NEW TOOLS FOR MANAGEMENT OF BEVERAGE FILLING HYGIENE

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Laboratory and process studies indicate that novel functional coatings in combination with physical disinfection improve process hygiene significantly.

Bacteria in aquatic environments are predominantly not in a free floating planktonic stage, but have a tendency to attach to surfaces, and form multi-species communities called biofilms. Aggregated in a biofilm, microorganisms have several advantages: they have a higher resistance to biocides and cleaning agents, nutrients are easier to access, and the creation of micro-niches (like anaerobic sites within an aerobic environment) facilitates the growth of organisms which normally could not grow outside the biofilm. The formation of a biofilm is a complex process since attachment mechanisms vary between different bacterial species and surface properties, also, environmental factors have an influence on the attachment.

Although some general concepts can be applied to the attachment of cells and formation of biofilms, much species-specific behaviour exists. The primary attachment may be aided by structures extending from the cell surface, like fimbriae, flagella and pili. Surface type may have an effect on the use of pili; it has, for instance, been shown that *Vibrio cholerae* uses different types of pili when attaching to biotic or abiotic surfaces. Also outer-membrane proteins may play a role in attachment. Cell surface hydrophobicity is considered known to predict bacterial adhesion to some extent. Generally, hydrophobic cells are more adherent than hydrophilic ones, and most bacteria preferentially adhere to hydrophobic surfaces, but hydrophobicity and adherence do not always correlate. In addition, the electric charge of the surface and cell has an influence on the attachment. Most bacteria and surfaces are negatively charged, but there are exceptions, like *Actinomyces naeslundii*, whose rod-shaped cells are bipolar.

It is assumed that most microbes first become irreversibly attached to a surface. If the environment and possible signalling molecules favour surface-attached growth, then the cells attach irreversibly by means of specific molecular mechanisms. The attachment is called irreversible when cells require mechanical force to be removed. After attachment, microbes start to produce extracellular polymeric substances (EPS), often referred to as slime. With these substances they anchor to each other and to the surface.

Open food and beverage contact surfaces do not normally provide a solid-liquid interface for microbial attachment, and true biofilms do therefore not form very often. Nevertheless, surfaces become soiled by drifting organic matter and microbes. Bottling or canning is the most microbiologically critical phase in the beverage industry, because the product is open for microbes coming from surfaces, air and the environment. The broad spectrum of products packaged means that there are lots of nutrients available and increasing product range has in itself been shown to increase microbiological risks in filling. Cleaning is costly in the beverage industry in terms of both production downtime and consumption of energy, water, chemicals and working hours, which is why long runs between cleaning and short cleaning programmes are economically favourable.

COATINGS REDUCING MICROBIAL ATTACHMENT

Process surface hygiene has traditionally been managed by controlling the roughness of stainless steel. Since microbial attachment is an unwanted process in many applications, material scientists have for some time worked with the aim of developing non-stick coatings, to which microbes would attach less easily than to non-coated surfaces. Photocatalytic titanium dioxide (TiO_2) coatings have been shown to be able to reduce microbial attachment. TiO_2-coatings become hydrophilic after exposure to short wave UV-irradiation (UVA), after which the sheeting water carries the dirt away. Photocatalytic reactions also kill cells directly, so, these coatings have a dual mechanism. The precise action of photocatalytic coatings on microbes is still not completely clear. Most attachment studies have been made with a single bacterial species, which does not reflect actual process conditions. The reductions in microbial numbers have varied from rather insignificant (10%) to very significant (90-99%). Development of coating properties is an evolving field, and one way to improve the protection properties of coatings against microbial attachment may be doping with nitrogen to increase the visible light activity of the coatings.

Many metals, like copper and silver, have antimicrobial or antibacterial properties. Even though these metals are not typical components of stainless steel, different antimicrobial steels have been developed based on controlled dissolution and interaction of these metals with bacteria. To obtain bactericidal activity under weak UV-light, TiO_2 films with very small concentrations (e.g. 0.02 µg cm^{-2}) of antibacterial metals like copper or silver have also been developed. The advantage of silver is that it forms an even precipitation layer on steel, does not weaken its corrosion tolerance, and is harmless to humans. On the other hand, silver ions in waste waters may disturb the action of biological waste water treatment plants.

A third promising group of coatings to reduce microbial attachment or facilitate their removal are low energy hydrophobic coatings. Because these are hydrophobic, water forms almost spherical droplets on them, carrying the dirt away as seen in nature on lotus plants. These coatings work best in an inclined position, which is why they could be applicable to splash areas, where inclined surfaces are used. Like with photocatalytic coatings, the action of low energy coatings is not yet fully understood and varying results of their actions against bacterial attachment have been reported.

PRACTICAL EXPERIENCES WITH FUNCTIONAL COATINGS

The action of functional coatings has been studied in laboratory and process studies in recent projects coordinated by the Finnish malting and brewing industry (Figure 1). The antimicrobial function of the photocatalytic coatings varied. It seems that TiO_2 coatings may be more active against bacteria than against eukaryotic yeasts and moulds. In laboratory studies, TiO_2 coatings, where antibacterial silver had been added (TiO_2+Ag), were shown to significantly decrease attached microbial numbers, but the same effect was not seen with TiO_2 coatings without added silver. In actual brewery

Figure 1. Sample coupons on filling machines.

packaging process studies, TiO_2 and TiO_2+Ag coatings caused 1-4 log reductions in attached microbial numbers in some places after three to six months of installing the coatings. The results were, however, not statistically significant, and in most places no effect of the coatings on attached microbes could be seen. There are many experimental variables on filling machines affecting results, like cleaning procedures and changes in the product range. In addition, as the number of samples analysed is low for practical reasons, statistically significant differences are hard to observe. Material characterisations at the end of the study showed that TiO_2-coatings endured process conditions in dry places, but, in wet places, damaged areas were formed on the coatings. Silver had mostly dissolved from the coatings during one year under production conditions, meaning that a more long-lasting way to incorporate silver to the coatings should be found.

Also, various hydrophobic low energy coatings have been studied in these projects. Low energy coatings were shown in laboratory studies to significantly reduce the area coverage of microbes, but no effect on live microbial numbers was seen in process studies. In addition to decreasing microbial attachment (non-stick), coatings may also improve process hygiene by being easy-to-clean. In laboratory cleaning studies, low energy coatings were slightly easier to clean than stainless steel. However, in the studies, low energy coatings did not endure process conditions, as parts of them were found to have peeled off during one-year process studies. Furthermore, they had changed from hydrophobic to hydrophilic. In any case, the novel coatings will probably require regular regeneration – it just has to be economically feasible.

There may be several reasons for the varying functionality of the coatings. The amount of UVA light is critical for the efficacy of photocatalytic coatings, and is a limiting factor under process conditions. In laboratory studies, different UVA exposures were used, but, so far, under process conditions, it has not been possible to control the amount of light. Also, the chemical form of TiO_2 influences the action of coatings, and material development may improve the activity of coatings. Inclined installation enabling water and dirt to roll off the surface is central for the action of low energy coatings. Possibly the 10° inclination used under process studies was not sufficient for the optimal functioning of the coatings. It was also noted that amorphous silica precipitates may form on the surfaces under process conditions, and these probably also affect the functionality of the new materials. Laboratory cleaning tests for artificial and process-formed precipitates showed that different detergents vary in effect towards different types of dirt, which favours the use of alternating detergents in cleaning.

PHYSICAL DISINFECTION DURING PRODUCTION

Coatings that reduce microbial attachment may help to control the hygiene during process without downtime needed for cleaning. Another possible means to control microbial numbers during production is physical disinfection. The germicidal action of UVC-irradiation has been known for long, and UVC disinfection is utilised e.g. in disinfection of drinking water. UVC has also been used successfully in combination with air rinsing for disinfection of empty cans prior to filling.

UVC inactivates microorganisms through breaking of chemical bonds in their DNA chains. In laboratory studies, UVC irradiation killed even 99.999 per cent of the microbes on surfaces, when the dose was high enough. Wet surfaces needed higher doses than dry surfaces. Biofilm, cell aggregates and dirt decrease the penetration of UVC, so it is most suitable for prevention of microbial attachment and biofilm formation, and not for removing already formed biofilms.

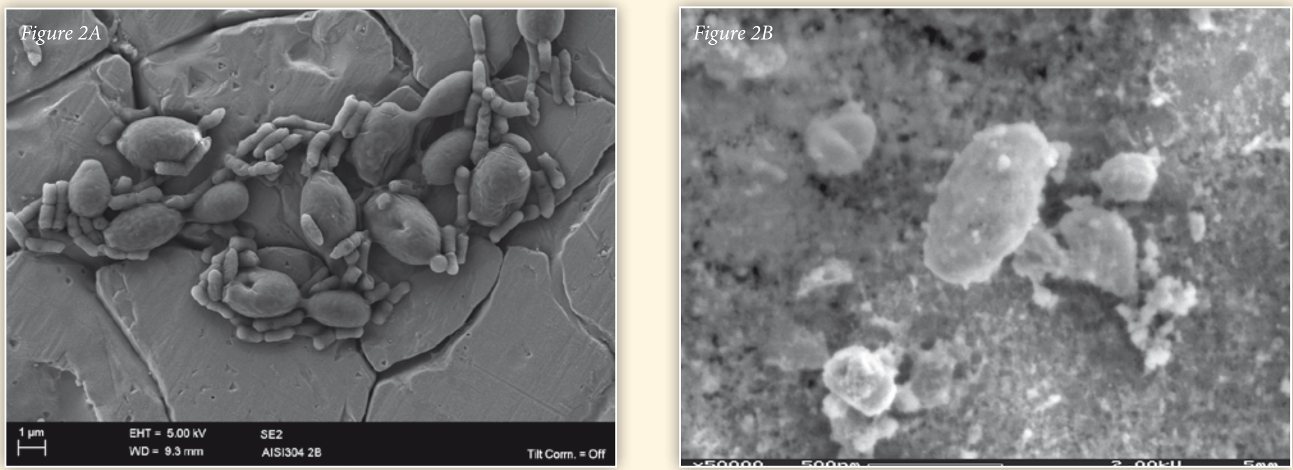

Figure 2. Microbes on stainless steel surface A) in the laboratory and B) on a sample coupon from a one-year process study.

The conditions of the air in processing areas are important to brewery hygiene. Aerosols formed during cleaning and microbes removed from surfaces by air currents are present in the air, and act as contaminants after settling down onto surfaces again. Air ionisation has been shown to have potential for killing microbes in the air, but the phenomenon is not totally understood, and results are partly contradictory. The effect of air ions varies with different microbial species, and several other environmental factors like aerosol size may also affect the action of ions. Low concentrations of ions are naturally present in the air, but effective numbers can be produced by ionisators. In laboratory studies, approximately 10,000 negative ions per cm^3 and $>10^6$ positive ions per cm^3 were produced in the air. This so-called active oxygen worked best against Gram-negative bacteria, but did not affect Gram-positive bacteria and yeasts. Ionisation may also produce ozone, which is why safety issues have to be considered when installing ionisators.

FUTURE

The novel means described above have potential for improvement of brewery hygiene, but at least part of them need more development in order to really function under process conditions. The activity and durability of functional coatings need to be improved. The action of UVC irradiation and air ionisation was confirmed in laboratory studies, but it would be useful to have information of their effectivity under process conditions. It is worthwhile to perform both controlled laboratory studies and process studies, because conditions in these may be quite different. In most natural and industrial environments, multispecies and not single species microbial communities are found. Starvation and production of protective extracellular polymeric substances (slime) are common among these communities, leading to a stronger stress response and increased resistance against chemical and physical disinfection (Figure 2). Therefore, results obtained under controlled laboratory conditions can never be directly ➜

applied to industrial environments. Laboratory experiments, on the other hand, are essential to obtain comparable under standardised conditions.

SUMMARY

Hygiene and cleaning are of paramount importance to the beverage industry, but they also cause considerable costs in terms of production interruptions and consumption of energy, water, chemicals and working hours. At the same time, environmental demands call for reductions in water consumption and the use of more environmentally friendly chemicals. With the help of novel functional coatings, the attachment of microbes on process surfaces can possibly be reduced, or their removal facilitated. Another means to improve process hygiene could be physical disinfection. VTT Technical Research Centre of Finland and Tampere University of Technology have in close co-operation with the brewing industry and providers of coating and disinfection technology applied new coating techniques to process surfaces, as well as UVC disinfection and air ions as physical disinfection methods during production. In addition, the aim has been to adapt cleaning chemicals to function along with new surfaces and disinfection methods. Different photocatalytic TiO_2 coatings and hydrophobic low energy coatings have been studied in laboratory and process studies. Microbiological results from photocatalytic coatings have been variable, and it seems that these may be more active against bacteria as compared to eukaryotic yeasts and moulds. Coatings supplemented with Ag ions reduced numbers of attached microbes significantly in laboratory studies, and this effect was also seen in some process tests. Low energy coatings reduced microbial coverage significantly in laboratory studies, but similar effects on attached microbial numbers were not seen in process studies. In laboratory cleaning studies, low energy coatings were cleaned slightly more efficiently than stainless steel. UVC killed 99.999 per cent of microbes on the surfaces when the dose was high enough in laboratory experiments. Air ions worked most efficiently on Gram-negative microbes. These novel means have potential for facilitating improvements in the management of brewery hygiene, but at least functional coatings need more development in order to make them withstand process conditions and be really functional there.

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REFERENCES

The authors of this article have a complete list of references for the background statements and results referred to in this article which can be obtained by contacting the authors.