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Research Paper

Effect of storage conditions on shelf stability of undiluted neutral electrolysed water

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Abstract

Neutral electrolysed water (NEW) is an oxidising sanitiser that can be made locally on-site but is often stored in ready-to-use format in order to accumulate the large volumes required for periodic or seasonal use. The shelf stability of NEW sanitiser was therefore assessed under various storage conditions to guide the development of protocols for its industrial application. To that end, fresh NEW with an available chlorine concentration (ACC) of 480 mg/L, pH 6.96 and oxidation reduction potential (ORP) of 916 mV was stored under different conditions. These were open or sealed polypropylene bottles, three different surface area-to-volume (SA:V) ratios (0.9, 1.7 and 8.7) and two temperatures (4, and 25 °C). NEW stored at 4 °C was significantly more stable than NEW stored at 25 °C, where ACC and pH decreased by 137 mg/L and 0.7, respectively, while ORP increased by 23 mV after 101 days of storage. At 25 °C, ACC decreased to <0.01 mg/L after 52 days in bottles with a SA:V ratio of 8.7 with a similar decrease after 101 days in bottles with a SA:V ratio of 1.7. However, pH decreased by up to 3.7 pH units but ORP increased by up to 208 mV. Antimicrobial efficacy of 'aged' electrolysed oxidising (EO) water with different ACC and ORP, but the same pH (i.e., 3.4 ± 0.2) was evaluated against *Escherichia coli* and *Listeria innocua* to determine any differences in residual antimicrobial activity. EO water with an ACC of ≥7 mg/L and ORP of 1,094 mV caused at least a 4.7-log reduction, whereas EO water with non-detectable ACC and considerably high ORP (716 mV) had little antimicrobial effect (<1-log reduction). Results from this study indicate that the efficacy of NEW as a sanitiser for large scale applications like horticulture can be maintained for at least 3 months when stored in closed containers with low SA:V ratio at low temperatures.

HIGHLIGHTS

- NEW sanitiser (480 mg/mL) was more stable to storage at 4 °C than at 25 °C.
- Containers with a lower SA:V ratio improved the stability of NEW.
- Antimicrobial effects of NEW were significant at chlorine concentration > 7 ppm.

Electrolysed water, referred to as electrolysed oxidising (EO) water, is a non-specific broad-spectrum sanitiser *(28)*. It is typically generated by the electrolysis of pure water with added hydrochloric acid (HCl) and/or NaCl in an electrolytic cell *(3)*. Water molecules and chloride ions can then be electrolytically reacted to form different chlorine species such as hypochlorous acid (HOCl), hypochlorite ions (ClO^-) and chlorine (Cl_2). The proportion of these species in EO water is mainly dependent upon the pH of the EO water generated *(28)*. Accordingly, different types of EO water exist and can be produced by changing the composition of the feed stream, the reaction conditions, and the arrangements of the electrolytic cell. These include acidic, slightly acidic, neutral and alkaline EO water. Among all types, neutral EO water (NEW) has gained increasing attention. The neutral pH minimises corrosion potential and is physiologically compatible. The pH of NEW also minimises safety issues from Cl_2 off-gassing and maximises the availability of the hypochlorous acid species and therefore maximises antimicrobial efficacy *(22)*.

EO water has notable biocidal activity, which is mainly due to its physicochemical properties such as available chlorine concentration (ACC), pH and oxidation reduction potential (ORP) *(2, 22)*.There are numerous studies demonstrating that EO water could be used as a surface disinfectant of different materials commonly found in food processing facilities including cutting boards *(17)*, stainless steel, vitreous china, ceramic tiles, and glass *(21)*. Other studies have also reported their potential as an effective sanitiser for a range of fresh fruits and vegetables *(12-14)*, barley grains *(10)*, and a variety of meat and produce *(5, 9, 21)*. Reviews by Huang *et al*., *(10)* and Rahman *et al*., *(22)*, indicated that the antimicrobial efficacy of EO water can range from no effects to at least 6-log reduction in viable count. This variation has been suggested to be related to both intrinsic and extrinsic factors, including the type of EO water used, temperature, ACC, food composition and surface characteristics, water hardness, organic load, and bacterial type *(10)*. Therefore, understanding these factors, their interactions, and how they affect the antimicrobial effects of NEW would aid its development as an effective and reliable antimicrobial application in the food industry.

In addition to the need to better characterise the factors affecting EO efficacy, improved studies to determine the best storage conditions for EO water are important. In horticulture, produce that is eaten raw requires sanitiation after harvest and possibly in the field pre-harvest to moderate the presence of pathogens in harvested produce and/or control phytopathogens that can reduce crop yield. These large-scale applications require large volumes of sanitiser, which may be mixed or made on-site or made in ready-to-use format and transported to the point of application. EO water is typically produced in an electrolytic cell and accumulated in a holding vessel until used. EO water once produced, needs to be stored under the conditions that best preserve its stability as a sanitizer.

Previous studies have identified a range of storage factors including material type, light conditions, temperature, agitation level, and opened or closed container to influence the shell stability of EO water *(3, 11, 23, 24, 32, 33)*. However, these studies examined EO water with a low ACC (i.e., ≤100 mg/L).,. Such low levels of ACC do not reflect the large-scale application of NEW as a sanitiser in commercial settings, in which ACC > 400 mg/L is commonly produced *(7, 18, 15)*. Arguably, freshly made NEW with a higher initial ACC may result in longer shelf stability but this remains unclear.

The aim of this study was, therefore, to assess the changes in physicochemical properties (i.e., ACC, pH, and ORP), of NEW at a high level of ACC (i.e., that is relevant to commercial use, initial ACC of 480 mg/L, pH 6.96, ORP 916 mV) stored under various conditions. Another aspect of this study was to specifically characterise the antimicrobial efficacy of 'aged' EO water (with different ACC and ORPs at the same pH) to provide an insight into its potential mode of action and an assurance that degradation mechanisms or products do not alter the relative efficacy of the antimicrobial activity.

MATERIAL AND METHODS

Preparation of electrolysed water solution. NEW was generated by the electrolysis of a saturated sodium chloride solution using an Envirolyte® ELA-400 (Envirolyte Industries International OÜ, Tallinn, Estonia) *(4, 22)*. ACC (i.e. free chlorine) was analysed colorimetrically, using a Compact

CLO2+ meter (Palintest, Australia; with a detection limit of 0.01 mg/L), which determines ACC by a colourimetric reaction of free chlorine with di-ethyl-p-phenyl diamine (DPD). pH and ORP were analysed with an Orion 250A pH meter (Orion, USA) and a MW 500 ORP meter (Milwaukee, USA), respectively. The anolyte produced had an initial ACC, pH and ORP of 480 mg/L, 6.96 and 916 mV, respectively.

Effect of storage conditions on stability of NEW. The changes in physicochemical properties of NEW were measured when stored under two different conditions. These included: (i) small volume bottles (50, 250 and 500 mL) with container surface area-to-volume ratios (SA:V ratios) of 8.7, 1.7, and 0.9 stored at 4 and 25 °C; and (ii) opened or closed bottles (500 mL) stored at 25 °C (with a SA:V ratio of 0.9). The surface area-to-area volume ratio was determined by the surface area of a cylindrical bottle (436.4 cm^2) divided by the volume of each respective solution (Fig. S1). All experiments were undertaken in the dark. This is because light is well known to decompose HClO and hypochlorite anions, which absorb energy in the region of 292 – 380 nm [24-26].

For Experiment One, NEW was produced and dispensed into 25 L polyethylene containers and stored sealed at 4 °C for 1 day. NEW was transferred into 18 x 500 mL, wide-mouth polypropylene screw cap bottles (LP1403PP-500, Wiltronics, VIC, Australia), which were tightly capped and stored at 4 or 25 °C in the dark (three replicates per treatment of 50, 250 and 500 mL with SA:V ratios of 8.7, 1.7, and 0.9 respectively, stored at either 4 or 25 °C). For each treatment, the ACC, pH and ORP of the NEW was measured periodically from the same bottles over a period of storage up to 101 days.

For Experiment Two, NEW was produced and dispensed into 25 L polyethylene containers and transferred into 42 x 500 mL polypropylene screw cap bottles (three replicates per treatment per time point). In the first treatment ‘closed’ bottles were filled with NEW such that there was no head space and were tightly capped. For the second treatment: ‘opened’ bottles were filled, and the mouth of the bottles were covered loosely with aluminium foil. All bottles were stored in the dark at

25 °C. For each treatment, ACC, pH and ORP were measured periodically from different bottles over a period of storage up to 62 days.

Evaluating antimicrobial efficacy of aged EO water. *Escherichia coli* K12, MG1655 and *Listeria innocua*, ATCC33090 were used to assess the antimicrobial efficacy of the EO water after storage. Cultures were obtained from the culture collection of the Tasmanian Institute of Agriculture, University of Tasmania, Australia.

Preparation of bacterial inocula. Bacterial cultures, previously maintained at -80 °C, were resuscitated by streaking onto Brain-Heart Infusion (BHI) (AM11, AMYL, VIC, Australia) agar and incubated at 37 °C for 24 hours. A well-isolated single colony from each culture was aseptically transferred into 10 mL of BHI broth and incubated at 37 °C for 24 hours to achieve a cell density of approximately 10^9 CFU/mL. The cultures were stored at 4°C and used as 'stock' cultures within a week.

To prepare the inocula, stock cultures of *E. coli* and *L. innocua* were diluted 1:10^3 in 10 mL of BHI broth to prepare working cultures. These cultures were incubated at 37 °C for 20 hours to achieve a cell density of approximately 10^9 CFU/mL. A 1 mL aliquot of each of these working cultures was then harvested by centrifugation at 5,000 *g* for 5 minutes (Eppendorf 5417R) at 20 °C. The pellet was resuspended and washed twice with 1 mL of 0.1% peptone water (LP0037, Oxoid LTD., Basingstoke, Hampshire, England) with subsequent centrifugation at 5,000 *g* for 5 minutes at 20 °C. The final washed pellet was resuspended in 1 mL of 0.1% peptone water and used as the inoculum (approximately 10^9 CFU/mL) for trials to assess antimicrobial efficacy.

Antimicrobial evaluation. EO water with different SA:V ratios that had been stored at 25 °C for 87 and 101 days in Experiment One was used to specifically study the antimicrobial effects of 'aged' EO water. After measurement of ACC, ORP and pH, an aliquot (0.9 mL) of the aged EO water was

transferred into a sterile microcentrifuge tube and combined with 0.1 mL of either an *E. coli* or *L. innocua* suspension outlined above. Bacterial numbers, before and after exposure to treatments for 1, 5 and 10 minutes at approximately 20 °C, were determined by spread plating 0.1 mL aliquots of appropriately diluted samples on BHI agar. After incubation at 37 °C for 18 hours, bacterial colonies were enumerated.

Statistical analysis. All experiments were set up in a completely randomised design with three replications per treatment. The effects of temperature, opened/closed storage, volume, time and their interactions were analysed by two-way analysis of variance (ANOVA) within a repeated measures framework using a Kenward-Rogers degrees of freedom adjustment. For the antimicrobial study, treatment effects were analysed by one-way ANOVA for each exposure time. The assumptions of ANOVA, such as homogeneity of variance and the Gaussian distribution, were confirmed by the use of quantile-quantile plots and residual plots for all variables. Significant differences were established at a level of $P < 0.05$. All statistical analyses were done using SAS version 9.3.

RESULTS

Effect of surface area to volume ratio and temperature on the stability of NEW. pH and ORP, but not ACC, were significantly influenced by the interaction between volume, temperature and time (Table S1). When NEW was stored at 4°C for 101 days, the ACC decreased for all three SA:V ratios (Fig. 1a). The observed decrease also occurred faster with an increase in SA:V. The three treatments (SA:V ratios of 8.7, 1.7, and 0.9) had a final ACC of 343, 400, and 420 mg/L, respectively (Fig. 1a). The initial pH of 7.0 remained relatively stable, decreasing to 6.5, 6.4, and 6.3, for treatments with SA:V ratio of 8.7, 1.7, and 0.9, respectively (Fig. 1b). The ORP also remained relatively stable throughout the duration of the experiment. From an initial ORP of 916 mV, final ORP levels were 955, 961, and 939 mV for SA:V ratios of 8.7, 1.7, and 0.9, respectively (Fig. 1c).

When stored at 25°C, the ACC decreased from 480 mg/L to <0.01 mg/L for bottles with a SA:V ratio of 8.7 and 1.7 by day 53 and 101, respectively (Fig. 1a). This was in contrast to bottles with a SA:V ratio of 0.9 in which the ACC decreased from 480 to 59 mg/L over 101 days (Fig. 1a), reinforcing the influence of the SA:V ratio on retention of ACC. The pH of all NEW treatments declined over the first 65 days of storage and then remained relatively stable. Bottles with SA:V ratios of 8.7, 1.7, and 0.9 had final pH of 3.6, 3.2, and 3.3, respectively (Fig. 1b). The ORP of all treatments initially increased from 916 to 1,130 mV within the first 52 days (Fig. 1c). This was followed by a period in which the ORP of all bottles, except for the bottles with a SA:V ratio of 8.7, remained constant above 1,100 mV. On days 31 and 80, the ORP of NEW in bottles with a SA:V ratio of 8.7 and 1.7 decreased from above 1,000 mV to 493 mV and 716 mV, respectively.

Effect of opened or closed containers on the stability of NEW at 25 °C. In these experiments, ACC, pH and ORP were significantly influenced by the interaction between treatment and time (Table S2). The ACC declined more quickly in opened bottles when compared to closed bottles (Fig. 2a). The initial ACC of 480 mg/L for both opened and closed treatments decreased to 170 and 253 mg/L over the course of 62-day storage, respectively. The pH of NEW with both opened and closed treatments decreased from 7.0 to 4.8 and 3.6, respectively (Fig. 2b). For the ORP data, there was an increase in ORP for both treatments within the first 50 days of storage (from 916 mV to approximately 1,100 mV; Fig. 2c). This was followed by a period in which their ORP appeared to be unchanged.

Antimicrobial efficacy of aged EO water. Aged EO water with different ACC and ORP, but at the same pH (pH 3.4±0.2), was found to significantly influence the mean log reduction of *E. coli* and *L. innocua* for each exposure time (Table 1; $P < 0.05$). The initial inoculum level of *E. coli* and *L. innocua* was 9.09 and 9.21 log CFU/mL, respectively. EO water with an ACC of 63 mg/L, and ORP of 1,121 mV reduced *E. coli* and *L. innocua* by to below the detection limit following one minute of exposure. EO

202 water with an ACC of 7 mg/L and ORP of 1,094 mV, however, reduced *E. coli* only by 4.76 ± 0.09 log
203 CFU/mL within one minute. Thereafter, no further effects were observed with longer exposure
204 times. For EO water with non-detectable ACC (<0.01 mg/L) and ORP of 716 mV, reduced *E. coli* and
205 *L. innocua* by 0.98 ± 0.08 log CFU/mL and 1.02 ± 0.02 log CFU/mL, respectively after one minute with
206 no further effects observed after 10 minutes. This was similar to our observation for EO water with
207 much lower ORP (364 mV) but with the same ACC (i.e. < 0.01 mg/L) and comparable pH (i.e. 3.66). It
208 was found that *E. coli* and *L. innocua* populations were reduced by 0.97 ± 0.07 log CFU/mL and 1.05 ±
209 0.03 log CFU/mL, after one minute, respectively.
210
211 **DISCUSSION**
212 Our study showed that the physicochemical properties of NEW with an ACC concentration of 480
213 mg/mL were more stable at lower temperature (4 °C *versus* 25 °C) in closed (*cf*. opened) containers,
214 and with lower SA:V ratios (0.9>1.7>8.7) over a storage period of 101 days (Fig. 1). The observed
215 increase in stability of NEW at 4 °C is consistent with previous studies. For instance, Meireles *et al*.
216 *(17)* reported that the ACC of NEW (with an initial ACC of 100 ppm) was more stable at 5°C than at
217 25 and 30°C after 200 days. A similar observation was also made for Xin *et al. (32)* when NEW (with
218 an initial ACC of 98 ppm, ORP of 875 mV and pH 6.5) was stored at 4 °C compared to 20 and 35 °C for
219 over a period of three weeks.
220 The observed effects of temperature on NEW also agree well with those on acidic EO water.
221 Previous studies showed that acidic EO water (pH <3) stored in glass bottles for up to 398 days was
222 more stable at 4 °C than at ≥20 °C *(6, 24)*. However, comparison of the observed shelf-stability of
223 NEW with that previously observed for acidic EO water revealed that NEW might have a longer shelf-
224 stability than acidic EO water *(16, 24, 27)*, particularly at low storage temperatures and in sealed
225 containers with low SA:V ratios. Of particular note was a long stability study (i.e., 398 days) that
226 showed acidic EO water to be unstable at both 4 and 25 °C with ACC decreasing to <1 mg/L within
227 the first 65 days *(24)*. The longer shelf-stability of NEW than acidic EO water also agrees well with

228 the idea that the loss of ACC is due to volatilisation of the chlorine gas (Cl_2) into the headspace. This
229 reaction would most likely occur faster at higher temperatures and lower pH values; and potentially
230 increase degradation of HClO under near neutral pH conditions at the higher temperatures *via* a
231 chlorine dioxide intermediate with release of a proton *(1)*. This might explain the observed changes
232 in pH and ORP (Fig. 1b and 1c).

233 The effects of the SA:V ratio on the ACC of NEW were more noticeable at 25 °C than at 4 °C (Fig
234 1a). The ACC decreased faster in bottles with an increase in the SA:V ratio (0.9<1.7<8.7). To our
235 knowledge, the effect of container shape on shelf stability has not been examined previously but our
236 results suggest that the size of the headspace of containers may potentially effect shelf stability of
237 NEW rather than SA:V ratio per say. In practical terms, shelf stability of NEW could be improved by
238 storing it in containers that are filled to maximum capacity to minimise head space. Similarly, the
239 physicochemical properties of NEW were more stable in closed bottles than in opened bottles (Fig.
240 2). This is consistent with a previous study in which there were little changes in the ACC and ORP of
241 NEW, acidic, or slightly acidic EO water in closed bottles and not in opened bottles when stored at
242 20°C *(3, 8, 33)*. . Taken together, the higher stability of EO water observed in bottles with lower SA:V
243 ratio and in closed bottles observed might be because the evaporation rate of the dissolved Cl_2
244 increases with an increase in the SA:V ratio *(1)*, thereby driving the decomposition of HClO in the EO
245 water. Furthermore, the decomposition of HClO leads to hydrochloric acid and oxygen, resulting in a
246 decrease in the pH *(29)*.

247 In addition, aged EO water with different ACC and ORP levels but at the pH of 3.4±0.2 exhibited
248 different antimicrobial effects on *E. coli* and *L. innocua* (Table 1). EO at an ACC of 63 mg/L and ORP of
249 1,121 mV caused a >9 log CFU/mL reduction of both test organisms within one minute at ~20 °C. In
250 contrast, EO water with low ACC (7 ppm) but similar ORP (1,094 mV) was able to reduce *E. coli* by
251 approximately half (~4.8-log reduction). EO water with a much lower ACC and ORP (ACC of <0.01
252 mg/L and ORP of either 364 and 716 mV) produced the least antimicrobial effects, reducing both
253 bacterial species by only ~1 log CFU/mL within one minute. These results, taken together, indicate

254 that ACC plays a major role in the antimicrobial effects of EO water while pH and ORP contribute
255 much less. Similarly, Waters & Hung *(28)*, showed that EO water with pH of 2.8 and ACC of 0 mg/L
256 had little effects on *E. coli* (<0.3-log reduction), whereas treatment with EO water with pH of 7.0 but
257 higher ACC (34 mg/L) caused a 4.8-log reduction of *E. coli*. It also should be noted that the lack of
258 efficacy of EO water with non-detectable ACC, but considerably high ORP (716 mV), was inconsistent
259 with previously published data which ORP values of ≥650 mV were effective in eliminating bacteria
260 within a few seconds although ACC was not reported *(26, 34)*.
261 We determined the effects of various storage conditions on the stability of NEW in the context of
262 commercial use (i.e., with high ACC) as a sanitiser. Our data revealed that the stability of the
263 physicochemical properties of NEW (i.e., ACC, pH and ORP) was higher at low temperature (4 °C
264 *versus* 25 °C) in a closed container and with a low SA:V ratio (0.9>1.7>8.7). Results from this study
265 indicate that the antimicrobial effects of aged EO water is primarily dependent upon ACC rather than
266 ORP and pH. The results of this study provide a useful insight for the food industry to accumulate
267 and store NEW under the conditions that best preserve its stability as a sanitizer (i.e., maintaining
268 ACC). However, further experiments to measure the stability of NEW when produced and stored on
269 an industrial scale (e.g., 1,000 – 10,000 L closed polyethylene holding tanks of NEW at 500 mg/L ACC)
270 for use as a sanitiser should be considered. A predictive model for NEW stability should also be
271 developed to provide a tool for industry to better manage EO water.
272
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278

Table S1. Summary of analysis of variance of Experiment 1 showing the effects of volume, temperature and sampling day, and their interactions on ACC, ORP and pH of NEW stored for up to 101 days.

pH

Effect	Num DF	Den DF	F Value	P Value
Volume	2	6	50.63	0.0002
Days	17	210	16976.0	<.0001
Volume × Days	34	210	67.92	<.0001
Temp	1	210	300354	<.0001
Temp × Volume	2	210	55.20	<.0001
Temp × Days	17	210	10585.2	<.0001
Temp × Volume × Days	34	210	57.49	<.0001

ORP

Effect	DF	DF	F Value	Pr > F
Volume	2	6	4257.57	<.0001
Days	17	210	313.82	<.0001
Volume × Days	34	210	856.94	<.0001
Temp	1	210	194.15	<.0001
Temp × Volume	2	210	7690.14	<.0001
Temp × Days	17	210	307.41	<.0001
Temp × Volume × Days	34	210	866.43	<.0001

ACC

Effect	Num DF	Den DF	F Value	P Value
Volume	2	23.2	3.01	0.0686
Days	1	306	1490.38	<0.001
Days × Volume	2	306	7.39	0.0007
Temp	1	306	61.08	<0.001
Temp × Volume	2	306	2.92	0.0556

Days × Temp	1	306	460.08	<0.001
Days × Temp × Volume	2	306	1.71	0.1827

Table S2. Summary of analysis of variance of Experiment 2 showing the effects of treatment (open vs closed), sampling day, and their interactions on ACC, ORP and pH of NEW stored for up to 101 days.

pH

Effect	Num DF	Den DF	F Value	P Value
Treatment	1	30	271.02	<.0001
Days	7	30	2161.16	<.0001
Treatment × Days	7	30	80.56	<.0001

ORP

Effect	Num DF	Den DF	F Value	P Value
Treatment	1	30	7.14	0.0105
Days	7	30	695.79	<.0001
Treatment × Days	7	30	40.57	<.0001

ACC

Effect	DF	DF	F Value	Pr > F
Treatment	1	30	0.00	0.9961
Days	7	30	314.29	<.0001
Treatment × Days	7	30	5.39	0.0249

334 **Figure S1.** Representative bottles showing surface-area to volume ratios (SA:V 8.7, 1.7 and 0.9) used

335 to store the neutral electrolysed water.

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337

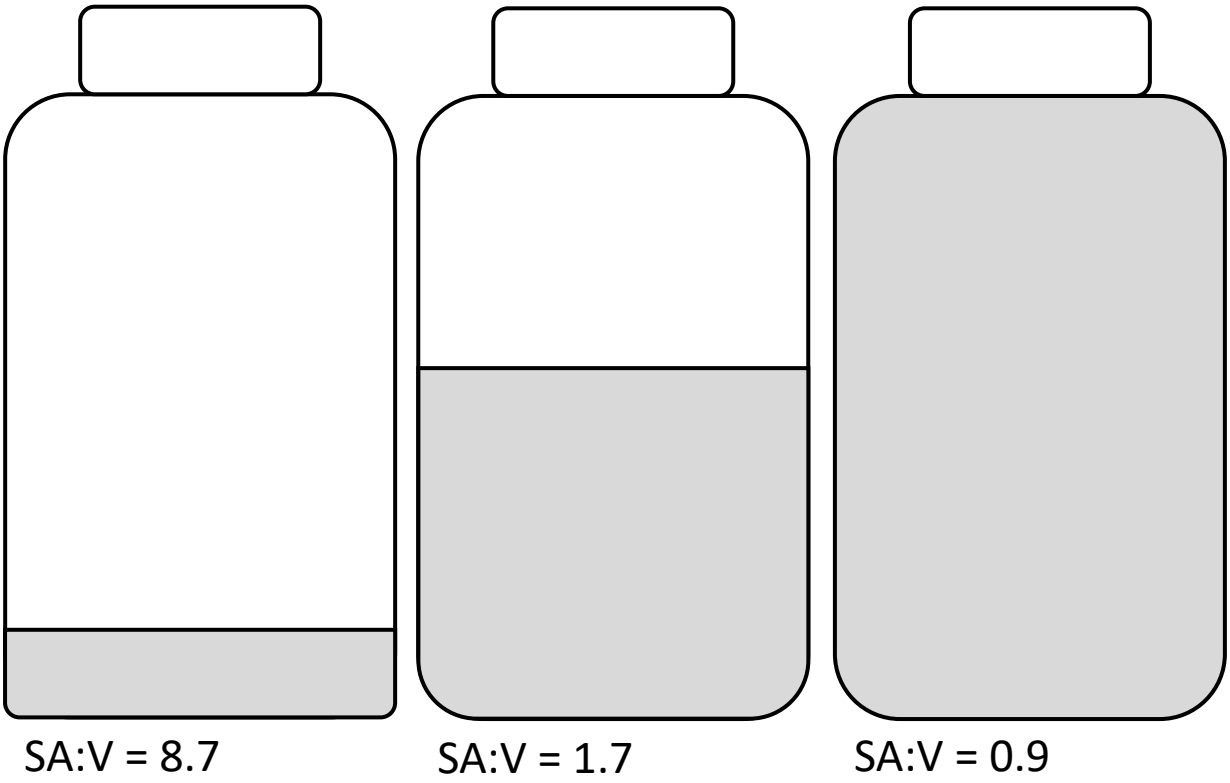

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Fig. 1: Effect of temperature, 4 °C (black) and 25 °C (grey) on A) concentration of available chlorine (ACC), B) pH and C) ORP of NEW with SA:V ratios of 8.7 (square), 1.7 (circle), and 0.9 (triangle) stored for up to 101 days. Error bars equal to 1 S.D.

Fig. 2: Effect of 'open' (black) or 'closed' (grey) containers on the rate of change of the A) concentration of available chlorine B) pH and C) the ORP of NEW stored at 25 °C overtime. Error bars equal to 1 S.D.

TABLE 1. Antimicrobial effects of stored EO water on *Escherichia coli* and *Listeria innocua.* Different letters (a, b, c) denote a significant treatment (α = 0.05) effect within each exposure time. One S.D is shown in parentheses. n=3 for each EO water treatment within each exposure time.

				Mean Log reduction‡ (log CFU/mL)		
				Exposure time (minutes)		
Bacteria	**ACC (mg/L)**	**pH**	**ORP (mV)**	**1**	**5**	**10**
E. coli	<0.01*	3.66	364	0.97 (0.07) a	1.03 (0.04) a	0.96 (0.05) a
	<0.01*	3.24	716	0.98 (0.08) a	0.93 (0.08) a	0.94 (0.07) a
	7	3.35	1,094	4.76 (0.09) b	4.81 (0.07) b	4.72 (0.13) b
	63	3.26	1,121	>9+ (0.00) c	>9+ (0.00) c	>9+ (0.00) c
L. innocua	<0.01*	3.66	364	1.05 (0.03) a	1.01 (0.02) a	1.05 (0.02) a
	<0.01*	3.24	716	1.02 (0.02) a	0.99 (0.04) a	0.99 (0.02) a
	63	3.26	1,121	> 9 (0.00) b	>9+ (0.00) b	>9 (0.00) b

‡) Mean Log reduction = initial counts – final count at each sampling time point (data not shown)

*) Below detection limit of Compact ClO_2+ meter (0.01 mg/L)

+) Below detection limit of enumeration method < 1 log CFU/mL

ACC, available chlorine concentration.

ORP, oxidation reduction potential.