Soil Films in the Beverage Industry: A Review

T. A. Mamvura[1,*], S. E. Iyuke[1], J. D. Cluett[2] and A. E. Paterson[3]

ABSTRACT

J. Inst. Brew. 117(4), 608–616, 2011

This article provides an overview of the formation of soil films, their composition, effects and control measures that can be applied in the beverage industry, focusing on the negative aspects of their formation. The positive aspects utilised in bioreactors and fermentation reactions will not be reviewed here. Soils gain access to equipment surfaces and these form soil films that are an assembly of microbial cells, organic and inorganic foulants, irreversibly attached to a surface, and enclosed in an extracellular polymeric substance (EPS) matrix. The EPS is vital in the structure and functioning of different soil film communities. Soil films are responsible for the deterioration of water and corrosion in water distribution systems, food contamination, reduced product quality in the beverage industry, infection on medical surfaces, plaque build up on teeth, increase in fuel costs on ships, decrease in heat transfer in heat exchangers and deterioration of metal due to microbial activity during microbiologically influenced corrosion (MIC).

Key words: beverage industry, extracellular polymeric substance, soil film.

INTRODUCTION

In the brewing industry soil films are a real, practical threat to hygiene and product quality, particularly in the demanding world of draught beer[35]. Residual matter derived from brewing materials, processes and the environment develop into soil films on the surfaces of equipment and these become a threat throughout the brewing process[9]. It is estimated that soil film formation costs approximately €250 million annually to the German brewing industry[51]. Soil films are a well-organised and cooperating community of microorganisms attached together with organic and inorganic substances to a surface by the help of extracellular polymeric substances (EPS), which are produced by the microorganisms to form a single layer or three-dimensional structure[13,28,34]. Soil films have been encountered in the medical industry (e.g. dental plaques on teeth, films on contact lenses, catheters, endotracheal tubes, prosthetic joints and mechanical cardiac valves), in the marine environment (e.g. fouling of ship hulls, ship and marine platforms, offshore rigs) and in the chemical process industry (fouling of membranes, heat exchangers, process vessels, pipes and product dispensing lines), resulting in poor hygiene and reduced product quality or sometimes food contamination[5,10,13,16,20,29,34,55].

Materials often used in the food and beverage industry include plastics, rubber, glass, cement and stainless steel with the capacity to support soil film growth increasing from glass, stainless steel, polypropylene, chlorinated PVC, unplasticized PVC, mild steel, polyethylene, ethylene-propylene to latex[49]. Stainless steels, in particular austenitic grades 304 and 316, are the most commonly used food contact surfaces. This is because of their chemical and mechanical or physical stability at various food-processing temperatures, cleanability and high resistance to corrosion, with 316 having a higher resistance to corrosion by foods, detergents and disinfectants[49] due to its anticorrosive properties from the added molybdenum as compared to 304.

Electron microscopy work has shown that soil films can attach onto all types of surfaces e.g. plastic, soil particles, wood, medical implant materials, tissue, food products, stainless steel, aluminium, glass, Buna-N and teflon seals and nylon materials and that this attachment is facilitated by fimbriae, pilli, flagella and that EPS acts to form a bridge between microorganisms and the conditioning film. Teflon, glass and nylon surfaces are smooth surfaces and the microorganisms appear to be attached, while stainless steel surfaces have a rough appearance due to cracks and crevices that can be sufficient to trap microorganisms[28,29,48]. Such a microstructure allows the escape of entrapped microorganisms from the shear forces of the bulk liquid and even the mechanical methods of cleaning may be inadequate to remove the trapped microorganisms[29].

SOIL FILMS

Soil films in the brewing industry

All of the major raw materials used for brewing are potential sources of unwanted microorganisms, some of which could possibly be pathogenic. In addition, brewing aids such as finings, primings and filtration media, and containers (casks, bottles, etc.) can contribute contaminants[23]. Figure 1 shows the brewing process and potential sources of microbial contamination.

Both wort and beer are prone to spoilage organisms, the former especially so since it provides a nutrient-rich, oxygenated environment required for microbial growth[23], while beer has less nutrients, a low pH (3.8–4.7), contains alcohol from 3–7% by volume, which makes it less susceptible to bacterial growth[23,33,37,43].

[1] School of Chemical and Metallurgical Engineering, Faculty of Engineering and the Built Environment, University of the Witwatersrand, Wits 2050, Johannesburg, South Africa.
[2] JDC Consulting, PO Box 1457, Lonehill 2062, South Africa.
[3] Independent consultant – Jacklin Paterson Consulting cc.
[*] Corresponding author. E-mail: atmamvura@yahoo.com

Publication no. G-2012-0120-1164
© 2011 The Institute of Brewing & Distilling

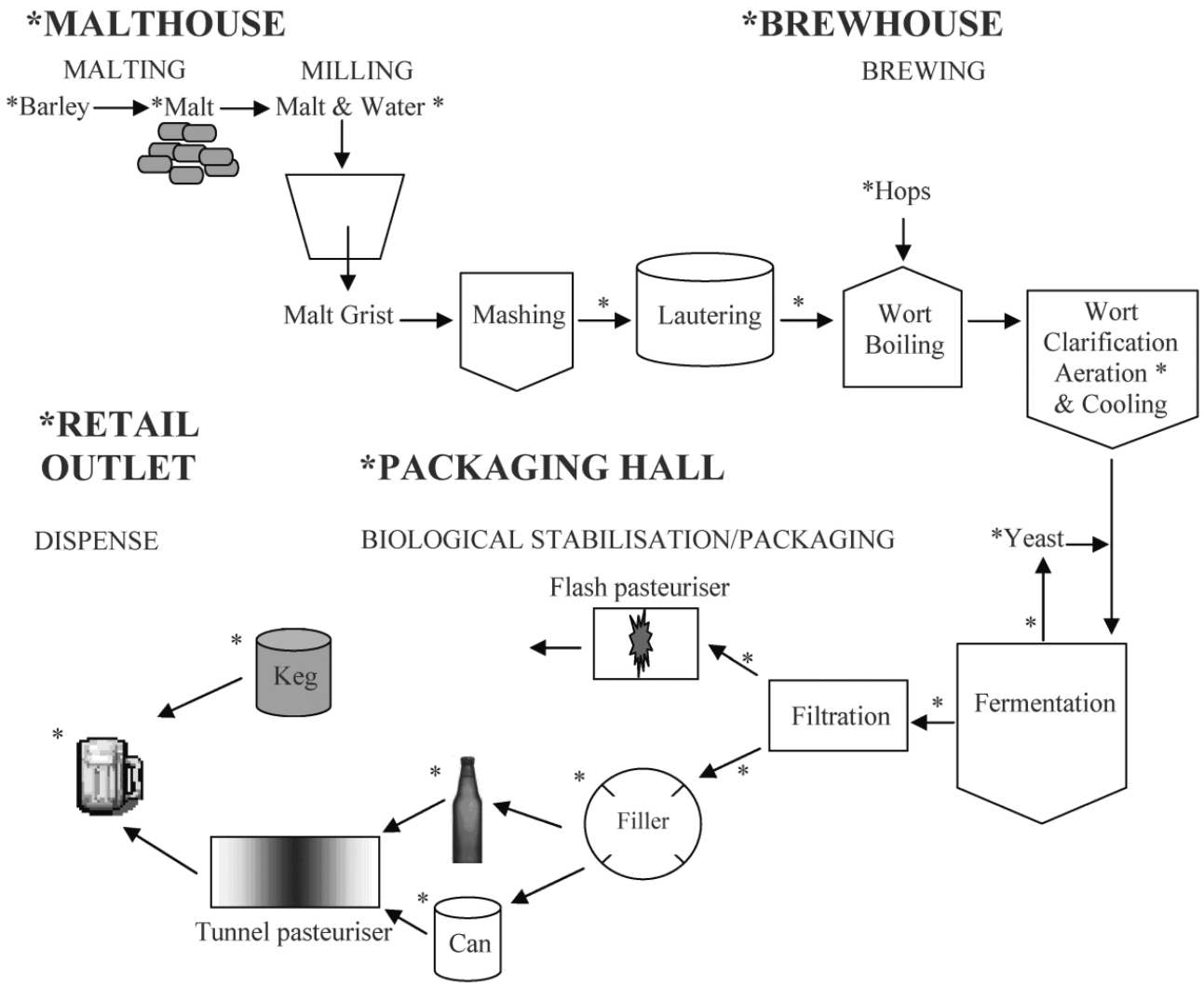

Fig. 1. Basic scheme representation of the brewing process from Vaughan et al.[51] Potential sources of microbiological contamination are indicated by * (figure reproduced with permission from the JIB).

Primary contaminants may originate from the environment, raw materials (e.g. malt, hops and adjuncts which can carry their own microbial contaminants), fermentation vessels and piping systems and brew house; and these may have a hand in soil film formation, while secondary contaminants are introduced during bottling, canning or kegging[51]. Water as a brewing raw material is boiled during wort boiling, but later additions should be sterile to prevent contamination[51]. Microbes emanating from brewing liquor, malt and hops, and those found in wort, will not survive the boiling stage, and together with the sterilisation of potable water to be used, means that there are relatively few species of bacteria and fungi that cause spoilage problems; the list is restricted to a few wild yeasts and certain Gram-positive and Gram-negative bacteria[23].

Equipment used in the beer filling process is particularly prone to soil film formation due to large volumes of water used for bottle rinsing, thereby creating an environment suitable for microbial attachment and accumulation on surfaces[35,51]. Table I shows the beer spoilage microorganisms and the stage of the brewing process at which they can occur.

Many bacterial strains have been shown to retain their viability for extended periods in beer, but as long as they are not able to grow in the product, they are not harmful and are therefore not considered spoilage organisms[30]. With improvements in technology being introduced in modern breweries, the importance of various specific spoilage organisms has changed, with well-known spoilage organisms, such as the aerobic acetic acid bacteria (*Gluconobacter oxydans* and *Acetobacter* spp.), no longer appearing to be as great a problem[25]. Improvements in beer handling and bottling technology have resulted in a significant reduction in the oxygen content during the process and in packaged beer, thereby permitting the growth of strictly anaerobic microorganisms such as *Pectinatus* spp. and *Megasphaera cerevisiae,* which have caused increased spoilage in packaged beer within the past few decades[26,37].

Pectinatus and *Megasphaera* both have three genera. *P. haikarae,* together with the two genera mentioned in Table

Table I. Beer spoilage microorganisms and the stage at which they occur from Vaughan et al. [51] (table reproduced with permission from the JIB).

Stage	Spoilage microorganisms	
Barley in the field/malting	*Aspergillus*	*A. fumigatus*
	Fusarium	*F. culmorum; F. graminearum*
Mashing and wort separation	*Pediococcus*	*Ped. pentosaceus; Ped. inopinatus*
	Bacillus	*B. coagulans*
	Rahnella	*R. aquatilis*
	Citrobacter	*C. freundii*
	Klebsiella	*K. terrigena; K. oxytoca*
Fermentation	Wild yeasts	non-*Saccharomyces*; *Saccharomyces* spp.
	Pediococcus	*Ped. inopinatus*
	Selenomonas	*S. lacticifex*
	Zymophilus	*Z. raffinosivorans*
	Rahnella	*R. aquatilis*
	Obesumbacterium	*O. proteus*
Biological stabilisation and packaging	*Pectinatus*	*P. cerevisiiphilus; P. frisingensis*
	Megasphaera	*M. cerevisiae*
	LAB[a]	*Lactobacillus* spp.; *Pediococcus* spp.
Contaminants of finished beer	LAB	*L. brevis; L, backi; L. brevisimilis; L. casei; L. paracollinoides; L. coryniformis; L. curvatus; L. lindneri; L. plantarum; L. buchneri*
	Pediococcus	*Ped. damnosus; Ped. inopinatus*
	Pectinatus	*P. cerevisiiphilus; P. frisingensis*
	Megasphaera	*M. cerevisiae*
	Zymomonas	*Z. mobilis*
	Micrococcus	*K. kristinae*
Dispense	Acetic acid bacteria	*Acetobacter aceti; Acetobacter pasteurianus; Gluconobacter oxydans*
	LAB	
	Wild yeasts	

[a] Lactic acid bacteria.

I (*P. cerevisiiphilus; P. frisingensis*), are strains of *Pectinatus,* while *Megasphaera* has *M. paucivorans* and *M. sueciensis* together with *M. cerevisiae* mentioned above and in Table I[37,43]. *P. haikarae, M. paucivorans* and *M. sueciensis* have only recently been proposed as new species[43]. From Table I, these species were identified in packaging right up to the finished product. Beers spoiled by *Pectinatus* exhibit heavy sediments, hazes, small clots, and an extremely unpleasant taste and odour due to the hydrogen sulphide produced[25]. *Megasphaera* forms only slight hazes in beer and almost unnoticeable sediments, but causes severe off-flavours with unpleasant smelling compounds, including butyric acid, caproic acid and hydrogen sulphide being formed, such that the beer is undrinkable[26]. *Megasphaera* is a low ethanol tolerant genus and thus it mainly affects no- and low-alcohol beers[26].

L. paracollinoides and *L. backi* are strains of lactic acid bacteria (LAB) and have recently been proposed as new species[3,24,33] and their frequency in beer spoilage incidents is not well known. The genetic characterization indicates that *L. paracollinoides* and *L. backi* are closely related to *L. collinoides* and *L. coryniformis*, respectively and some of the strains belonging to *L. paracollinoides* and *L. backi* might have been misidentified as *L. collinoides* and *L. coryniformis* respectively in the past[3,43,44].

Ped. claussenii, a strain of *Pediococcus*, has also been reported as a new species and some of its strains produce exopolysaccharides[14,43]. All the strains of *L. paracollinoides*, *L. backi* and *Ped. claussenii* characterized to date have been isolated from beer brewing environments, making them unique LAB species to the brewing industry[43].

The genus *Micrococcus* includes one species, *Kocuria kristinae* (previously known as *M. kristinae*) relevant to breweries. It is very sensitive to the concentration of ethanol and hop bitters in beer and is capable of anaerobic growth and growth above pH 4.5[25] and this is why it has been reported in the finished product (Table I).

Table II. Specific spoilage bacteria capable of growth in beer, modified from Jespersen and Jakobsen[25] (and reproduced with permission from Elsevier)

Gram-positive	Gram-negative
Lactobacillus spp.	*Pectinatus* spp.
Pediococcus spp.	*Megasphaera* spp.
Micrococcus spp.	*Zymomonas* spp.
	Selenomonas spp.
	Zymophilus spp.

Pediococcus species (especially *Ped. inopinatus*) are observed from the mashing and wort separation processes, right up to the fermentation stage and packaging stage, showing that the species may have survived the processing stages and recovered, and these may result in soil film formation when the conditions are favourable. This is because *Pediococcus* species can tolerate high temperatures and changes in pH[14]. Most wild yeasts from the fermentation stage can survive up to the dispensing section. Because of the difficulty in discriminating them from brewing yeasts[37], these can also result in soil film formation especially in dispensing lines. The beer spoilage organisms in Table I can be further grouped as either Gram-positive or Gram-negative and this is shown in Table II.

Formation of soil films

In order to have a thorough understanding of the dynamics and effects of soil films, and to be able to design appropriate preventative or control measures, it is of significance to understand the natural processes of soil film formation[21]. This formation can be grouped into four key stages: (i) initial attachment of organic and inorganic foulants, and charged ions resulting in a conditioned surface

to neutralize surface charge that is likely to repel approaching microorganisms; (ii) attachment of microbial cells to the surface: this involves reversible attachment of microorganisms caused by weak interaction forces (van der Waals attraction, electrostatic, hydrophobic) between microbial cells and the surface followed by irreversible attachment of microorganisms caused by permanent bonding (dipole-dipole interaction, hydrophobic, ion-dipole, hydrogen, ionic bonding, covalent bonding and hydrophobic interaction) to the surface aided by the production of EPS; (iii) entrapment of inorganic and organic debris and nutrients in the system creating soil films; and (iv) detachment of soil films due to fluid shear stresses and aging of microbial cells[5,6,8,21,29,34,50,55]. The irreversible attachment can take from 20 minutes to a maximum of 4 hours at 4–20°C[8].

The conditioning process alters the physico-chemical properties of the surface including electrostatic charges, surface free energy and surface hydrophobicity[21,29,34]. Maximum attachment of soil films depends upon high surface free energy and electrophoretic mobility or wettability of surfaces. Surfaces with high surface free energies such as stainless steel and glass are more hydrophilic, generally showing greater bacterial attachment than hydrophobic surfaces such as teflon, Buna-N rubber and fluorinated hydrocarbon[6,8,28]. The presence of multivalent cations (Mn^{2+}, Ca^{2+}, Mg^{2+}, Na^{+} and Fe^{3+}) has been shown to influence the attachment process, possibly by altering the surface characteristics or by bridging cellular anionic polyelectrolytes to anionic polyelectrolytes adsorbed on the wetted surface[6,17]. Moreover, microorganisms tend to attach uniformly in a monolayer to hydrophilic surfaces (such as glass), while on hydrophobic surfaces (such as nylon and tin) they tend to adhere in clumps[8].

Soil films have also been examined under various hydrodynamic conditions such as laminar and turbulent flow. Films formed under laminar flow are found to be patchy with round cells, consisting of rough cell aggregates separated by interstitial voids, while those formed under turbulent flow are patchy, with elongated structures with streamers that oscillate in the bulk fluid[8,11,12]. Furthermore, soil films formed in laminar flow have a low tensile strength and easily break, but soil films formed in turbulent flow are remarkably strong and resistant to mechanical breakage[18].

Another interesting point to note is that soil films formed from single species in vitro and those produced in nature by mixed species consortia have been shown to exhibit similar overall structural features with some minor differences[12,18], with soil films from mixed species being thicker, more compact and stable to environmental stress[8,17]. This may be due to the production of a variety of EPS materials that result from the activity of different microorganisms[29].

Composition of soil films

Soil films are composed primarily of microcolonies of different species of microbial cells (+15% v/v) and of matrix material (+85% v/v)[11,18,28]. Researchers have been able to show that the EPS is mainly made up of polysaccharides (~24.5%) and proteins (~82.8%), polysaccharides existing in nature either as neutral or as polyanionic, as in the case for the EPS of Gram-negative bacteria[6,17,20,28,34,45]. The EPS also contains teichoic acid (secondary cell wall polymers), nucleic acids, phospholipids and other polymeric substances[7,8]. Intermolecular interactions between the various functional groups within the EPS macromolecules serve to strengthen the overall mechanical stability of the EPS and, hence the survival of the enclosed microorganisms[7].

The composition of soil films differ depending on the environment they are formed in, but the difference is usually small. The structure of soil films, particularly the EPS, need to be understood as the mechanical stability of the EPS matrix has to be overcome in the cleaning process to reduce and/or control soil films[20]. The structure of the soil film may be composed of the following:

- Scaling or particle foulants: these may be deposits of inorganic precipitates of magnesium, calcium carbonate, iron oxides, silica, clay, mineral crystals, corrosion particles and other particles[6,17,20,28,29].
- Organic foulants: these may be deposits of organic substances e.g. oil, proteins, lipids, humic substances, EPS[17,20,28,29,54].
- Biologically active foulants: these can be yeasts, moulds, viruses, fungi, spores, Gram-positive and Gram-negative bacteria that can adhere to surfaces and facilitate soil film development[2,13,16,20,55]. The microorganisms observed are almost in every physiological state known, from aerobic to anaerobic and from exponential to stationary phases of growth[11] and they have a net negative surface charge usually behaving as hydrophobic particles[8].

Scaling and organic foulants usually originate from water and sometimes from other raw materials (e.g. proteins from the mashing process in the brewery industry; calcium and magnesium from brewing water) and fouling can be controlled by eliminating these foulants from the source. However, microorganisms are pseudo-particles, which can multiply, and pretreatment to be done has to remove all the microorganisms, otherwise they will reattach, grow and actively multiply to form a colony of cells entrapping inorganic and organic debris, nutrients and other microorganisms, with their EPS resulting in soil film formation again[20,29]. The EPS has several functions. This includes facilitation of initial attachment of bacteria to a surface, formation and maintenance of microcolony and soil film structure, enhanced soil film resistance to environmental stress and antimicrobial agents, and enabling the bacteria to capture nutrients[34]. Most of the microorganisms in soil films are aerobic, but anaerobic microorganisms are also present[34].

One researcher showed that soil films studied were mainly composed of water (85.6%), followed by inorganic foulants, mainly cations (11.7%) to stabilise the EPS and the film, and the rest was the organic fraction (2.7%)[6]. The composition of the inorganic fraction showed that manganese ions had the highest ratio (59.5%), followed by iron ions (18.5%), and aluminium ions (7.5%)[6].

Biologically active foulants

Soil films consist of a complex consortia of microorganisms that provide niche environments for additional microbial communities[51]. Bacteria are the best-studied

microorganisms with respect to colonization of surfaces and subsequent soil film formation, although fungi, yeasts, algae, protozoa and viruses have all been isolated from soil films in industrial and medical settings[31]. The cell surfaces of most microorganisms are anionic and this is normally due to the presence of negatively charged phosphate, carboxylate or sulphate groups in the cell wall or capsular or polysaccharide macromolecules[56]. Most Gram-negative bacteria have long polysaccharide regions of their lipopolysaccharide and proteins exposed, resulting in a hydrophilic surface, while Gram-positive bacteria have the lipid portion of lipoteichoic acid extending outward from the cell, resulting in a hydrophobic surface[48]. Gram-negative bacteria produce neutral or negatively charged soil films, whereas Gram-positive organisms develop positively charged cationic matrices[35].

In vitro experiments have demonstrated that primary colonisers enable secondary bacteria to become part of the soil films and in brewery soil films, slime-producing *Acetobacteraceae* are considered as the primary colonisers, providing favourable conditions for the aerotolerant, anaerobic and acidophilic *Lactobacillaceae* and the anoxic environment indispensable for *Pectinatus* and *Megasphaera*[1].

It is common practice to differentiate brewery associated yeasts into *Saccharomyces* and non-*Saccharomyces* yeasts; and cultivation of independent analysis of brewery soil films from bottling plants and adjacent areas has shown that yeasts are the dominating organisms of most soil films[47]. Soil films formed by *Pseudomonas*, *Enterobacteriaceae*, *Lactobacillus*, *Acetobacter* and *Saccharomyces* have been frequently isolated and are well known in breweries[55].

In another study conducted, most of the *Candida pelliculosa* strains showed soil film formation on a microplate assay, but no *Saccharomyces cerevisiae* isolates were observed. Therefore, it was assumed that the *C. pelliculosa* species were involved in attachment and primary soil film formation in beer bottling plants, while *S. cerevisiae* was a late coloniser of a preformed soil film, and increased the beer spoiling potential of the soil film[47]. Yeasts unable to multiply in beer can also cause problems, as they can attach to surfaces and become pioneer organisms for the development of soil films. The species *Candida albicans* is a well-studied soil film-forming yeast[46].

Also, other researchers have concluded that the primary colonisers could be non-beer spoiling Gram-negative bacteria (e.g. *Pseudomonas* species) and the secondary colonisers could be wild yeasts, particularly *Pichia anomala*, *Candida sake* and *Debaryomyces hansenii*[35,47]. Identity of wild yeasts recovered from soil films, in and around the bottle filler of one brewery, showed that most were wild non-brewing strains of *S. cerevisiae* and *Pichia anomala*. In terms of attachment to surfaces, *P. anomala* can form soil films, whereas the *S. cerevisiae* isolates are non-soil film forming[35].

Acetic acid bacteria and enterobacteria can reproduce in areas where residues of process intermediates, beer or other products collect. Although these bacteria may not be harmful by themselves, they protect accompanying microorganisms from dehydration and disinfectants by forming slime (EPS)[51]. If product residues are undisturbed for longer periods of time, yeasts begin to grow along with the acetic acid bacteria, producing metabolites that encourage lactic acid bacteria (LAB) to grow[51]. The LAB produces lactic acid that can be metabolised to propionic acid by anaerobic species such as *Pectinatus* species and the cycle can result in a mixed species soil film formation at the site[1].

Outside of packaging, barley-associated soil films have attracted attention as they have an important role in poor wort and in beer filtration after using contaminated grain[35]. Beer that is 'commercially sterile' on leaving a brewery and which is then dispensed at draught beer outlets can contain low levels (~10^3/mL) of planktonic or free cells (lactic acid and acetic acid bacteria and diverse yeasts) and this is a reflection of the soil film within the dispense tubing, which slowly colonises lines from entry at the dispensing tap or the container end[35,46].

Structure of soil films

The presence of high levels of nutrients, macroscopic and microscopic deposits of food residues, and frequent stress from cleaning, sanitizing or processing treatments in the beverage industry influences the soil film structure[8]. In the past, soil films were thought to be a compact structure, but recently studies have shown that they have a porous structure with capillary water channels to distribute water and nutrients and removal of secreted waste[10]. The structure and architecture of the soil film is dependent on the flow rate of the bulk fluid, the profile of the surface, the presence of poor and unhygienic welds and the number of different species involved[34].

The architecture of the soil film is species-specific for single cultures and substrate-specific for multi-cultures. In heterogenic soil films, the architecture is often irregular, probably due to the different growth and adherence patterns of the microorganisms[34]. The thickness of soil films is dependent on the flow rate, with maximum thickness obtained between laminar and turbulent flows[34]. Furthermore, the thickness in the laminar zone is dependent on the substrate accessibility and on erosion in the turbulent zone[34].

Characterisation of soil films

Soil film development and structure can be analysed using various methods. This enumeration process helps to confirm the source and extent of contamination and the type(s) of microbial cells involved as contaminating agents[29]. The methods available can be divided into two broad categories, cultivation and microscopy.

Cultivation methods

These are conventional methods involving resuspending and redispersing soil film cells, plating them onto a solid medium, incubating and counting[28,53]. The different methods used for sampling soil films on external and internal surfaces are swabbing, rinsing, agar flooding, agar contact methods and sometimes scraping, vortexing or sonication[18,29]. These are quantitative methods that have been used frequently for years and they are generally inexpensive and easy to use, but they require appropriate media and culturing methods to be devised. Vortexing and sonication have the advantage of sampling internal and

external soil film cells, but the recovery efficiency is unknown[18]. However, these techniques can be abrasive to attached cells and may result in injury, which could, in turn, result in viable but non-culturable cells[31]. Thus, most of these techniques normally incorporate a resuscitation step of several minutes to allow for cell recovery[31].

Swab plating. In this method, moistened swabs or sponges are used to remove microbial cells from the surfaces. The sample liquid is then plated onto an agar plate or a selective medium, incubated, and then colonies are enumerated and identified if desired. The advantage of this method is that with selective media, specific bacteria, yeast, and moulds can be isolated and identified, but it is time consuming and also microorganisms may be selectively removed from the surface[8].

Contact plating. Contact plating directly samples a surface by pressing a plate of solidified agar against the surface. This method is simpler than swabbing, but it is not possible to sample irregular or rough surfaces. The limitations of the method depend on how much pressure is applied to the agar, contact time, presence of soil, and if the agar picks up the contaminating microbial cells. In addition, microorganisms do not quantitatively adhere to the agar surface upon application, again resulting in selection for a specific microorganism or underestimating microbial numbers on the sampled surface[8].

ATP bioluminescence test. The universal presence of ATP in living organisms and the reaction of this adenosine compound, with the substrate luciferin and the enzyme luciferase, with the subsequent emission of visible light (562 nm) have provided a means of rapidly assessing the microbial status of a plant. Many foodstuffs, including beer, naturally contain ATP as a biological residue and this has to be allowed for when interpreting results[23]. The biochemical reaction is based on the enzyme that causes the tails of fireflies to glow, and requires the presence of oxygen and Mg^{2+}:

$$\mathrm{ATP} + \mathrm{O_2} + \mathrm{Mg^{2+}} \xrightarrow{\textit{luciferase}} \mathrm{AMP} + \mathrm{CO_2} + \text{oxyluciferin} + \mathrm{PPi} + \text{(592 nm)}$$

where AMP is adenosine monophosphate and PPi is inorganic pyrophosphate.

This is a rapid biochemical method for estimating the total ATP collected by swabbing a surface, which is related to the amount of food residues and microorganisms collected by the swab, with a result obtained within 10 minutes. However, the ATP bioluminescence test cannot detect low levels of microorganisms. More than 10^3 bacteria or 10^1 yeast cells must be collected by the swab to have positive results[52]. The amount of light produced is measured using an illuminometer and is directly proportional to the amount of ATP (or microorganisms) present. ATP presence can also be due to dead organic material as well as viable organisms, and so the bioluminescence technique can also evaluate a general lack of hygiene[23].

Microscopy methods

Microscopy methods involve using light, epifluorescence, differential interference contrast (DIC), transmission electron (TEM), scanning electron (SEM), atomic force (AFM) and confocal scanning laser microscopy (CSLM)[8,17,28,29,48]. The use of CSLM and epifluorescence microscopy requires the organisms in soil films to be stained with fluorescent stains[28]. Before the use of CSLM, electron microscopes were the preferred method of choice to examine microbial soil films under high resolution. Unfortunately, sample preparation for electron microscopy resulted in dehydrated samples and as a result this provided a deceivingly simplistic view of soil film structure, since the soil film collapsed when water was removed[12].

Epifluorescence microscope. The applications of this method include determination of viable cells, soil film cell arrangement, microcolony formation, cell morphology, soil film pH, and distribution of chemicals in soil films[48].

CSLM. This is an improved version of an epifluorescence microscope, where viability can be determined by staining the transformed cells with a membrane impermeable fluorescent dye[48]. CSLM needs digital imaging methods to create computer reconstructions of the chemical and physical conditions within the microenvironment of the bacterial communities[31]. CSLM has been used to greater effect to characterise soil films, as it allows direct observation of hydrated samples, producing three-dimensional structures.

AFM. This is a non-invasive microscopic technique capable of imaging surfaces at nanometre resolutions. Furthermore, as no stains or coatings are needed in this method, soil films may be observed in situ[31].

There is no practical method for quantitative determination of soil film microorganisms in the beverage industry environment. This is because swabs and sponges do not quantitatively detach firmly adherent microorganisms. However, they provide useful information on the extent of microbial growth on a surface and on the extent to which cleaning has been effective[8]. The effectiveness of soil film removal and control can be monitored using ATP-bioluminescence for rapid results or plate count procedures for sensitive results.

TREATMENT AND PREVENTION OF SOIL FILMS

The primary objective of a treatment process is the removal of product residues. Indirect removal of these residues is also a first critical point in the removal, killing and control of soil films[49]. On the other hand, equipment design is important to control or eliminate soil film formation on contact surfaces, but poor design can make the task difficult, especially when using mechanical treatments in cleaning, which have proved to be efficient in the removal of soil films on good designs[29].

Several strategies to remove unwanted soil films exist that may be applied to a particular system, depending on its characteristics, and these include: (i) mechanical cleaning, (ii) the use of antimicrobial agents, (iii) stopping soil film growth by removing essential nutrients, (iv) inhibiting microbial attachment to a surface and (v) promoting biomass detachment[21,54]. Mechanical cleaning and antimicrobial agents are the most-used methods; however, mechanical cleaning can be costly, as it typically involves equipment down time or a significant labour expendi-

ture[54]. Also, it may not be applicable due to inaccessibility of the fouled surface.

Many countermeasures have been proposed in the literature, but the focus of this paper is on two methods: (i) surface modification (biomimicry), and (ii) use of silver ions.

Surface modification (Biomimicry)

The term biomimicry deals with bio-inspired based designs, implying the use of the natural world as a model to base an engineering development or device for hierarchical structures[5]. Innovations in materials science have led to a range of new products that can hinder microbial attachment to surfaces[35]. Surface modification techniques have greatly improved in recent years with the ability to produce discrete, highly ordered surface features on the micro- or nano-scale, to more accurately define this biological-surface interface, as this allows for the isolation of specific dimensions of feature geometry that may be dominant in inhibiting settlement of microorganisms[40]. However, dimensional differences between target species makes combating microbial fouling a challenging task and necessitates the individual examination of each fouling organism for species-specific fouling control[40].

There are natural surfaces that are able to resist accumulation of soil films using a combination of chemical and physical structures. Marine organisms such as sharks, mussels and crabs have natural antifouling defences, as does the endothelium of a healthy artery. However, these surfaces can lose their antifouling characteristics due to age, injury or disease[32]. Sharks have placoid scales, which have a vascular core of dentine surrounded by an acellular enamel layer similar to teeth[32]. A bio-inspired surface, Sharlet AF™, a design containing 2 μm wide rectangular-like ribs, periodic features (4, 8, 12 and 16 μm) in length and spaced at 2 μm, managed to reduce Ulva settlement by 86% as compared to a smooth surface[4]. This design was inspired by the nano- and microscopic patterns of a shark skin. These dimensions are smaller than the average diameter of the Ulva spore body (~5 μm), showing that the width and spacing of a topographical feature necessary to deter soil film formation can be tailored to the size of the organism[32]. This means that antifouling strategies that exploit surface topography can typically be based on a consideration of the length scale of the settling body of each targeted fouling organism[40].

It can be hypothesised that narrower channels and pillar spacing smaller than the dimensions of the microorganisms involved may be effective in reducing the settlement of the microorganisms[41]. This indicates that an interaction exists between roughness measurements and feature spacing that must be considered when designing topographic surfaces[41]. These engineered surface topographies have defined and ordered structural features that are tailored to the identified critical dimensions (i.e. feature width, spacing and height) of the fouling microorganisms of interest[40]. The proposed criteria to identify topographical feature limits of a surface are: (i) the microorganisms must be forced to remain on top of the protruded topographical features and not be able to settle between features (feature spacing), (ii) the microorganisms must not be able to stabilise its entire mass on one single feature (feature size), and (iii) if the microorganism is bridged between two topographical features, it must not be able to contact the floor between features, i.e. the number of attachment points must be minimised[38].

On the other hand, surfaces can be designed to facilitate removal of a soil film when it does occur. Surfaces may foul at the same rate, but the force required to remove fouling microorganisms can be markedly different[39]. Research has shown that the only surface parameter that strongly correlates with fouling removal is waviness; with the highest waviness profiles having the weakest fouling adherence[39]. Researchers have also suggested that there is an inverse relationship that exists between the percentage of soil film cells inactivated after exposure to antimicrobials and the width of crevices (i.e. roughness) on attachment surfaces[31].

Use of silver

Several attempts have been made over the years to protect materials, instruments and equipment by plating, painting and application of enamel. Protection of surfaces include: addition of a non-adhesive or antimicrobial coating, release of a toxic agent at the surface, introduction of surface modifying additives and addition of biocidal substances[13]. Introduction of organic or inorganic compounds to superficial coatings or biocidal agents within the surface material to create antimicrobial surfaces may directly inactivate the microorganisms attached to the surface and/or prevent their adhesion. This entails that a suitable biocide should be selected to provide a broad activity against the microorganisms commonly found in the target application area and may be added to polymers during its manufacture[13]. Some natural materials such as silver are non-selective antimicrobials that are active against a broad range of aerobic, anaerobic, Gram-negative and Gram-positive bacteria, yeasts, filamentous fungi and viruses[15].

One researcher reported that a titanium-silver alloy showed high antibacterial activity and good biocompatibility. However, although silver alloyed stainless steels showed less bacterial adhesion than their silver-free counterparts, the low solubility (<0.03%) of silver in stainless steel can be an obstacle to bulk alloying. In addition, all these approaches produce a thin silver-containing layer (<0.2 μm) on soft substrates, which makes it difficult if not impossible to ensure high durability and anti-bacterial properties simultaneously[15]. With silver (0.042%), antimicrobial steels were shown to reduce the number of adhering bacteria by 99% compared to normal stainless steel[35]. Regrettably this effect declined with time and further work is required to explore this opportunity further[35].

Developments in the synthesis of nanoparticles (NPs), and their numerous potential applications, has opened up new possibilities for engineering. Various nanometre-sized metal and metal oxide particles can provide antibacterial properties, and silver NPs seem to be particularly attractive, due to excellent antimicrobial efficiency of silver itself that has known for a long time[36]. The antimicrobial mechanism of the NPs is not known, but it can be assumed that it is similar to the ions, and this is due to their small size and high specific surface area, which makes them extremely reactive with the environment[27]. Layers of silver produced by sputtering silver metal in a

Table III. Comparison of the two technologies of soil film control.

Technique	Surface modification	Use of silver
Primary objective	Inhibition of soil film formation on surfaces	Control and elimination of already formed soil films
Mode of operation	Surface features are smaller than the average diameter of the target microorganisms inhibiting their settlement and adhesion	Release of silver ions that target vital activities to cells resulting in inactivation of the microorganisms
Cost	Very expensive to construct the mould with the designed surface profile	Relatively inexpensive to produce silver nanoparticles but expensive to plate, coat or alloy onto the surface. Low silver solubility into stainless steel affects alloying process
Cost in relation to plant	Applicable to new plants or plant upgrades	Used on existing and new plant equipment and vessels
Toxicity	Zero toxicity levels to humans and the environment	Toxicity levels should be adhered to
Ease of scale up	Difficulty in scale up due to surface designs with nano-patterns	Relatively ease to scale up

controlled atmosphere under different conditions to produce silver NPs species have been produced and studied and significantly higher bactericidal properties than simple Ag^+ solutions have resulted. This is due to the hypothesis that Ag^+ ions are responsible for the antimicrobial activity of silver, even as NPs[19].

Silver exerts its antimicrobial effect by progressive elution from the devices. A silver concentration of 100 µg/litre, achieved by adding silver nitrate in solution, is deemed safe for human consumption by the World Health Organization and the Environmental Protection Agency[42] and sub-millimolar concentrations of $AgNO_3$ are reported to be lethal to a range of bacterial species, both Gram-positive and Gram-negative. When silver ions bind to biological molecules containing thio, amino, carboxylate, imidazole, or phosphate groups, they inhibit activities that are vital to the microorganisms' regulatory processes and cause inactivation of microorganisms or they can displace other essential metal ions[7] such as Ca^{2+} and Zn^{2+}. The exact mode of action is unknown, but Ag^+-treated bacteria exhibit a characteristic initial stimulation in respiration before cell death occurs[22].

Summary

The two control strategies mentioned above have their pros and cons and comparisons are made (Table III).

CONCLUSIONS

Planktonic cells become sessile cells when nutrients become limited. The microorganisms attempt to locate organic material attached to the process equipment to which they can adhere and live on. When this is achieved, a reversible and irreversible attachment process starts and the formation of soil film takes place. The microorganisms in soil films are resistant towards various countermeasures and it is therefore important to prevent soil film formation by using the correct surface design methods, detergents and disinfectants. Cleaning should be performed frequently, and in addition, the choice of material of construction has an important influence on the inclination of soil film formation. Soil films are often found in cracks, corners, gaskets, joints and crevices in the pipe material or in dead ends in the pipe system. When determining the numbers and species in the soil films, standard methods such as plate counts or microscopy can be used. The countermeasures employed depend largely on the costs involved and to date the most effective solution has yet to be determined.

ACKNOWLEDGEMENTS

The work presented was made possible with the assistance of the Andrew W Mellon Foundation, WITS University and FoodBev Seta.

REFERENCES

1. Back, W., Secondary contaminations in the filling area. *Brauwelt Int.*, 1994, **4,** 326-333.
2. Baehni, P. C. and Takeuchi, Y., Anti-plaque agents in the prevention of biofilm-associated oral diseases. *Oral Dis.*, 2003, **9,** 23-29.
3. Bohak, I., Thelen, K. and Beimfohr, C., Description of *Lactobacillus backi* sp. nov., an obligate beer-spoiling bacterium. *Monatsschr. Brau.*, 2006, **59**, 78-82.
4. Carman, M. L., Estes, T. G., Feinberg, A. W., Schumacher, J. F., Wilkerson, W., Wilson, L. H., Callow, M. E., Callow, J. A. and Brennan, A. B., Engineered antifouling microtopographies: correlating wettability with cell attachment. *Biofouling*, 2006, **22,** 11-21.
5. Chambers, L. D., Stokes, K. R., Walsh, F. C. and Wood, R. J. K., Modern approaches to marine antifouling coatings. *Surf. Coat. Technol.*, 2006, **201,** 3642-3652.
6. Characklis, W. G., Fouling biofilm development: a process analysis. *Biotechnol. Bioeng.*, 2009, **102,** 1923-1960.
7. Chaw, K. C., Manimaran, M. and Tay, F. E. H., Role of silver ions in destabilization of intermolecular adhesion forces measured by atomic force microscopy in *Staphylococcus epidermidis* biofilms. *Antimicrob. Agents Chemother.*, 2005, **49,** 4853-4859.
8. Chmielewski, R. A. N. and Frank, J. F., Biofilm formation and control in food processing facilities. *Compr. Rev. Food Sci. Food Saf.*, 2003, **2,** 22-32.
9. Cluett, J. D., Cleanability of certain stainless steel surface finishes in the brewing process. *MPhil Dissertation*, 2001, Rand Afrikaans University, Johannesburg, South Africa.
10. Costerton, J. W., The predominance of biofilms in natural and engineered ecosystems. In: The Biofilm Primer., J. W. Costerton, Ed., Springer-Verlag: Berlin, 2007, pp. 5-14.
11. Costerton, J. W. and Wilson, M., Introducing biofilms. *Biofilms*, 2004, **1,** 1-4.
12. Davey, M. E. and O'Toole, G. A., Microbial biofilms: from ecology to molecular genetics. *Microbiol. Mol. Biol. Rev.*, 2000, **64,** 847-867.
13. de Carvalho, C. C. C. R., Biofilms: recent developments on an old battle. *Recent Pat Biotechnol.*, 2007, **1,** 49-57.
14. Dobson, C. M., Deneer, H., Lee, S., Hemmingsen, S., Glaze, S. and Ziola, B., Phylogenetic analysis of the genus *Pediococcus*, including *Pediococcus claussenii* sp. nov., a novel lactic acid bacterium isolated from beer. *Int. J. Syst. Evol. Microbiol.*, 2002, **52,** 2003-2010.
15. Dong, Y., Li, X., Sammons, R. and Dong, H., The generation of wear-resistant antimicrobial stainless steel surfaces by active screen plasma alloying with N and nanocrystalline Ag. *J. Biomed. Mater. Res. B Appl. Biomater.*, 2010, **93,** 185-193.

16. Donlan, R. M., Biofilms and device-associated infections. *Emerging Infect. Dis.*, 2001, **7,** 277-281.
17. Donlan, R. M., Biofilms: microbial life on surfaces. *Emerging Infect. Dis.*, 2002, **8,** 881-890.
18. Donlan, R. M. and Costerton, J. W., Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev.*, 2002, **15,** 167-193.
19. Fan, F. R. F. and Bard, A. J., Chemical, electrochemical, gravimetric, and microscopic studies on antimicrobial silver films. *J. Phys. Chem. B*, 2002, **106,** 279-287.
20. Flemming, H. C., Biofouling in water systems: cases, causes and countermeasures. *Appl. Microbiol. Biotechnol.*, 2002, **59,** 629-640.
21. Francolini, I. and Donelli, G., Prevention and control of biofilm-based medical-device-related infections. *FEMS Immunol. Med. Microbiol.*, 2010, **59,** 227-238.
22. Holt, K. B. and Bard, A. J., Interaction of silver (I) ions with the respiratory chain of *Escherichia coli*: an electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar Ag^+. *Biochemistry*, 2005, **44,** 13214-13223.
23. Hornsey, I. S., Microbiology in the brewery. In: Brewing., I. S. Hornsey, Ed., The Royal Society of Chemistry: Cambridge, 1999, pp. 194-223.
24. Iijima, K., Suzuki, K., Asano, S., Kuriyama, H. and Kitagawa, Y., Isolation and identification of potential beer-spoilage *Pediococcus inopinatus* and beer-spoilage *Lactobacillus backi* strains carrying the *horA* and *horC* gene clusters. *J. Inst. Brew.*, 2007, **113,** 96-101.
25. Jespersen, L. and Jakobsen, M., Specific spoilage organisms in breweries and laboratory media for their detection. *Int. J. Food Microbiol.*, 1996, **33,** 139-155.
26. Juvonen, R. and Suihko, M-L., *Megasphaera paucivorans* sp. nov., *Megasphaera sueciensis* sp. nov. and *Pectinatus haikarae* sp. nov., isolated from brewery samples, and emended description of the genus *Pectinatus. Int. J. Syst. Evol. Microbiol.*, 2006, **56,** 695-702
27. Kaali, P., Strömberg, E. and Karlsson, S., Prevention of biofilm associated infections and degradation of polymeric materials used in biomedical applications. In: Biomedical Engineering, Trends in Materials Science, A. N. Laskovski, Ed., InTech: Rijeka, 2011, pp. 513-540.
28. Kokare, C. R., Chakraborty, S., Khopade, A. N. and Mahadik, K. R., Biofilms: importance and applications. *Indian J. Biotechnol.*, 2009, **8,** 159-168.
29. Kumar, C. G. and Anand, S. K., Significance of microbial biofilms in food industry: a review. *Int. J. Food Microbiol.*, 1998, **42,** 9-27.
30. Lawrence, D. R. and Priest. F. G., Identification of brewery *cocci*. Proc. Eur. Brew. Conv. Congr. Copenhagen, IRL Press : Oxford, 1981, pp. 217-227.
31. Lindsay, D. and von Holy, A., Bacterial biofilms within the clinical setting: what healthcare professionals should know. *J. Hosp. Infect.*, 2006, **64,** 313-325.
32. Magin, C. M., Cooper, S. P. and Brennan, A. B., Non-toxic antifouling strategies. *Mater. Today*, 2010, **13,** 36-44.
33. Manzano, M., Iacumin, L., Vendrame, M., Cecchini, F., Comi, G. and Buiatti, S., Craft beer microflora identification before and after a cleaning process. *J. Inst. Brew.*, 2011, **117,** 343-351.
34. Poulsen, L. V., Microbial biofilm in food processing. *Lebensm. Wiss. Technol.*, 1999, **32,** 321-326.
35. Quain, D. and Storgårds, E., The extraordinary world of biofilms. *Brewer & Distiller International*, 2009, **5,** 31-33.
36. Radetić, M., Ilić, V., Vodnik, V., Dimitrijević, S., Jovančić, P., Šaponjić, Z. and Nedeljković, J. M., Antibacterial effect of silver nanoparticles deposited on corona-treated polyester and polyamide fabrics. *Polym. Adv. Technol.*, 2008, **19,** 1816-1821.
37. Sakamoto, K. and Konings, W. N., Beer spoilage bacteria and hop resistance. *Int. J. Food Microbiol.*, 2003, **89,** 105-124.
38. Scardino, A. J., Harvey, E. and de Nys, R., Testing attachment point theory: diatom attachment on microtextured polyimide biomimics. *Biofouling*, 2006, **22,** 55-60.
39. Scardino, A. J., Hudleston, D., Peng, Z., Paul, N. A. and de Nys, R., Biomimetic characterisation of key surface parameters for the development of fouling resistant materials. *Biofouling*, 2009, **25,** 83-93.
40. Schumacher, J. F., Aldred, N., Callow, M. E., Finlay, J. A., Callow, J. A., Clare, A. S. and Brennan, A. B., Species-specific engineered antifouling topographies: correlations between the settlement of algal zoospores and barnacle cyprids. *Biofouling*, 2007, **23,** 307-317.
41. Schumacher, J. F., Carman, M. L., Estes, T. G., Feinberg, A. W., Wilson, L. H., Callow, M. E., Callow, J. A., Finlay, J. A. and Brennan, A. B., Engineered antifouling microtopographies: effect of feature size, geometry, and roughness on settlement of zoospores of the green alga *Ulva*. *Biofouling*, 2007, **23,** 55-62.
42. Silvestry-Rodriguez, N., Bright, K. R., Slack, D. C., Uhlmann, D. R. and Gerba, C. P., Silver as a residual disinfectant to prevent biofilm formation in water distribution systems. *Appl. Environ. Microbiol.*, 2008, **74,** 1639-1641.
43. Suzuki, K., 125th anniversary review: Microbiological instability of beer caused by spoilage bacteria. *J. Inst. Brew.*, 2011, **117,** 131-155.
44. Suzuki, K., Koyanagi, M. and Yamashita, H., Genetic characterization and specific detection of beer-spoilage *Lactobacillus* sp. LA2 and related strains. *J Appl. Microbiol.*, 2004, **96,** 677-683.
45. Tian, Y., Behaviour of bacterial extracellular polymeric substances from activated sludge: a review. *Int. J. Environ., Pollut*, 2008, **32,** 78-89.
46. Timke, M., Wang-Lieu, N. Q., Altendorf, K. and Lipski, A., Fatty acid analysis and spoilage potential of biofilms from two breweries. *J. Appl. Microbiol.*, 2005, **99,** 1108-1122.
47. Timke, M., Wang-Lieu, N. Q., Altendorf, K. and Lipski, A., Identity, beer spoiling and biofilm forming potential of yeasts from beer bottling plant associated biofilms. *Antonie van Leeuwenhoek*, 2008, **93,**151-161.
48. Trachoo, N., Biofilms and the food industry. *Warasan Songkhla Nakharin.*, 2003, **25,** 807-815.
49. van Houdt, R. and Michiels, C. W., Biofilm formation and food industry, a focus on the bacterial outer surface. *J. Appl. Microbiol.*, 2010, **109,** 1117-1131.
50. van Loosdrecht, M. C. M., Lyklema, J., Norde, W. and Zehnder, A. J. B., Influence of interfaces on microbial activity. *Microbiol. Rev.*, 1990, **54,** 75-87.
51. Vaughan, A., O'Sullivan, T. and van Sinderen, D., Enhancing the microbiological stability of malt and beer: a review. *J. Inst. Brew.*, 2005, **111,** 355-371.
52. Verran, J. and Jones, M., Problems of biofilm in the food and beverage industry. In: Industrial Biofouling., J. Walker., S. Suramn. and J. Jass, Eds., John Wiley: New York, 2000, pp. 145-173.
53. Wilson, M., Susceptibility of oral bacterial biofilms to antimicrobial agents. *J. Med. Microbiol.*, 1996, **44,** 79-87.
54. Xavier, J. B., Picioreanu, C., Rani, S. A., van Loosdrecht, M. C. M. and Stewart, P. S., Biofilm-control strategies based on enzymic disruption of the extracellular polymeric substance matrix: a modelling study. *Microbiology*, 2005, **151,** 3817-3832.
55. Zottola, E. A. and Sasahara, K. C., Microbial biofilms in the food processing industry: should they be a concern. *Int. J. Food Microbiol.*, 1994, **23,** 125-148.
56. Škvarla, J., A physico-chemical model of microbial adhesion, *J. Chem. Soc., Faraday Trans.*, 1993, **89,** 2913-2921.

(Manuscript accepted for publication December 2011)