An evaluation of ozonated water as an alternative to chemical cleaning and sanitisation of beer lines

Louise M. Fielding, Andrew Hall and Adrian C. Peters

Food Research & Consultancy Unit, Cardiff School of Health Sciences, University of Wales Institute Cardiff, Llandaff Campus, Western Avenue, Cardiff CF5 2YB, UK

Correspondence:
Louise M. Fielding, Food Research & Consultancy Unit, Cardiff School of Health Sciences, University of Wales Institute Cardiff, Llandaff Campus, Western Avenue, Cardiff CF5 2YB, UK.
Tel: +44-29-20416456;
Fax: +44-29-20416306;
E-mail: lfielding@uwic.ac.uk

Keywords:
beer line, biofilm, chemical cleaner, ozonated water, sanitiser

Abstract

A reduction in beer quality may arise from biofilm development in beer lines. Regular cleaning is, therefore, essential. A comparison between traditional chemical cleaner and ozonated water was made using a model dispense system, challenged with organisms isolated from a brewery (*Enterobacter* and yeast). The effect of each treatment on survival and biofilm formation at four sites in the model was investigated. Both systems reduced biofilm from initial levels by 3 (ozone) and 2.7 logs (chemical). Non-recirculation of media mimicked the effect of biofilm formation upon the microbial load of dispensed beer. Ozonated water gave a significantly higher reduction than chemical cleaner. Ozonated water also resulted in a reduction in microbial counts, with the exception of the fob detector, during a dispense protocol that mimicked normal-use conditions. Ozonated water has the potential for use as a cleaning and sanitisation agent for commercial beer lines as it leaves no residues, preventing possible product taint.

Introduction

Beer spoilage has been a long-standing problem for the brewing industry and its retailers. Problems may be caused by wild yeasts, such as *Candida*, *Brettanomyces* and *Zygosaccharomyces*, which cause the beer to become turbid, ropey or develop a yeasty aroma. Bacteria associated with beer spoilage include lactic acid bacteria such as *Lactobacillus brevis*, or acetic acid bacteria such as *Acetobacter aceti*. Production of diacetyl and dimethyl sulphide by these organisms can also give beer a stale odour and an off flavour (Campbell 1997). Spoilage by other bacteria such as *Enterobacter cloacae* is also important as these organisms can survive the fermentation process (Jespersen & Jakobsen 1996).

Problems may also occur at retail outlets such as pubs, bars and hotels. Contamination of beer lines occurs as beer contaminated with low levels of bacteria and yeast passes over the tubing surfaces as a drink is dispensed. In low numbers, the organisms and their by-products do not have any perceivable effect on the beer. Under commercial flow conditions, however, organisms can accumulate on the pipe surfaces in a structure called a biofilm. Biofilm formation is essentially a simple process comprising four key stages.

- Conditioning of the surface (adsorption of organic molecules onto the surface);
- Adhesion of cells to the surface;
- Production of glycocalyx and extracellular polysaccharide, which help the cells to stick firmly

Journal compilation © 2007, Blackwell Publishing Ltd *Journal of Foodservice*, **18**, pp. 59–68

to the surface and allow other cells to join the biofilm;

- Cell dissociation (Zottola & Sasahara 1994).

It is known that certain bacteria do cause problems with biofilm formation on food-contact surfaces. These organisms include *Pseudomonas* sp., *Klebsiella* sp. and *Enterobacter* sp. (Madigan & Martinko 2006). It is well reported that cells within a biofilm are more resistant to antimicrobial compounds such as commercially available cleaning solutions (Lewis 2001). In addition to this, many of the cells within the biofilm are physically protected from the effects of cleaning agents by the structure of the biofilm, and so are able to carry on growing normally after cleaning (Holah *et al.* 1994; Lewis 2001). It is the biofilm, therefore, which allows cells and their by-products to accumulate to such a level that they are able to spoil the product. A protocol that would destroy microorganisms and remove the biofilm would, therefore, be beneficial. It is important to define the terms cleaning and sanitisation as they are often misinterpreted. Cleaning is the removal of soil or dirt from a surface, and usually employs a detergent to achieve this. A detergent does not kill microorganisms. A disinfectant is a treatment that will kill microorganisms, reducing them to an acceptable level. A sanitiser is a chemical that consists of a detergent, and removes dirt, and a disinfectant, which reduces the number of microorganisms present. In terms of biofilm, the act of cleaning with a detergent alone to remove the biofilm will also remove a proportion of the microorganisms present. A sanitiser may have the added advantage of further reducing the level of microorganisms.

Ozone (O_3) is already used to disinfect drinking and waste water (Cho *et al.* 2003), and has proved effective against a number of organisms including *Cryptosporidium parvum* oocysts and *Clostridium perfringens* spores, which are relatively resistant to chlorine (Finch *et al.* 1993; Venczel *et al.* 1997). Ozone's ability to destroy microorganisms is due to its strong oxidising effect. The hydroxyl radicals (·OH), which are formed as a product of ozone decomposition, can have a similarly powerful biocidal effect (Cho *et al.* 2003). The biocidal properties of ozone are discussed in more detail by Staehelin & Hoigné (1985). The oxidising potential of ozone can also break down the biofilm structure and may be more effective at removing biofilm from surfaces. Better control of the biofilm could increase the length of time needed between cleaning operations.

The organisms used in this study were all isolated from a local brewery in Cardiff (UK) and were comprised of brewers yeast (*Saccharomyces cerevisiae*) and three unknown isolates, which were subsequently identified, using the API system (a series of biochemical tests to differentiate between different microorganisms), as wild yeast, *Enterobacter cloacae* and *Enterobacter* sp. As all of the organisms are naturally present in beer, their use should give a representative example of a biofilm likely to form in a beer line. The ozone used takes the form of ozonated water produced by a WL2 Advanced Oxidant Generator (AOG, Ozone Pollution Technology, Australia/IMI Cornelius, UK). The commercial chemical beer line cleaner (PROSAN plus, Proton Group Ltd., Normanton, UK) is actually a sanitiser as it contains a disinfectant component (6.5% sodium hydroxide and 2.5% sodium hypochlorite).

The aim of this research was to evaluate the use of ozonated water as an alternative to a traditional, chemical beer line cleaning agent against the consortium of microorganisms in a model beer line.

Materials and methods

Model beer line system

A model beer line system, based on that encountered in commercial premises, was designed and constructed. Two identical systems were created in parallel to allow direct comparison of two different cleaning methods. Identical conditions were maintained in each side of the system. All experiments were performed at ambient temperature (approximately 18°C). Schematic diagrams of the equipment can be seen in Figs. 1 and 2 and show the system configuration during normal media flow through the system or cleaning with the chemical cleaner. Figure 2 shows the system configuration during cleaning with the ozone.

The medium (the fluid pumped around the system representing beer) for the experiment was

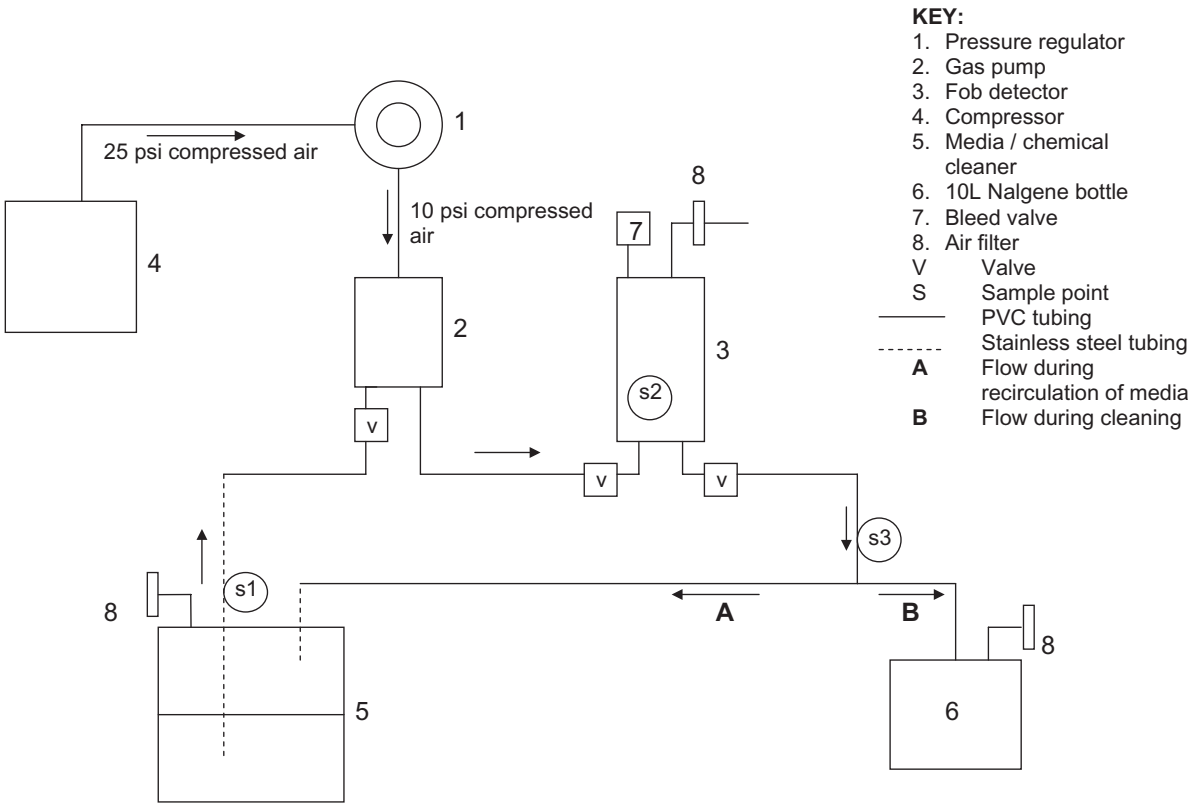

Figure 1 Schematic of the model system configuration during normal media flow through the system or cleaning with chemical cleaner.

housed in a 10-L jar and was circulated using a gas pump powered by an air compressor regulated to 25 pounds per square inch (psi). Stainless steel tubes connected the media jar to the system. The tubing used was braided flexible tubing, 3/8-in. internal diameter food grade polyvinyl chloride (PVC) tubing, typical of that found in the industry (IMI Cornelius). To allow recirculation, the air compressor was set to 25 psi, with the two gas pumps set at 10 psi to ensure that the flow rate through each system was the same. The system also incorporated a fob detector, which is a standard device allowing excess gas to be removed from the media, as in a typical beer line.

Culture selection and maintenance

All microorganisms were isolated from beer line systems and stored in Wallerstein Laboratory Nutrient agar (WLN agar, Oxoid, Basingstoke, UK), which is commonly used in the brewing industry. A mixture of organisms was prepared by inoculating one loopful of each organism into a flask containing 100 mL of tryptone soya broth (TSB, Oxoid) and incubated on a shaking platform (100 rpm) at 30°C for 18 h. Duplicate WLN agar streak plates were prepared to ensure that all organisms would grow on the medium and to identify the colonies formed by each organism. A typical colony was sampled and inoculated onto a WLN agar slope and refrigerated to form the stock culture. A working culture for each experiment was subcultured from the stock culture by removing a colony, using a sterile plastic loop, and releasing it into 100 mL of TSB in a 250-mL conical flask. This was incubated on a shaking platform as described earlier. This process ensured that exactly the same organisms, in the same phase of growth, were used in each experiment. Each organism was inoculated at a level of 50 mL in 5 L of WLN broth containing 185-mL alcohol and 320-mg calcium oxalate (modified WLN broth) to give a total initial inoculum level

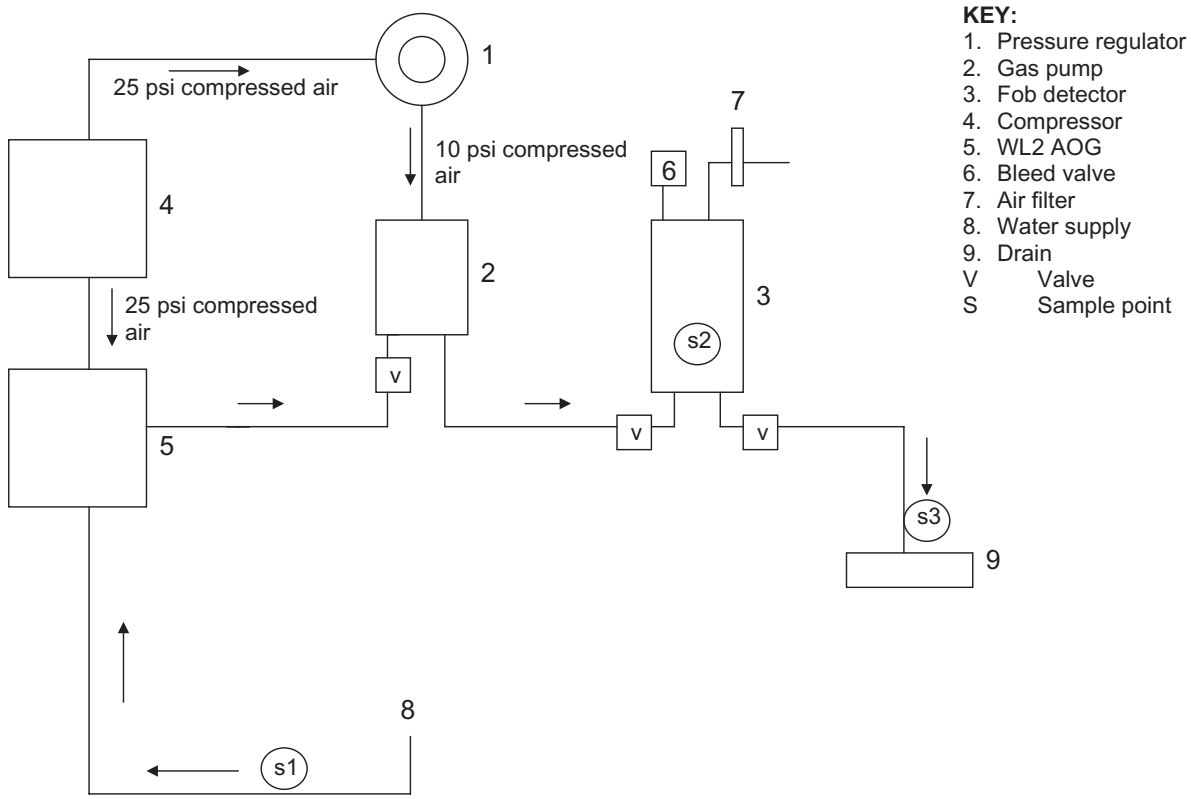

Figure 2 Schematic of the model system configuration during cleaning with ozone.

of approximately 1.6×10^7 colony forming units (cfu)/mL.

Growth of biofilm within the model system

The inoculated medium was recirculated through the system for an initial period of 2 h to allow attachment of the organisms to the surface of the tubing. This is demonstrated by flow path A in Fig. 1. The inoculated medium was then pumped completely through the system and was removed to waste to prevent excessive growth within the system. The stainless steel tubes were removed from the contaminated media and cleaned using an alcohol wipe to remove excess organisms, and were placed into 5 L of sterile-modified WLN broth, which was then recirculated for the duration of the experiment. Replacement of the inoculated media with sterile media was necessary because of the high numbers of bacteria present in the system after the 2-h recirculation of the inoculated medium. Sampling of the tubing occurred at 0, 24, 48 and 72 h.

Sampling of tubing

A 10-cm length of the effluent tubing and a pipe cutter were cleaned using an alcohol wipe. A pair of forceps was dipped in 70% w/v industrial methylated spirits and was flamed. The beer line tubing was removed from the connector on the fob detector, and three 3-cm lengths were cut from it aseptically, using the previously cleaned pipe cutter, and were placed into a sterile Petri dish. The end of the tubing was again wiped using an alcohol wipe and was inserted back into the connector. Each of the three samples of tubing was aseptically removed from the Petri dish using flamed forceps, was placed into a universal bottle containing 10 mL of maximum recovery diluent (MRD, Oxoid BM0204) and was mixed on a vortex mixer for 10 s to remove any unattached cells. To ensure that the entire internal surface was swabbed, the following protocol was used, swabbing the upper surface only each time. The tubing sample was removed from the MRD, and a pre-moistened swab was pushed through the

tubing and pulled back through twice. The tubing was then rotated through 90°, and the swabbing was repeated. The swab was then rotated through 180°, and the tube was rotated another 90°, and the swabbing was repeated. The tubing was rotated by 90° one last time and was swabbed.

The swab was placed into the 10-mL MRD and vortexed for 10 s to remove the cells from the swab tip. Serial 10-fold dilutions were prepared and 0.1 mL of these dilutions was used to prepare duplicate aerobic spread plates on WLN agar, which were incubated at 30°C for 24 h. Anaerobic plates were also prepared as described earlier, and incubated in a gas jar with a Campygen sachet (Oxoid) to remove the oxygen.

Sampling of the fob detector and intake tubing

At 72 h, the fob detector and intake tubing were also sampled. The intake was swabbed in the same way as the rest of the tubing. A grid was marked onto the outside of the fob detector bowl dividing the front into three 5-cm^2 areas. Each of these was swabbed with a pre-moistened swab using a standard technique (the swab was moved over the area, left to right, rotating the swab a quarter turn every five movements. The swab was then moved over the area, top to bottom, rotating as previously described). This technique was used for both systems to ensure comparable results.

Ozone generation

The ozonated water was generated by a WL2 AOG at a flow rate of 1.7 L/min. To demonstrate the 'ozone demand' of the system, a Hach kit (Hach Lange Ltd, Manchester, UK) was used to measure the ozone remaining in the water after it had passed through the dirty system. It is important that there is an ozone left in the water at the end to ensure that the ozone was present during the entire cleaning process. A 72-h biofilm was developed to give a high ozone demand. The WL2 AOG was switched on, and three replicates of the water exiting the system were sampled at 0, 5, 10, 30 and 60 min. The ozone concentration was also measured at the point of production using an Orbisphere Analyzer (Model 3600/313E, Orbisphere Laboratories, Neuchatel/Geneva, Switzerland).

The effect of cleaning on biofilm

The two pieces of intake tubing were detached from the stainless steel connectors. One was attached to the WL2 AOG and the other was placed into a Nalgene bottle Fisher Scientific, Loughborough, UK containing a 10% solution of the commercial chemical beer line cleaner made according to the manufacturer's instructions (100 mL/L). The two systems were then cleaned.

For the chemical cleaning system, the intake was attached to a stainless steel tube and the chemical cleaner was pumped through until 1 L had been collected at the outlet. The system was left for 10 min, flushed through for 20 s and allowed to stand for 10 min. It was flushed through for a further 20 s and allowed to stand for 10 min. The remaining cleaner was also flushed through. The flow is shown by path B in Fig. 1.

When using the ozonated water to clean, the WL2 AOG was connected to the water supply and the air compressor was regulated to 25 psi. The outlet of the WL2 AOG was connected to the intake of system 1 and water was run off to waste, controlled by a valve. The WL2 AOG was turned on and run at a flow rate of 1.7 L/min for 60 min. The flow path is shown in Fig. 2.

Following cleaning, each system was sampled as described earlier. Before sampling, 5 L of sterile deionised water was run through each system. This was found to be sufficient in removing all residual chlorine from the system (effluent samples were taken and tested using a free-chlorine test kit). Serial dilutions and spread plates were prepared as described earlier. To evaluate the potential for regrowth, 5 L of fresh media was recirculated for 72 h, and all areas of the system were sampled as described earlier. The effect of numerous cleaning cycles upon the microbial populations of the two systems was also evaluated. A 72-h biofilm was established as described earlier and, during this time, samples were taken to create a second set of growth curve data. The system was then cleaned, and the fresh medium was recirculated for 72 h. After 72 h, the system was sampled and then cleaned again. This sequence was repeated so that the system was cleaned and allowed to 'recover' for 72 h for a total of four cycles.

The same sampling techniques were used throughout.

The effect of cleaning on media contamination

The effect of cleaning on the microbiological quality of the beer (represented by media) in the period between cleaning was also investigated. The medium was not, therefore, recirculated and was sampled and drained directly to waste as shown by flow path B in Fig. 1. This allowed the media to be sampled and gave a true reflection of the number of organisms that could be present in the beer during the week following cleaning.

To avoid excessive growth, a new medium was designed to represent the beer, which would not provide excess nutrients to the organisms. The new medium was based on a typical keg bitter and consisted of 9.7-L distilled water, 300-mL absolute ethanol, 115-g glucose, 15-g peptone and 2.5-g yeast extract. All components, with the exception of the alcohol, were mixed in a 10-L Nalgene bottle and autoclaved at 121°C for 15 min. Immediately before the experiment, the alcohol was added aseptically and the bottle was inverted twice to mix thoroughly.

The beer lines were designed to mirror the conditions experienced by in situ beer line equipment. The flow rate was controlled, using a flow regulator, at the rate of 0.568 L (1 pint) in 20 s. The activity of the gas pumps was controlled using a solenoid valve and a timed mains connector. The solenoid valve switched the system on for 20 s and then off for 14 min, 40 s. The solenoid was controlled using a 240-v timer to operate for 4 h every day, pumping 9.1 L (16 pints) in 24 h. Duplicate 1-mL pour plates using tryptone soya agar (CM0313) were prepared.

Data analysis

Data were collated using Excel (Microsoft, Redmond, WA), and plate counts were calculated. All counts were transformed into log values, and the log reduction values were calculated (mean log control data–mean log sample data). This allows us to see more clearly the effect of the different cleaning regimes as 1 log indicates a 90% reduction, 2 log, a 99% reduction and so forth. Log reduction results were imported to Minitab12 (Minitab Inc., State College, PA), a statistical analysis package. The data were analysed using one-way and two-way analysis of variance (ANOVA) as appropriate. Significant differences are reported where $P < 0.05$.

Results

Growth of biofilm in the model system

The growth curve data (not presented) showed an increase in microbial growth in the tubing from 0 h (2.85 log cfu/mL aerobic and 2.90 cfu/mL anaerobic) to 72 h (7.39 log cfu/mL aerobic and 7.10 cfu/mL anaerobic). The fob detector and the intake tubing were also examined, and these displayed considerable growth of biofilm during the 72-h period (fob detector, 6.94 log cfu/mL aerobic and 5.88 cfu/mL anaerobic; intake, 7.36 log cfu/mL aerobic and 7.27 cfu/mL anaerobic). This demonstrated that the organisms were able to grow well under experimental conditions. There was no significant difference between aerobic and anaerobic counts.

WL2 AOG ozone levels

The Hach kit (Hach Lange Ltd) was used to test the ozonated water exiting the beer line at various times into the cleaning schedule over three cycles. The results (not presented) demonstrated that, over the three runs carried out, the level of ozone in the water increased from 0.1 mg/L at time 0 min until it exceeded 2.3 mg/L after the 60-min cleaning. This indicated that, as cleaning progressed, there was a reduction in the amount of oxidisable material present, reducing ozone depletion in the water over time. The Orbisphere analyser demonstrated that the ozone concentration at the point of production was 3.5 mg/L (equivalent to 3.5 ppm).

The effect of cleaning on biofilm

Table 1 shows the results obtained following cleaning with ozonated water or with chemical beer line cleaner. In all cases, the ozone led to a greater reduction in the number of bacteria present, achieving a reduction of between 0.58 and 1.15 log (aerobic), and 1 and 1.42 log (anaerobic) greater than the chemical cleaner.

Table 1 The log cfu/mL organisms recovered following cleaning with ozonated water or chemical cleaner after initial biofilm development. The figures in brackets denote the log reduction

	System 1, ozone (log cfu/mL)		System 2, chemical cleaner (log cfu/mL)	
Sample type	**Aerobic plates**	**Anaerobic plates**	**Aerobic plates**	**Anaerobic plates**
Intake (S1)	3.12 (4.24)	3.10 (4.17)	4.23 (3.13)	4.10 (3.17)
Fob detector (S2)	3.85 (3.09)	3.12 (2.76)	4.43 (2.51)	4.17 (1.71)
Tubing (S3)	3.62 (3.77)	3.60 (3.50)	4.77 (2.62)	5.02 (2.08)

Table 2 The log cfu/mL organisms recovered from the three sample points following recirculation with fresh media after cleaning and subsequent regrowth of the biofilm

	System 1, ozone (log cfu/mL)		System 2, chemical cleaner (log cfu/mL)	
Sample type	**Aerobic plates**	**Anaerobic plates**	**Aerobic plates**	**Anaerobic plates**
Intake (S1)	6.49	6.09	6.03	6.02
Fob detector (S2)	5.77	n.d. at 10^{-4}	5.78	n.d. at 10^{-4}
Tubing (S3)	7.13	6.30	7.09	6.43

n.d., not detected.

Following cleaning, the potential for regrowth in the system was investigated. The results for this are presented in Table 2, and show that after cleaning with either ozonated water or chemical cleaner, there was a considerable regrowth of biofilm in the system. There was, however, an overall reduction in microbial load of the components tested compared with the initial levels, before any cleaning operation.

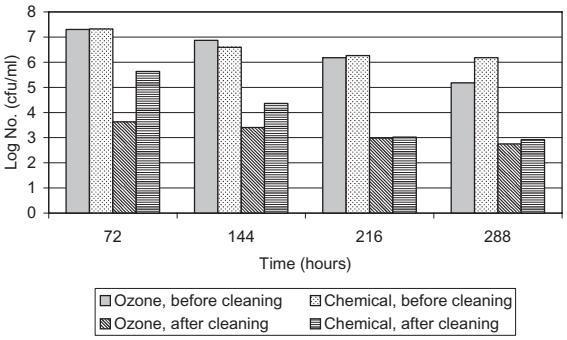

Figure 3 The effect of repeated cleaning cycles with ozonated water or chemical beer line cleaner on the aerobic colony count of the tubing.

The effect of repeated cleaning cycles on biofilm development

The results obtained using a repeated cycle of cleaning and regrowth on the aerobic and anaerobic colony count of the tubing can be seen in Figs. 3 and 4 respectively. A two-way ANOVA showed that the use of ozonated water resulted in a significantly greater reduction overall, compared with the chemical cleaner. This was particularly noticeable in the first and second cleaning cycles. There was no difference between the two types of cleaning on the third cleaning cycle, but after the fourth cycle, the use of the chemical cleaner gave a greater log reduction than the ozonated water. When the intake tubing was analysed, there was a difference between the types of cleaner on the first cleaning only, with the ozonated water giving a greater log reduction. There were no differences between the ozonated water and the chemical cleaner for the fob detector.

The two-way ANOVA also determined the interaction between the type of cleaning and cleaning time (i.e. 72, 144, 216, 288 and 360 h). The analysis showed that there was a reduction in the number of organisms present after each successive cleaning. There was a significant difference in the log reduction between the second and third cleaning cyctes. Between the third and the fourth

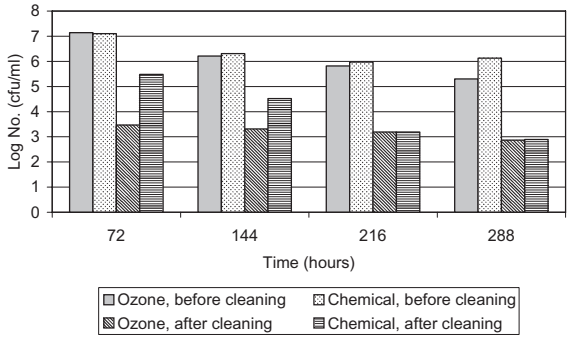

Figure 4 The effect of repeated cleaning cycles with ozonated water or chemical beer line cleaner on the anaerobic colony count of the tubing.

cleaning cycles, the reduction is diminished, but one factor that should be considered is that there were fewer organisms present before cleaning.

Similar trends were seen when the intake tubing was analysed. The two-way ANOVA showed that the first and third cleaning cycles gave significantly greater log reductions than the second and fourth cleans ($P \leq 0.05$). Again, the fourth cleaning gave the lowest log reduction from the aerobic plate count data. When the fob detector was cleaned, the two-way ANOVA showed that there was no significant difference between the log reduction of the first two cleaning cycles, but the reduction increased significantly for the third and fourth cycles ($P \leq 0.05$). Overall, the reduction at this site was significantly lower than the other sites.

The effect of cleaning on contamination of media

The two-way ANOVA showed that there was a significant difference in the results obtained for the ozone and the chemical cleaning agents. This was true over the duration of the experiment with the log cfu/mL being significantly lower for the ozone compared with the chemical cleaner after both the first and second cleaning cycles ($P \leq 0.05$ after both cleaning cycles). After just two cleaning cycles (at 0 and 168 h), the ozone cleaning regime resulted in a significantly lower level of contamination in the dispensed medium. This demonstrates that ozone cleaning has the potential to reduce beer contamination and to increase the period between cleaning cycles.

Discussion

The use of ozone as an antimicrobial agent has been known for a number of years, including its role in water decontamination (Gurley 1985). The application of the technology to beer lines is, however, novel, and the effect of ozone on biofilm has received little attention. Although there are health and safety occupational exposure limits for ozone in air (0.2 ppm) (Anon 1998), these do not apply to ozonated water. This allows the highly effective level of ozone produced by the WL2 AOG to be used safely and legally.

The model system mimicked a commercial beer line system in that the major components of such a system were present. It was not designed to be a replica of a commercial system so it did not include features such as a chiller unit or dispense head. The components of the system that were tested were those that were perceived to be at most risk from biofilm development – the intake, the internal tubing and the fob detector. A traditional medium used in the brewing industry (WLN broth) was employed in the initial experiments where the medium was recirculated. This was also tested to assess the effects of biofilm formation and cleaning on the microbial load of the medium. A new medium was designed to represent the characteristics of beer and was used to determine the effect of cleaning on media contamination. This set of experiments more closely mirrored the conditions found in a commercial beer line system, where the medium was dispensed and no recirculation occurred.

The growth curve experiments demonstrated that the consortium of organisms used was able to colonise the system and form a biofilm within 72 h. This supports previous work (Peters *et al.* 2002), which showed a significant biofilm development in a chilled water line after 72 h. Once the system was cleaned with either the ozonated water or the chemical beer line cleaner, there was a reduction in the number of both aerobic and anaerobic organisms. The log reductions observed were greater for the system that had been cleaned with the ozonated water than that which had been cleaned with the chemical beer line cleaner during the initial cleans only, when the system was at its most contaminated. Beyond the second clean, when the number of organisms

had been reduced, there was no significant difference between the two types of cleaner. The chemical cleaner achieved a better reduction on the fourth cleaning of the tubing. It should be remembered that both systems had fewer organisms present during the later cleaning stages so it is not unexpected that the reduction in numbers was lower. There was no significant difference between the two cleaning protocols when the fob detector was tested. One explanation for this is that there is a little flow of cleaning agent (either ozonated water or chemical) through the fob detector during cleaning. The agents, therefore, have little impact upon this area.

When the system was recirculated with new, uninoculated medium to assess the potential of the biofilm to regrow within the system, the regrowth occurred in each of the four areas tested (tubing, intake, fob detector and media), but the initial level before cleaning was not attained in the majority of the areas. In each case, there is a progressive downward trend over the four cleaning cycles. The specific log reductions after each cleaning have been discussed, and there does not seem to be a pattern regarding which cleaning gave the overall best results at each sample point. The downward trend is, however, apparent at all sample points. Repeated cleaning, beyond four cleaning and regrowth cycles, may result in a 'tailing off' of this downward trend as the microorganisms left in the system may be more resistant to the cleaning agents used. Alternatively, the number present after regrowth may continue to decline until the presence of a biofilm is undetectable, if there is not a resistant population.

When the medium was not recirculated, the use of ozonated water as a cleaning agent gave rise to a significantly greater reduction in the microorganisms present than the chemical treatment. In this case, only the medium was sampled. It is apparent then that the recirculation of the medium leads to continual recontamination as the medium passes through the system. When the fresh medium was used, a system more closely resembling real-life conditions, any contamination of the medium had arisen from one pass through the system. The real effect of ozone can, therefore, be seen as the medium is not becoming recontaminated by repeated passage through the system. It would be useful to see the effects of repeated cycles of cleaning and regrowth in a commercial system with beer as the medium.

Conclusion

The potential for the use of ozonated water as a replacement for chemical beer line cleaner has been demonstrated as it results in a significantly better or comparable reduction in the microbial load in the majority of the samples tested, and it leaves no residue in the system, leading to a reduction in product taint. This could result in improved cleaning of beer lines and potentially other drink dispensers, and offer a better quality product for the consumers.

Acknowledgements

The authors would like to thank IMI Cornelius for designing and constructing the model beer line system, and the University of Wales Institute Cardiff UWIC Research Opportunity for funding the project.

References

Anonymous (1998) Ozone: health hazards and precautionary measures. Guidance Note EH38 (revised). Health and Safety Executive: UK.

Campbell I (1997). Beer. In: *Food Microbiology – Fundamentals and Frontiers* (eds M Doyle, L Beuchat & T Montville), pp. 662–7. ASM Press: Washington DC.

Cho M, Chung H, Yoon J (2003). Disinfection of water containing natural organic matter using ozone-initiated radical reactions. *Applied and Environmental Microbiology* **69**:2284–91.

Finch G, Black E, Gyürék L, Belosevic M (1993). Ozone inactivation of *Cryptosporidium parvum* in demand free phosphate buffer determined by in vitro – excystation and animal infectivity. *Applied and Environmental Microbiology* **59**:4203–10.

Gurley B (1985). Ozone: pharmaceutical sterilant of the future? *Journal of Parenteral Science and Technology* **39**:256–61.

Holah J, Bloomfield S, Walker A, Spenceley (1994). Control of biofilms in the food industry. In: *Bacterial Biofilms and Their Control in Medicine and Industry* (eds J Wimpenny, W Nichols, D Stickler & H Lappin-Scott), pp. 163–8, Bioline: Cardiff, UK.

Jespersen L, Jakobsen M (1996). Specific spoilage organisms in breweries and laboratory media for their detection. *International Journal of Food Microbiology* **33**:139–55.

Lewis K (2001). Riddle of biofilm resistance. *Antimicrobial Agents and Chemotherapy* **45**:999–1007.

Madigan MT, Martinko JM (2006). *Brock. Biology of Microorganisms*. Pearson Education Inc.: Upper Saddle River, NJ.

Peters A, Drake J, Saltmarsh M (2002). Heterotrophic bacteria and biofilm development in vending machines. The NSF International/WHO Symposium on HPC Bacteria in Drinking Water. Public Health Implications? Conference proceedings, pp. 377–93, 22–24 April 2002, Geneva, Switzerland.

Staehelin J, Hoigné J (1985). Decomposition of ozone in water in the presence of organinc solutes acting as promoters and inhibitors of radical chain reactions. *Environmental Science and Technology* **19**:1206–13.

Venczel L, Arrowood M, Hurd M, Sobsey M (1997). Inactivation of *Cryptosporidium parvum* oocysts and *Clostridium* spores by mixed oxidant disinfection and by free chlorine. *Applied and Environmental Microbiology* **63**:1589–601.

Zottola E, Sasahara K (1994). Microbial biofilms in the food industry – should they be of concern? *International Journal of Microbiology* **23**:125–48.