Molybdenum doped titanium dioxide photocatalytic coatings for use as hygienic surfaces - the effect of soiling on antimicrobial activity

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Abstract

Titanium dioxide (TiO_2) surfaces doped with molybdenum (Mo) were investigated to determine if their photocatalytic ability could enhance process hygiene in the brewery industry. The ability of the TiO_2-Mo surfaces to reduce the number of brewery microorganisms was examined with and without the application of a brewery soil (beer). Doping TiO_2 with Mo showed a 5-log reduction in bacterial counts and a 1-log reduction in yeast. The presence of dried beer resulted in microbial growth over time being apparent however, a more dilute soil did not interfere with antimicrobial activity. The TiO_2-Mo surface was also active in the dark suggesting it could have a dual function, being antimicrobial and photocatalytic. The study highlights the importance of assessing the impact soiling can have on antimicrobial/photocatalytic ability and suggests the TiO_2-Mo coating could act as a secondary barrier in helping prevent microbial proliferation between cleaning/disinfection cycles within a brewery setting.

Keywords: photocatalytic surfaces; titanium dioxide; molybdenum; antimicrobial activity; brewery microorganisms; soiling

Introduction

The photocatalytic properties of titanium dioxide (TiO_2) are well documented (Fujishima et al. 2000; Foster et al. 2011) and its uses in the degradation of pollutants in water and air are well established (Fujishima & Honda 1972; Frank & Bard 1977). Titanium dioxide is a semiconductor with a wide band gap (3.2 eV), which can be excited by ultraviolet (UV) irradiation (λ <380 nm). Photons with energy equal to or higher than its band gap can promote an electron from the valence band to the conduction band, producing electron-hole pairs. The photogenerated electrons react with molecular oxygen (O_2) to produce superoxide radical anions ($^{\bullet}O_2^-$), and the photogenerated holes react with water to produce hydroxyl ($^{\bullet}OH$) radicals (Fujishima et al. 2000; Daviðsdóttir et al. 2013). UV light and reactive radical species are able to decompose organic compounds and inactivate microorganisms. Immobilized TiO_2 particles deposited as thin films on substrates can also become superhydrophilic after UV illumination, making residual material easily displaced with water. Thus, TiO_2 surfaces can also have a self-cleaning effect (Fujishima et al. 2000) as well as being able to degrade pollutants and microorganisms through photocatalysis.

It is widely recognised that environmental surfaces can act as reservoirs for microbial fouling and in recent years there have been many publications regarding photocatalytic disinfection with reports referring to the destruction of Gram positive and Gram negative bacteria as well as fungi, algae, protozoa, endospores and viruses (Foster et al. 2011). Both the photocatalytic disinfection and self-cleaning properties of TiO_2 surfaces make them potentially applicable to areas where microbial fouling can arise, for example, in hospital settings or in the food and beverage industries. Within a brewery environment, the hygienic status of process surfaces plays a major role in ensuring the quality of beer. Beer production and dispensing often takes place in closed systems, where in-place cleaning procedures are applied (Storgards 2000). The cleaning and disinfection regimes employed are essential for removing product deposits and microbial populations. However, long production runs between cleaning cycles

are typical, making such systems more susceptible to bacterial accumulation. The accumulation of bacteria on surfaces can lead to the development of biofilms, which may consist of multiple communities of microorganisms (Costerton et al. 1995). The secretion of an extracellular matrix by these organisms keep them protected against disinfection, thus biofilms once established can be difficult to eradicate from surfaces (Costerton et al. 1995). Hard to reach areas can also be problematic. The use of photocatalytic surfaces under such circumstances could help towards discouraging the build-up of microbial populations during long production runs and in between disinfection cycle. However, since UV light is required to activate photocatalytic surfaces, the use of TiO_2 in indoor situations is limited.

Doping TiO_2 with transition metals causes a shift reduction in the band gap, enabling surfaces to become active under visible light (Wilke & Breuer 1999). Titanium dioxide doped with a transition metal can be deposited as a thin film coating onto surfaces such as stainless steel, typically used in breweries. In a study by Ratova et al. (2013), thin films of TiO_2 were doped with different amounts of the transition metals molybdenum (Mo), niobium (Nb), tungsten (W) and tantalum (Ta) onto glass substrates using reactive magnetron co-sputtering. The films were analysed in terms of their composition and structure by energy dispersive X-ray spectroscopy (EDX) and Raman spectroscopy and their photocatalytic activity was assessed by the degradation of the organic dye methylene blue under UV and fluorescent light sources. The study reported that, after annealing in air at 600 °C, TiO_2 coatings doped with Mo exhibited the highest photocatalytic activity under both light sources. This was attributed to the presence of an anatase crystalline phase and a 'red-shift' in band gap energy towards the visible spectrum (Ratova et al. 2013). However the photocatalytic effect against bacteria was not examined.

The aim of this study was to develop a novel functional surface by applying a Mo-doped TiO_2 coating and to determine whether process hygiene in the brewery industry could

be improved. The first stage was to identify an optimal doping level. Following this, the ability of TiO_2-Mo thin films deposited onto stainless steel to firstly reduce numbers of *Escherichia coli* (a model organism) and then brewery organisms in the absence and presence of a brewery soil was investigated.

Materials and Methods

Production of photocatalytic thin films

Stainless steel (AISI 304-2B) was used as a substrate upon which thin films of TiO_2 doped with Mo were deposited by a closed field unbalanced magnetron sputter ion plating technique (Teer Coatings Ltd, Droitwich, UK) (Laing et al. 1999). The production of the thin films was as described by Ratova et al. (2013). In brief, three 300 mm × 100 mm vertically opposed unbalanced planar magnetrons and one blanking plate were installed in the chamber and two magnetrons were fitted with titanium targets and one with the Mo dopant metal target. All targets were of 99.5% purity. The magnetrons with the titanium targets were in the closed field configuration and driven in pulsed DC sputtering mode using a dual channel Advanced Energy Pinnacle Plus supply at a frequency of 100 kHz and a duty of 50% (in synchronous mode). The Mo metal target was driven in a continuous DC mode (Advanced Energy MDX). The Ti targets were operated at a constant time-averaged power of 1 kW and the dopant target was operated at powers in the range of 0-240 W, selected to produce a range of dopant levels in the coatings. The base pressure of the sputtering chamber was 1×10^{-3} Pa. The flow rate of argon was controlled through a mass flow controller and the flow rate of oxygen was controlled by an optical emission monitor set to 15% of the full metal (Ti) signal, which had been previously determined to produce stochiometric TiO_2 coatings (Ratova et al. 2013). Stainless steel samples (2 × 1 cm) were mounted on a substrate holder, which was rotated between the magnetrons at 4 rpm during deposition. The target to substrate separation was 8 cm. The titanium and Mo

targets were cleaned by pre-sputtering in a pure argon atmosphere for 10 minutes. Deposition times were adapted to obtain a film thickness of 800 nm - 1 μm. The sputtered films were post deposition annealed at 600 °C for 30 minutes in air.

Coating characterization

Energy dispersive X-ray spectroscopy (EDX – Edax Trident) was employed to analyse the coating compositions and determine the dopant level. Previous experience had shown that the as-deposited coatings would be amorphous. Thus, the TiO_2 and TiO_2-Mo thin films were annealed at 600 °C and then analysed by X-ray diffraction (XRD) in the θ-2□ mode (URD6 Seiferd & Co diffractometer with CuKα_1 radiation at 0.154 nm) and by Raman spectroscopy (Renishaw Invia, 514 nm laser) to obtain information regarding their crystalline structure.

Photocatalytic activity

Photocatalytic activity levels were determined via the degradation of the organic dye methylene blue (MB) (Alfa Aesar, UK), since MB is a dye often used as a model organic compound to measure photoreactivity (Ratova et al. 2013). Aqueous MB absorbs light most strongly at about 660 nm with a given molar absorptivity of 10^5 dm^3 mol^{-1} cm^{-1}. The absorbance at 660 nm versus UV or fluorescent irradiation time can be translated into a graph of peak height absorbance against irradiation time, which has an exponential form (Houas et al. 2001). MB concentrations were calculated using the measured absorbance peak around 663 nm. The formula below was used to calculate the photocatalytic activity of each of the films. Two parameters were defined: P_{aUV} for UV irradiation and P_{aFL} for fluorescent light irradiation.

$$P_a = 1 - [C_0e^{-mx} / C_0e^{-cx}]$$

Where:

C_0 = peak height at time = 0 (ie initial concentration)

C_0e^{-mx} = decay rate of methylene blue with no sample present

C_0e^{-cx} = decay rate of methylene blue in contact with a photocatalytic coating

The decomposition of MB was assessed using an initial concentration of 0.105 mmol l^{-1}. Annealed TiO_2 and TiO_2-Mo samples were placed in 10 ml of MB solution, which was irradiated at an integrated power flux of 4 mW cm^2 with 2 x 15 W 352 nm UV tubes (Black Ray, Cambridge, UK) or at an integrated power flux of 6.4 mW cm^2 with 2 x 15 W fluorescent tubes, of which the UV component (300 - 400 nm) was 1.3 mW cm^2. A 10 cm distance between the light source and MB solution was used and a UV/Vis spectrophotometer (PerkinElmer, UK) was employed to measure the absorbance peak height. Measurements were taken every hour over a total of 5 hours.

Using the above approach, photoactivity values were recorded for all the TiO_2 and TiO_2-Mo coatings and from those results, the optimal dopant level was identified. Samples with that composition were then tested with soiling (20 µl dried on undiluted beer) and, following removal of the soil (swabbed clean with acetone, methanol, ethanol and distilled water), under UV and fluorescent light.

Antimicrobial activity

Microorganisms

Escherichia coli (ATCC 8739) was used as a model organism in the first instance. Microorganisms isolated from brewery surfaces were obtained from VTT Culture Collection and included *Pseudomonas rhodesiae* E-031889, *Serratia marcescens* E-031888, and *Wickerhamomyces anomalus* C-02470. *E.coli*, *P.rhodesiae* and *S.marcescens* were cultured on Nutrient agar (Oxoid, UK) and *W. anomalus* on YM agar (Difco, USA). Incubation was at 37

^{0}C for *E.coli*, 30 °C for *P.rhodesiae* and *S.marcescens* and 25 °C for *W. anomalus* for 24 to 48 hours.

Light source

For the antimicrobial assays, surfaces were illuminated (wavelength range of 300-700 nm) in a cooled incubator (Gallenkamp, Loughborough, UK) fitted with six fluorescent lamps (Sylvania, Ontario, Canada) with an energy output of 6.4 mW cm^2. The temperature was set at 20 °C.

Antimicrobial assay

Measurements of the antimicrobial activity of the TiO_2-Mo photocatalytic surfaces were performed using ISO 27447 as guidance (with minor modifications). A single colony of each organism was placed in 10 ml Nutrient broth (Oxoid, UK) or YM broth (Difco, USA) for *W. anomalus* and incubated for 18-24 hours at the appropriate temperature in a shaker incubator set at 150 rpm. Suspensions were centrifuged at 1560g for 10 minutes and cells were washed in Ringers solution (Oxoid, UK) prior to re-centrifugation. An optical density of 0.08 at 540 nm (Jenway 6305 Spectrophotometer) in Ringers solution was obtained and a 1:10 dilution performed prior to inoculating 50 µl onto the 2 × 1 cm surfaces (approx 10^5 cfu ml^{-1}) contained within a petri dish. A 2 × 1 cm polyethylene film (Scientific Laboratory Supplies, UK) was placed over the bacterial suspension to ensure even distribution and the surfaces were placed in a tissue culture tray (Corning , UK) with the lid on and placed under fluorescent light set up in a 20 °C incubator under static conditions. Humidity was carefully maintained to avoid suspensions drying on the surface. At selected time points (0, 12, 24 and 48 hours), surfaces were removed and vortexed for 1 minute in neutralizing broth (20 g l^{-1} Soya Lectin (Holland and Barrett, UK) and 30 g l^{-1} Tween 80 (Sigma Aldrich, UK)) to remove attached bacteria

(Caballero et al. 2010). Bacteria remaining on the surface were diluted 1:10 in 0.9% NaCl (Fisher Scientific) as appropriate and 100µl plated and spread onto an appropriate pre poured agar plate. Colony counts were performed after overnight incubation. All tests were carried out in triplicate. Stainless steel was used as a light control and a set of coated surfaces were also kept in dark conditions to serve as further controls.

Tests were performed on TiO_2-Mo coatings immediately after annealing and on coatings which had been soiled with either undiluted beer or diluted beer (10%). Beer used to simulate soiling was a pasteurised, commercial product. 20 µl of undiluted or beer diluted in sterile distilled water was dried onto the surfaces prior to the application of the 10^5 cfu ml^{-1} bacterial suspension and the effect of soiling on microbial numbers was examined over an irradiation period of up to 96 hours.

Minimum inhibitory concentration determination

In order to determine the minimum inhibitory concentrations (MICs) of Mo, an agar incorporation method was used (Wiegand et al. 2008). Stock solutions (1000 mg l^{-1}) of Mo standard for AAS (Fluka Analytical, UK) in liquid form were serially diluted to cover concentrations ranging from 0.25-5 mg l^{-1}. Since stock solutions of Mo also contained 10% hydrochloric acid, serial dilutions were also performed as above using hydrochloric acid (Fisher Scientific) alone (1.02 Mol) to ensure MICs were due to the metal and not the acid. A 0.5 McFarland standard suspension (approximately 1.5 x 10^8 cfu ml^{-1}) of each of the test cultures was made and a swab stick was dipped into the suspension and spread onto the surface of a portion of the Mo incorporated agar. The bacterial suspension was left to dry prior to incubation at the appropriate temperature for 24 hours. The test was performed three times and results showing the concentration where no visible microbial growth was evident that agreed on two or more occasions were adopted as the MIC.

Molybdenum ion release by inductively coupled plasma atomic emission spectroscopy

A Varian Vista AX CCD inductively coupled plasma atomic emission spectrometer (ICP-AES) (Varian Inc) was used to assess any Mo ion release from the deposited thin film coatings. Surfaces were individually placed into a petri dish to which 20 mL of HPLC grade water (Fisher Scientific, UK) was added and incubated under fluorescent lighting in a 20 ^{0}C incubator. At 2, 4, 8, 24, 48, 72, 96 and 168 hours surfaces were transferred to fresh HPLC grade water whilst the previous sample was kept for analysis by ICP-AES. All samples were frozen at -85 ^{0}C prior to analysis. Molybdenum ion release was calculated from calibration curves (0.1-5 ppm) and all tests were carried out in triplicate.

Statistics

A 2 tailed homoscedastic Student's t-test was performed using Microsoft Excell 2010 to compare data sets. If the p value was less than 0.05 then results were statistically significant.

Results

Coating characterization and photocatalytic activity

Titanium dioxide and TiO_2-Mo coatings were deposited as thin films onto stainless steel substrates using magnetron sputtering. The Mo dopant levels, determined by EDX analysis, increased with increasing power delivered to the Mo target and ranged from 2.69 to 11.81 at% (Table 1). Photocatalytic activity levels (P_{aUV} and P_{aFL} values) for the annealed coatings, determined from the methylene blue tests, are also listed in Table 1. From these data, it can be seen that the highest activity under both light sources was achieved by the coatings doped at 6.95 at% of molybdenum.

The annealed TiO_2-6.95at% Mo coatings were analysed by XRD (Figure 1) and Raman spectroscopy (Figure 2). The XRD pattern showed the presence of the (1 0 1) anatase peak at $2\theta = 25.4°$ and the (1 1 0) rutile peak at $2\theta = 27.4°$ and several other minor peaks. The sample also showed the presence of some monoclinic β-titania. In contrast, the Raman spectrum showed only anatase peaks at 144, 389, 515 and 638 cm^{-1}. Based on these analyses, the assumption must be that the structure of the TiO_2-6.95at% Mo coatings after annealing in air at 600°C is a mixed phase, anatase/rutile structure, but the relative proportions of each phase were not determined.

Photocatalytic activity of soiled surfaces

Photocatalytic activity of the TiO_2-Mo surface in the absence and presence of soiling (undiluted beer) and following removal of the soil were demonstrated via the degradation of MB (Table 2). Doping TiO_2 with Mo considerably improved photoactivity under fluorescent light. The apparent increase in photoactivity of soiled surfaces under fluorescent irradiation will require further investigation. Following cleaning to remove the soil prior to assessing photocatalytic activity, a slight decrease in P_{aUV} and P_{aFL} values was observed.

Antimicrobial activity

The antimicrobial activity demonstrated by the TiO_2-Mo surfaces was investigated against *E.coli* and the brewery organisms; *P.rhodesiae*, *S.marcescens* and *W.anomalus*. The TiO_2-Mo coated surface proved effective against the model organism *E.coli* with counts from an unsoiled surface reducing to below the limit of detection (<10 cfu ml^{-1}) within 1-4 hours (Figure 3). *P. rhodesiae* (Figure 4a) counts were also reduced to <10 cfu m^{-1} and *S. marcescens* (Figure 5a) to <15 cfu ml^{-1} within 24 hours. Further tests need to be conducted against *P. rhodesiae* and *S. marcescens* to investigate the rate of kill over shorter irradiation times. *W. anomalus* (Figure

6a) counts at 72 hours were reduced by 1 log to $>10^3$ cfu ml^{-1}. The presence of brewery soil in the form of undiluted beer on the TiO_2-Mo surfaces reduced the antimicrobial effectiveness, and growth of all brewery organisms rather than inhibition was seen (Figure 4b - 6b). The presence of dilute beer (10%) on the surfaces did not reduce the antimicrobial activity of coatings. No viable *P. rhodesiae* (Figure 4c) were recovered from the TiO_2-Mo coatings after 24 hours irradiation and <15 cfu ml^{-1} of *S. marcescens* (Figure 5c) remained. *W. anomalus* (Figure 6c) counts after 72 hours irradiation were $>10^3$ cfu ml^{-1}, although this was a significant ($p = <0.05$) decrease compared to the non-antimicrobial surfaces. Bacteria were inactivated more readily than the yeast and the TiO_2-Mo surfaces were also as active in dark conditions as in the light.

Minimum inhibitory concentration determination and Molybdenum ion release by inductively coupled plasma atomic emission spectroscopy

Minimum inhibitory concentrations showed that *E.coli*, *P.rhodesiae* and *S.marcescens* were more susceptible to Mo than *W.anomalus* (Table 3). Inductively coupled plasma atomic emission spectrometry was used to detect Mo ion release from the thin film coating over a duration of 7 days (Figure 7). An initial burst of Mo ions (0.73 ppm) over the first 2 hours was recorded followed by a sudden decline and then a steady low concentration release. The low concentration of Mo ions (<0.04 ppm) being released after the initial 48 hours suggests that Mo is retained on the surface and is not readily leachable.

Discussion

The development of novel functional surfaces could play an important role in aiding the reduction of microbial contamination within the brewing industry. It is well known that microbial numbers decrease when in contact with a TiO_2 surface which has been illuminated

with UV light. For example, Kikuchi et al. (1997), found a suspension of *E. coli* (3×10^4 cfu ml^{-1}) deposited onto TiO_2 coated glass was eradicated within 1 hour of exposure to UV light (1 mW cm^{-2}). The potential of such photoactive surfaces may not only help prevent the build up of microbial numbers during long production runs but also reduce the cleaning costs to the beverage industry in terms of production stoppages and consumption of energy, water and chemicals (Priha et al. 2011). This would be beneficial economically, environmentally and also in terms of reducing human exposure to harsh chemical disinfection regimes. The continually acting nature of the surface could also make the brewing industry surfaces more efficient since microbial loads may be lower when in use compared to a non bioactive surface, which in turn could improve beer quality. The use of UV lighting in indoor environments can, however be hazardous but in this work by doping TiO_2 with Mo, activity using fluorescent lighting was enhanced. The addition of Mo at 6.95 at.% to TiO_2 improved the photocatalytic activity as determined by the degradation of methylene blue under fluorescent light. It was also noted that the crystal structure of the TiO_2-Mo coating was in the mixed phase anatase/rutile form. Despite the anatase form being known as the most photoactive, in this instance it appeared that the mixed phase structure helped improve photoactivity. Other researchers have also shown that mixtures of anatase and rutile show a synergistic effect and enhanced photocatalytic activity (Boehme & Ensinger 2011). Indeed, the Degussa P-25 TiO_2 powder (Evonik Degussa GmbH), a standard material used for photocatalytic reactions, has enhanced activity due to having a rutile/anatase mixed phase ratio of 1:4 (Ohno et al. 2001).

The antimicrobial effect of the TiO_2-Mo photocatalytic coatings were first tested using *E.coli* as a model organism. Since counts were reduced to <10 cfu ml^{-1} within 1-4 hours, tests were performed to look at the effect on brewery organism numbers. Within 24 hours of irradiation under fluorescent light, *P. rhodesiae* and *S. marcescens* counts decreased by 5-logs. Longer irradiation times were investigated to establish if complete bacterial kill could be

achieved. *W. anomalus* counts after longer fluorescent light exposure (72 hours) were $>10^3$ cfu ml^{-1}; however this was 1-log less than the non-antimicrobial surface. The fact that higher numbers of yeast remained attached to the surface could be due to differences in the cell wall structure and the larger size and increased complexity of a eukaryotic cell which could make them more resistant in nature to oxidative agents, compared to bacterial cells and more resistant to killing. It was also noted that the Mo MIC for *W. anomalus* was considerably higher than for the bacterial strains used. The TiO_2-Mo surfaces were also active in the dark. This suggests that the coating could have a dual function, being both photoactive and antimicrobial.

The photocatalytic mode of killing through the formation of radical species targets no particular site within a microbe and avoids the potential of resistance development (Page et al. 2009). However, since the Mo doped surface proved to be innately antimicrobial and with a long disinfection time through exposure to 24 hours or more irradiation, phenotypic change in microorganisms and the induction of resistance development should be taken into consideration. With the ability of microorganisms to adapt to a variety of environmental situations including chemical conditions, it is not surprising that resistance to broadly used disinfectants has been reported (McDonnell & Denver Russell 1999). However, to our knowledge no case of microbial resistance to Mo metal has been reported, but the importance of monitoring for signs of the emergence of resistance to Mo should be highlighted and always considered when introducing new strategies for disinfection.

In the dispensing and bottling section of a brewery, it is common for process surfaces to become covered by a conditioning film formed by the absorption of various organic materials (Jullien et al. 2008) from the spilt beer. The antimicrobial efficacy of the TiO_2-Mo surface was also examined following the deposition of soil in the form of undiluted beer onto the surface. Higher numbers ($>10^5$ cfu ml^{-1}) than the original inoculum of all microorganisms remained on the surface following exposure to fluorescent light for 48 - 96 hours. It would

therefore appear that the presence of soil had a significant impact on the antimicrobial effectiveness of the photocatalytic surface compared to without soil. With the contaminating population remaining viable, and possibly even multiplying, it may be that the concentration of soil was masking the effects of TiO_2-Mo and acting as a nutrient source. That said, photocatalytic activity determined by MB degradation appeared to be greater on surfaces soiled with undiluted beer under fluorescent light compared to without soil. However, it is likely that the soil was washed away during immersion in MB and the apparent enhanced activity could have been attributed to merely variability within the experimental set up. Further investigations will explore this anomaly.

As the TiO_2-Mo surfaces were also active in the dark, it was difficult to distinguish whether photocatalysis or the innate antimicrobial nature of the Mo had the greatest effect on reducing microbial numbers on the unconditioned surfaces. To therefore suggest that organisms remaining viable on the conditioned surfaces because of soil blocking the light cannot be validated without further studies, since microbial numbers also failed to decline on the conditioned surfaces in the dark. Fujishima et al. (2000), state that photocatalysts are not especially useful at breaking down large volumes of soilage, but they are capable of destroying it as it accumulates. The heavy dried on soil may therefore have been too great for photocatalysis to have had a significant effect on microbial numbers and therefore inappropriate for the intended application.

In a study by Yasuyuki et al. (2010), metals including titanium and molybdenum were tested for their antibacterial properties against two bacterial strains using a film contact method. The results showed titanium did not exhibit antibacterial properties, but molybdenum did. TEM images showed that metal accumulation resulted in the disruption of the bacterial cell wall and other cellular components. In terms of the innate antimicrobial ability of molybdenum, the soil could also have prevented bacteria from coming into close contact with the surface and retarded

Mo ion release, despite the lower concentration (below MIC), to inhibit the growth of the organisms. Further tests would be required to validate this. Numerous articles have been published with conflicting data regarding the effects of conditioning films on bacterial attachment. Barnes et al. (1999), treated stainless steel coupons with skimmed milk and subsequently challenged them with bacteria. They found milk reduced the adhesion of bacteria compared to untreated surfaces. Conversely, Verran et al. (2006), found significantly higher numbers of cells were retained on stainless steel surfaces treated with bovine serum albumin than on those without. Araújo et al. (2013), report on how in the presence of interfering substances, the effectiveness of quaternary ammonium compounds mildly to severely reduced the antimicrobial activity depending on the type of interfering substance. To compare results with other publications is, however, difficult due to differences in the types of microorganisms used, and the surfaces, conditioning films and operating conditions (Jullien et al. 2008). To our knowledge, no other reports have been published relating to the effect of soiling of TiO_2-Mo photocatalytic surfaces on microbial numbers.

The contamination of surfaces with heavy dried on soil did, however, present an unlikely exposure scenario. The application of dilute beer to the surface more closely mimics real brewery dispensing and bottling processes since automated rinsing with jets of water may be in place, which would cause spilt beer to become weaker. Dilute beer applied to the TiO_2-Mo surface resulted in a 5-log reduction of *P.rhodesiae* and *S.marcescens* and a 2-log reduction of *W.anomalus*. The diluted soil would not have formed such a carbohydrate rich thick layer, and this may have enabled photocatalysis and the innate antimicrobial effect to breakdown organic products and microbes. Ahmed et al. (2011), examined the photocatalytic degradation of human serum albumin (HSA) on TiO_2 and TiO_2-Ag films by immersing the surfaces in 0.5% w/v HSA in distilled water for 30 minutes. Changes in protein conformation consistent with denaturation and enhanced binding and oxidation, thought to be induced through a

photocatalytic mechanism, were found by Raman spectroscopy. The ability of photocatalysis to break down a low concentration of HSA may indicate a similar process with the dilute beer placed onto the TiO_2-Mo surface, as opposed to the decomposition of the heavier soil.

A similar study carried out by Priha et al. (2011) to determine whether process hygiene in the beverage industry could be improved by applying TiO_2 to stainless steel with or without added antimicrobial compounds was investigated in laboratory attachment tests and in a 15-month process study. Photocatalytic coatings containing silver (Ag) reduced microbial coverage in laboratory studies and in some process samples, but some of the TiO_2 coatings were damaged and most of the precipitated Ag had dissolved. From the TiO_2-Mo coatings, an initial burst of Mo ions followed by a decline to low steady state concentrations (< 0.04 ppm) was revealed. The lack of leaching of Mo ions from the surface would be of benefit in process trials, and it would that ensure undesirable chemicals would not reach the end product. Investigations to enhance the hygienic status of process surfaces in the brewing industry by application of the TiO_2-Mo coatings in comparison with stainless steel are underway.

In conclusion, the study highlights the development of a functional surface consisting of a thin film coating of TiO_2 doped with Mo deposited by magnetron sputtering, for use in enhancing the hygienic status of surfaces in environments where microbial fouling and surface conditioning is likely. The photocatalytic and discovery of the innate antimicrobial nature of the surface proved to significantly reduce microbial numbers even with the application of a dilute brewery soil. In addition to the exploitation of functional surfaces that may reduce fouling, the importance of regular cleaning still needs to be highlighted. Nevertheless, the surface may act as a secondary level defence against microbial populations and proliferation between disinfection/cleaning cycles. Ongoing brewery process studies will provide more information on the hygienic status of TiO_2-Mo compared to stainless steel and investigations into developing a photoactive but not innately antimicrobial surface are underway.

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Table 1. Dopant levels, predominant crystalline structure and photocatalytic activity levels under UV and fluorescent light irradiation for Mo-doped titania coatings deposited onto stainless steel substrates

Sample type	**Power to Mo target (W)**	**At.% Mo**	**Annealing temperature (°C)**	**Crystal structure**	**P_{aUV}**	**P_{aFL}**
TiO_2	0	0	600	Anatase	0.69	0.41
TiO_2-Mo	100	2.69	600	Anatase	0.73	0.54
TiO_2-Mo	150	5.48	600	Anatase/rutile	0.77	0.47
TiO_2-Mo	180	6.95	600	Anatase/rutile	0.88	0.72
TiO_2-Mo	240	11.81	600	Amorphous	0.57	0

Pa = photocatalytic activity values under UV light and fluorescent (FL) light

Table 2. Photocatalytic activity values of TiO_2 and TiO_2-Mo surfaces as determined by the degradation of methylene blue, under UV and fluorescent light illumination

Surface coating	**Treatment**	**P_{aUV}**	**P_{aFL}**
TiO_2	Unconditioned	0.69	0.41
TiO_2-Mo	Unconditioned	0.88	0.72
TiO_2-Mo	Conditioned	0.77	0.77
TiO_2-Mo	Cleaned following conditioning	0.70	0.54

Pa = photocatalytic activity values under UV light and fluorescent (FL) light

Table 3. Minimum inhibitory concentration of molybdenum against *Escherichia coli*, *Pseudomonas rhodesiae*, *Serratia marcescens* and *Wickerhamomyces anomalus*

Microorganism	**MIC (mg l^{-1})**
Escherichia coli	2 - 3
Pseudomonas rhodesiae	2 - 3
Serratia marcescens	2 - 3
Wickerhamomyces anomalus	10 - 15

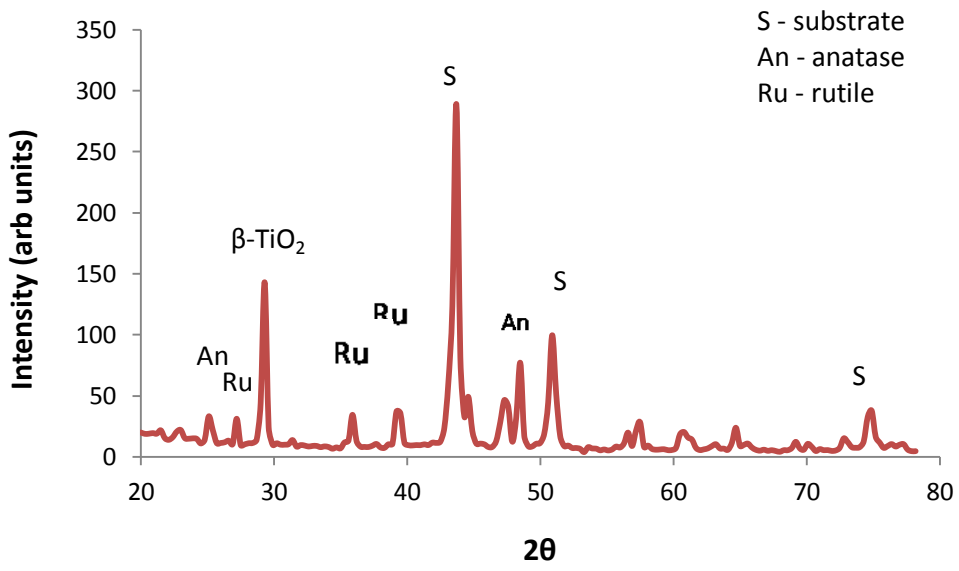


Figure 1.

XRD pattern of 6.95 at.% TiO_2-Mo surface showing the anatase/rutile mix structure

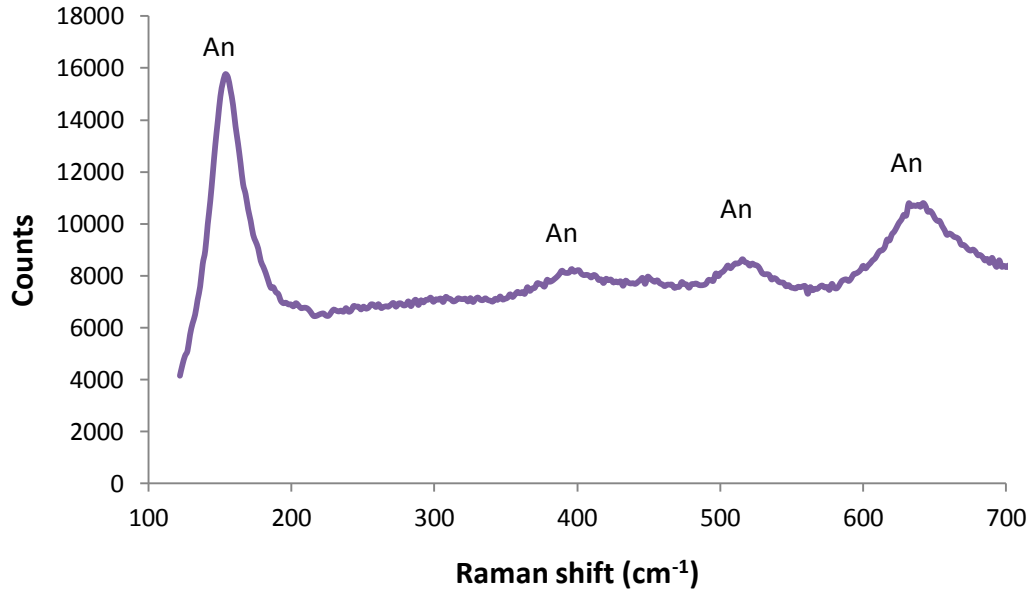


Figure 2. Raman spectra of 6.95 at.% TiO_2-Mo surface annealed at 600 ºC showing the crystal (anatase) structure of the coating

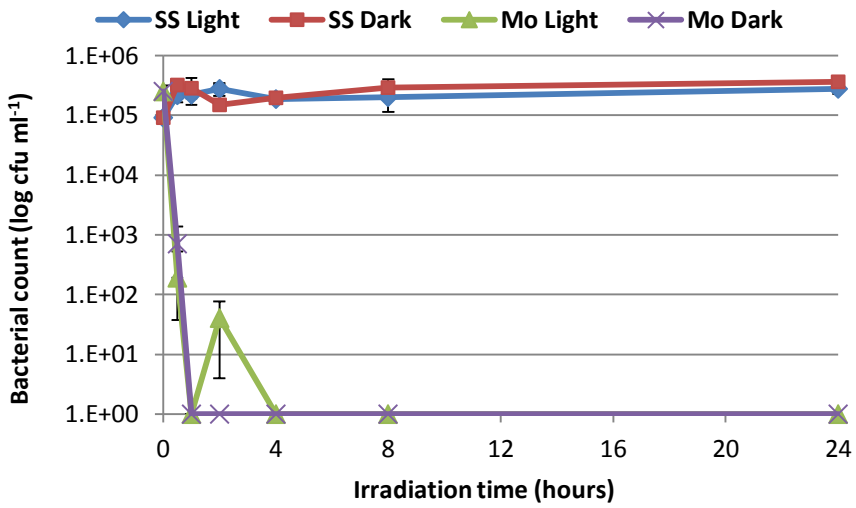
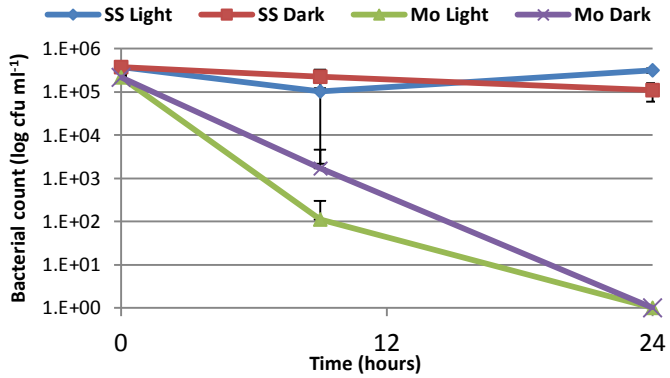
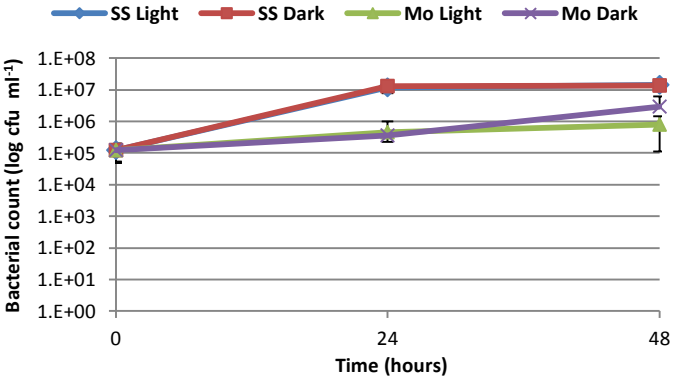


Figure 3. Antimicrobial effect of TiO_2-Mo surfaces on *Escherichia coli* without soil. Stainless steel surfaces were used as controls and light and dark conditions were investigated. The assay was performed in triplicate and each point represents the mean with standard deviation (SD). SD may not be visible at every point as they were very small and some lines may superimpose each other



(a)



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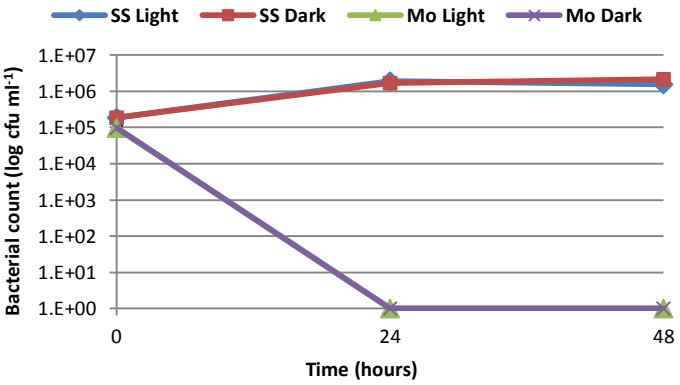
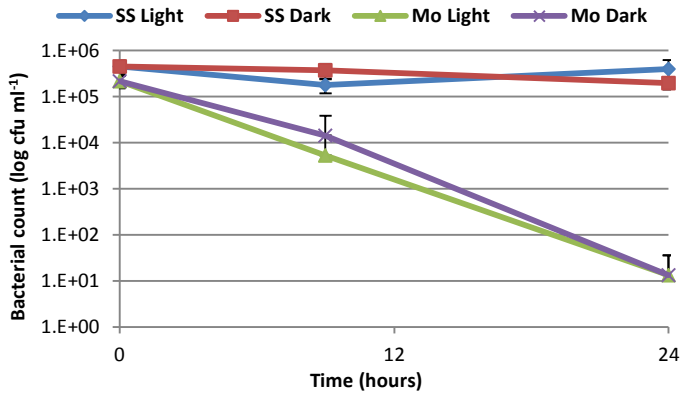


(c)

Figure 4. Antimicrobial effect of TiO_2-Mo surfaces on *Pseudomonas rhodesiae* **(a)** without soil, **(b)** with undiluted soil (beer), **(c)** with dilute soil (10% beer). Stainless steel surfaces were used as controls and light and dark conditions were investigated. The assay was performed in triplicate and each point represents the mean with standard deviation (SD). SD may not be visible at every point as they were very small



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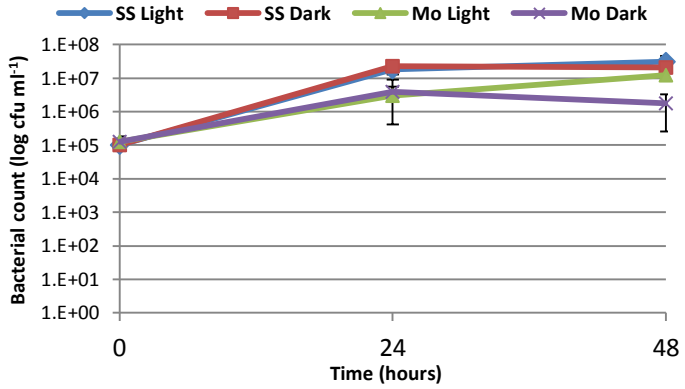

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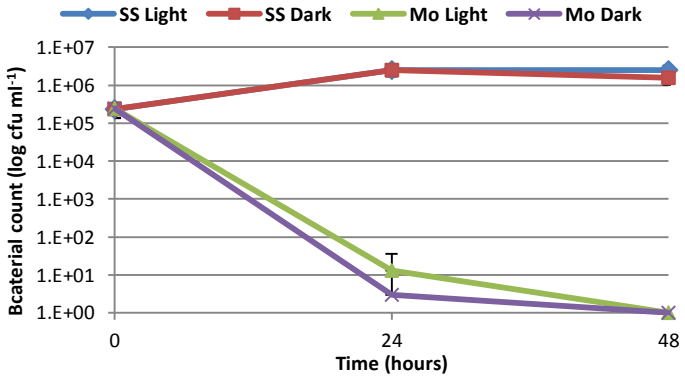
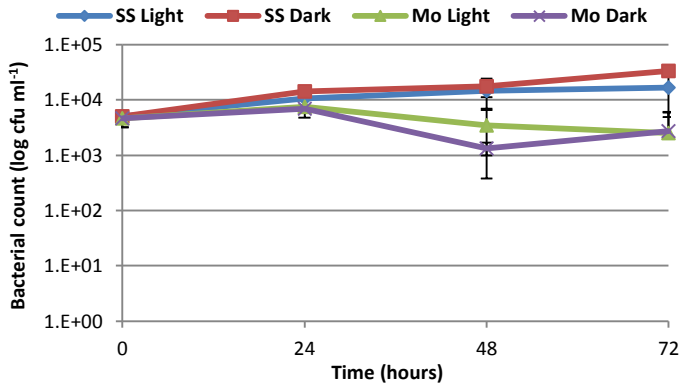
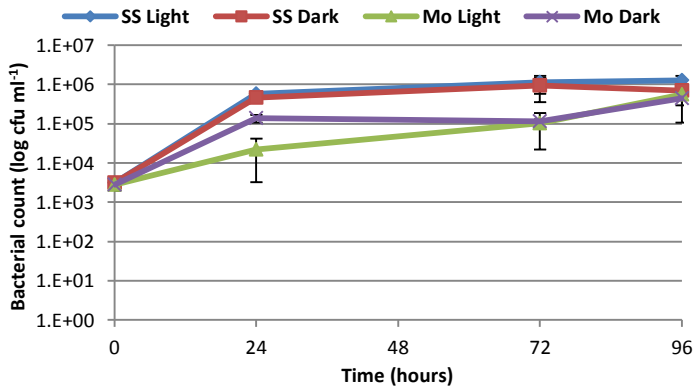


(c)

Figure 5. Antimicrobial effect of TiO_2-Mo surfaces on *Serratia marcescens* **(a)** without soil, **(b)** with undiluted soil (beer), **(c)** with dilute soil (10% beer). Stainless steel surfaces were used as controls and light and dark conditions were investigated. The assay was performed in triplicate and each point represents the mean with standard deviation (SD). SD may not be visible at every point as they were very small and some lines may superimpose each other



(a)



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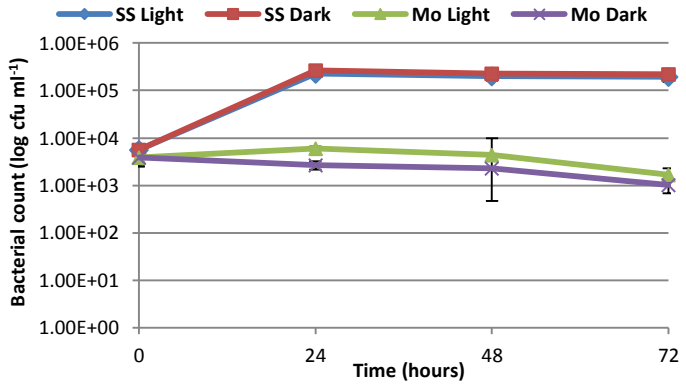

(c)

Figure 6. Antimicrobial effect of TiO_2-Mo surfaces on *Wickerhamomyces anomalus* **(a)** without soil, **(b)** with undiluted soil (beer), **(c)** with dilute soil (10% beer). Stainless steel surfaces were used as controls and light and dark conditions were investigated. The assay was performed in triplicate and each point represents the mean with standard deviation (SD). SD may not be visible at every point as they were very small and some lines may superimpose each other

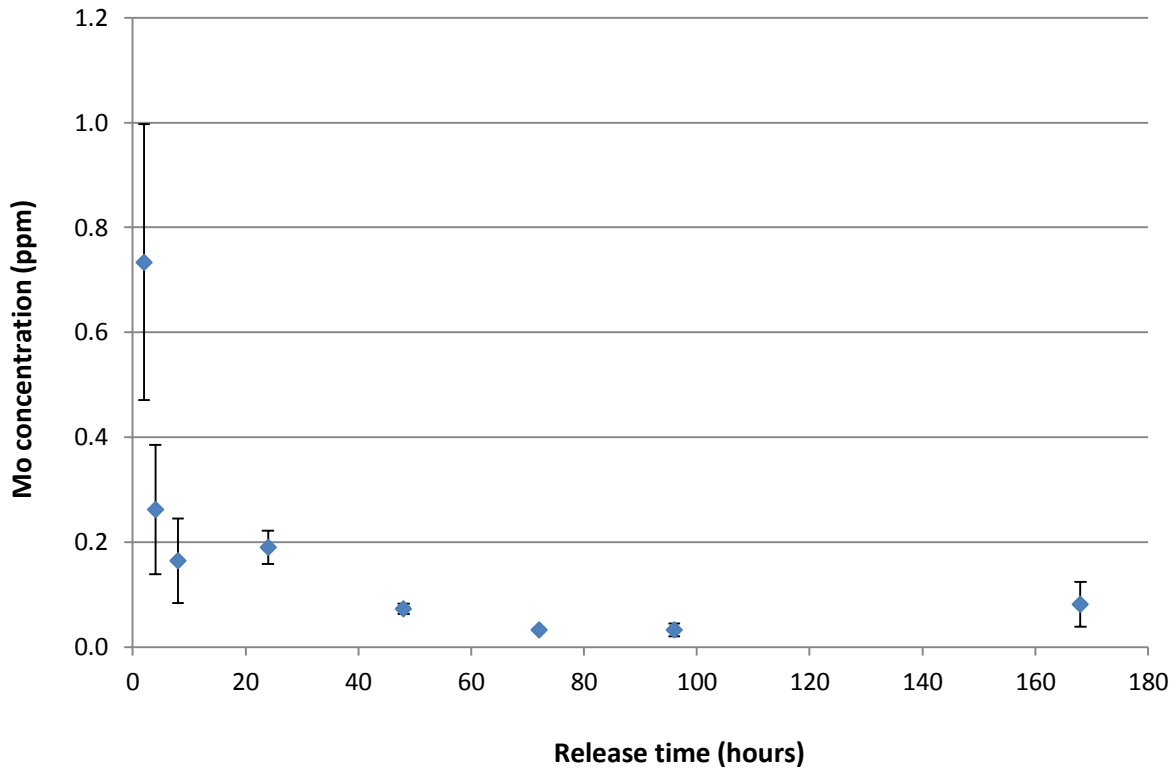

Figure 7. Molybdenum ion release determined by ICP-AES over 168 hours. Tests were performed in triplicate and each point represents the mean with standard deviation (SD)