

Development of Portable Flow-Through Electrochemical Sanitizing Unit to Generate Near Neutral Electrolyzed Water

Jufang Zhang, Hongshun Yang , and Joel Zhi Yang Chan

Abstract: We developed a portable flow-through, electrochemical sanitizing unit to produce near neutral pH electrolyzed water (producing NEW). Two methods of redirecting cathode yields back to the anode chamber and redirecting anode yields the cathode chamber were used. The NEW yields were evaluated, including: free available chlorine (FAC), oxidation-reduction potential (ORP), and pH. The performances of 2 electrodes ($\text{RuO}_2\text{-IrO}_2/\text{TiO}_2$ and $\text{IrO}_2\text{-Ta}_2\text{O}_5/\text{TiO}_2$) were investigated. The unit produced NEW at pH 6.46 to 7.17, an ORP of 805.5 to 895.8 mV, and FAC of 3.7 to 82.0 mg/L. The NEW produced by redirecting cathode yields had stronger bactericidal effects than the NEW produced by redirecting anode yields or NEW produced by mixing the commercial unit's anode and cathode product ($P < 0.05$). Electron spin resonance results showed hydroxyl free radicals and superoxide anion free radicals were present in the NEW produced by developed unit. The NEW generator is a promising sanitizing unit for consumers and the food industry to control foodborne pathogens.

Keywords: *Escherichia coli* O157:H7, free radical, *Listeria monocytogenes*, safety, sanitization

Practical Application: Current commercial NEW-producing units are quite large and are not convenient for family using. The developed portable flow-through, NEW-producing unit has great potential in a wide range of applications, such as organic farm, households, and small food industries. The examined sanitizing treatments showed effective control of *Escherichia coli* O157:H7 and *Listeria monocytogenes*.

Introduction

Adequate sanitizing treatments should be applied during the processing and preparation of foods in family kitchens and in the food industry. Recently, the consumption of organic foods has increased worldwide (Yu, & Yang, 2017). U.S. regulations have established that some sanitizing agents are permitted to process organic foods and equipment. However, these chemical sanitizers have limited availability and sanitizing effects (Zhang, & Yang, 2017). Therefore, it is important to develop sanitizers that are suitable for family kitchens and the food industry, especially organic food industry.

Electrolyzed water (EW) has been approved as a food sanitizer by the Food and Drug Administration (Hsu, 2003, 2005; Huang, Hung, Hsu, Huang, & Hwang, 2008; Liu, Tan, Yang, & Wang, 2017a; Liu, Wu, Lim, Yang, & Wang, 2017b; Park, Guo, Rahman, Ahn, & Oh, 2009; Zhang, & Yang, 2017; Zhang, Zhou, Chen, & Yang, 2017). It has been reported to possess antimicrobial activity against a variety of microorganisms (Hao et al., 2012; Huang et al., 2008; Liu et al., 2017a; Pang, & Hung, 2016; Sow, Tirtawinata, Yang, Shao, & Wang, 2017; Zhang, Li, Jadeja, & Hung, 2016). However, at low pH, EW is corrosive, has a short shelf life, might

be toxic to operators, and cause corrosion of different types of metals. A feasible solution is the use of a nearly neutral EW (NEW; pH 6.0 to 7.5; Ayebah, and Hung, 2005; Cao, Zhu, Shi, Wang, & Li, 2009; Gil, Gómez-López, Hung, & Allende, 2015; Len, Hung, Erickson, & Kim, 2000; Waters, Tatum, & Hung, 2014; Xuan et al., 2016).

In general, NEW is produced by mixing the catholyte with EW produced by a divided electrolytic cell, with diluted NaCl solution as the electrolyte (Yang, Feirtag, & Diez-Gonzalez, 2013; Zhao, Zhang, & Yang, 2017). With same electrolyte, another method to produce NEW involves using 3 tubs with 2-membrane partitions and 4 sheets of electrodes in the electrolytic cells (Umimoto, Fujiwara, Nagata, & Yanagida, 2013). Additionally, NEW can be produced by electrolysis of hydrochloric acid (HCl) or diluted NaCl solution in a nonflow-through undivided electrolytic cell (Cao et al., 2009). Some NEW is produced using a developed circulating EW generating system with an undivided electrolytic cell using a NaCl/HCl solution (Xuan et al., 2016). However, an undivided electrolytic cell is limited by cell resistance, which results in low-current and low-power efficiencies, and at the same time, introduces rapid chlorate accumulation from the electrolysis of stagnant electrolytes (Grinberg, Skundin, & Tuseeva, 2001; Nath, Wang, Torrens, & Langdon, 2011).

There are some reports of the continuous production of NEW using a commercial electrolysis unit (Gil et al., 2015; Guentzel, Lam, Callan, Emmons, & Dunham, 2008; Hao et al., 2012; Monnin, Lee, & Pascall, 2012). However, these current commercial NEW-producing units are quite large and are not convenient for households and small food industries (Yang et al., 2013).

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A portable, user-friendly NEW generator is necessary to meet market demand and improve food safety.

In our previous study, we developed an electrolytic sanitizing unit that can produce NEW noncontinuously (Zhang et al., 2017). In the present study, we aimed to develop a flow-through electrochemical sanitizing unit that can produce NEW continuously through further development of the previous unit. NEW produced through two different methods using this portable flow-through electrolytic sanitizing unit were analyzed, and their sanitizing effects were tested. Two kinds of widely used electrodes ($\text{RuO}_2\text{-IrO}_2/\text{TiO}_2$ [Ru/Ir] and $\text{IrO}_2\text{-Ta}_2\text{O}_5/\text{TiO}_2$ [Ir/Ta]) were investigated to produce NEW, electron spin resonance (ESR) was used to check the free radicals in the NEW. Based on these results, a pathway to produce NEW using the flow-through electrolytic cell was provided.

Materials and Methods

Portable flow-through NEW producing unit

Figure 1(A) shows an overview of the developed portable flow-through NEW producing unit, which consists of electrolyte containers, a peristaltic pump, a controller (Nanjing Runze Fluid Control Equipment CO., Ltd, Nanjing, Jiangsu, China), two serially connected electrolytic cells (Dongguan Sunrise Environmental Technology Co., Ltd, Guangzhou, Guangdong, China; $L \times W \times H$, 100 mm \times 50 mm \times 10 mm), and 2 direct current (DC) power supplies (KXN-305D, Shenzhen Zhaoxin Electronic Instrument Equipment Co., Ltd, Shenzhen, Guangdong, China). Figure 1(B) and 1(C) show schematic illustrations of the portable flow-through NEW producing unit configured in 2 different ways: redirecting the cathode yield of 1st electrolytic cell back to the anode chamber of the 2nd electrolytic cell of the unit (B); and redirecting the anode yield of the 1st electrolytic cell back to the cathode chamber of the 2nd electrolytic cell of the unit (C). NEW was generated from the 2nd electrolytic cell for both the 2 methods.

Analytical measurements of NEW

All chemicals used in this study were of analytical grade. Deionized water (DI) was used for cleaning and to dissolve solutes. The free available chlorine (FAC) concentration was determined using the iodometric method (Hao et al., 2012; Hsu, 2005). Briefly, potassium iodide was mixed with a sample of EW. Chlorine was reduced by potassium iodide, resulting in the formation of an equivalent amount of iodine, which was titrated with sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$). The oxidation-reduction potential (ORP) was measured using a Mettler Toledo Seven compact ORP meter (Metrohm Singapore Pte, Ltd, Singapore), and pH was measured using a Thermo Orion 410 pH meter (Thermo Scientific, Waltham, MA, U.S.A.).

Electrochemical characterization of $\text{RuO}_2\text{-IrO}_2/\text{TiO}_2$ and $\text{IrO}_2\text{-Ta}_2\text{O}_5/\text{TiO}_2$ electrodes

Linear sweep voltammetry (LSV) curves of the $\text{RuO}_2\text{-IrO}_2/\text{TiO}_2$ (Ru/Ir) and $\text{IrO}_2\text{-Ta}_2\text{O}_5/\text{TiO}_2$ (Ir/Ta) anodes were carried out using an Autolab type III potentiostat/galvanostat. A platinum plate was used as the counter electrode, and an Ag/AgCl electrode as the reference. The fast linear scanning method was employed over a potential range of 0 to 1.5 V, at a scanning speed of 20 mV/s. The test sample was covered with epoxy resin except for a round exposed area of 1 cm². LSV measurements were performed in saturated NaCl solution and 0.5 M H_2SO_4 for different purposes (Ren et al., 2015; Tang, Li, Li, Chen, & Zeng, 2016).

Efficacy of the sanitizing effect

The sanitizing effects of NEW samples (20 mL) generated from the developed portable electrolytic unit were compared with those of NEW samples generated from a commercial electrolytic unit (Hoshizaki ROX-10WB3-EW, Smitech (Asia) Pte Ltd, Singapore). *Escherichia coli* O157:H7 (strain C7927), and *Listeria monocytogenes* (strain ATCC BAA-839) were used in this study. The bactericidal activity of the NEW samples was determined as previously reported, with slight modifications (Yang et al., 2013). Briefly, 24-hr bacterial suspensions (10 mL each in TSB) were centrifuged ($3000 \times g$, 4 °C) for 10 min, and the resulting pellets were rinsed with 10 mL of sterile 0.1% peptone water (PW), centrifuged, and resuspended in 10 mL of PW. Subsequently, 1 mL of each bacterial suspension was mixed with 9 mL of each NEW sample for 30 s. Aliquots (1 mL) were added to 9 mL of neutralizing buffer solution (5.2 g/L; Becton, Dickinson and Comp., Sparks, Md., U.S.A.). The neutralized mixture was serially diluted for plating on petri dishes with Tryptic Soya Agar (TSA) as media. Following incubation at 37 °C for 24 hr, bacterial colonies were counted. For each bacterial strain, two separate experiments were performed independently. For each experiment, independent parallel groups were carried out in duplicate, resulting in 4 observations for each strain.

Identification of free radical species by ESR

The free radicals were examined according to the methods detailed in previous studies, with slight modifications (Mokudai, Nakamura, Kanno, & Niwano, 2012; Stan, Woods, & Daeschel, 2005). Aliquots (180 μL) of NEW/EW produced by the portable electrolytic sanitizing unit and CNEW (commercial neutral EW; produced by mixing the anode and cathode products) were mixed with 40 μL of 1 M 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) for 10 s. Immediately after mixing, the mixture was transferred to an ESR spectrometry cell, and the ESR measurements were started after 30 s on an X-band ESR spectrometer. In this study, ethanol (50%) was used as an OH radical scavenger, mixed with aliquot (180 μL) of NEW and 40 μL of 1M DMPO. The measurement conditions were as follows: field modulation frequency, 100 kHz; field modulation width, 0.1 mT; amplitude, 2; sweep time, 30 s; time constant, 0.03 s; microwave frequency, 9.192 GHz; microwave power, 1 mW.

Statistical analysis

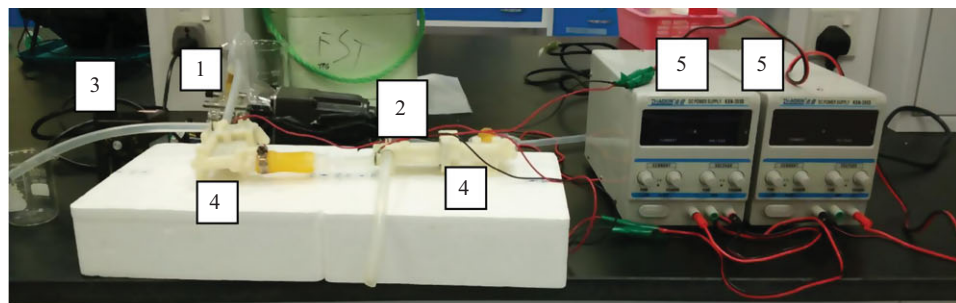
Data were reported as the mean \pm standard deviation. Analysis of variance (ANOVA) and Duncan's test were performed using SAS software (SAS Inst. Inc., Cary, N.C., U.S.A.). Statistical significance was set at $P < 0.05$.

Results and Discussion

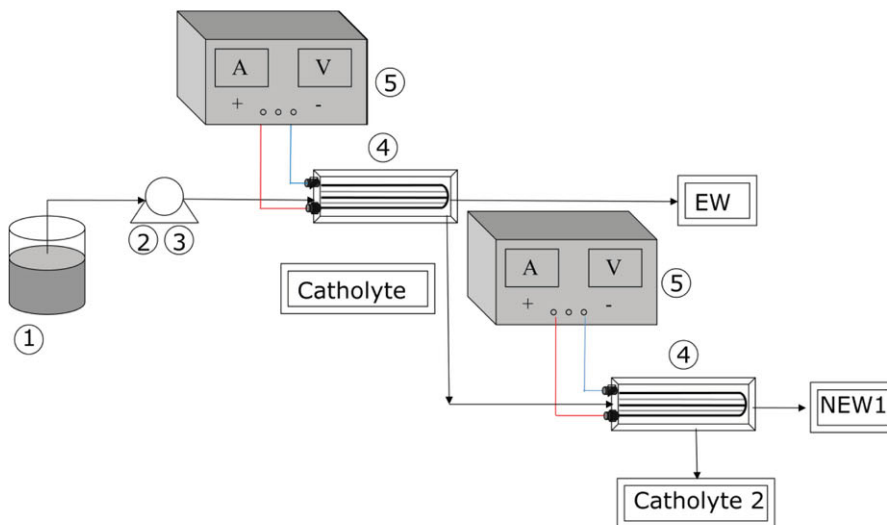
Development of the electrolytic sanitizing unit

Producing NEW by redirecting the cathode product.

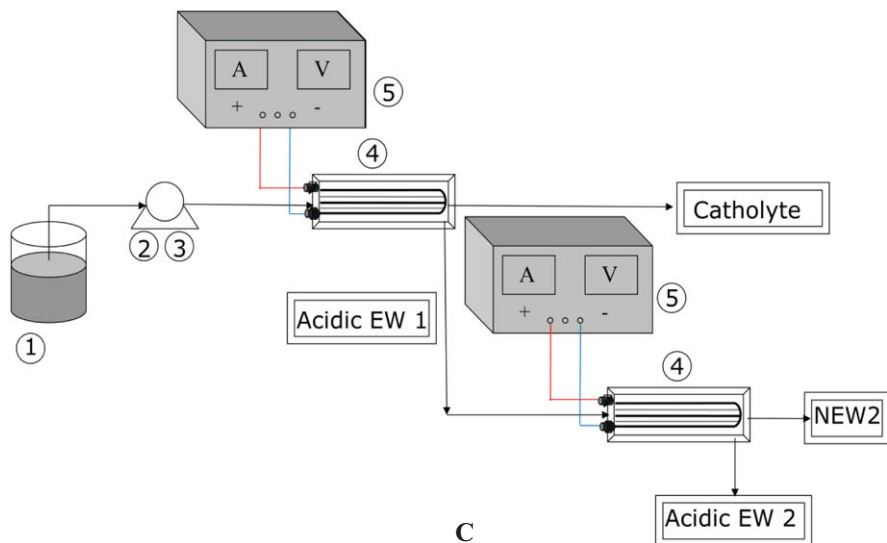
Dimensionally stable anodes (DSA), especially low-cost Ru/Ir electrodes, are widely used to produce EW because of their lower corrosion rates and higher selectivity and efficiency in the electrochemical oxidation of Cl ions to Cl_2 (Le Luu, Kim, & Yoon, 2015; Tang et al., 2016). Recently, the study of the chlorine evolution reaction of Ir/Ta electrodes has demonstrated high electrode catalytic activity and chlorine production (Ren et al., 2015). In this section, the electrolytic cell equipped with Ir/Ta electrodes was investigated.



A



B



C

Figure 1—(A) Overview of the portable flow-through neutral electrolyzed water (NEW) producing unit. (B) Schematic illustration of the portable flow-through NEW producing unit, in which NEW is produced by redirecting the cathode yields back to the anode chamber. (C) Schematic illustration of the portable flow-through NEW producing unit, in which NEW is produced by redirecting the anode yields back to the cathode chamber. 1, electrolyte; 2, pump; 3, controller; 4, electrolytic cell; 5, power supply.

LSV analysis was first performed to investigate the electrocatalytic activity of these two kinds of electrode. Similarly, the EW produced by a single electrolytic cell (Zhang et al., 2017) with these two different electrode materials was checked. Figure 2 shows the representative LSV curves (Figure 2A, 2B) and half-wave potentials (Figure 2C, 2D) of Ru/Ir and Ir/Ta electrodes in

an acidic electrolyte (0.2 M H₂SO₄) and in saturated NaCl solution. In H₂SO₄, the half-wave potential of Ru/Ir was significantly larger than that of Ir/Ta (Figure 2C). Accordingly, in Figure 2A, above the threshold potentials for the oxygen evolution, at constant current densities, the electrode potential of Ir/Ta was greater than that of Ru/Ir. This finding clearly demonstrated higher

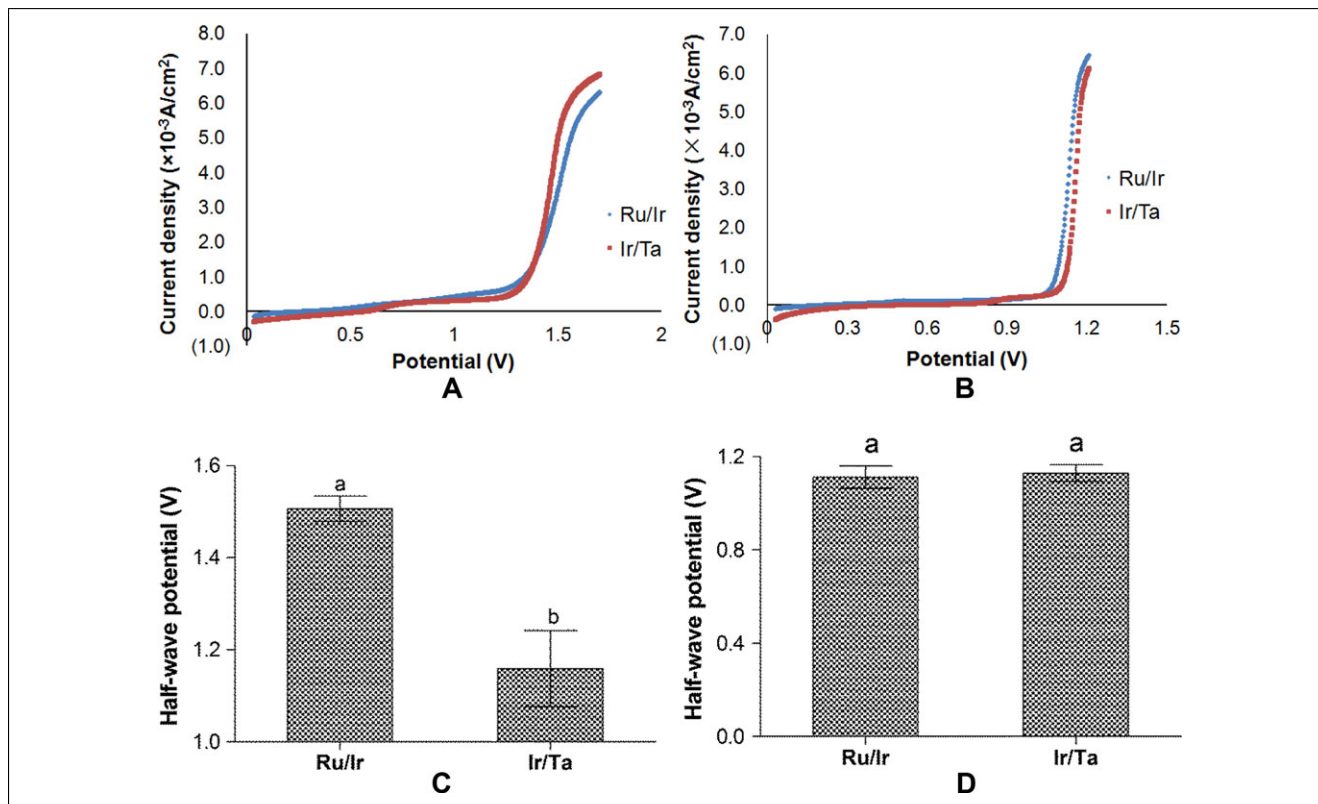


Figure 2—Linear sweep voltammetry curves and half-wave potential of RuO₂-IrO₂/TiO₂ (Ru/Ir) and IrO₂-Ta₂O₅/TiO₂ (Ir/Ta) electrodes in 0.5 mol/L H₂SO₄ (A, C) and saturated sodium chloride solution (B, D) at a sweeping rate of 20 mV/s.

electrocatalytic activity toward the reaction of oxygen evolution (OER) for the Ir/Ta electrode, which was in consistent with previous studies (Comminellis et al., 2008). However, the differences in the electrocatalytic activity toward the reaction of chlorine evolution were not significant, according to the LSV and half-wave potential in the NaCl solution (Figure 2B and 2D).

Figure 3 shows FAC, ORP, and pH trends produced by a single electrolytic cell with two different electrode materials at flow rates of 900, 800, 700, 600, 500, and 400 mL/min, with NaCl concentrations of 10 and 2 g/L. There was no significant difference in the FAC, ORP and pH between the two electrolytic cells with different electrodes at a high NaCl concentration. However, at a low NaCl concentration, the ORP was significantly lower for electrolytic cell with an Ir/Ta electrode compared with that for the Ru/Ir electrode. These results were in agreement with the electrochemical property results, indicating a higher electrode stability of Ir/Ta electrode under oxygen evolving conditions, such as using an alkaline solution as the electrolyte (Comminellis et al., 2008). Thus, in the following experiments, we only used a Ru/Ir electrode in the second electrolytic cell.

Based on these results, 2 and 10 g/L NaCl solutions were tested to produce NEW by connecting the 2 portable electrolytic cells with different electrodes serially at different flow rates. FAC, pH and ORP of the NEW produced were investigated, as shown in Figure 4A–C. For the Ru/Ir–Ru/Ir unit (electrolytic cell equipped with a Ru/Ir electrode connected with another Ru/Ir electrode), all groups achieved near neutral pH, from 6.46 to 7.17 (Figure 4B) except at the flow rate of 400 mL/min and an NaCl concentration of 2 g/L. However, for the Ru/Ir–Ir/Ta unit (an electrolytic cell equipped with a Ru/Ir electrode connected to an Ir/Ta electrode), except for the groups at high flow rate (500

to 900 mL/min) and at a high NaCl concentration (10 g/L), the other NEWs all had an acidic pH. This was probably because of the stronger OER activity of Ir/Ta than the Ru/Ir electrode.

For the Ru/Ir–Ru/Ir unit, the FAC ranged from 3.7 to 82.0 mg/L, with the volume from 52 to 122 mL/min. For the Ru/Ir–Ir/Ta unit, with 2 g/L NaCl solution, the FAC concentration was lower than 1.5 mg/L under all flow rates. Accordingly, under these conditions, the ORP of NEW produced was also very low, ranging from 147.2 to 238.5 mV (range from 813.4 to 974.6 mV for NEW produced from Ru/Ir–Ru/Ir unit). It is probable that such conditions induced the selectivity for oxygen formation reducing the production of chlorine (Karlsson, & Cornell, 2016).

In general, compared with the portable flow-through electrolytic NEW generator equipped with the Ru/Ir electrode, the one with the Ir/Ta electrode did not enhance the FAC production or ORP value of NEW. Thus, in later part of this study, we only investigated the developed electrolytic unit with a Ru/Ir electrode.

Producing NEW by redirecting anode products. We next assessed NEW production by redirecting the anode yields back to the cathode chamber during electrolysis. Figure 5 shows the trend of FAC (A, C), ORP, and pH (B, D) during NEW electrolysis using the developed unit at different NaCl concentrations: A and B, 10 g/L; C and D, 2 g/L. For the 10 g/L NaCl solution, the current density of the 1st electrolytic cell was kept stable at 30 mA/cm², and the 2nd electrolytic cell changed from 7.5 to 20.625 mA/cm² to maximize FAC production in NEW during electrolysis. Similarly, for the 2 g/L NaCl solution, the current density of the 2nd electrolytic cell was kept stable at 30 mA/cm², while that of the first cell changed from 22.5 to 30 mA/cm². For both concentrations of NaCl, at different current densities, there

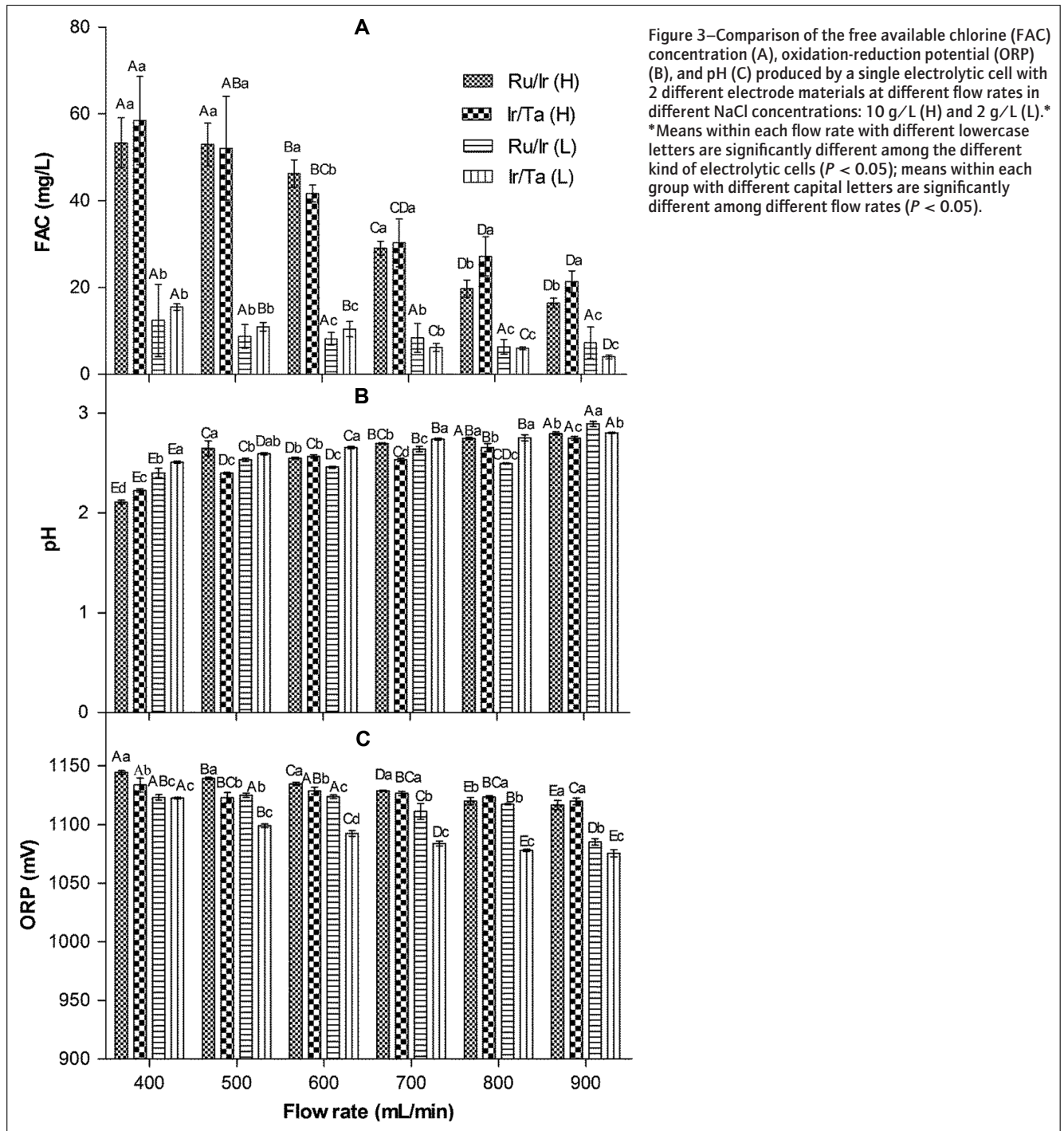


Figure 3—Comparison of the free available chlorine (FAC) concentration (A), oxidation-reduction potential (ORP) (B), and pH (C) produced by a single electrolytic cell with 2 different electrode materials at different flow rates in different NaCl concentrations: 10 g/L (H) and 2 g/L (L). * Means within each flow rate with different lowercase letters are significantly different among the different kind of electrolytic cells ($P < 0.05$); means within each group with different capital letters are significantly different among different flow rates ($P < 0.05$).

was no significant change in FAC, while the pH could be adjusted to neutral. The ORP was inversely proportional to the pH (Figure 5), such that decreasing the pH increased the ORP. Taking into account of the results of redirecting the cathode product, a hypothetical proposed portable NEW (NEW1) sanitizing unit for domestic and small-scale industry especially organic food industry use is shown in Figure 6.

Considerably lower FAC and ORP values were observed in this experiment compared with those in section Producing NEW by redirecting the cathode product. Even at the higher NaCl concentration (10 g/L) and low flow rate (400 mL/min), the FAC concentration was no larger than 25 mg/L and ORP was lower

than 200 mV. This indicated not only pH neutralization happened in the cathode chamber of 2nd electrolytic cell, but also the reaction which can reduce ORP and FAC also happened. It is probable that, in addition to OH^- and H_2 , some other elements with antioxidant properties were produced in the cathode part, which reacted with the oxidants produced from the anode chamber of the first electrolytic cell. This was in line with a previous study in which an unstable transient H_2 element was involved in alkaline water production (Henry, & Chambron, 2013). It may cause a low sanitizing effect compared with the NEW produced by redirecting the cathode yields back to the anode chamber, which will be discussed more in the next section.

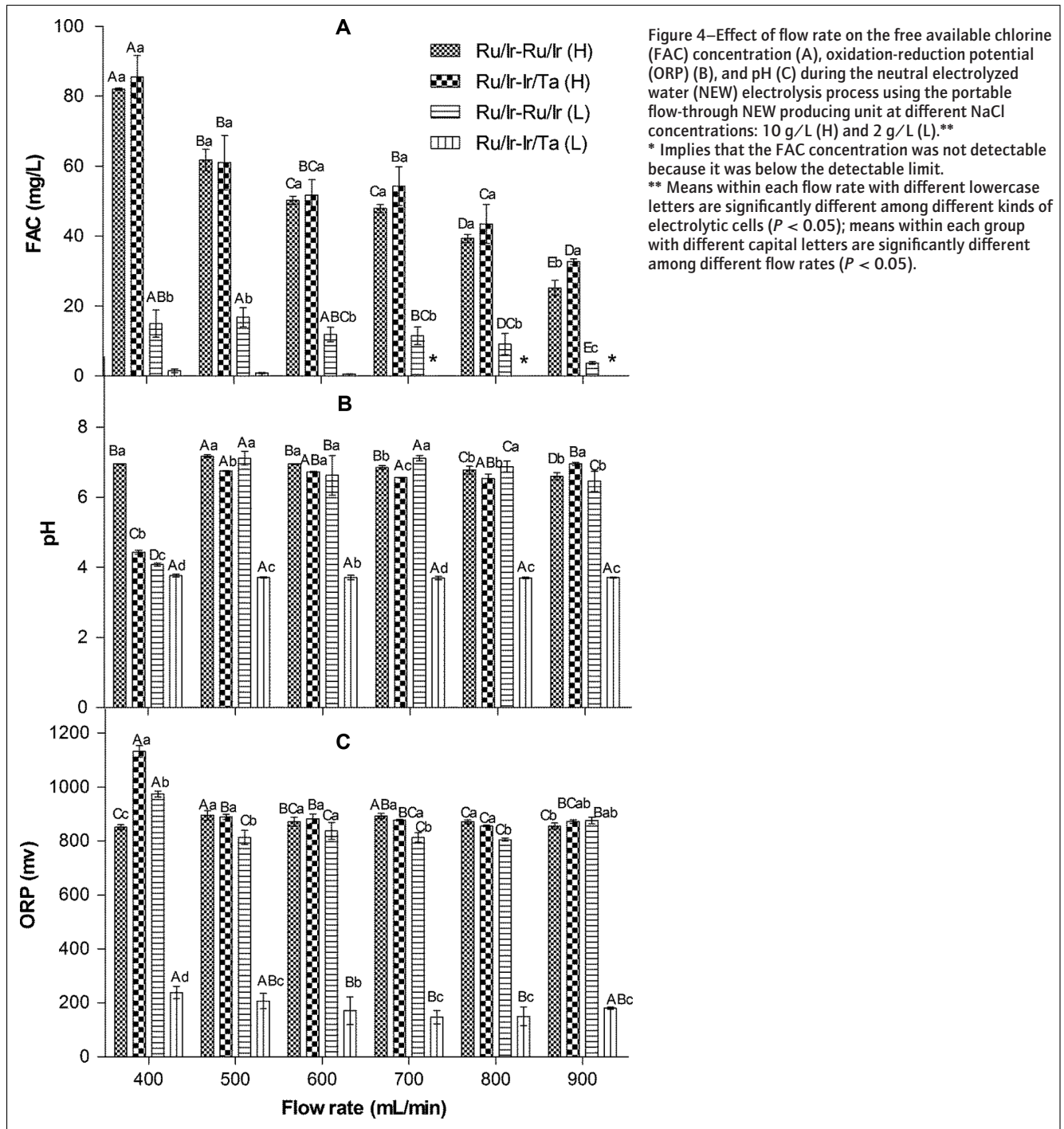


Figure 4—Effect of flow rate on the free available chlorine (FAC) concentration (A), oxidation-reduction potential (ORP) (B), and pH (C) during the neutral electrolyzed water (NEW) electrolysis process using the portable flow-through NEW producing unit at different NaCl concentrations: 10 g/L (H) and 2 g/L (L).** * Implies that the FAC concentration was not detectable because it was below the detectable limit. ** Means within each flow rate with different lowercase letters are significantly different among different kinds of electrolytic cells ($P < 0.05$); means within each group with different capital letters are significantly different among different flow rates ($P < 0.05$).

Efficacy of sanitizing effect

Two kinds of NEW with the same FAC but produced via different principles (NEW1, NEW2, produced by redirecting the cathode product and anode products, respectively) were evaluated. To make the comparison, NEW produced by mixing the anode and cathode products of commercial EW generator (CNEW) was also involved.

The properties of different NEWs used to inactivate *E. coli* O157:H7 and *L. monocytogenes* in this study are shown in Table 1. The pH values of the NEWs produced from different generators and principles ranged from 5.78 to 6.77. The ORP values ranged from 103.0 to 876.6 mV.

Table 1 shows the surviving populations of *E. coli* O157:H7 C7927 and *L. monocytogenes* BAA-839 after treatment with the NEW solutions. There was no significant difference between the sanitizing effects on different strains treated with the same NEW, while the sanitizing effect increased with the increasing FAC concentration in the different NEW. At a FAC concentration of 40 mg/L, the populations of *E. coli* O157:H7 and *L. monocytogenes* in the treated samples decreased to undetectable levels (as determined by both plating and enrichment procedures) after 30 s of exposure to NEW. For a FAC concentration of 4 mg/L, the antimicrobial effect of different NEW products was in the order of NEW1 > CNEW > NEW2. NEW1 inactivated *E. coli*

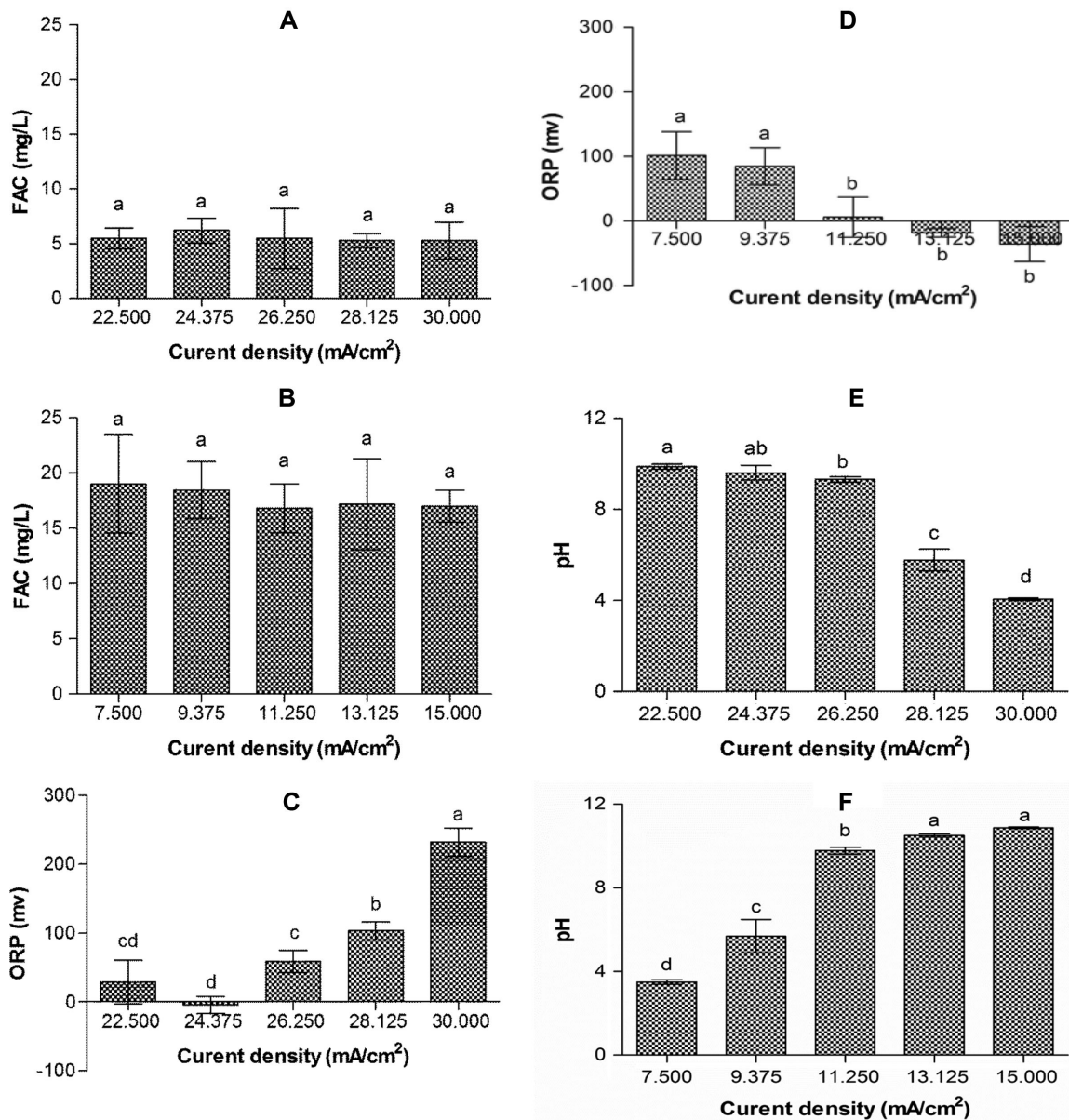


Figure 5—Effect of current density* on the free available chlorine (FAC) concentration (A, B) oxidation-reduction potential (ORP) (C, D), and pH (E, F) during the neutral electrolyzed water (NEW) electrolysis process using the portable flow-through NEW producing unit with a flow rate of 400 mL/min at different NaCl concentrations: 2 g/L (A, C, E), 10 g/L (B, D, F)**

*At a NaCl concentration of 10 g/L, to attain the highest FAC concentration in the produced NEW, the current density of the 2nd electrolytic cell was maintained at a stable 30 mA/cm², and the current density of 1st cell was changed from 22.5 to 30 mA/cm²;

At a NaCl concentration of 2 g/L, to attain the highest FAC in the produced NEW, the current density of the 1st electrolytic cell was kept stable at 30 mA/cm², and the current density of the 2nd cell was changed from 7.5 to 20.625 mA/cm².

** Means within each current density with different lowercase letters are significantly different ($P < 0.05$).

O157:H7 by around 2.75 log CFU/mL reductions, which was significantly higher than 0.72 and 1.89 log reductions achieved by NEW2 and CNEW, respectively. Similar results were observed for *L. monocytogenes*.

The EW's sanitizing effects depend on the FAC (include hypochlorous acid [HClO], hypochlorite ion [ClO] and chlorine gas [Cl₂]), ORP, and pH. The pH values of NEWs (around 4 mg/L) produced from different generators and principles ranged from 5.78 to 6.45 in our study. At this pH range, HClO is the

chlorine species with the highest proportion in solution (Len et al., 2000). NEW1, NEW2, and CNEW all contained similar amount of FAC (4 mg/L); therefore, this sanitizing result can probably be explained by the ORP order: NEW1 > CNEW > NEW2 (Table 1). The ORP of a solution is an indicator of its oxidizing or reducing strength, with higher positive ORP values indicating a greater oxidizing strength. Previous reports (Liao, Chen, & Xiao, 2007) suggested that the ORP of EW might be the primary factor affecting microbial inactivation, which

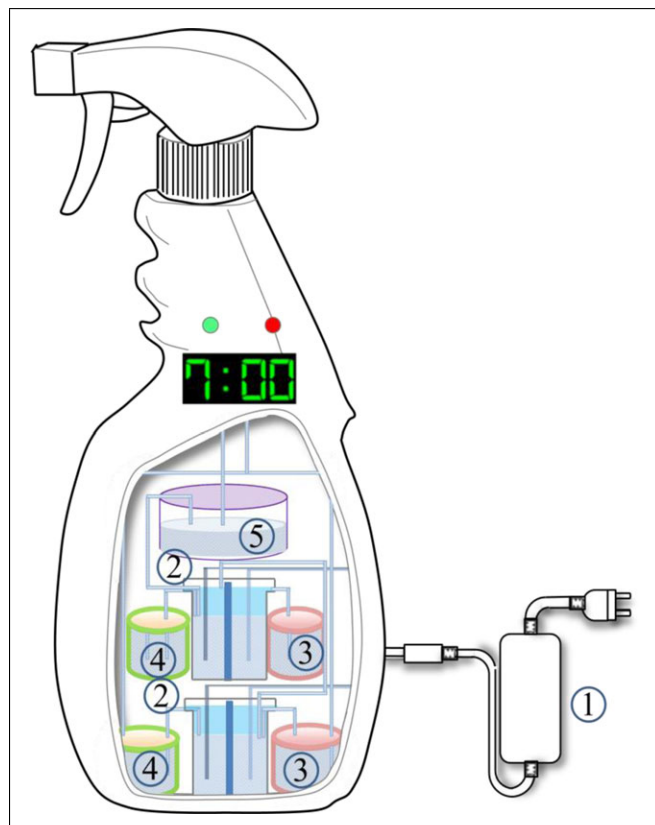


Figure 6—A scheme depicting proposed portable neutral electrolyzed water sanitizing unit. 1, power supply; 2, electrolytic cell; 3, EW tank; 4, catholyte solution tank; 5, NEW tank.

reacts synergistic with HClO and pH to inactivate microorganisms. In the present study (with similar FAC and pH), the higher ORP values in NEW appear to be responsible for the greater antimicrobial effect at the same FAC concentration (Table 1).

Identification of free radicals in EW

Free radicals are also viewed as antimicrobial components of EW. Figure 7 shows the representative ESR spectra of DI (A), NEW1 (B), NEW1 + 2.5% Ethanol C), NEW2 (D), EW (E), CNEW (F). In NEW1 (obtained by redirecting the cathode yield), a DMPO-OH and DMPO-OOH-like signal were detected (Figure 7B). The

presence of the 2 spin adducts were confirmed by hyper fine coupling constants (HFCC) of $\alpha_N = \alpha_H = 1.5$ mT for DMPO-OH (Saito, Kohno, Yoshizaki, & Niwano, 2008) and $\alpha_N = 1.41$ mT, $\alpha_{H\beta} = 1.13$ mT, and $\alpha_{H\gamma} = 0.15$ mT for DMPO-OOH (Clément et al., 2005). After adding 2.5% ethanol ($\cdot\text{OH}$ and $\text{O}_2\cdot^-$ -scavengers), no clear signal was detected, which again proved the existence of $\cdot\text{OH}$ and $\text{O}_2\cdot^-$. Comparing Figure 7A and 7D with Figure 7B, it can be seen that there was no clear signal from DMPO-OH and DMPO-OOH in the NEW2 sample or the DI sample, probably because of the unstable transient anti-oxidizing materials produced in the cathode side (Henry, & Chambron, 2013). For Figure 7E and 7F, although there were some signals in the EW sample (produced from a single portable electrolytic unit) and CNEW sample, the signals were not specific. Figure 7E also shows that the signal intensity of the EW sample was lower compared with that in Figure 7A.

The hydroxyl radicals ($\cdot\text{OH}$) in EW were investigated by ESR in recent studies (Table 2). A conflicting observation was reported in terms of the presence of $\cdot\text{OH}$, although all the products were produced by electrolyzing diluted NaCl solution. It is interesting that all the previously reported electrolytic cells that could produce $\cdot\text{OH}$ were batch units (Mokudai et al., 2012; Suzuki et al., 2002; Xiong, & Liu, & Liu, 2010). In the present study, redirecting the cathode products in the flow-through equipment produced NEW that contained $\cdot\text{OH}$. The lower signal intensity in EW (Figure 7E) indicated that by redirecting the catholyte, especially the hydroxide ions, amplified the production of $\cdot\text{OH}$ and $\text{O}_2\cdot^-$.

Based on these results, and combined with the electro-catalysis mechanism, we proposed a schematic pathway of the HClO in NEW1 produced by redirecting catholyte yield to the anode chamber, as shown in Figure 8. It starts by the electrolysis of the mixture of NaCl solution and the redirecting catholyte, producing chlorine (reaction 1a), $\cdot\text{OH}$ (reaction 1b) and $\text{O}_2\cdot^-$ (reaction 1c, electrolysis of O_2). The $\cdot\text{OH}$ may react with Cl^- to form $\text{Cl}\cdot$ (reaction 2), which might be produced by electrolysis itself (reaction 3) or the decomposition of HClO (reaction 4) (Hao et al., 2012; Saran, Beck-Speier, Fellerhoff, & Bauer, 1999). Thus, the system can follow different reaction pathways to produce Cl_2 (reaction 5). Alternatively, the $\cdot\text{OH}$ enters numerous chemical reactions, yielding H_2O_2 (reaction 6), O_2 (reaction 7), and H_2O (reaction 8) (Hao et al., 2012; Mokudai et al., 2012; Xiong et al., 2010). The $\text{O}_2\cdot^-$ is known to react with water to produce H_2O_2 (reaction 9). It may also react with O_2 to form O_3 (reaction 10), which might be formed by water electrolysis (Hao et al., 2012; Xiong et al., 2010).

Table 1—The surviving population of *Escherichia coli* O157:H7 (C7927) and *Listeria monocytogenes* (BAA-839) after treatment with neutral electrolyzed water (NEW) solutions.*

NEW group	FAC (mg/L)	ORP (mV)	pH	Surviving population (log CFU/mL)	
				<i>E. coli</i> O157:H7 C7927	<i>L. monocytogenes</i> BAA-839
DI	0.0 ± 0.0 ^c	326.1 ± 13.6 ^c	7.12 ± 0.11 ^a	8.58 ± 0.15 ^a	8.31 ± 0.14 ^a
4NEW1	3.7 ± 0.3 ^b	876.6 ± 12.6 ^a	6.45 ± 0.29 ^{bc}	5.83 ± 0.33 ^d	6.15 ± 0.34 ^d
4NEW2	4.3 ± 0.8 ^b	103.0 ± 13.2 ^d	5.78 ± 0.50 ^d	7.96 ± 0.04 ^b	7.94 ± 0.05 ^b
4CNEW	3.9 ± 0.3 ^b	841.3 ± 11.2 ^b	6.29 ± 0.19 ^{bc}	6.69 ± 0.11 ^c	6.82 ± 0.16 ^c
40 NEW1	39.3 ± 1.1 ^a	871.6 ± 8.1 ^a	6.77 ± 0.11 ^{ab}	ND	ND
40CNEW	39.7 ± 1.0 ^a	867.9 ± 7.6 ^a	6.09 ± 0.20 ^{cd}	ND	ND

*Different lowercase letters within a column represent significant differences ($P < 0.05$).

DI, Deionized water.

4NEW1, 4NEW2, and 4CNEW (commercial neutral electrolyzed water) have a free available chlorine (FAC) concentration of 4 mg/L; 40NEW and 40CNEW have a FAC concentration of 40 mg/L.

4NEW1 and 40NEW1 were generated by redirecting the cathode product; 4NEW2 was generated by redirecting the anode product; 4CNEW and 40 CNEW were generated by mixing the anode and cathode products of a commercial generator.

ND, not detectable by direct plate count or negative on enrichment media.

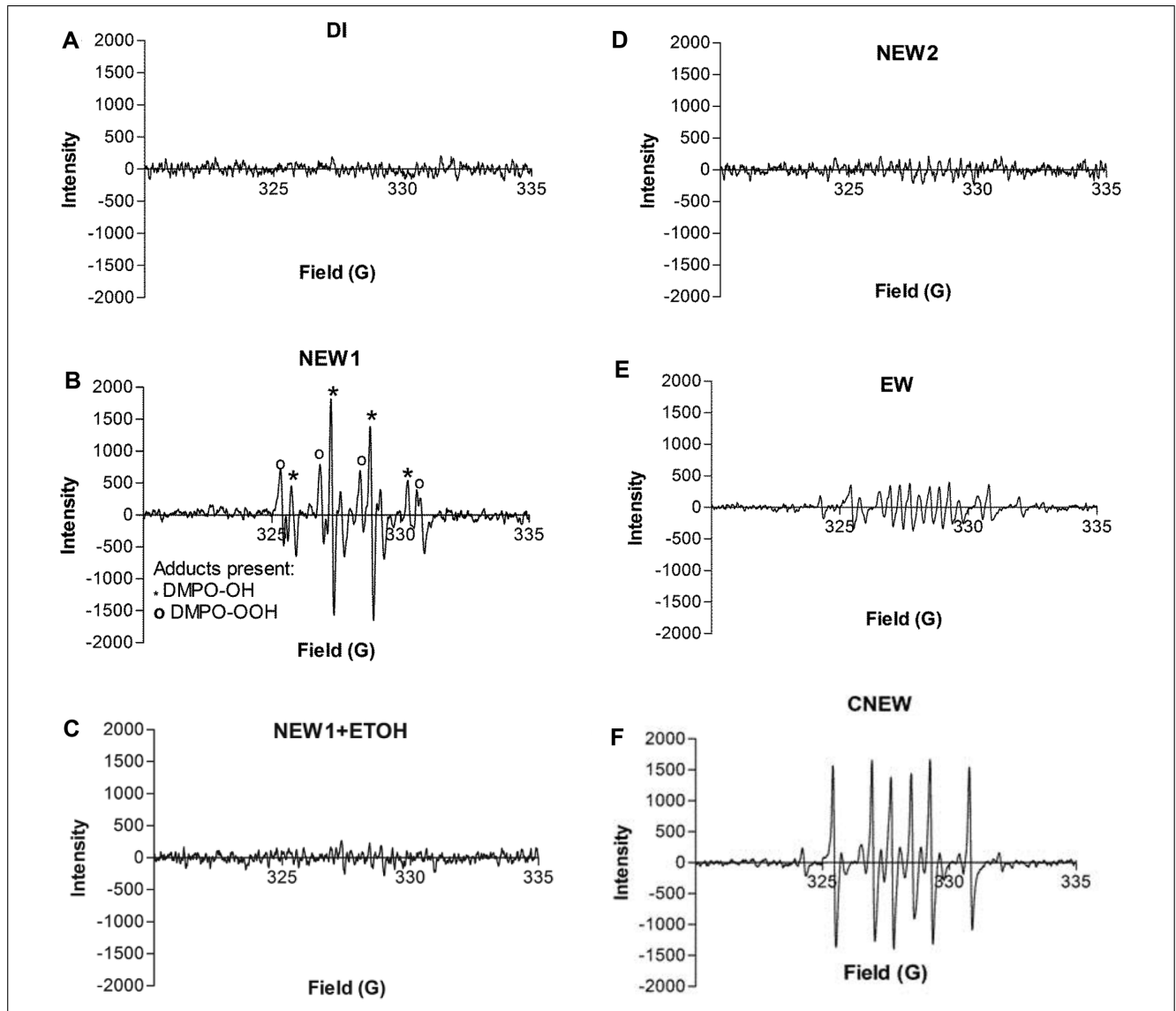
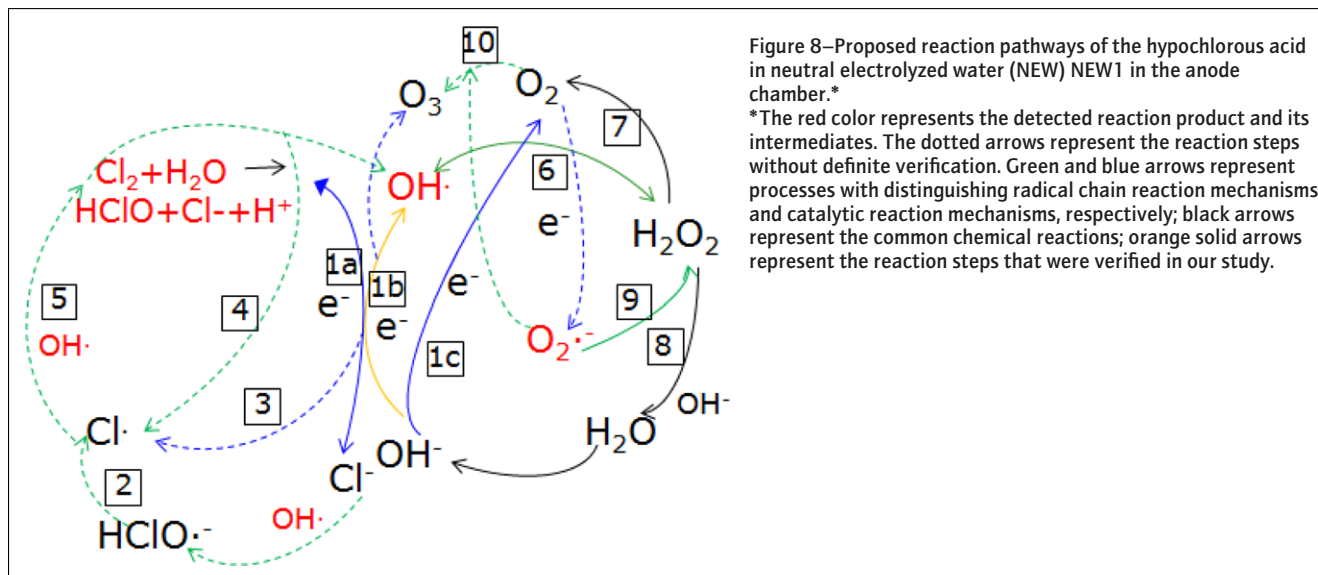


Figure 7—Electron spin resonance (ESR) spectra of neutral electrolyzed water (NEW) produced by the portable flow-through NEW producing unit. (A) DI, (B) NEW1 (produced by redirecting cathode yields back to the anode chamber), (C) NEW1 + 2.5% ETOH (ethanol), (D) NEW2 (produced by redirecting anode yields back to the cathode chamber), (E) Electrolyzed water (EW, produced by the developed single electrolysis unit without redirecting), (F) CNEW (commercial neutral electrolyzed water; produced by mixing the commercial unit’s anode and cathode product after electrolysis).

Table 2—Different views on the production of OH in electrolyzed water (EW)

Statement	Type of electrolytic cell		Type of EW	Note	Reference
	Batch/ continuous	Membrane /nonmembrane			
Presence of OH·	Batch-type generator,	Nonmembrane	AEW	1%, 0.6 A, 15 min electrolysis	(Mokudai et al., 2012)
		Nonmembrane With membrane	NEW, AEW AEW	N.V. 0.1%, 10 min electrolysis	(Xiong et al., 2010) (Suzuki et al., 2002)
	Continuous generator (developed by ourselves)	With membrane	NEW	1%, 0.8 A, 400 mL/min	This study
Nonpresence of OH·	Batch-type generator	With membrane	AEW	0.05%; 10 min electrolysis	(Stan et al., 2005)
		With membrane	AEW	0.05%; 10 min electrolysis	(Stan, & Daeschel, 2005)
	Continuous generator	With membrane	AEW	13.6%, 14.4 ± 0.4 A	(Stan, & Daeschel, 2005)
		With membrane	AEW	13.6%, 14.4 ± 0.4 A	(Stan et al., 2005)

AEW, acidic electrolyzed water; N.V., not available.



The most accepted mechanism of NEW production would be the direct electro-catalysis of Cl^{-1} to Cl_2 , such as the Volmer–Krisalik–Tafel mechanism (Le Lunn et al., 2015; Trasatti, 1987). However, the indirect oxidation of Cl^{-1} mediated by $\cdot\text{OH}$ and some other radical reactions were also investigated recently for the strong antimicrobial effect of these free radicals (Hao et al., 2012; Mokudai et al., 2012; Saran et al., 1999; Xiong et al., 2010). We proposed a pathway for chlorine production from both perspectives. Our portable, flow-through electrolytic sanitizing unit improved the presence of $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ in NEW. In particular, the existence of hydroxide ions can amplify the production of these free radicals, which is different from a common chlorine sanitization (Mohamed, Parveen, & Oscar, 2015).

Conclusions

A portable flow-through electrolytic sanitizing unit was developed to produce NEW. It provided NEW with a FAC ranging from 3.7 to 82.0 mg/L, near neutral pH between 6.46 and 7.17, and an ORP between 805.5 and 895.8 mV. The produced NEW (formed by redirecting of the catholyte solution back to the anode chamber) had strong bactericidal activity: a FAC concentration of 40 mg/L achieved >6 log CFU/mL reductions, and a FAC concentration of 4 mg/L achieved >2 log CFU/mL reductions. Moreover, the NEW produced by this method had stronger bactericidal effects on both *E. coli* O157:H7 and *L. monocytogenes* compared with NEW produced by a CNEW. In addition, $\cdot\text{OH}$ (a strong antimicrobial agent) was present in NEW produced by redirecting the cathode product in the portable flow-through electrochemical sanitizing unit. The results suggest that the developed portable flow-through electrolytic unit promises to produce NEW that could control foodborne pathogens.

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Author Contributions

Jufang Zhang collected the experimental data, interpreted the results and drafted the manuscript. Joel Zhi Yang Chan collected the data of comparing different solutions generations. Hongshun Yang designed the experiment and revised the manuscript.

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