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Development of a portable electrolytic sanitising unit for the production of neutral electrolysed water

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ABSTRACT

The aim of this study was to develop and evaluate the characteristics and performance of a portable electrolytic sanitising unit. Free available chlorine (FAC), oxidation-reduction potential, and pH of electrolysed water were measured. Response surface methodology coupled with a Box-Behnken design was used to describe the input-output relationship and optimise FAC production. A partial catholyte solution was reintroduced to electrolysis for generating neutral electrolysed water. The result found that RuO_2-IrO_2/TiO_2 electrode was very effective. A FAC concentration of 4 mg/L achieved >2 log CFU/mL reduction, while a FAC concentration of 40 mg/L achieved >6 log CFU/mL reduction in *Escherichia coli* O157:H7 and *Listeria monocytogenes* BAA-839. The developed sanitiser had a pH of 7.08 ± 0.08, and the commercial sanitiser had a pH of 3.77 ± 0.18. The developed sanitiser had similar bactericidal effects as the commercial sanitiser. The results revealed that the developed sanitising unit is promising for the control of foodborne pathogens.

1. Introduction

Adequate sanitising treatments should be applied during the processing and preparation of fruits and vegetables in family kitchens and food industries. Current household sanitisers are not favored by consumers because of the presence of harmful chemicals. Recently, the consumption of organic foods has increased worldwide, especially in developed countries (Li et al., 2015; Liu, Tan, Yang, & Wang, 2017; Yu & Yang, 2017). U.S. regulations have established that ozone and few other sanitising agents are allowed to clean organic foods and equipment used in organic food processing. However, these chemical sanitisers have limited availability and sanitising effects (Zhang & Yang, 2017; Ölmez & Kretzschmar, 2009). Therefore, it is important to develop sanitisers that are suitable for family kitchens and food industries. With increasing consumption of organic foods and increased awareness of food safety, the market for this sanitiser will be significant.

EW, also referred to as acidic EW or hypochlorous acid, is produced by electrolysing a diluted NaCl solution with direct current (DC) in an electrolytic cell containing a cation ion-exchange membrane that separates the anode side and the cathode side (Hsu, 2003, 2005; Liao, Chen, & Xiao, 2007). Compared to chlorine (clorox), EW has competitive advantages including being environmentally-friendly because it only uses water and salt as resources. It doesn't involve production, handling and transportation of using conventional chlorine (Hricova, Stephan, & Zweifel, 2008), economical because the EW production only involves water, salt and electricity. It can be generated on site when needed, being much less costly than conventional chlorine aspect of sanitiser generation, transporting and handling (Hricova et al., 2008; Huang, Hung, Hsu, Huang, & Hwang, 2008), safety thus it has been approved as a food additive in Japan, and the application on food was also approved by both U.S. Food and Drug Administration (FDA) and U.S. Department of Agriculture (USDA) (Hricova et al., 2008), and having strong sanitising effect because of major component being hypochlorous acid and there are some other effective components including free radicals, active oxygen, hydrogen peroxide and ozone gas, which are not existed in clorox and with higher oxidation-

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reduction potential (ORP) (Yang, Feirtag, & Diez-Gonzalez, 2013). However, at low pH, EW is corrosive, has a short shelf-life, and may be toxic to the operator (Ayebah & Hung, 2005; Waters, Tatum, & Hung, 2014; Xuan et al., 2016). A feasible solution is the use of a nearly neutral EW (NEW; pH ~6).

Even though several studies have reported the bactericidal effects of both EW and NEW (Luo, Kim, Wang, & Oh, 2016; Park, Guo, Rahman, Ahn, & Oh, 2009; Thorn, Lee, Robinson, Greenman, & Reynolds, 2012; Waters & Hung, 2014; Zhang, Li, Jadeja, Fang, & Hung, 2016), few researchers have investigated the effects of processing factors on the performance of EW/NEW generators. Current commercial EW-producing units are quite large and not convenient for applications in households and small food industries (Yang et al., 2013). A portable, user-friendly NEW generator is necessary to meet the market demands and improve food safety.

The aim of this study was to develop and evaluate the characteristics and performance of a portable and affordable electrolytic sanitising unit. We evaluated the effects of NaCl solution flow rate, NaCl concentration, and current density on pH, ORP, and free available chlorine (FAC) using a mathematical model. We investigated the optimum conditions of FAC production from this small-scale unit. Under optimised conditions, a reflux experiment was performed with different reflux ratios of catholyte to produce NEW. Finally, we studied the sanitising effects of EW and NEW generated from this system.

2. Materials and methods

2.1. Development of a small-scale electrolytic unit

A small-scale electrolytic unit was developed (Fig. 1A and B). It consisted of an electrolyte container, a peristaltic pump, a controller (Nanjing Runze Fluid Control Equipment Co., Ltd, Nanjing, China), an electrolytic cell (10 cm × 5 cm × 1 cm, length × width × height; Dongguan Sunrise Environmental Technology Co., Ltd, Guangdong, China), and a DC power supply (KXN-305D, Shenzhen Zhaoxin Electronic Instrument Equipment Co., Ltd, Shenzhen, China). The anode and cathode consisted of RuO_2-IrO_2/TiO_2 electrodes separated by a polytetrafluoroethylene membrane, which allowed for the separate production of EW and catholyte. The geometric area of the electrode was 27 cm^2.

2.2. Analytical measurements of EW

All chemicals used in this study were of analytical grade. Deionised water (DI) was used for cleaning and dissolving solutes. FAC concentration was determined by the iodometric method (Hsu, 2005; Qin, Li, Chen, & Russell, 2002). 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 ($Na_2S_2O_3$). The concentration of FAC was calculated using following equation:

$$FAC = \frac{(V_2 - V_1)*C_{Na_2S_2O_3}*M}{V_E}$$

where $C_{Na_2S_2O_3}$ represents the concentration of the $Na_2S_2O_3$ titrating solution (mol/L), V_2 represents the volume of the $Na_2S_2O_3$ titrating solution consumed in the treated sample (mL), V_1 represents volume of the $Na_2S_2O_3$ titrating solution consumed in the blank sample (mL), V_E represents the volume of EW/NEW (mL), M represents the molar mass of chlorine (35,453 mg/mol).

ORP was measured with a Mettler Toledo Seven compact ORP meter (Metrohm Singapore Pte, Ltd, Singapore), and pH was measured with a Thermo Orion 410 pH meter (Thermo Scientific, Waltham, MA, USA). The yield was obtained based on average volume/min of generated EW solution in 20 min.

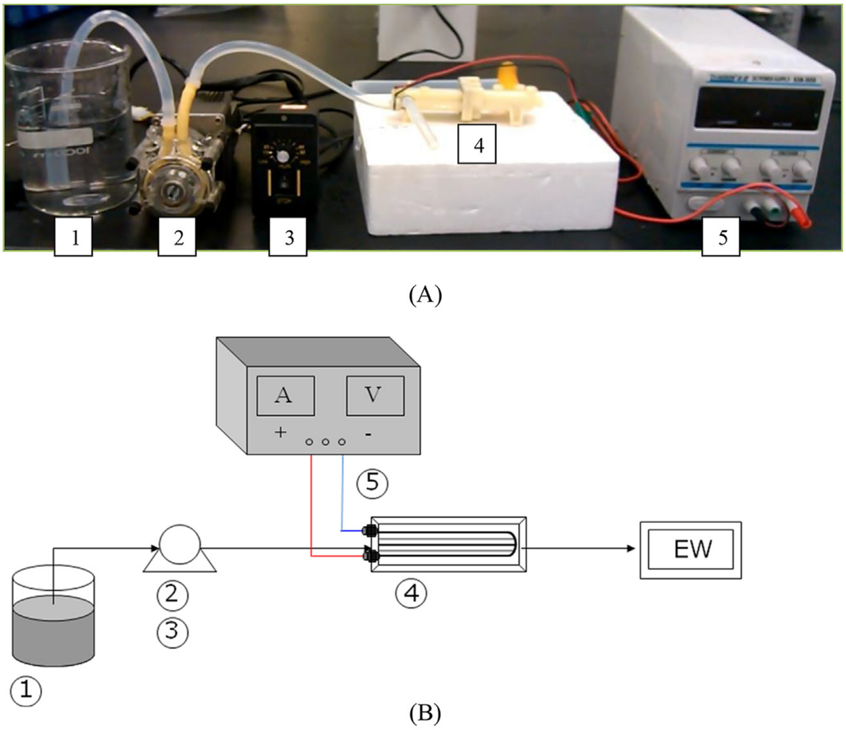


Fig. 1. (A) Overview of the developed small-scale electrolytic unit. (B) Schematic representation of the small-scale electrolytic unit. 1, electrolyte; 2, pump; 3, controller; 4, electrolytic cell; 5, power supply.

2.3. Experimental design and optimisation by response surface methodology

Response surface methodology (RSM) and Box-Behnken design (BBD) were applied to evaluate the effects of three independent variables (flow rate, NaCl concentration, and current density) on FAC production, ORP, pH, energy consumption, and EW yield. Based on preliminary data, BBD had three levels (−1, 0, and +1; Table 1). The obtained results were fitted into an empirical second degree polynomial model (Thirugnanasambandham, Sivakumar, & Maran, 2014):

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ij} X_i^2 + \sum_{i=1}^{3} \sum_{i<j}^{3} \beta_{ij} X_i X_j \tag{1}$$

where Y is the predicted response; X_i and X_j are input variables, which affect the response variable Y; β_0 is a constant; β_i is the ith linear coefficient; β_{ii} is the quadratic coefficient, and β_{ij} is the linear by-linear interaction between X_i and X_j.

A desirability function approach, which finds operating conditions that provide the "most desirable" response values, was performed (Standards, Technology, Croarkin, Tobias, & Zey, 2001):

$$D = \left[\left(\frac{\widehat{Y_1}(x) - L_1}{T_1 - L_1} \right)^s \left(\frac{\widehat{Y_2}(x) - U_2}{T_2 - U_2} \right)^t \left(\frac{\widehat{Y_3}(x) - U_3}{T_3 - U_3} \right)^u \right]^{1/3} \tag{2}$$

where L_i, U_i, and T_i (i is 1, 2, and 3) represent the lower, upper, and target values, respectively, that are desired for each response; Y_i, $\widehat{Y_1}$, $\widehat{Y_2}$, and $\widehat{Y_3}$ represent the estimated response models of FAC production, energy consumption, and EW yield, respectively; s, t, and u represent the weighting factor of FAC production, energy consumption, and EW yield, respectively. In this study, the desirability function was used to optimise process variables to achieve the highest FAC production and EW yield at the lowest energy consumption. Energy consumption was calculated as reported by Zaviska, Drogui, and Pablo (2012) with a slight modification:

$$E = \frac{U * I}{v} \tag{3}$$

where E is the energy consumption (kWh × m^{-3}), I is the current intensity (A), U is the electrical potential (V), and v is the flow rate of NaCl solution (m^3/h). I and U were recorded using a power supply (KXN-305D, Shenzhen Zhaoxin Electronic Instrument Equipment Co., Ltd, Shenzhen, Guangdong, China). The conditions to achieve a specific standard were based on high concentration of FAC and EW yield while low energy consumption.

Data were analysed by analysis of variance (ANOVA) using Design-Expert 8.0 program software (Stat-Ease, Inc. Minneapolis, MN, USA).

2.4. Sanitising effects

The sanitising effects of EW samples (20 mL) generated from the small-scale electrolytic unit were compared to those of EW samples generated from a commercial electrolytic unit (Hoshizaki ROX-10WB3-EW, Smitech (Asia) Pte Ltd, Singapore). *Escherichia coli* (strain ATCC 25922), *E. coli* O157:H7 (strain C7927), and *Listeria monocytogenes* (strain ATCC BAA-839) were used in this study. The bactericidal activity of the EW samples was determined as previously reported with slight modifications (Waters & Hung, 2014; Yang et al., 2013). Briefly, 24-h bacterial suspensions (10 mL each) were centrifuged (3000×*g*, 4 °C) for 10 min, and the resulting pellets were rinsed with 10 mL of sterile 0.1% peptone water (PW), centrifuged, and re-suspended in 10 mL of PW. Subsequently, 1 mL of each bacterial suspension was added to 9 mL of each of the EW samples and votex for 30 s. Aliquots (1 mL) were added to 9 mL of neutralising buffer solutions for another 40 s to stop the reaction (5.2 g/L; Becton, Dickinson and Company, Sparks, MD, USA) (Yang et al., 2013). The neutralised mixture was serially diluted for plating in petri dishes. Following incubation at 37 °C for 24 h, bacterial colonies were counted (Chong, Lai, & Yang, 2015). For each bacterial strain, two separate experiments were independently performed. For each experiment, parallel groups were carried out in duplicate resulting in four observations for each strain.

2.5. Statistical analysis

Each experiment was repeated in triplicates independently, and every time one 15 mL sample was collected. Data were reported as mean ± standard deviation. ANOVA and Duncan's test were

Table 1
Box-Behnken design and experimental responses.

Run Number	Experimental conditions			Output parameters				
	Flow rate (mL/min)	NaCl concentration (g/L)	Current density (mA/cm^2)	FAC[a] (mg/L)	ORP[b] (mV)	pH	Energy consumption (kWh/m^3)	Yield (mL/min)
1	200 (−1)	8 (0)	30.0 (1)	39.7	1119.4	2.93	4.32	85.3
2	400 (0)	8	22.5 (0)	16.8	1123.7	2.68	1.17	142.0
3	400	10 (1)	15.0 (−1)	19.3	1115.2	2.82	0.71	152.7
4	600 (1)	8	15.0	11.2	1103.9	3.00	1.25	220.0
5	200	10	22.5	37.3	1109.9	3.14	2.72	85.3
6	200	6 (−1)	22.5	24.3	1106.9	3.09	2.92	85.3
7	200	8	15.0	28.7	1100.7	3.23	1.56	85.3
8	400	10	30.0	33.9	1128.7	2.54	1.86	152.7
9	400	8	22.5	18.0	1123.6	2.67	1.20	158.0
10	400	8	22.5	18.0	1124.1.	2.68	1.20	142.0
11	600	8	30.0	20.8	1118.0	2.72	1.21	220.0
12	400	8	22.5	14.4	1123.5	2.68	1.17	152.7
13	400	8	22.5	16.8	1123.7	2.67	1.20	158.0
14	400	6	30	24.8	1127.0	2.61	1.77	152.7
15	600	6	22.5	10.3	1110.3	2.90	0.80	220.0
16	400	6	15	11.9	1116.8	2.90	0.73	152.7
17	600	10	22.5	21.2	1113.3	2.90	0.73	220.0

[a] FAC: free available chlorine.
[b] ORP: oxidation-reduction potential.

performed using SAS software (SAS Institute Inc., Cary, NC, USA). Statistical significance was set at $P < 0.05$.

3. Results and discussion

3.1. Development and analysis of the mathematical models

3.1.1. Model design

The BBD design (Table 1) was investigated using design expert software. Multiple linear regression analyses of the experimental data yielded second order polynomial models for predicting FAC production, pH, ORP, and energy consumption and a linear model for predicting EW yield:

$$\text{FAC} = 16.80 - 8.32A + 5.06B + 6.01C - 0.52AB - 0.35AC + 0.4BC + 4.55A^2 + 1.93B^2 + 3.74C^2 \tag{4}$$

$$\text{pH} = 2.68 - 0.11A - 0.012B - 0.14C - 0.013AB + 5.000E - 003AC + 2.500E - 003BC + 0.29A^2 + 0.039B^2 + 2.000E - 003C^2 \tag{5}$$

$$\text{ORP} = 1123.72 + 1.08A + 0.76B + 7.06C + 0.000AB - 1.15AC + 0.82BC - 12.52A^2 - 1.10B^2 - 0.70C^2 \tag{6}$$

$$\text{Energy consumption} = 1.17 - 0.94A - 0.025B + 0.61C + 0.032AB - 0.70AC + 0.028BC + 0.72A^2 - 0.099B^2 + 0.19C^2 \tag{7}$$

$$\text{EW yield} = 152.04 + 67.35A \tag{8}$$

where A represents the coded flow rate, from −1 to 1; B represents the coded NaCl concentration, from −1 to 1; and C represents the coded current density, from −1 to 1.

The adequacy of the mathematical models was evaluated by ANOVA (Table 2). Based on the low probability value ($P < 0.0001$) of Fisher's test, all mathematical models were highly significant. Regression coefficients (R^2), which should be ≥ 0.80 for a good model fit (Joglekar & May 1987), were 0.9792 for FAC production, 0.9920 for pH, 0.9839 for ORP, 0.9922 for energy consumption, and 0.9924 for EW yield; therefore, only 2.08, 0.8, 1.61, 0.78, and 0.76% of the total variables, respectively, were not explained by the models.

Only EW yield had a linear model, probably because yield is mostly affected by flow rate of sodium chloride solution. One of the main objectives of our study was to enhance FAC production in the small-scale electrolytic unit. In subsequent experiments, FAC production was analysed, and pH and ORP were compared.

3.1.2. Model analysis of FAC production

The response surface curves representing the effect of different factor levels (A: flow rate, B: NaCl concentration, and C: current density) on the response (FAC production) are shown in Fig. 2. FAC increased with decreasing flow rate and increasing current density and NaCl concentration in the range of 10.27–39.67 mg/L. This result was consistent with the findings of Hsu (2003, 2005).

Among the electrocatalytic anode, especially the dimensionally stable anode (DSA) electrodes, titanium (Ti) electrodes coated with porous layers of Ir/Ru oxide catalyst have lower corrosion rates and higher selectivity and efficiency in the electrochemical oxidation of Cl ions to Cl_2 in the order $Ti/IrO_2 > Ti/RuO_2 > Ti/Pt–IrO_2 > BDD > Pt$ (Choi, Shim, & Yoon, 2013; Khelifa, Moulay, Hannane, Benslimene, & Hecini, 2004; Tang, Li, Li, Chen, & Zeng, 2016). In this study, the anode and cathodes used were RuO_2-IrO_2/TiO_2 electrodes.

There is little information on the effects of processing factors on the performance of EW generators and on the performance of different technologies and systems (Thorn et al., 2012). Decreasing flow rate increased residence time of the electrolyte in the electrolytic unit, thereby increasing the degree of electrolysis which would enhance the following reactions on the anode (Choi et al., 2013; Hsu, 2005):

$$2Cl^- \rightarrow Cl_2 + 2e- \tag{9}$$

$$Cl_2\,(aq) + H_2O \leftrightarrow HClO + HCl \tag{10}$$

FAC production was significantly affected by flow rate and current density (Table 2). When taking into account the coded variables of FAC in the polynomial equation (Eq. (4)), flow rate (A) was the most critical parameter affecting FAC production, because the flow rate coefficient was 8.32, which was higher than the NaCl

Table 2
ANOVA results for the response surface models for FAC concentration, pH, ORP, energy consumption, and EW yield.[a]

Source	FAC			pH			ORP			Energy consumption			EW yield		
	Sum of squares	d.f[a]	*P*-value	Sum of squares	d.f.	*P*-value	Sum of squares	d.f.	*P*-value	Sum of squares	d.f.	*P*-value	Sum of squares	d.f.	*P*-value
Model	1227.07	9	<0.0001	0.64	9	<0.0001	1103.19	9	<0.0001	14.49	9	<0.0001	36288.18	3	<0.0001
A	553.28	1	<0.0001	0.095	1	<0.0001	9.25	1	0.1000	7.09	1	<0.0001	36288.18	1	<0.0001
B	204.63	1	0.0001	0.001	1	0.2334	4.65	1	0.2210	0.005	1	0.5957	0.000	1	1.0000
C	288.84	1	<0.0001	0.17	1	<0.0001	399.03	1	<0.0001	3.01	1	<0.0001	0.000	1	1.0000
AB	1.07	1	0.6086	0.0006	1	0.3871	0.00	1	1.0000	0.004	1	0.6252			
AC	0.49	1	0.7277	0.0001	1	0.7231	5.29	1	0.1950	1.96	1	<0.0001			
BC	0.65	1	0.6893	0.00002	1	0.8589	2.72	1	0.3382	0.003	1	0.6786			
A^2	87.17	1	0.0019	0.36	1	<0.0001	660.27	1	<0.0001	2.18	1	<0.0001			
B^2	15.72	1	0.0792	0.007	1	0.0202	5.07	1	0.2034	0.041	1	0.1562			
C^2	58.74	1	0.0054	0.00002	1	0.8839	2.05	1	0.4022	0.16	1	0.0167			
Residual	26.11	7		0.005	7		18.04	7		0.11	7		277.80	13	
Lack of fit	17.47	3	0.1810	0.005	3	0.0010	17.83	3	0.0002	0.11	3	0.0026	15.97	9	1.000
Pure error	8.64	4		0.0001	4		0.21	4		0.001	4		261.83	4	
R^2	0.9792			0.9920			0.9839			0.9922			0.9924		

[a] FAC: free available chlorine, ORP: oxidation-reduction potential, EW: electrolysed water, d.f.: Degrees of freedom.

concentration (5.06) and current density (6.01) coefficients (Eq. (4)). The coefficients of the linear terms of the second order polynomial equation obtained from BBD represent an estimation of the principal effect of those factors (Thirugnanasambandham et al., 2014; Zaviska et al., 2012).

In this study, pH first decreased and subsequently increased with decreasing water flow rate (Fig. S1). The low flow rate resulted in a longer residence time of the ions in the electrolytic cell; therefore, more hydrogen ions moved to the cathode side (Hsu, 2003). ORP was inversely proportional to pH (Fig. S2). These results were consistent with the findings of Rahman, Ding, and Oh (2010a, b), who reported that at similar FAC concentrations, ORP increased with decreasing pH.

The effects of salt concentration, voltage and flow rate on the final properties of EW generated were consistent with previous reports from commercial large scale units. As voltage increased, FAC and ORP increased while pH decreased (Ezeike & Hung, 2004). High salt concentration and low flow rate enhanced the FAC result due to more time for electrolytic reactions or more electrolysis of the salt solution within the system (Hsu, 2005). The effect of salt concentration and flow rate on pH was reported, might be due to stronger electrolytic reactions generating more chlorine gases and hypochlorous acid (Umimoto, Fujiwara, Nagata, & Yanagida, 2013).

3.1.3. *Optimisation of FAC production*

The optimum conditions consisted of a flow rate of 362.6 mL/min, a NaCl concentration of 10 g/L, and a current density of 30 mA/cm^2. Under these conditions, 35.81 mg/L FAC was produced with a yield of 139.4 mL/min and an energy consumption of 2.21 kWh/m^3. The desirability was 0.593. If desirability is close to zero, it means that some compromises are necessary to satisfy the criteria.

A validation experiment was performed under the optimised conditions. Under these conditions, the concentration of FAC produced was 39.73 ± 0.92 mg/L, which was very close to the optimised result (35.81 mg/L); therefore, the model was effective.

The small-scale electrolytic unit used in this study was asymmetrical, which revealed that EW production was not equal to catholyte production. Therefore, when determining the optimal

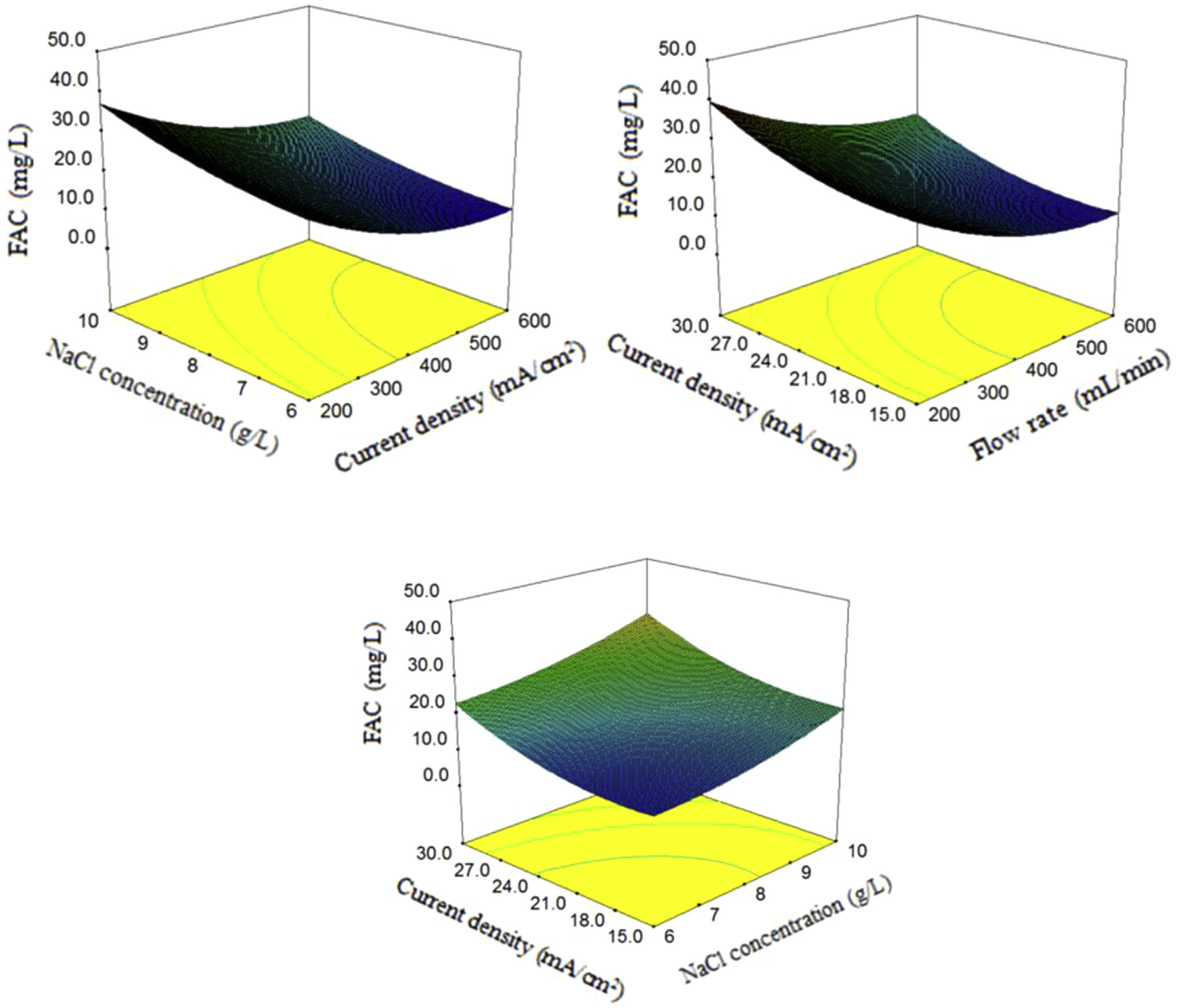


Fig. 2. Response surface plots representing the effect of process variables (A: flow rate, B: NaCl concentration, and C: current density) on FAC* production.
* FAC: free available chlorine.

conditions for FAC production, EW yield and energy consumption should be taken into account. However, their percentage was lower than FAC production (we set a weighting factor of 1/5 for volume and energy consumption and a weighting factor of 5/5 for FAC production). In our study, we used BBD to investigate the main and interaction effects of different factors on FAC production. Additionally, we optimised the process to have the highest concentration of FAC and EW yield with the lowest energy consumption.

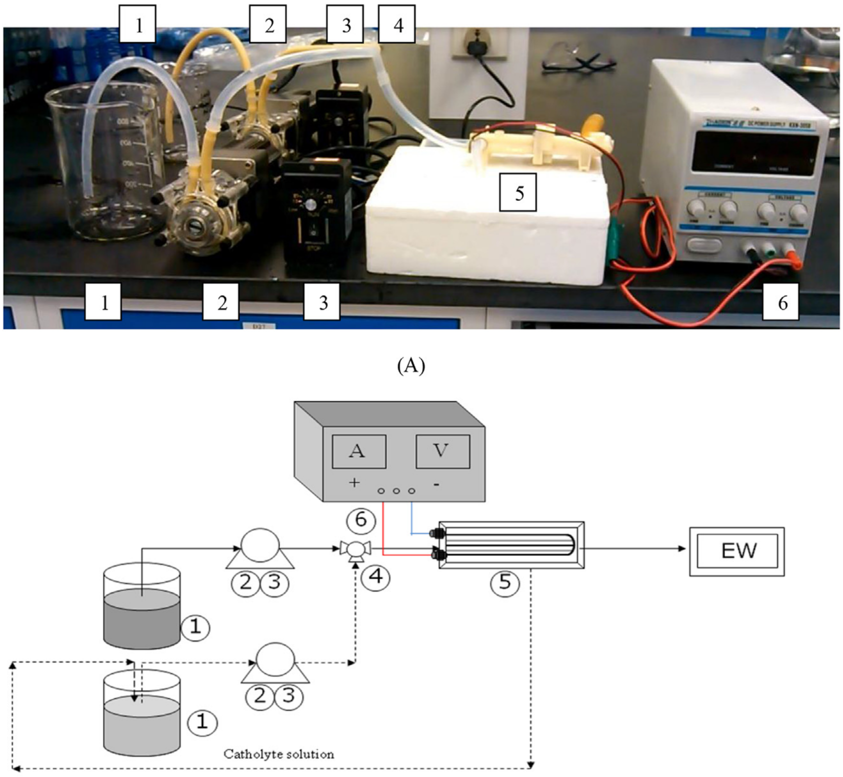
3.2. Modification of the unit to produce NEW

Some studies have reported that EW significantly increases the surface roughness of carbon steel, aluminum, and copper and that pH of EW is a significant factor in the corrosion rate of different types of metals (Ayebah & Hung, 2005; Waters et al., 2014). In our study, we attempted to increase the pH of EW by reintroducing catholyte. Fig. 3A and B shows the modified unit. There were two containers and two pumps in this unit, of which one container and one pump belonged to the recycling system. This recycling system (represented by a dash line in Fig. 3B) was open through the T-connector only during the reflux experiment. The reflux experiment was conducted under two different conditions: 1) optimised conditions and 2) NaCl concentration of 2 g/L, current density of 7.5 mA/cm^2, and flow rate of 800 mL/min. The catholyte solution ratios for each condition were 20%, 40%, 60%, 80%, and 100%. At each reflux ratio, the total inlet flow rate was fixed.

There were no significant changes in FAC production when the catholyte solution ratio changed from 0% to 100% (Fig. 4 A–D). Under optimised conditions, with increasing reflux ratio of catholyte, pH increased from 2.66 to 3.79, and ORP decreased from 1130.6 to 1106.6 mV. However, with 2 g/L NaCl concentration, 7.5 mA/cm^2 current density, and 800 mL/min flow rate, pH was 7.08 ± 0.08 with 100% catholyte solution ratio and 3.77 ± 0.18 with 0% catholyte solution ratio.

There are several methods to produce NEW. In general, NEW is produced by mixing catholyte with EW to increase the pH of EW (Monnin, Lee, & Pascall, 2012; Posada-Izquierdo et al., 2014; Zhao, Zhang, & Yang, 2017). Another method involves using three tubs with two-membrane partitions and four sheets of electrodes in the electrolytic cells (Umimoto et al., 2013). Additionally, NEW can be produced by electrolysis of hydrochloric acid (HCl) or diluted NaCl solution in an electrolytic cell without any membranes (Issa-Zacharia, Kamitani, Miwa, Muhimbula, & Iwasaki, 2011; Ding et al., 2015; Luo et al., 2016; Xuan et al., 2016). Even though we only generated NEW with a NaCl concentration of 2 g/L, a current density of 7.5 mA/cm^2, a flow rate of 800 mL/min and a catholyte ratio of 100%, under the optimised conditions, pH increased by 1.13, while FAC was not affected. Therefore, it is possible to increase the pH value to neutral by redirecting the cathode product into the electrolytic cell. A hypothetical proposed portable NEW sanitising unit for domestic and small-scale industry especially organic food industry use is shown in Fig. 5. Further modification of this system can be performed to achieve this purpose.

Since EW has not been widely used in both Singapore and China, the current regulations in both countries are on chlorine especially chlorine residue only. There are no specific regulations on applying the concentration for electrolysed water. In Singapore, the residue of chlorine should be 1–3 mg/L while in China the residue chlorine should be greater than 0.5 mg/L. For processing fruits and vegetables, the concentration of chlorine should be within 50–100 mg/L. This developed portable unit meets these requirements.



(A)

(B)

Fig. 3. (A) Overview of the small-scale electrolytic unit. (B) Schematic representation of the small-scale electrolytic unit. 1, electrolyte; 2, pump; 3, controller; 4, T-connector; 5 electrolytic cell; 6, power supply.

3.3. Sanitising effects

We tested the sanitising effects of EW produced by this small-scale electrolytic unit. Four types of EW produced by this small-scale electrolytic unit were used and compared to EW produced by a commercial electrolytic unit.

The properties of EW and the surviving populations of *E. coli* ATCC 25922, *E. coli* O157:H7 C7927, and *L. monocytogenes* BAA-839 in the EW solutions are presented in Tables 3 and 4, respectively. At similar FAC concentrations, there were no significant differences in the sanitising effects of EW produced by the different systems. The sanitising effects were greater with increasing FAC concentrations. A FAC concentration of 4 mg/L achieved >2 log CFU/mL reductions while a FAC concentration of 40 mg/L achieved >6 log CFU/mL reductions. It should be noted that there were no significant differences in the bactericidal effects of different EW samples with 4 mg/L FAC. The bactericidal effect of 4 mg/L FAC at pH 7.08 was similar to that of 4 mg/L FAC at pH 3.55 or 3.77. Researchers have reported that the bactericidal effect of EW on *E. coli* and *L. monocytogenes* varies significantly. Previous studies have shown that EW achieves 2–7 log CFU/mL reductions of these bacterial strains (Thorn et al., 2012; Yang et al., 2013; Awad & Tong, 2016). In general, the sanitising effects of EW depend on the amount of hypochlorous acid (HClO) produced in the solution (Thorn et al., 2012). At pH 5.0–6.5, the most dominant form of chlorine compounds in NEW is HClO, which has 80 times more disinfection effect than an equivalent concentration of the hypochlorite ion (OCl^-) (Len, Hung, Erickson, & Kim, 2000; Hao et al., 2012; Thorn et al., 2012). At acidic pH (2.3–2.8), as the main form of chlorine is Cl_2, the volatilisation and loss of chlorine lead to lower microbicidal efficacy (around 1 log CFU/mL) compared with the one at pH 5.0–6.5 (Hao, Wu, Li, & Liu, 2017). However, in our study there were no significant differences between the sanitising effects of NEW and EW, probably due to limited concentrations we selected (4 and 40 mg/L) which could not show the influence of pH (Len et al., 2000; Hao et al., 2012). Further in-depth investigations on the effect of developed sanitisers from this new unit on the metabolic pathway of foodborne pathogens' and corresponding application in foods like fresh-cut vegetables should be conducted (Liu, Wu, Lim, Aggarwal, Yang, & Wang, 2017; Sow, Tirtawinata, Yang, Shao, & Wang, 2017).

For the other small size commercial equipment, such as Ionator EXP and Lotus sanitising system, filtered tap water was applied instead of NaCl solution, thus can not produce solution with reasonable amount of sanitisers, mainly due to that the electrochemical reactions were not strong enough without effective chlorine ions there (Yang et al., 2013). An EW electrode was reported by Umimoto and others (2013); however, the size (12 cm × 20 cm × 10 cm) was pretty large and yield was much limited (<80 mL/min). Our current system was small enough (10 cm × 5 cm × 1 cm) while with relatively high yield (85–220 mL/min).

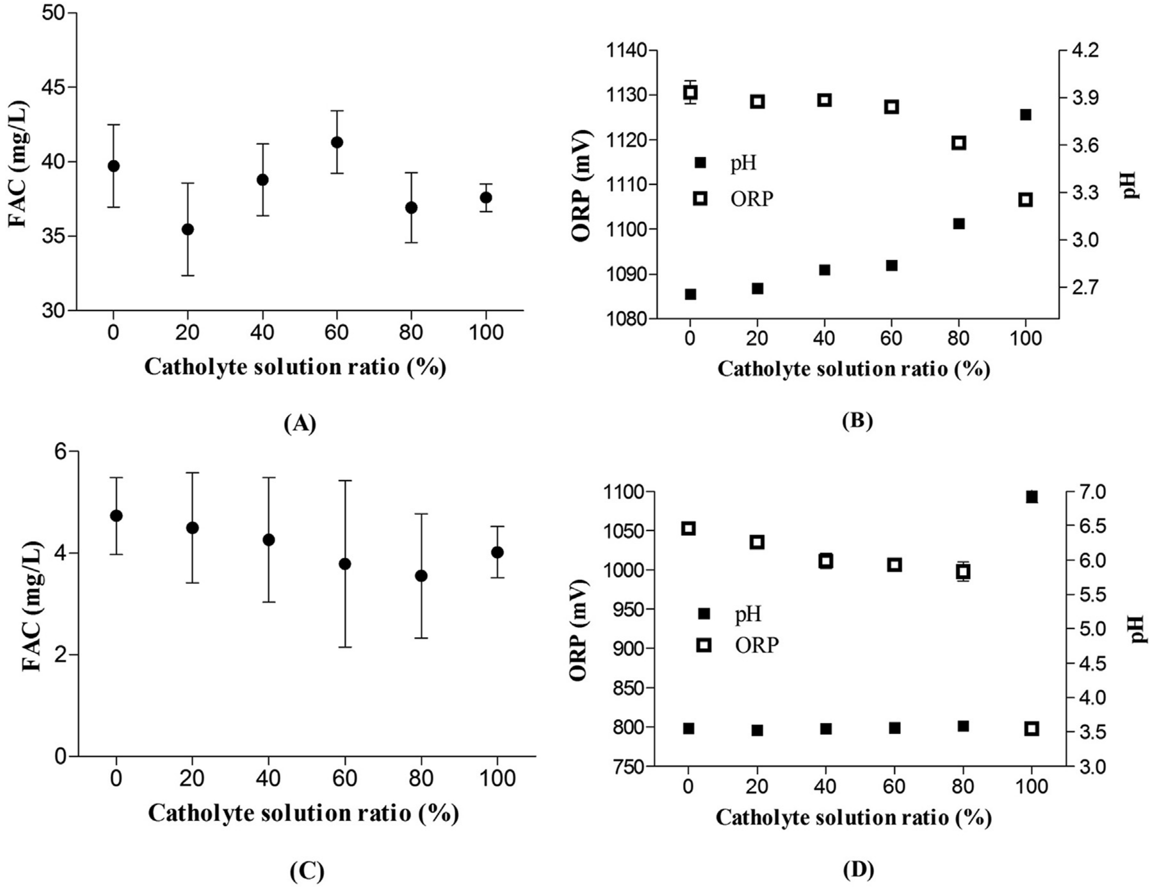


Fig. 4. Effects of catholyte solution ratio on EW production. (A) FAC concentration, (B) pH and ORP* of EW produced under optimised condition; (C) FAC concentration, (D) pH and ORP of EW produced under low FAC production conditions**.

* EW: electrolysed water, FAC: free available chlorine, ORP: oxidation-reduction potential.

** Optimised conditions: NaCl concentration, 10 g/L; current density, 30 mA/cm^2; flow rate, 400 mL/min. Low FAC conditions: NaCl concentration, 2 g/L; current density, 7.5 mA/cm^2; flow rate: 800 mL/min.

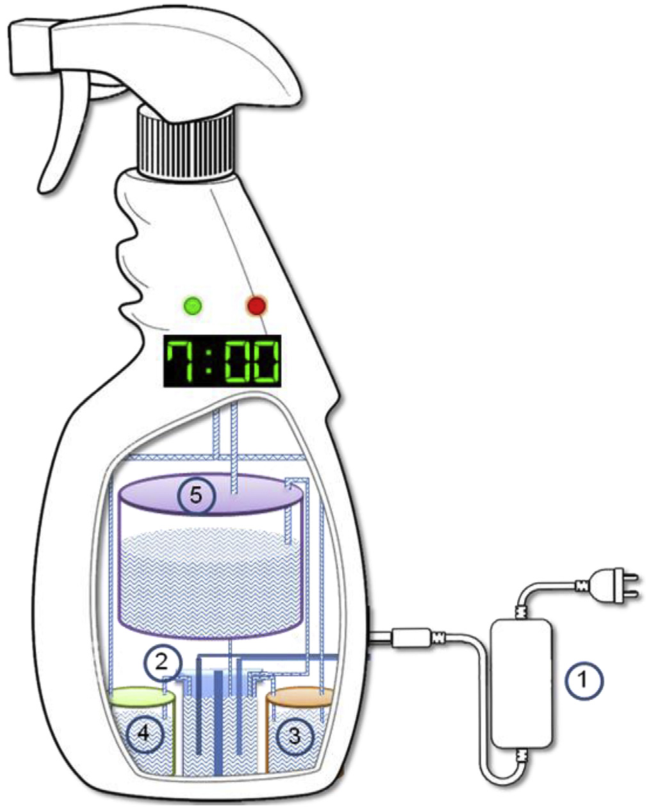


Fig. 5. A scheme depicting proposed portable neutral electrolysed water sanitising unit. 1, power supply; 2, electrolytic cell; 3, EW tank; 4, catholyte solution tank; 5, NEW tank.

Table 3
Physicochemical properties of electrolysed water solutions.[a]

EW group	FAC (mg/L)	ORP (mV)	pH
DI	0.0 ± 0.0^{c}	319.8 ± 11.9^{e}	7.11 ± 0.13^{a}
4CEW	3.6 ± 0.6^{b}	910.0 ± 28.4^{c}	3.77 ± 0.18^{b}
4SEW1	4.7 ± 0.8^{b}	1053.0 ± 3.4^{b}	3.55 ± 0.06^{c}
4SEW2	4.0 ± 0.5^{b}	797.6 ± 3.1^{d}	7.08 ± 0.08^{a}
40CEW	36.6 ± 6.4^{a}	1124.8 ± 0.7^{b}	2.89 ± 0.03^{d}
40SEW1	39.7 ± 1.0^{a}	1130.6 ± 0.6^{a}	2.66 ± 0.01^{e}
40SEW2	37.6 ± 2.8^{a}	1106.6 ± 2.6^{a}	3.79 ± 0.01^{b}

EW: electrolysed water, FAC: free available chlorine, ORP: oxidation-reduction potential, DI: Deionised water, 4CEW: FAC 4 mg/L generated from the commercial electrolyser, 4SEW1: FAC 4 mg/L generated from our small-scale electrolytic unit, 4SEW2: FAC 4 mg/L generated from our small-scale electrolytic unit with reflux electrolysis of 100% catholyte, 40CEW: FAC 40 mg/L generated from the commercial electrolyser, 40SEW1: FAC 40 mg/L generated from our small-scale electrolytic unit, 40SEW2: FAC 40 mg/L generated from our small-scale electrolytic unit with reflux electrolysis of 100% catholyte.

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

4. Conclusion

A continuous portable electrolytic unit was developed. RSM analysis indicated good agreement between the experimental and predicted values. The critical parameter affecting FAC production was flow rate. The optimised conditions consisted of a flow rate of 362 mL/min, a current density of 30 mA/cm^2, and a NaCl concentration of 10 g/L. Under these conditions, a reflux experiment was conducted to generate NEW. Only EW produced with low FAC concentrations had a pH of 7.08 ± 0.08. EW generated with this unit showed 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 reduction. Moreover, the developed sanitiser had similar bactericidal effects on both *E. coli* O157:H7 and *L. monocytogenes* as a commercial sanitiser. The results suggest that the developed prototype unit is promising for controlling foodborne pathogens.

Table 4
Surving population of *Escherichia coli* (ATCC 25922), *E. coli* O157:H7 (C7927), and *Listeria monocytogenes* (BAA-839) following treatment with EW.[a]

EW group	Surviving population (log CFU/mL)		
	E. coli ATCC 25922	*E. coli* O157: H7 C7927	*L. monocytogenes* BAA-839
DI	8.29 ± 0.05^{a}	8.29 ± 0.07 ^{a}	8.26 ± 0.09 ^{a}
4CEW	5.52 ± 0.17^{b}	5.88 ± 0.31^{b}	5.75 ± 0.23 ^{b}
4SEW1	5.53 ± 0.14^{b}	5.68 ± 0.12 ^{b}	5.58 ± 0.33 ^{b}
4SEW2	5.71 ± 0.05^{b}	5.81 ± 0.21 ^{b}	5.70 ± 0.09 ^{b}
40CEW	ND	ND	ND
40SEW1	ND	ND	ND
40SEW2	ND	ND	ND

EW: electrolysed water, DI: Deionised water.
ND: not detectable by direct plate count or negative on enrichment media.
FAC: free available chlorine, ORP: oxidation-reduction potential, DI: Deionised water, 4CEW: FAC 4 mg/L generated from the commercial electrolyser, 4SEW1: FAC 4 mg/L generated from our small-scale electrolytic unit, 4SEW2: FAC 4 mg/L generated from our small-scale electrolytic unit with reflux electrolysis of 100% catholyte, 40CEW: FAC 40 mg/L generated from the commercial electrolyser, 40SEW1: FAC 40 mg/L generated from our small-scale electrolytic unit, 40SEW2: FAC 40 mg/L generated from our small-scale electrolytic unit with reflux electrolysis of 100% catholyte.

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

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2017.04.020.

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