, **92** (Suppl. 1): 98–110.

Guðbjörnsdóttir B., Einarsson H., Thorkelsson G. (2005): Microbial adhesion to processing lines for fish fillets and cooked shrimp: influence of stainless steel surface finish and presence of gram-negative bacteria on the attachment of *Listeria monocytogenes.* Food Technology and Biotechnology, **43**: 56–61.

Hilbert L.R., Bagge-Ravn D., Kold J., Gram, L. (2003): Influence of surface roughness of stainless steel on microbial adhesion and corrosion resistance. International Biodeterioration and Biodegradation, **52**: 175–185.

Holah J.T. (1992): Industrial monitoring: Hygiene in food processing. In: Melo L.F., Bott T.R., Fletcher M.,

Capdeville B. (eds): Biofilms – Science and Technology. Springer, New York: 645–659.

Holah J.T., Gibson H. (1999): Biofouling: Cleanability problems in food processing? In: Wirtanen G., Salo S., Mikkola A. (eds): Proceedings of 30th R3 – Nordic Contamination Control Symposium, VTT Symposium 193, Espoo: 177–186.

Jang A., Szabo J., Hosni A.A., Coughlin M., Bishop P.L. (2006): Measurement of chlorine dioxide penetration in dairy process pipe biofilms during disinfection. Applied Microbiology and Biotechnology, **72**: 368–376.

Johnston M.D., Simons E.A., Lambert R.J. (2000): One explanation for the variability of the bacterial suspension test. Journal of Applied Microbiology, **88**: 237–242.

Jones M.B., Miller J.J., Brown W.E. (1986): Effectiveness of a quaternary ammonium compound in the presence of hard water and organic soil. Developments in Industrial Microbiology, **25**: 771–777.

Keener K.M., Bashor M.P., Curtis P.A., Sheldon B.W., Kathariou S. (2004): Comprehensive review of *Campylobacter* and poultry processing. Comprehensive Reviews. Food Science and Food Safety, **3**: 105–116.

Krönig B., Paul T. (1897): Die chemischen Grundlagen der Lehre von der Giftwirkung und Desinfection. Zeitschrift für Hygiene, **25**: 1–112.

Kumar C.G., Anand S.K. (1998): Significance of microbial biofilms in food industry: a review. International Journal of Food Microbiology, **42**: 9–27.

Langsrud S., Sidhu M.S., Heir E., Holck A.L. (2003): Bacterial disinfectant resistance – a challenge for the food industry. International Biodeterioration and Biodegradation, **51**: 283–290.

Larson E.L., Morton H.E. (1991): Alcohols. In: Block S.S. (ed.): Disinfection, Sterilization, and Preservation. 4th Ed. Lea & Febiger, London: 204–224.

Lawrence J.R., Korber D.R., Hoyle B.D., Costerton J.W., Caldwell D.E. (1991): Optical sectioning of microbial biofilms. The Journal of Bacteriology, **173**: 6558–6567.

Le Chevallier M.W., Cawthon C.D., Lee R.G. (1988): Factors promoting survival of bacteria in chlorinated water supplies. Applied and Environmental Microbiology, **54**: 649–654.

Lelieveld H.L.M. (1985): Hygienic design and test methods. Journal of the Society of Dairy Technology, **38**: 14–16.

Lomander A., Schreuders P., Russek-Cohen E., Ali L. (2004): Evaluation of chlorines impact on biofilms on scratched stainless steel surfaces. Bioresource Technology, **94**: 275–283.

Luppens S.B.I., Reij M.W., van Der Heijden R.W.L., Rombouts F.M., Abee T. (2002): Development of a standard test to assess the resistance of *Staphylococcus aureus* biofilms cells to disinfectants. Applied and Environmental Microbiology, **68**: 4194–4200.

Mafu A.A., Roy D., Goulet J., Magny P. (1990): Attachment of *Listeria monocytogenes* to stainless steel, glass, polypropylene, and rubber surfaces after short contact times. Journal of Food Protection, **53**: 742–746.

Mattila-Sandholm T., Wirtanen G. (1992): Biofilm formation in the food industry: a review. Food Reviews International, **8**: 573–603.

Mettler E., Carpentier B. (1998): Variations over time of microbial load and physicochemical properties of floor materials after cleaning in food industry premises. Journal of Food Protection, **61**: 57–65.

Møretrø T., Hermansen L., Holck A.L., Sidhu M.S., Rudi K., Langsrud S. (2003): Biofilm formation and the presence of the intercellular adhesion locus *ica* among staphylococci from food and food processing environments. Applied and Environmental Microbiology, **69**: 5648–5655.

Morwood K. (2006): Step by step disinfectant evaluation. Cleanroom Technology Magazine (The European Journal of Contamination Control), 16th February 2006. Available at http://www.cleanroom-technology.co.uk/story.asp?storyCode=38475

Ntsama-Essomba C., Bouttier S., Ramaldes M., Dubois-Brissonet F., Fourniat J. (1997): Resistance of *Escherichia coli* growing as biofilms to disinfectants. Veterinary Research, **28**: 353–363.

Oulahal N., Martial-Gros A., Bonneau M., Blum L.J. (2004): Combined effect of chelating agents and ultrasound on biofilm removal from stainless steel surfaces. Application to *Escherichia coli* milk and S*taphylococcus aureus* milk biofilms. Biofilms, **1**: 65–73.

Peng J.S., Tsai W.C., Chou C.C. (2001): Surface characteristics of *Bacillus cereus* and its adhesion to stainless steel. International Journal of Food Microbiology, **65**: 105–111.

Peters A. (2003): Control of biofilm in the food industry: a microbiological survey of high-risk processing facilities. In: Lens P., O'Flaherty V., Moran A.P., Stoodley P., Mahony T. (eds): Biofilms in Medicine, Industry and Environmental Biotechnology: Characteristics, Analysis and Control. IWA Publishing, Cornwall: 554–567.

Phelps E.B. (1911): The application of certain laws of physical chemistry in the standardization of disinfectants. The Journal of Infectious Diseases, **8**: 27–38.

Rayner J., Veeh R., Flood J. (2004): Prevalence of microbial biofilms on selected fresh produce and household

surfaces. International Journal of Food Microbiology, **95**: 29–39.

Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities, No L 31, 01/02/02: 1–24.

Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Union, No L 226, 25/06/2004: 3–21.

Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. Official Journal of the European Union, No L 226, 25/06/2004: 22–82.

Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official control on products of animal origin intended for human consumption. Official Journal of the European Union, No L 226, 25/06/2004: 83–127.

Rodgers M., Zhan X.M. (2003): Moving-medium biofilm reactors. Reviews in Environmental Science and Biotechnology, **2**: 213–224.

Salo S., Mattila-Sandholm T., Wirtanen G. (2001): Do we need enhanced low pressure cleaning procedures for open process surfaces? In: Proceedings of 32nd R3 – Nordic Symposium on Contamination Control: 185–193.

Sequiera C.A.C., Carrasquinho P.M.N.A., Cebola C.M. (1988): Control of microbial corrosion in cooling water systems by the use of biocides. In: Sequiera C.A.C., Tiller A.K. (eds): Microbial Corrosion 1. Elsevier Applied Science, Essex: 240–255.

Sharma M., Anand S.K. (2002): Characterization of constitutive microflora of biofilms in dairy processing lines. Food Microbiology, **19**: 627–636.

Sidhu M.S., Langsrud S., Holck A. (2001): Disinfectant and antibiotic resistance of lactic acid bacteria isolated from food industry. Microbial Drug Resistance, **7**: 73–83.

Somers E.B., Wong A.C. (2004): Efficacy of two cleaning and sanitizing combinations on *Listeria monocytogenes* biofilms formed at low temperature on a variety materials in the presence of ready-to-eat meat residue. Journal of Food Protection, **67**: 2218–2229.

Springthorpe S. (2000): Disinfection of surfaces and equipment. Journal of the Canadian Dental Association, **66**: 58–60.

Stewart P.S., Rayner J., Roe F., Rees W.M. (2001): Biofilm penetration and disinfection efficacy of alkaline hypochlorite and chlorosulfamates. Journal of Applied Microbiology, **91**: 523–532.

Stoodley P., Hall-Stoodley L., Lappin-Scott H.M. (2001a): Detachment, surface migration, and other dynamic behavior in bacterial biofilms revealed by digital time-lapse imaging. Methods in Enzymology, **337**: 306–319.

Stoodley P., Wilson S., Hall-Stoodley L., Boyle J.D., Lappin-Scott H.M, Costerton J.W. (2001b): Growth and detachment of cell clusters from mature mixed- species biofilms. Applied and Environmental Microbiology, **67**: 5608–5613.

Suihko M.L., Salo S., Niclasen O., Guðbjörnsdóttir B., Torkelsson G., Bredholt S., Sjöberg A.M., Gustavsson P. (2002): Characterization of *Listeria monocytogenes* isolates from meat, poultry and seafood industries by automated ribotyping. International Journal of Food Microbiology, **72**: 137–146.

Tamine A.Y., Robinson R.K. (1999): Yoghurt: Science and Technology. Woodhead Publishing Ltd, Cambridge.

Templeton C. (2005): Choosing the right cleaning and disinfection program for your operation. In: Proceedings of the 5th London Swine Conference – Production at the Leading Edge. 6–7 April 2005.

Troller J.A. (1986): Water relations of foodborne bacterial pathogens – an update review. Journal of Food Protection, **49**: 656–670.

Troller J.A. (1993): Sanitation in food Processing. Academic Press, San Diego: 30–70, 263–286.

Türetgen I. (2004): Comparison of the efficacy of free residual chlorine and monochloramine against biofilms in model and full scale cooling towers. Biofouling, **20**: 81–85.

Van Loosdrecht M.C.M., Lyklema J., Norde W., Zehnder A.J.B. (1989): Bacterial adhesion: A physicochemical approach. Microbial Ecology, **17**: 1–15.

Vidal D.R., Ragot C., Thibault F. (1997): Bacterial biofilms and resistance to disinfectants. Annales Pharmaceutiques Françaises, **55**: 49–54.

Watson H.E. (1908): A note on the variation of the rate of disinfection with change in the concentration of the disinfectant. Journal of Hygiene, **8**: 536–542.

Wirtanen G. (1995): Biofilm Formation and its Elimination from Food Processing Equipment. VTT Publications 251, Espoo: 106.

Wirtanen G., Husmark U., Mattila-Sandholm T. (1996): Microbial evaluation of the biotransfer potential from surfaces with *Bacillus* biofilms after rinsing and cleaning procedures in closed food-processing systems. Journal of Food Protection, **59**: 727–733.

Wirtanen G., Juvonen R. (2002): Disinfectant efficacy on spoilage yeasts isolated from various food proc-

esses. In: Wilson D.I., Fryer P.J., Hastings A.P.M. (eds.): Fouling, Cleaning and Disinfection in Food Processing. City Services Design and Print, Cambridge: 189–196.

Wirtanen G., Langsrud S., Salo S., Olofson U., Alnås H., Neuman M., Homeid J.P., Mattila-Sandholm T. (2002): Evaluation of Sanitation Procedures for Use in Daires. VTT Publication 481, Espoo: 96.

Wirtanen G., Saarela M., Mattila-Sandholm T. (2000): Biofilms – Impact on hygiene in food industries. In: Bryers J. (ed.): Biofilms II: Process Analysis and Aplication. John Wiley – Liss, New York: 327–372.

Wirtanen G., Salo S., Allison D.G., Mattila-Sandholm T., Gilbert P. (1998): Performance evaluation of disinfectant formulations using poloxamer-hydrogel biofilm-constructs. Journal of Applied Microbiology, 85: 965–971.

Wirtanen G., Salo S. (2003): Disinfection in food processing – Efficacy testing of disinfectants. Reviews in Environmental Science and Biotechnology, **2**: 293–306.

Wirtanen G., Salo S. (2005): Improper dairy hygiene – Case studies on poor microbial ecology in the Nordic DairyNET project. In: 36th R3 – Nordic Symposium and 5th European Parenteral Conference of ESPC, 23–25 May 2005, Linköping: 171–182.

Wirthlin M.R., Chen P.K., Hoover C.I. (2005): A laboratory model biofilm fermenter: design and initial trial on a single species biofilm. Journal of Periodontology, **9**: 1443–1449.

Zoltai P.T., Zottola E.A., McKay L.L. (1981): Scanning electron microscopy of microbial attachment to milk contact surfaces. Journal of Food Protection, **44**: 204–208.

Zottola E.A., Sasahara K.C. (1994): Microbial biofilms in the food processing industry – Should they be a concern? International Journal of Food Microbiology, **23**: 125–148.

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Corresponding author:

RNDr. Jarmila Schlegelová, Výzkumný ústav veterinárního lékařství, v.v.i., Hudcova 70, 621 00 Brno, Česká republika
tel.: + 420 533 331 619, fax: + 420 541 211 229, e-mail: schlegelova@vri.cz