Research article

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Cleaning in place with onsite-generated electrolysed oxidizing water for water-saving disinfection in breweries

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The use of acid electrolysed water (AcEW) is a relatively new sanitizing technique for brewery equipment. Experiments showed that a 30% AcEW (a free chlorine concentration of above 17 mg/L) was a sufficient and effective alternative to conventional sanitizers such as peracetic acid (2%). On the basis of the results of industrial-scale clean-in-place tests, an effective AcEW-based clean-in-place procedure, which requires only 10 min of cleaning and does not require final water rinsing after sanitation, was established for the bright beer tank. Copyright © 2013 The Institute of Brewing & Distilling

Keywords: brewery; cleaning in place (CIP); electrolysed oxidizing water; acid electrolysed water (AcEW); sanitizer

Introduction

For most brewers obtaining a product with consistent characteristics is important, but this objective is difficult to achieve given that effective production is frequently impeded by bacterial contaminants present in breweries *(1)*. Biofilm control in brewery manufacturing plants generally involves a process called clean-in-place (CIP), defined as the 'cleaning of complete items of plant or pipeline circuits without dismantling or opening of the equipment and with little or no manual involvement on the part of the operator' *(2)*. CIP systems generally involve the sequential use of caustic and acid wash steps and a sanitizer step, which is crucial for removing or killing beer spoilage bacteria. Most sanitizers commonly used in brewery CIP consist of diluted condensed solutions, such as hydrogen peroxide (H_2O_2), chlorine dioxide (ClO_2) or peracetic acid *(3,4)*. The dilution process can involve risks and can be difficult to manage. In addition, some sanitizers contain additional ingredients such as heavy metals, intended to enhance stability, and which require thorough washing with running water to avoid the release of toxic product residues. An alternative sanitizer, which is not produced from the dilution of hazardous condensed solutions, is therefore required for practical use *(5,6)*.

Electrolysed oxidizing water is made by electrolysing dilute (0.1–0.2%, w/w) sodium chloride (NaCl) solution on a commercially available apparatus. Electrolysis is based on the preparation of a disinfectant called an anolyte, produced by membrane electrodialysis with the NaCl solution *(7–9)*. In the anode cell, water reacts with the anodic electrode, thereby producing oxygen and hydrogen ions. Chlorine ions also react with the electrode, generating chlorine gas and hypochlorous acid (HOCl) *(10)*. This solution is called acidic electrolysed water (AcEW). AcEW normally has a pH of 2.7 or lower, an oxidation–reduction potential (ORP) greater than 1100 mV, and a free chlorine concentration of 10–80 mg/L in the form of HOCl. Researchers have previously evaluated the effectiveness of AcEW in inactivating many food-borne pathogens, including *Escherichia coli*, *Salmonella enteritidis* and *Listeria monocytogenes (11–13)*. The researchers obtained a considerable reduction in logarithmic units of colony-forming units (CFU) when compared with the initial population.

Although AcEW has been used as a disinfectant against bacterial pathogens in a variety of applications, such as in the medical testing area and in food processing *(13–15)*, studies on the use of AcEW with brewery equipment have not been extensively reported on. In the current work, the effectiveness of AcEW as a sanitizing agent for internal CIP systems of breweries was evaluated with the focus on the bright beer tank. An effective AcEW-based CIP procedure, which does not require a final water rinse after sanitation, was established.

Materials and methods

Acidic electrolysed water

Laboratory scale. AcEW was prepared in a laboratory-scale apparatus (GRDJ-1000, ZHOUJI, China). To prepare 1 L AcEW stock solution, 2 L of 0.05% (w/w) NaCl in softened water was electrolysed under a current of 15 A for 1 min at room temperature.

Industrial scale. The industrial generator comprised several components including a water softener, brine tank, electrochemical cell, oxidant tank and metering pumps, which feed the AcEW into the process stream (Fig. 1). To prepare 1.7 L AcEW stock solution, 3.4 L of 0.05% (w/w) NaCl in softened water was electrolysed under a current of 15 A for 1 min at room temperature.

The ORP and pH were measured using an electrometer (PB-20, Sartorius) equipped with an ORP and pH sensor. Free chlorine

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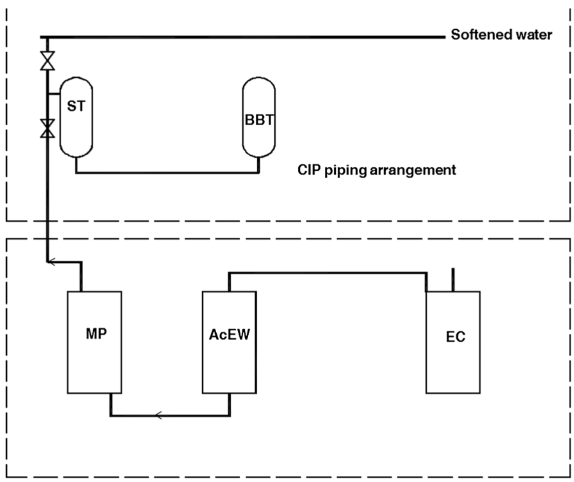

Figure 1. Industrial generator combined with CIP piping arrangement. BBT, Bright beer tank; ST, sanitizer tank; EC, electrochemical cell; MP, metering pump.

content was measured with a chlorine meter (Chlorometer Duo, Palintest, England).

Strains and growth parameters

The following microorganisms were used: *Lactobacillus brevis* (*L. brevis*, DSMZ 20054), *Saccharomyces diastaticus* (*S. diastaticus*, ATCC 28338) and *Bacillus subtilis var. niger* (*B. subtilis*, ATCC 9732). *L. brevis* was cultured in de Man-Rogosa-Sharpe medium for 48 h at 26°C. *S. diastaticus* was cultured in yeast mould broth for 48 h at 25°C. *B. subtilis* was cultured for 3 days to enable spore formation in heart infusion broth (Difco Laboratory, Detroit, MI, USA). The microbial colonies were washed twice in phosphate-buffered saline (PBS) and re-suspended in PBS by adjusting the concentration to McFarland #1.

Assay for bactericidal activity

The AcEW stock solution, directly made using the electrolysis apparatus, was diluted with water to produce working solutions of different concentrations of free chlorine. Bactericidal activity was determined by mixing 10 mL of microbial suspension with 90 mL of AcEW working solution, and the mixture was incubated for a designated time period. Peracetic acid solution (2%, v/v) was used as the control assay. After incubation, free chlorine or peracetic acid was inactivated by adding 150 mL of 0.5 % (w/w) sodium thiosulfate solution ($Na_2S_2O_3$).The final mixtures were cultured to determine the CFUs of the residual viable microorganisms. The results for the mixtures with sanitizers were compared with those for the original microbial suspension to evaluate the bactericidal activity. The results were expressed as CFU/mL.

Corrosion studies

Corrosion in CIP systems is a major concern in the brewery manufacturing processes. An extensive corrosion research programme was developed in this study to prevent the AcEW from causing corrosion in equipment made from stainless steel 304 or carbon steel A36 (two alloys commonly used in brewing plants).Stainless steel 304 and carbon steel A36 were immersed in an AcEW stock solution and 2% (v/v) peracetic acid solution, respectively. Samples were removed from the solutions at various points during the 15-day immersion period; the samples were then cleaned and weighed. The changes in weight were used to calculate uniform corrosion rates. The solution was changed every 3 days to ensure an active ingredient concentration. Corrosion rate was calculated using the following equation *(16)*:

$$R = \frac{m_1 - m_2}{m_1} \times 100\%$$

where R is the corrosion rate; m_1 is the weight before immersion; m_2 is the weight after immersion.

Forcing test

Bottles of beer were stored at 60°C for 6 days, and then at 0°C for 2 days. The contents of a bottle were poured into a glass cuvette (without degassing), and haze was measured with an Lg-automatic model ApS haze meter. The measurement was expressed in European Brewery Convention (EBC) units. A haze below 1 EBC was considered acceptable.

Beer aging conditions and freshness evaluation

Fresh beer was aged at 37°C for 7 days in a dark room. After aging, the beer was stored at 4°C until use. A trained panel of five members evaluated the freshness level of the aged beer samples, presented randomly in one session to the panellists. Freshness was evaluated according to a scale of 1–10: >8 = fresh; 6–8 = slightly aged; 4–5 = moderately aged; 1–3 = fully aged.

Results and discussion

Free-chlorine concentration, pH, and ORP of AcEW

The free chlorine concentration, pH, and ORP of the AcEW were measured, and the results are shown in Table 1. The dilution of the stock solution decreased the free chlorine concentration, increased the pH and decreased the ORP. The concentration of free chlorine in the stock solution was 10-fold higher than that in the working solution (10% of the original proportion). No significant difference between the pH and ORP of the stock and working solutions was found.

Laboratory-scale studies

Comparison of bactericidal effects of the AcEW and peracetic acid. Table 2 illustrates the effects of AcEW on the survival of beer spoilage bacteria under different concentrations of free

Table 1. Free chlorine concentration, pH, and oxidation-reduction potential (ORP) of acid electrolysed water (AcEW)

	Original proportion	Free chlorine (mg/L)	pH	ORP (mV)
Stock solution	100%	52	2.44	1178
Working solution	70%	38	2.67	1159
	50%	27	2.99	1134
	30%	17	3.19	1105
	20%	10	3.24	1025
	10%	5	3.30	998

Table 2. Effect of AcEW and peracetic acid on microorganism growth

Sanitizer	Working solution	Growth (CFU/mL)[a]		
		B. subtilis	*S. diastaticus*	*L. brevis*
AcEW	10%	1910	988	0
	20%	872	0	0
	30%	0	0	0
	50%	0	0	0
	70%	0	0	0
	100%	0	0	0
Peracetic acid	2%	0	0	0
Original suspension[b]		6.3×10^6	2.7×10^6	3.2×10^6

[a]Duplicate experiments were performed, and almost identical results were obtained.
[b]The CFU of the original suspension in the assay was calculated from that of the original bacterial suspension.

chlorine. The results showed that 30% of the original proportion of AcEW completely inhibited the growth of *B. subtilis* (approximately 6.3×10^6 CFU/m) within 5 min. According to Delgado *et al. (17)*, an effective disinfectant solution should reduce the population by 5.0 log CFU. As a result, 30% of the original proportion of AcEW, with a free chlorine concentration of above 17 mg/L, was evaluated as an effective sanitizer in internal CIP systems of breweries.

Corrosion studies. A comparison of the corrosion rates of AcEW and peracetic acid is shown in Table 3. The corrosion rate of the 30% AcEW was similar to that of deionized water and it was considerably lower than that of 2% peracetic acid.

Industrial-scale CIP tests

Industrial-scale CIP tests enable a more extensive investigation of the effectiveness of AcEW in bacterial decontamination, biofilm removal and the CIP of lines. All aspects of the CIP process can be readily controlled in the system (Fig. 1). This process includes rinsing duration, rinse solution temperature and AcEW concentration. The efficacy of AcEW-based CIP depends on the chemical aspects of the process, such as AcEW concentration, contact time and frequency of water rinsing. This efficacy was evaluated by measuring microbial contamination, as well by conducting forcing tests and sensory analyses. Our results showed that bacterial growth was completely inhibited within 10 min by contact with 30% AcEW, and within 20 min by contact with 2% peracetic acid (Table 4). Boal *(6)* performed pilot tests at five beverage manufacturing facilities and achieved similar results that showed that a 10–15 min contact time of AcEW was enough to inhibit microbial contamination. Accordingly, a 10 min AcEW treatment should be sufficient to ensure an efficacious sanitizing effect.

Given that AcEW immediately becomes inert after contact with organic matter *(5)*, little residue is retained. Therefore, a practical economic application of AcEW was developed by shortening the duration of final water rinsing, which can take up to 20 min to complete in a traditional CIP procedure with 2% peracetic acid. Figure 2 shows the effects of different water rinsing durations on the forcing test results. The forcing test showed that haze formation in the beer was readily distinguished with different water rinsing durations under the 2% peracetic acid treatment, whereas no obvious differences were observed for the 30% AcEW treatment. Thus, no final water rinsing after 30% AcEW treatment is needed to guarantee good beer clarity.

The results of the freshness evaluation (Fig. 3) indicated that a good freshness score of the aged beer samples could be achieved with a 5 min 30% AcEW treatment, regardless of the subsequent water rinsing durations (0–20 min).

Conclusions

The use of electrolysed water is a relatively new sanitizing technique for brewery products. The advantage of using AcEW as a sanitizer is that it can be prepared by the electrolysis of a dilute saline solution, without the use of chemicals other than NaCl. Moreover, it can immediately become inert after contact with organic matter. On the basis of these advantages, the application of electrolysed water for sanitizing in brewery equipment has been described in this study. The focus was on the prevention of bacterial contamination and water conservation via CIP with electrolysed water. It was concluded that 30% of the original proportion of AcEW with a free chlorine concentration of above 17 mg/L is a sufficient and effective alternative to conventional sanitizers such as peracetic acid (2%). On the basis of the results of the industrial-scale CIP tests, an effective AcEW-based CIP procedure, which takes only 10 min of cleaning and

Table 3. Mean values of corrosion rates on day 15

Coupon	Deionized water	30% AcEW	2% Peracetic acid
Stainless steel 304	0.014%	0.016%	0.601%
Carbon steel A36	0.002%	0.007%	0.028%

Table 4. Comparison of bactericidal effects of AcEW and peracetic acid under different contact times

		Population (CFU/mL)[a]	
Treatment	Time(min)	Anaerobic bacteria	Aerobic bacteria
30% AcEW	5	+ and +	+ and −
	10	− and −	− and −
	15	− and −	− and −
	20	− and −	− and −
	Original suspension[b]	+ and +	+ and +
2% peracetic acid	5	+ and +	+ and +
	10	+ and +	+ and −
	15	+ and −	− and −
	20	− and −	− and −
	Original suspension[b]	+ and +	+ and +

[a]+, Positive for culture with visible colony; −, negative for culture without visible colony. Duplicate experiments were performed and results are represented.
[b]The CFU of the original suspension in the assay was calculated from that of the original rinse water before sanitation.

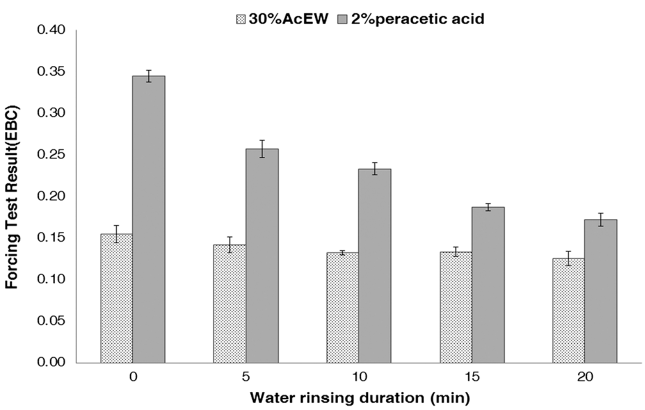

Figure 2. Effect of water rinsing duration on forcing test results. Data are expressed as means ± SD ($n = 5$).

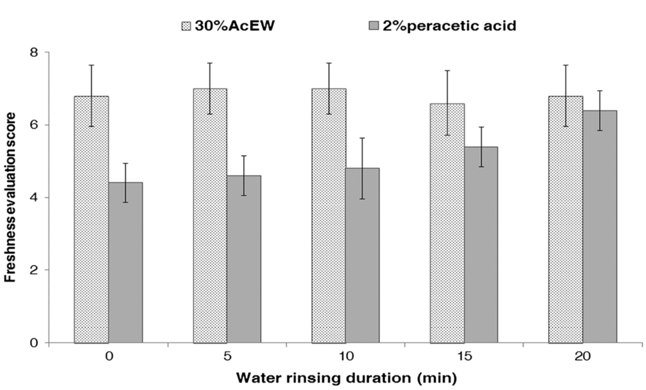

Figure 3. Effect of water rinsing duration on beer freshness. Data are expressed as means ± SD ($n = 5$).

does not require final water rinsing after sanitation, was established for the bright beer tank. From the results of this study, it is suggested that electrolysed water presents promising potential as an alternative sanitizer for breweries.

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