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Integrated Management of Cleaning and Disinfection Programs for Bio-adhesion Control and Biofilm Removal in Food Industries – a review*

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Introduction

Bacteria attachment onto surfaces, also referred as bio-adhesion, and biofilm formation are well-recognized, wide-spread and naturally occurring phenomena in a variety of environments, including food industries. Knowing that food product contaminations may occur from environmental routes, the **surface route** has to be definitely considered **as important as people and air routes.** Thus, as part of the Hazard Analysis Critical Control Point (HACCP) program for pathogens and environmental risk assessment for other spoilage micro-organisms, relevant and effective management of process streams cleaning and disinfection needs to systematically integrate/take into account bio-adhesion and biofilm principles and specificities.

Bio-Adhesion and Biofilm – Background Information

Mineral, organic and microbiological soils

In the absence of specific cleaning and disinfection sequences, surface scaling is a natural and irreversible phenomenon. Different types of soils can accumulate onto surfaces. **Mineral** soils are mineral particles, generally coming from the water used in process or from the products themselves. These mineral deposits may, as a carrier, help micro-organisms to adhere easily onto surfaces. **Organic** soils are fragments of products (proteins, sugar, and grease) which may be used as nutrients by micro-organisms. **Microbiological** soils are defined as accumulation of micro-organisms onto surfaces. When organized as an ecosystem, they are also referred as "Biofilm".

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Biofilm definition

In most environmental ecosystems, the microbial planktonic (individual free floating organisms) way of life is seldom encountered or does generally represent an intermediate and transitory stage. **More than 99 % of microbial life is estimated to be fixed onto solid surfaces.**

Natural microbial biofilms are **mixed populations** of **matrix-enclosed micro-organisms** that are concentrated at an interface (usually solid/liquid) and typically **surrounded** by an extra-cellular polymeric substance **(EPS)** matrix. This definition includes microbial aggregates, flocculates and population adherent to membrane pores. As stated by Sutherland (1) "the biofilm matrix is a dynamic environment in which the component microbial cells appear to reach homeostatic are optimally organized to make use of all available nutrients".

Description of the biofilm structure

Biofilms are composed primarily of **microbial cells** and **EPS.** EPS may account for 50 % to 90 % of the total organic carbon of biofilms. Biofilms are very heterogeneous, containing micro-colonies of bacterial cells encased in this EPS matrix and separated from other micro-colonies by interstitial voids (also called water channels).

Stages for biofilm formation

The development of a mature biofilm may take several hours to several weeks, depending on the system, initial stages (conditioning layer, initial adhesion) occurring as soon as the system is back into production. For any wet surface, the formation of biofilm onto a surface happens in **5** well-defined and well-described **stages.**

- Stage 1: The conditioning layer. The first substances associated with the surface are not bacteria but trace organics form a "conditioning layer". The layer aims **to neutralize excessive surface charge and surface free energy** which may prevent bacteria cells from approaching near enough to initiate attachment. In addition, the adsorbed organic molecules often serve as an initial nutrient source.
- Stage 2: The bacterial initial adhesion. Once a conditioning layer is formed, the next step in biofilm formation is the adhesion of bacteria to this layer. This adhesion mainly based on **electrostatic and non electrostatic** (such as Van der Waals) physico-chemical properties is not permanent.
- Stage 3: The consolidation of bacterial adhesion. To ensure **permanent adhesion onto the surface** to be colonized, bacterial attachment is strengthened by the use of fimbriae, pili, flagella and EPS that act to form bridge between bacteria and the conditioning film.
- Stage 4: The bacterial colonization. Responding to favourable nutrients conditions, bacteria will rapidly multiply by binary fission. Bacteria organize themselves as a community, **optimizing exchanges and cross-communication** to ensure survival and expansion.

- Stage 5: The detachment and sloughing off of bacteria within biofilms. As soon as consolidation and colonization are finished, the biofilm is considered as mature. A dynamic equilibrium between the biofilm itself and the environmental conditions (mainly flow conditions) is achieved. Nevertheless, the equilibrium is dynamic: when some new bacteria arrived or appear, some of them are released into the environment.

Based on this kinetic description and for this review, "bio-adhesion" corresponds to the 3 earliest stages of biofilm formation – conditioning layer, bacterial initial adhesion, and consolidation of bacterial adhesion – and "biofilm growth" to the 2 latest stages – bacterial colonization, and detachment/sloughing off.

Roles of the biofilm

Being a "strong and sticky framework" (2), the hydrated extracellular EPS acts simultaneously as a spider's web and as a protective shield against environmental stresses. According to Mittelman (3), this "polymeric material, or glycocalyx, that not only **facilitate attachment** but also act as an ion-exchange system for **trapping and concentrating trace nutrients** from the overlying water. The glycocalyx also acts as a **protective coating** for the attached cells which mitigates the effects of biocides and other toxic substances."

Micro-organisms involved in biofilm formation

A well-matured biofilm community can include bacteria, fungi, yeasts, protozoa, and other micro-organisms. Nevertheless, **bacteria** play a key and central role in installing and developing biofilms. They are considered as primary or **pioneering colonizers,** whereas fungi, yeasts, and protozoa will be considered as secondary colonizers.

Bacteria such as *Alcaligenes, Bacillus,* Enterobacteriacea such as coliforms, *Flavobacterium, Pseudomonas* and *Staphylococcus* have been reported by many scientific publications to have a significant capability on developing biofilms. Capability of pathogens, such as *Campylobacter,* entero-pathogenic *Escherichia coli, Listeria, Salmonella, Vibrio cholerae,* to initiate biofilm formation is still unclear and challenged. Most of the scientists agree on the fact that **pathogens,** due to their fastidious growth requirements and their limited capability to compete with indigenous flora, have to be considered, for process streams of food industries, as **secondary colonizers.**

Key environmental factors leading to biofilm formation

In the food processing environments, the overall global environmental conditions favour microbial attachment and biofilm formation. Colonisation of a surface by micro-organisms and subsequent development of the biofilm depends upon factors that influence metabolism of the cells, such as the availability of nutrients and trace elements. Nevertheless, it is absolutely not realistic to foresee control of bio-

adhesion and biofilm growth by removing all nutrients. Within the water business, it has been proven that oligotrophic bacteria, such as *Pseudomonas,* have the capability of multiplication within environments containing traces of assimilable organic carbon (less than few µg/L), in mainly using carbon dioxide and carbonates always present in water. From this initial microbial growth, autotropohic bacteria can then colonize surfaces.

The physical conditions within the flowing system, such as flow velocity, temperature, and the nature and physico-mechanical aspects (crevices, holes, ...) of solid surface to which the micro-organisms will attach, also exert an influence on biofilm growth.

Integrated and global management of bio-adhesion and Biofilm for cleaning and disinfection

Management of finished products contamination related to bio-adhesion and biofilm formation can be described with the "Iceberg" concept (4). This model describes the sequence of events leading to out-of-control impacts on finished products safety and quality. The top part of the iceberg corresponds to a level of contamination which already visibly (to our consumers) impacts our finished products. Consequences of such management type are high level of non quality costs, withdrawing, recall and even crisis. Most of the times, short term and expensive actions plans are needed to resolve related issues. This is most of the time the mode of food industries for biofilm related issues. In better understanding acceptable quality deviation/threshold which will not impact on finished products quality, out-of control factors, operators and management staff judgement and decisions processes, pro-active and cost-effective management of contaminations becomes possible.

For the food industry, not knowing bio-adhesion principles and processes leads systematically to over-conservative and reactive corrective actions. On the contrary, understanding biofilm growth in process stream allows proactive and fine-tuned management of issues related to bio-adhesion. Unfortunately, this straightforward and simple consideration is seldom taken into account by food industries professionals as knowledge building up and mastering is only possible with long term and costly Research and Development programs. But it needs to be convinced that outcomes of such programs are outstanding. In the same order of ideas, limited and partial understanding of biofilm may result to mis-interpretations in case of safety and quality issues. The biofilm concept is then considered as a "magic black box" containing any explanations, a Pandora box which is better not to open and an easy excuse not to move forward for improvements: *"You know – This is still related to bio-adhesion and biofilm. It is always impossible to completely manage this. What we have reached is already good enough ..."*

Thus, all scientific details are considered important for long term action plans and improvements but may not be necessary needed or useful for the food industries on a routine basis. Knowing that a specific gene *(algC* gene for *Pseudomonas aeruginosa*

– (5)) could be involved in the exo-polysaccharides production and excretion, that bacteria can communicate between each other (the "quorum sensing" concept), that disinfection kinetic can be modelled by log-survivor/time curves do not really help to validate daily product batches and process parameters or to know if the process stream is in control and stable. And this crucial link between science and applied and practical cleaning and disinfection procedures often lack at corporate or factory levels. Being able to link bio-adhesion, cleaning and disinfection procedures and finished products quality becomes mandatory. Bacteria able to induce biofilm are always present in our process streams. They will sooner or later induce biofilm and impact on our products. But when and how often remain unknown for most of our factories? In order to optimize cleaning and disinfection with both quality and economical objectives in mind, knowledge and know-how on bio-adhesion and biofilm growth are required for any agents of change in the food industry.

Impact of hygienic design and cleaning/disinfection procedures on bio-adhesion and biofilm growth

Interactions between micro-organisms, liquid, and surface to be colonized are numerous and complex. Many scientific teams are currently working on better understanding these relationships and on evaluating how it can impact on food industries cleaning and disinfection procedures.

Microbial surface properties

Overall negative electrostatic charge

As in other organisms, the **bacterial cell wall** provides structural integrity to the cell. Moreover, due to its content, it is also **responsible** for the normal **negative** overall bacterial **electrostatic charge.**

Gram negative cell wall contains on the outside part a thin peptidoglycan (poly-*N*-acetylglucosamine and *N*-acetylmuramic acid) layer adjacent to the cytoplasmic membrane. In addition to the peptidoglycan layer, the Gram negative cell wall also contains an additional outer membrane composed by phospholipids and lipopolysaccharides (also referred as LPS) which face into the external environment. **The highly charged nature of lipopolysaccharides confer an overall negative charge to the Gram negative cell wall.**

The Gram positive cell wall is characterized by the presence of a very thick peptidoglycan layer. Imbedded into the peptidoglycan layer, polyalcohols called teichoic acids, some of which are lipid-linked to form lipoteichoic acids, are present. Because lipoteichoic acids are covalently linked to lipids within the cytoplasmic membrane, they are responsible for linking the peptidoglycan to the cytoplasmic membrane. **Teichoic acids give the Gram positive cell wall an overall negative charge** due to the presence of phosphodiester bonds between teichoic acid monomers.

Extra-cellular polymeric substance

EPS may vary in chemical and physical properties, but it is primarily composed of polysaccharides. In the case of biofilms produced by **Gram-negative bacteria,** polysaccharides are **neutral or polyanionic.** In allowing association with common divalent cations such as calcium and magnesium, these biofilms are known to provide greater binding force and being more difficult to remove. In the case of some **Gram-positive bacteria,** the chemical composition of EPS may be quite different and may be **primarily cationic.**

EPS is also highly hydrated because it can incorporate large amounts of water into its structure by hydrogen bonding. Most types of EPS are both **hydrophilic and hydrophobic.** EPS may also vary in its solubility. EPS may associate with metal ions, and other macromolecules (such as proteins, DNA, lipids, and even humic substances).

Other bacterial surface structures

Fimbrae are protein tubes that extend out from the outer membrane in many bacteria. Short in length but present in high numbers about the entire bacterial cell surface, they usually function to facilitate the attachment of a bacterium to a surface or to other cells.

Many bacteria secrete also **extra-cellular polymers** outside of their cell walls. These polymers are usually composed of **polysaccharides** (LPS O anti-gene) and sometimes **proteins.** Several studies have shown that treatment of adsorbed cells with proteolytic enzymes caused a marked release of attached bacteria, providing evidence for the role of proteins in attachment.

Flagella, complex structures generally involved in mobility capabilities, are also composed of many different proteins and evidence of the role of flagella in attachment processes have been also collected.

Modifications of bacterial surface properties

Very **limited R&D activities** are known to focus on modifications of microbial surface properties to limit bio-adhesion. Works done by Chavant et al. (6) on "positive" biofilm may be mentioned. A selective pressure allows to select bio-surfactant production which then covers surfaces to be colonized and to limit microbial adhesion of *Listeria* spp.

Colonized surface properties

General requirements

It is essential that all **surfaces that come into contact with food** are as **smooth** and **continuous** as possible. Dead legs (including probes or un-used T-pieces), non purgeable areas, bad welding, gaskets, corners, crevices, recesses, connecting pipes, non accessible for cleaning and disinfection parts are known to be locations of

microbial "growth pockets" and must be removed from process streams. A well-known example for the drinking water business is the capability of coliforms to survive and multiply on rubber-coated valves, leading to water quality deterioration (7). The European Hygienic Equipment Design Group (EHEDG) (8) has described in details how to hygienically designed equipment and select materials (document 8 – Hygienic equipment design criteria). Moreover, even if Cleaning In Place (CIP) system are set up, any equipment must be easily dismantled as there are no other solution to remove biofilm than a Cleaning Out of Place (COP). In trusting CIP process and designs too much, food industries often pay too little attention to this crucial factor.

Type of materials

The following materials of construction can be selected for pipes and equipments : (1) stainless steel (AISI-304 and AISI-316), (2) plastics (polypropylene – PP, Polyvinyl Chloride Unplasticized – PVC, Acetal Copolymer, Polycarbonate – PC, and High Density Polyethylene – PE). For seals, gaskets, and joint rings, the recommended choices are Ethylene Propylene Diene Monomer (EPDM), Nitrile rubber, Nitrile/Butyl rubber (NBR), silicon rubber, and fluoroelastomer (Viton).

All of these products are "corrosion resistant, non toxic, mechanically stable and such that the surfaces finish is not adversely affected under conditions of use" (8). Besides understanding **electrostatic charges, roughness** is the key parameter to select materials for process streams. Initial roughness of most of regular materials, such as stainless steel, PVC, polypropylene is equivalent. Nevertheless, their capabilities to resist to cleaning and disinfections procedures through time are important and need to be systematically evaluated before selection.

Moreover, the capability of any used material **to release nutrients** for microbial growth must be checked. Van der Kooij (9) are clearly demonstrated the impact of materials in assimilable organic carbon release (COA) on microbial re-growth.

Modifications of colonized surface properties

The key standard approach is to limit the roughness of surface. Reduction and inhibition of microbial adhesion is under constant evaluation by scientists. A standard treatment for stainless steel is expensive electrolytic polishing. Promising works described by *Ganapathy et al.* (10) investigate the use of cold plasma induced surface modification of materials used in the food industry to create surfaces that prevent bacterial attachment and biofilm formation. Amongst other possibilities to modify surface properties, physical (pulsed light, ionising treatments) and chemical (compounds graft) are also tested. The use of Teflon® is also considered as an interesting alternative.

In addition, "antimicrobial" surface consist in adding to the material (generally resins) chemical compounds known to have a microbial effect. The most well-known approach is to embed silver ions in resin. When bacteria adhere to the sur-

face, they are immediately killed. This approach does not reduce bio-adhesion, but avoid any biofilm formation. Besides huge expectations, drawbacks such as cost and limited action in time (maximum 1 week) make the actual interest for the food industries rather low.

Cleaning and disinfection approaches for biofilm removal

Defining the target "Acceptable Level"

The aim at all times must be to produce as consistent and as acceptable a product as possible, not only during a single production run but also daily from the commencement of the installed process. Defining the target **"acceptable level"** is key. In food industries, **targeting absence of biofilm into process streams is not a cost effective solution.** Preferably, the aim should be to ensure that the level of bio-adhesion and biofilm formation remains at an "acceptable level". This limit has to be driven by finished products safety and quality. It may vary from one product to another, from one process set up to another, and also from one micro-organism (or family of micro-organisms) to another.

With current cleaning and disinfection principles, **safety and quality issues** related to biofilm are seldom continuous. They are **more limited in time and space.** Contamination of finished products has to be considered as heterogeneous. To challenge "acceptable" limit only based on experience may be difficult. In these situations, QA managers may lack objective arguments to ensure that any modifications – mainly based on cost improvements – of cleaning and disinfection would not jeopardize the safety and quality of their products. By implementing Statistical Process Control (SPC) and new analytical tools such as direct analysis of biofilm by epifluorescence to define adequately this safety and quality threshold, knowledge and decisions are more scientifically-driven. And evaluation of the needed and optima cleaning and disinfection procedures becomes easier. Nevertheless, if already available for and routinely used by the Research & Development (R&D) community, the use of these new tools, still not really user-friendly and cost-affordable, remains limited in the food industries. Alternatively, evaluation of the actual biofilm contamination levels by swabbing methodology is common. This simple and available procedure has its pros and cons users. Nevertheless, well-standardized and validated swabbing instruction can be considered as a rough but robust way to estimate the "acceptable level".

Principles for optimizing Cleaning and Disinfection (C&D) procedure

Knowing the "acceptable" limit, 2 variables have to be clearly stated and defined: (1) what type of cleaning and disinfection sequence is optimum and (2) how often cleaning and disinfection has to be performed. An optimum cleaning and disinfection procedure should be set up to obtain the right balance between these 2 variables.

In case of sub-optimal cleaning and disinfection sequence, biofilms are slowly but surely building-up into the process streams. Process streams seem to be in control as there is no impact on the finished products. When the "acceptable" limit is reached and exceeded, only drastic solutions can ensure to bio-stabilize systems. If cleaning and disinfection frequency is too high, useless over-costs are generated. If frequency is too low, punctual and systematic quality deviations of the products in regards to microbial specifications occur.

The "Dr Sinner's circle" applied to biofilm removal

Dr Sinner's Circle describes an economical, ideal cleaning process with a perfect interaction of four parameters (**chemical action/detergency, temperature, time** and **mechanical action**) to ensure optimum removal of soil and maximum care of the load being processed. If one parameter is reduced, other factors must be increased to compensate. For issues related to bio-adhesion/biofilm formation, this concept can be applied for both cleaning and disinfection steps.

Cleaning step – Principles, limits and examples

Cleaning refers to the **physical removal of soils** (mineral and organic), thus exposing the micro-organisms (spoilage and pathogens) to the killing effect of the following disinfection step. Adequate cleaning may eventually reduce the bio-burden (population of micro-organisms fixed onto the surface). **Cleaning is the most essential stage in the cleaning and disinfection sequence.** Nevertheless, this stage of the cleaning and disinfection procedure is **seldom studied,** and not really considered by food industries operators as important. **Information from literature for validation purposes (within the HACCP perspective) is scarce.** Most of the time, food industries base their procedures on recommendations from suppliers, the exact figures being kept for internal use as it is often considered as a competitive advantage.

For bio-adhesion and biofilm formation issues, the main goal of the cleaning step is to **break down the EPS structure.** Depending of the product (mainly for water-based products), it may also be needed to **remove scaling residues,** which can be used by bacteria as attachment aids. In addition, cleaning is known to have a limited action in terms of removal of bacterial biofilm. *Gibson and al.* (11) has estimated that standard cleaning programs can achieve up to 1 log reduction.

Chemicals choice

Cleaning with water alone is possible. Nevertheless, some problems related to this option are well-known and described: (1) water does not naturally wet surface and access directly to soils, (2) water can not dissolve hydrophobic compounds (fats, proteins, and oils). In using surfactants, the surface tension of water is reduced. **Surfactants** are chemicals also called **surface-active agents.** They are bi-polar substances: one end is attracted by water and one end tends to attach to hydrophobic

compounds. In addition, water pH may be increased (alkaline solution) to improve solubility of proteins and oils or may be decreased (acidic solution) to improve solubility of minerals (scaling issues).

Chemicals Concentration and Time (CT concept) validation for cleaning

From literature review, the following basic information can be collected. **Alkaline cleaners** (targeting organic matter such as fats) have a pH above 11. They are normally caustic soda or potassium. The target concentration generally varies 0.5 to 2 %. The contact time will be **15–45 minutes,** depending of the process set up and the initial contamination level. **Acid cleaners** (targeting mineral deposits such as scale) have a pH below 2. They are normally phosphoric, nitric or organic acids, generally at a concentration between 0.5 and 1.5 %. The contact time will be **15–45 minutes,** depending also of the process set up and the initial contamination level.

Temperature

For **alkaline solution,** optimum temperatures are **between 60 and 80 °C. For acidic solution,** temperature should **never exceed 60 °C.**

Mechanical agents

To enhance the action of chemicals, a mechanical action is needed. If easily accessible, **wiping, brushing** and **spraying water under pressure** are acceptable solutions. As an example, *Gibson and al.* (11) describes optimum high pressure spraying conditions at 18 bar minimum for few seconds. Increase in pressure and spray time do not significantly increased the removal. For pipes cleaning, **flow** is **modified** to achieve turbulence in any part of the process and a minimum rate of 2–2.5 m/s should be reached. New pumping systems have the capability to create, by cavitations, micro-bubbles which is claimed to have the same effect as brushing.

Disinfection step – Principles, limits and examples

Disinfection is the **destruction of all vegetative forms of micro-organisms,** but spores may not be systematically destroyed. Disinfection step can only be effective if lead up by an adequate cleaning step. **Information from literature for validation purposes (within the HACCP and environmental risk assessment for spoilage perspectives) is numerous.** New chemicals are also proposed regularly by suppliers and relevant choice becomes more and more difficult, if not based on robust and complete scientific (and not marketing) reports.

Chemicals choice

Antimicrobial chemical agents for controlling environmental contaminations in food industries are generally classified into three categories: antiseptics, disinfectants, and sanitizers. An antiseptic is a substance that inhibits the growth and reproduction of disease-causing micro-organisms. Antiseptic are seldom selected. A dis-

infectant is a chemical killing or inactivating micro-organisms such as bacteria, viruses and/protozoans. A sanitizer is aimed to reduce the number of bacteria to a safe level, but does not completely eliminate them. As for other cleaning and disinfection objectives, "sanitizer" and "disinfectants" words are used interchangeably in regards to bio-adhesion and biofilm treatment.

Besides the simple use of hot water (85 °C), standard chemicals are categorized into four groups:

- **Chlorine-releasing compounds – 1st sub-family of halogens** such as hypochlorites, chlorine dioxide gas, chloramines are the most widely used of all disinfectants.
- **Iodophores – 2nd sub-family of halogens** consist in soluble mixture of iodine with surfactant which acts as a carrier for the iodine.
- **Quaternary ammonium** compounds, also known as "quaternaries", "quats" or "QACs" are essentially ammonium salts with some or all of the hydrogen atoms in $[NH4]^+$ ion substituted by alkyl or aryl groups, They are more effective against gram-positive than gram-negative bacteria. They are more active in the presence of small amounts of organic matter than any other class of disinfectants, but are inactivated by soaps, anionic detergents and inorganic polyphosphates.
- **Amphoteric** compounds are essentially alkyl or acyl amino acids. They combine detergent and disinfectant properties. They are of low toxicity, are non-corrosive and are expensive. They are effective against both gram-positive and gram-negative bacteria.
- A mixture of peracetic acid, hydrogen peroxide and acetic acid, which is stable and is effective against bacteria, spores, yeasts, moulds and viruses. The active agent is **peracetic acid.**

Disinfectant solutions are always used fresh – for most of them shelf lives of working solution are less than 2 days – and prepared as described and recommended by the suppliers. Selected chemical has to follow regulations related to chemicals used in food industries. The selection of the right disinfectant needs to take into account the following parameters: (1) active against a wide range of micro-organisms or targeting a specific family or specie, (2) able to sustain various environmental conditions (hardness of the water, organic matter, mineral content).

Chemicals Concentration and Time (CT concept) validation for disinfection

Chemicals Concentrations

In Europe, the **EN 1040/2005 standard** (12) – Chemical disinfectants and antiseptics/Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics – is a mandatory protocol to evaluate whether a disinfectant/sanitizer does or does not have a basic bactericidal activity. The test consists in adding the chemical to be tested into a suspension of *P. aerugi-*

nosa (ATCC 15442) and *S. aureus* (ATCC 6538). This European Standard applied to active substances and to formulations under development that are planned to be used in food, industrial, domestic, and institutional medical and veterinary areas. Equivalent tests such as the Chick-Martin test (yeast) and the Improved Kelsey-Sykes test *(S. typhi* and *S. aureus)* have been also developed and are widely used. The surface test, consisting in air drying micro-organisms which is then acted upon by the disinfectant is an alternative trying to better mimic the influence of adhesion on microbial resistance.

Chemicals suppliers base partially their recommendations in terms of concentrations and time on these laboratory tests. If useful to screen and compare new chemicals with standard ones, these methods do not take into account specificities related to microbial bio-adhesion and biofilm formation. Remaining (after the cleaning phase) EPS and microbial modified physiological activities linked to bio-adhesion processes definitely modify the capability of micro-organisms to resist to disinfectants. According to *Meylheuc et al.* (13), biofilms can be 10- to 1000-fold more resistant to disinfectants than suspended cultures. Sagripanti and Bonifacino (14) has estimated the resistance of *P. aeruginosa* is on average 300-fold more resistant when present on contaminated surfaces than in suspension. Thus, exact values to be applied for biofilm removal are seldom known or shared.

Contact times

To be effective, disinfectants must have sufficient contact time with the surfaces to which they are applied in order to allow them to kill target micro-organisms. Few disinfectants can kill micro-organisms instantaneously. The amount of contact time needed vary with the chemical used and the target micro-organism. Usually **20–30 minutes** is a sufficient contact time.

Temperature

All disinfectants, whether they are **spray, foams, aerosols** or **in liquid,** work best at temperature **higher than 40 °C.**

Mechanical agents

In the current emphasis on clean technology, numerous effective physical and mechanical processes to remove biofilms are available or under investigation and validation. As examples, the use of electric fields to maintain cells in suspension, the use of ultrasound to agitate water close to the solid surface with micro-bubbles, the use of turbulent promoters to interfere with the laminar sub-layer near the surface, the circulation of sponges, beads inside the process streams can be used. The drinking water industry, confronted to huge issues with biofilm, uses commonly "pigs", small rotating devices able to move inside water pipes in scrubbing internal surfaces.

Rotation of disinfectants

For the food industry, standard practice is to rotate regularly from one disinfectant to another. Main objective is to ensure limited development of resistant amongst the bacterial population found in factory environments. This may be challenged and **rotation should be avoided if possible** or, at least, **limited to a minimum.**

First of all, resistance to any microbial chemicals is seldom acquired and is mainly due to genetic changes. Under normal conditions of cleaning and disinfection procedures – high concentrations and contact times, the likelihood of having micro-organisms becoming resistant is low. It requires either high number of micro-organisms or many generations of the same microbial population. Unless strong evidence of such resistance is found, switch to another disinfectant may not be needed.

Secondly, incompatibility between the two disinfectants is common. Rinsing of surface to remove residuals is never perfect. On the contrary, it is accepted that chemicals residuals may remain onto surfaces, leading to a short-term remanant disinfection effect. Residues from a previous chemical may neutralize the new one and lead to an inadequate treatment.

Conclusions

Bio-adhesion and biofilm formation are essential know-how to be owned and mastered by the QA community in food industries. Within a competitive environment where cost reductions must be addressed in a balanced and soundly argued approach, the main objective is to scientifically validate both cleaning and disinfection steps taken into account all possible relationships between bacterial (considered as primary colonizers) contamination, surface to be colonized and the product itself.

Understanding and controlling bio-adhesion is still managed either internally or externally by the Research and Development community. This knowledge still need to be better transferred to factory floors. Activities on cleaning and disinfection improvement could be numerous in the food industries. From corporate to factory levels, simultaneous focus on both cleaning and disinfection steps are needed.

Summary

Bio-adhesion and biofilm formation are an essential know-how to be owned and mastered by the QA community in food industries. Based on this kinetic description, "bio-adhesion" corresponds to the 3 earliest stages of biofilm formation – conditioning layer, bacterial initial adhesion, and consolidation of bacterial adhesion – and "biofilm growth" to the 2 latest stages – bacterial colonization, and detachment/sloughing off. To have an impact on finished product, a mature biofilm does not need to be developed or present into process streams.

Within a competitive environment where cost reductions must be addressed in a balanced and soundly argued approach, the main objective is to scientifically vali-

date both cleaning and disinfection steps taken into account all possible relationships between bacterial (considered as primary colonizers) contamination, surface to be colonized and the product itself.

Understanding and controlling bio-adhesion is still managed either internally or externally by the Research and Development community. Solutions to limit bio-adhesion can be classified as hygienic design. Bacterial surface properties are well-known in general. Influence of polysaccharides, techoic acids, and external structures has been extensively studied. But, latest studies have demonstrated specificities related to species and even strains. This new field needs to be better evaluated. On the other hand, properties of surface to be colonized are better understood every day. Besides electrostatic charges and roughness, resistance to cleaning and disinfection procedures and capability to release organic matter have a real influence on biofilm formation. The main 2 alternative to improve surface properties are surface treatment (electrolytic polishing, cold-plasma, ...) and addition of antimicrobial substances onto or into surfaces. On the contrary, influence of the product itself is seldom studied.

Activities on cleaning and disinfection improvement could be numerous in the food industries. From corporate to factory levels, simultaneous focus on both cleaning and disinfection steps are needed. The main objective should be to find the optimum conditions to limit as much as possible biofilm growth between cleaning and disinfection procedures. In using the Dr. Sinner's circle as working frame, chemicals choice and concentration, contact time and temperature, mechanical actions have to be challenged to ensure that, if biofilm is not necessarily removed completely from process streams, it is maintained to an "acceptable" level. Concerning disinfection, focus of QA staff should not be done only on the choice of used chemicals and their concentrations, but definitely broader as alternative and cost-effective solutions exist.

Zusammenfassung

Für die Qualitätssicherung in der Lebensmittelindustrie ist es essentiell zu wissen, wie Bioadhesion und Biofilme sich bilden und wie diese kontrolliert werden können. Basierend auf der kinetischen Beschreibung beinhaltet die «Bioadhesion» die ersten drei Stadien der Bildung von Biofilmen – Konditionierungs-Schicht, erste Bakterien-Adhesion und Konsolidierung der bakteriellen Adhesion – und Biofilm-Wachstum die letzten zwei Stadien – bakterielle Besiedlung und Ablösung. Um auf das Endprodukt einen Einfluss zu haben muss der Biofilm nicht zwingend voll ausgebildet oder im Produktionsfluss vorhanden sein.

Innerhalb einer wettbewerbsorientierten Umwelt, wo Kostenreduktion in einer ausgewogenen und vernünftigen Art angegangen werden muss, ist das Hauptziel, die wissenschaftliche Validierung sowohl der Reinigungs – wie auch der Desinfektionsschritte unter Einbezug aller möglichen Beziehungen zwischen bakterieller Kontamination (Primärbesiedlung), den zu besiedelnden Oberflächen und dem Produkt.

Das Verständnis und die Kontrolle der Bioadhesion wird nach wie vor von den zuständigen Personen in Forschung und Entwicklung – intern oder extern – gemanaget. Die Lösung um Bioadhesion zu verhindern ist eine Frage des Hygiene-Design. Die Eigenschaften der Bakterienoberflächen sind in der Regel gut bekannt. Die Einflüsse von Polysacchariden und anderer externer Strukturen ist hinreichend erforscht worden. Allerdings haben neue Studien gezeigt, dass es Unterschiede bei den Spezies und sogar zwischen den Arten (Strains) gibt. Diese neuen Erkenntnisse müssen noch besser evaluiert werden. Andererseits werden die Oberflächeneigenschaften, welche besiedelt werden, immer besser verstanden. Neben der elektrostatischen Ladung und der Rauhigkeit, haben die Resistenz gegen Reinigungs- und Desinfektionsvorgänge und die Eigenschaft, organisches Material abzugeben, einen grossen Einfluss auf die Bildung von Biofilmen. Die zwei besten Alternativen zur Verbesserung der Oberflächeneigenschaften sind Oberflächenbehandlung (elektrolytisches Polieren, Kalt-Plasma, ...) und die Zugabe antimikrobieller Substanzen auf oder in die Oberfläche. Ein Einfluss auf das Produkt wird im Gegensatz dazu selten beobachtet.

Die Möglichkeiten zur Verbesserung der Reinigung und Desinfektion in der Lebensmittelindustrie sind zahlreich. Von der Konzernebene bis auf die Ebene der einzelnen Produktionsbetriebe ist der Schwerpunkt sowohl auf die Reinigungs- wie auch auf die Desinfektionsschritte zu legen. Das wichtigste Ziel sollte es sein, die optimalen Bedingungen zu finden um die Bildung von Biofilmen zwischen den Reinigungs- und Desinfektionsschritten so effizient als möglich zu verhindern. Durch den Gebrauch des Kreises von Dr. Sinner als Arbeitsgrundlage können die Wahl der Chemikalien und deren Konzentration, Konataktzeiten und Temperaturen, sowie die mechanischen Schritte optimiert werden. Es ist oftmals nicht notwendig, den Biofilm vollständig zu entfernen. Oft genügt es, diesen auf einem akzeptablen Niveau zu halten. Die Reinigungs- und Desinfektionsschritte beschränken sich oftmals auf die Wahl und die Konzentration der chemischen Produkte. Die Verantwortlichen der Qualitätssicherung sollten aber darüber hinaus unter Berücksichtigung der Biofilm-Bildung alternative und kostengünstige Lösungen finden.

Résumé

La connaissance des phénomènes de bio-adhésion et de formation de biofilm sont nécessaires et doivent être maîtrisé par la communauté AQ des industries agro-alimentaires. En se référant à la description de cinétique d'apparition et de croissance, le phénomène de « bio-adhésion » se rapporte aux 3 premières étapes – formation la couche conditionnant, adhésion initiale des bactéries et consolidation de l'adhésion bactérienne – et la « formation de biofilm » correspond aux 2 étapes ultimes – la colonisation bactérienne et la détachement et le re-largage dans l'environnement. L'impact des biofilms sur les produits finis ne nécessite pas un biofilm mature dans ou sur les équipements industriels.

En raison de la nécessité d'intégrer l'assurance qualité dans une démarche de compétition en réduisant les coûts associés, l'objectif principal est et doit rester une

validation scientifique des étapes de nettoyages et de désinfection (N&D) en prenant en compte les possibles relations entre les bactéries contaminantes, les surfaces à coloniser et le produit lui-même.

Comprendre et contrôler les phénomènes initiaux de bio-adhésion restent encore une prérogative des communautés de Recherche et Développement internes et externes. Les solutions envisagées à ce niveau sont souvent classés comme ingénierie hygiénique. Les propriétés de surface des bactéries sont bien connues. L'influence des polysaccharides, des acides téchoïques et des autres structures extérieures ont été étudiées de manière intensive. Néanmoins, les dernières études montrent clairement des variations significatives au niveau des espèces, voire des souches. Ceci reste à être précisé. Quant aux propriétés de surface des matériaux, leur connaissance s'améliore de jour en jour. A côté de l'influence des charges électrostatiques et de la rugosité, leur résistance aux procédures de nettoyage et désinfection dans le temps ainsi que leur capacité à re-larguer des matières nutritives semblent clés dans la formation des biofilms. Pour diminuer au mieux l'impact des matériaux, 2 types de recherche sont en cours et montrent des résultats intéressants : le traitement de surface (polissage électrolytique, plasma froid, ...) et l'ajout de substances biocides. A l'inverse de ces 2 facteurs (propriétés de surface des bactéries et des surfaces à coloniser) l'impact du produit lui-même est moins bien connu et compris.

Pour les industries agro-alimentaires, les activités d'amélioration des procédures de nettoyage et de désinfection peuvent être nombreuses. Que ce soit au niveau globale d'une compagnie ou usine par usine, des améliorations sont facilement envisageables, en particulier au niveau du nettoyage, le «parent» pauvre de notre procédures de N&D. L'objectif principal reste de trouver quel niveau de bio-adhésion et de biofilm il est possible de tolérer entre 2 séquences de N&D. En utilisant des notions simples comme le cercle de Sinner, il est possible de remettre en cause ou d'optimiser le choix et la concentration des produits chimiques, le temps de contact et la température d'utilisation de ces produits chimiques ainsi que les actions mécaniques. En effet, il n'est souvent pas nécessaire de supprimer complètement le biofilm de nos systèmes industriels, il suffit juste de le maintenir à un niveau acceptable. Finalement, les procédures de N&D se résument souvent au choix des produits chimiques. En intégrant les notions de bio-adhésion et biofilm dans leurs réflexions, les responsables AQ vont souvent trouver des solutions alternatives.

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