Surface microbiology – Biofilm formation and its elimination, sampling on surfaces, analysis and interpretation of results

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Surface microbiology – Biofilm formation and its elimination, sampling on surfaces, analysis and interpretation of results

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Extended Abstract
Microbes prefer attaching on solid surfaces rather than being in the liquid phase and therefore most of the microbial food contamination is related to biofilm formation. Biofilms are multi-layered cell clusters embedded in organic polymer matrices, which protect the microbial cells from environmental stresses, as well as from antibiotic and disinfectant attacks. It is also important to remember that as much as 96-98 % of a biofilm consists of water, which means that only 2-4 % of the total biofilm volume is detectable on dry surfaces. Microbes that inhabit contact and environmental sites in food processing are mostly harmful, because microbial communities of either spoilage microbes or pathogens in the wrong places lead to contamination of firstly the process surfaces and secondly the products produced in the process. A biofilm consists of microbial cell clusters with a network of internal channels or voids, which allows nutrients and oxygen to be transported from the bulk liquid to the cells, in the extracellular polysaccharide and glycoprotein matrix. Recent research has provided molecular insights into biofilm formation, the adhesion, which is influenced by the surrounding environment, involves complex interactions between the microbes and the substrate. Both laboratory studies with biofilms and hygiene surveys on surfaces in the industry have shown that surface-attached pathogens are more resistant to disinfectants containing e.g. chlorine or quaternary ammonium compounds, which are approved for use in food premises, than the planktonic cells are. Furthermore, these biofilms stick to and grow on commonly used materials e.g. elastomers and stainless steel. In the following subsections there are more examples of biofilm formation capability of the main foodborne pathogens of both Gram-negative and Gram-positive bacteria as well as of yeasts.

The cleanliness of surfaces, training of personnel and good manufacturing and design practices are the most important tools in combating biofilm problems in the food industry. Improper cleaning and disinfection procedures of food contact surfaces are contributing to many foodborne illness episodes. The elimination of biofilms is a difficult and demanding task and therefore cleaning and disinfection should be performed often enough so that mature biofilms are not formed in the food processing equipment, on the processing lines and on other surfaces in the processing facilities

so that safe products with an acceptable shelf-life and quality can be produced. An efficient cleaning procedure consists of a sequence of detergent and disinfectant applications at effective concentrations and correct temperatures and periods as well as of water rinses. The basic task of the detergents is to reduce the interfacial tensions of soils so that the soil including microbes in the biofilms becomes miscible in water. A prolonged exposure of the surfaces to the detergent makes the removal more efficient. Used cleaning solutions should only be re-used for prewashing, because the old cleaning solutions in the main cleaning phase can contaminate the equipment surfaces and thus destroy the cleaning result. The cleaning efficacy is also affected by the accessibility to and type of equipment cleaned. After cleaning the disinfectants kill the few microbes left on the surfaces. Residues of disinfectants must not be left on the process surfaces; they must be rinsed of the surfaces with water of potable quality. There is a photobacterial test, a luminescence inhibition method based on Vibrio fischeri, can be used, to test that the rinsing of surfaces has been proper. Finally the equipment and process lines should be left to dry in well ventilated areas, microbes do not grow on dry, clean surfaces.

In the food industry, the first step is to identify the biofilm problems in a particular process or site. Practical methods for assessing microbes and organic soil on processing surfaces are needed to establish the optimal cleaning frequency of the equipment and verify the efficiency of cleaning procedures. It is also important to use the best possible methods for isolation and detection of the biofilm for further characterisation in the laboratory. Methods for studying biofilm formation include microbiological, chemical, microscopy and molecular biological methods. The chemical methods used in the assessment of biofilm formation are indirect methods based on the utilisation or production of specific compounds, e.g. organic carbon, oxygen, polysaccharides and proteins, or on the biofilm microbial activity, e.g. living cells and adenosine 5'-triphosphate (ATP) content. Important tools in modern biotechnology-related research are based on microscopy techniques. Many different fluorochromes can be and have been used for the staining of microbes in food samples, biofilms and environmental samples. The three, major techniques applied in the molecular detection and identification of bacteria are the polymerase chain reaction (PCR), sequencing and the hybridisation technique. Next generation sequencing technologies (NGS) allow investigation in deeper layers of the microbial communities, whereas basic PCR technique is used for finding selected pathogens.

In testing the surface hygiene in food processing areas aerobic bacteria, yeasts, moulds and coliforms as indicators should be used. Specific microbes e.g. Listeria monocytogenes, Salmonella spp., Staphylococcus aureus and Escherichia coli can be added according to the microbial risks of the products produced. Hygiene samples are normally taken after cleaning, just before processing activities in the shifts begin, which means that at least the contact surfaces should be at a good hygienic level. The limits for the various scales of e.g. loose, normal and strict hygiene depending on the production activities in the area can be set independently for the various levels (good, adequate and poor hygiene) of each scale. Note that when the limits for the various scales are set the spoilage sensitivity of product should be kept in mind. The more prone the product is to spoilage the stricter limits should be used. The main focus of hygiene control should of course be on the food contact surfaces, but environmental

surfaces can also be part of the contamination routes e.g. through cross contamination. The initial levels can be based on either own survey results or values taken from literature. Information on aerobic bacteria is especially needed, when products with long self-life are produced. The microbes found on environmental surfaces can have an impact on the product quality especially on products produced in open or semi-open processes.

Training is needed to refresh the personnel about food safety and hygiene issues in the food processing. An important issue in risk assessment at the manufacturing level is that more quantitative and systematic procedures should be used and it should be based on scientific knowledge. Thus microbiological and chemical issues will be especially important for the safe production of feed, food and packaging material in the future. The hygienic design of process hygiene line features including cleaning systems must be improved using e.g. techniques based on e.g. 3D-printing, ultrasonics, dry-ice, ozone treatments and UVC-irradiation as well as combinations of these and other techniques. The efficacy of disinfectants used in food processing and in hand hygiene rubs can be improved by using compounds from essential oils.

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