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Microbial populations on brewery filling hall surfaces – Progress towards functional coatings

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ABSTRACT

Microbial populations on equipment surfaces of beverage filling lines were investigated as a function of surface coating type, location and time. Photocatalytic metal-ion doped (Ag or Mo) and non-doped TiO_2 coatings deposited using reactive magnetron sputtering and spray coating methods were studied as means to reduce microbial numbers accumulating on the surfaces. The coatings were applied to stainless steel coupons, which were mounted to one canning and one glass bottle filling line for 3–5 months. After exposure microbial numbers on the coupons were evaluated by culturing, and bacterial community profiles were characterised with PCR-DGGE (denaturing gradient gel electrophoresis). The results showed that the longer the run time after washes the higher microbial numbers were detected, and that the two filling lines each had their characteristic bacterial community. The major species identified were members of *Acinetobacter* sp., lactic acid bacteria and enterobacteria. No clear effect of the different coating materials on the microbial numbers or bacterial community composition on the surfaces was shown. In conclusion, functional coatings with sufficient mechanical and chemical durability for industrial surfaces have been developed. Although these coatings have been previously reported to reduce the number of microorganisms on the surfaces *in vitro*, their efficacy in the challenging beverage process conditions was not proven.

1. Introduction

Brewery packaging equipment surfaces are constantly exposed to moisture and nutrients during production, thus being susceptible to microbial attachment and growth on surfaces. Surface-attached bacteria are problematic to process industry: they cause contaminations, degrade and/or corrode materials and affect process efficiency (e.g., filtration units or heat exchangers). Equipment surfaces are cleaned on a regular basis to reduce the microbial load to a safe level. Washing procedures cost in terms of production interruptions and consumption of energy, water, chemicals and working hours. The process industry would thus benefit from novel means for managing microbes on process surfaces. Development of functional coatings, *i.e.* coatings reducing the number of adhering microbes on surfaces, has been a growing trend in recent years especially in clinical environments (Page, Wilson, & Parkin, 2009). Such coatings could have a valuable role in improving hygiene in industrial processes, including the beverage industry.

Coatings with photocatalytically active semiconductors, especially TiO_2, have attracted attention as a method for keeping surfaces free of dirt and microbes. TiO_2 photocatalysts have been shown to kill bacteria upon illumination by UVA light (315–400 nm) (Fujishima, Zhang, & Tryk, 2008). The presently accepted view on the mode of action is that $OH^{\bullet}$ produced by TiO_2 is

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the primary killing agent, but that other reactive oxygen species generated in the process, such as O_2^- and H_2O_2, may be partly responsible for inactivation of bacteria (Cho, Chung, Choi, & Yoon, 2005). Because of the short half-life of $OH^•$ and its low diffusion potential, bacterial cells to be oxidized must be close to the generation site. To obtain bactericidal activity under weak UV intensity, TiO_2 films doped with antibacterial metals such as copper (Cu), silver (Ag), and other elements have been developed for combined activity (Naik & Kowshik, 2014; Ratova, Kelly, West, & Iordanova, 2013; Sunada, Watanabe, & Hashimoto, 2003).

Photooxidation of bacteria has mostly been studied using single bacterial species, like *Escherichia coli, Staphylococcus aureus, Enterobacter cloacae, Bacillus subtilis, Pseudomonas aeruginosa, P. fluorescens, Deinococcus geothermalis* or *Burkholderia cepacia* (Allion et al., 2007; Ibáñez, Litter, & Pizarro, 2003; Keskinen et al., 2006; Kühn et al., 2003; Li & Logan, 2005; Pal, Pehkonen, Yu, & Ray, 2007; Raulio et al., 2006). The photocatalytic activity has varied from 10 to 99.9999 % reduction in the number of viable bacteria depending on the light intensity, wavelength and exposure time, as well as bacterial species and whether adhered or non-adhered bacteria have been studied. Yeasts appear to be considerably more resistant than bacteria against photooxidation (Kühn et al., 2003). A few studies using mixtures of microbial species have also been reported: Rajagopal, Maruthamuthu, Mohanan, and Palaniswamy (2006) oxidised biofilms formed by natural microbiota of pond water by photocatalysis, but failed to kill all microbes. Priha et al. (2011) used a mixed culture of bacterial and yeast strains to evaluate the efficacy of atomic layer deposited (ALD) TiO_2 and $TiO_2 + Ag$ coatings in reducing the number of adhering microbes on steel, and obtained a reduction in microbial numbers adhering onto $TiO + Ag$ coatings. Due to the inadequate mechanical durability of the coatings, however, their functionality in beverage process conditions could not be evaluated.

In this study the performance of doped (Ag or Mo) and non-doped TiO_2 coatings deposited using reactive magnetron sputtering and spray coating methods was investigated in process conditions at a brewery. In preceding studies executed in laboratory, similar coatings reduced the number of bacteria compared to the non-coated stainless steel (Fisher et al., 2014; Navabpour, Ostovarpour, & Hampshire, et al., 2014). Also, the mechanical durability and retention of photocatalytic properties of these coatings has been evaluated in brewery process conditions (Navabpour, Ostovarpour, & Tattershall, et al., 2014). The aim of this study was to verify the antimicrobial activity of the coatings in real process conditions. Our hypothesis was that Ag or Mo doping would enhance the photoactivity of TiO_2, enabling the reduction in the number of adhered microbes despite the low light intensity in process conditions.

2. Materials and methods

2.1. Installation of coupons to brewery filling machines

All coatings were applied to stainless steel with bright cold rolled finish (AISI 304 2B) coupons of 18.75 cm^2 (25 × 75 × 1 mm). The properties of coatings used in this study are presented in Table 1. Details of coating preparation are given in Navabpour, Ostovarpour, and Tattershall, et al. (2014). The coated and non-coated stainless steel coupons were disinfected prior to process studies by immersion to 70% ethanol for 2 h, and air-dried. The coupons were installed onto one canning line and one glass bottle line in a randomised block design, having three blocks with five replicates of each coupon type at both filling lines, totalling 15 replicates of each coating in each filling line. Filler surfaces were cleaned with saBesto spray (25–50% petroleum, 20–30% isopropanol, Würth, Riihimäki, Finland) and the coupons were fixed with HV350 glue (Valco, Cincinnati, OH). There was no special provision of UV lighting for the photocatalytic coatings; the process test took place under the usual brewery conditions, with coupons receiving varying amounts of light depending on their location in each machine. When installing the coupons, the light intensities of UVA (LP 471 UVA probe, spectral range 315–400 nm) and VIS (LP 471 RAD Probe, spectral range 400–1050 nm) were measured with a fluorometer (HD 2102.2, DeltaOhm, Padova, Italy), and were, on average, 0.5 (±1.0) μW cm^{-2} and 34 (±38) μW cm^{-2}, respectively. All coupons underwent the normal process conditions and cleaning regimes used while being in the process. Between production batches the washing programmes of the filling lines consisted of prerinsing with cold water, soaking with alkaline foam cleaner for 10 min with concurrent manual brushing where necessary, disinfection with chlorine based cleaner for 10 min, and final rinsing with cold water. For each microbiological sampling the time from the last washing cycle was documented. In addition, less-extensive washes without brushing were done during production at 4–24 h intervals.

Table 1
The studied materials. All coatings were deposited onto stainless steel with bright cold rolled finish (AISI 304 2B).

Coupon code	Material/coating	Concentration of dopant (%)	Deposition method
R	AISI 304 2B		Control, not coated
U1	TiO_2		Reactive magnetron sputtering + heat treatment
U2	TiO_2–Mo	7.0	Reactive magnetron sputtering + heat treatment
T2	TiO_2–Ag	0.5	Reactive magnetron sputtering
MC	TiO_2		Spray-coated with TiO_2 sol

2.2. Assessing microbial attachment and retention rates

Three samplings for the assessment of the number of microbes on the coupons between two weeks and five months from the installation of the coupons were performed (Table 2). The sampling times varied between the canning and glass bottle line due to production timetables. The coupons were swabbed with sterile 5 × 10 cm nonwoven gauzes (Mesoft, Mölnlycke Health Care, Gothenburg, Sweden) placed into 10 ml of Ringer's solution (Merck, Darmstadt, Germany) immediately after swabbing. Bacteria were detached from the gauze by homogenisation for 1 min (Stomacher), and the numbers of microbes were enumerated by plate counts. For enumeration of aerobic heterotrophic bacteria, samples were cultivated on Tryptic Soy Agar (BD, Becton, Dickinson and Company, New Jersey, USA) containing 10 mg l^{-1} cycloheximide (Sigma; Sigma-Aldrich, St. Louis, MO, USA) to prevent the growth of fungi, and incubated for 3 d at 30 °C. Moulds and yeasts were detected using Potato Dextrose Agar (BD, New Jersey, USA) containing 100 mg l^{-1} chloramphenicol (Sigma; Sigma–Aldrich, St. Louis, MO, USA) and 100 mg l^{-1} chlortetracycline (Sigma; Sigma–Aldrich, St. Louis, MO, USA) to prevent to growth of bacteria, and 20 mg l^{-1}

Table 2
The sampling times of the process study.

	Days from installation of coupons (days from last washing cycle)			
	First sampling	Second sampling	Third sampling	Coupon removal
Canning line	13 (1.5)	43 (1)	84 (3)	97 (1.5)
Glass bottle line	24 (2)	43 (1.5)	149 (1)	163 (1.5)