Assessment of Bioethanol Fermentation Performance Using Different Recycled Waters of an Integrated System Based on Food Waste

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To utilize the process water during ethanol fermentation from food waste saccharification broth, the water obtained after three types of technology—methane fermentation, electrodialysis, and microbial fuel cell—were utilized in recycle fermentation in food waste ethanol fermentation. The food waste methane water (FWS), electrodialysis water (FEW), and microbial fuel cell water (FWM), were compared with tap water in terms of ethanol fermentation, volatile fatty acid production, and other parameters. The results indicated that fermentation time was reduced by 50% using both FEW and FWM recycling. Among the different recycled water, FEW recycling in ethanol fermentation motivated yeast growth, yielding the highest ethanol value of 47 g/L. The pH changes in the fermentation systems during 60 h using the different recycled waters were within the optimal range of ethanol fermentation (pH 4.0 to 5.0). Moreover, the highest content of acids found in the fermentation systems were 15 g/L and 11 g/L for lactic and formic acid, respectively, which was less than the inhibition values reported. There was no significant inhibition of ethanol fermentation system due to the presence of VFAs. This study will aid the development of an integrated treatment plant for food waste and biofuel production.

Keywords: Recycled water; Ethanol fermentation performance; Food waste; Water and energy harvesting

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INTRODUCTION

Water recirculation technology in biofuel production is necessary to address the growing global water supply demand. The wastewater produced during biological conversion of food waste to biofuel contributes to environmental pollution. This wastewater contains more organic contents, is rich with amino acid content, and has higher levels of metal elements than tap water (Yang *et al.* 2016a). The cost of wastewater treatment increases with an increase in biofuel production rate, affecting the overall cost of biofuel (Zi *et al.* 2013). Among the existing substrates for ethanol production, food waste is abundantly available and easily degradable by bacteria or yeast. Consequently, a systematic approach for managing and treating it should be developed.

The significant variety in resource recovery has led to many developments in biofuel and electricity production from food waste including anaerobic digestion (AD), microbial fuel cell (MFC), and fermentation (Xin *et al.* 2018).

Anaerobic digestion for methane production from food waste is a well-known and established technology (Algapani *et al.* 2017; Ren *et al*. 2018). AD has the potential to produce 367 m^3 of biogas/ton (dry basis), of which methane represents about 65%, and about 6.25 kwh/m^3 of biogas energy (Curry and Pillay 2012). The solid residuals after AD have been treated either by incineration or by landfill. However, the aqueous solution from AD still needs to be further treated before being disposed of to the environment.

A microbial fuel cell (MFC) is an electrochemical cell (bioanode and biocathode) that diverts mechanical energy to electrical energy using microorganisms. It produces electricity of about 0.245 kwh/kg FW (Xin *et al.* 2018). In ethanol fermentation, food waste can produce 54.4 g/L ethanol under optimum conditions (Ma *et al.* 2008), but an insufficient quantity of stillage produced after ethanol distillation is an obstacle for its implementation. Electrodialysis with bipolar membrane (EDBM) has wide applications in wastewater treatment (Strathmann 2010), as shown by research recovering lactic acid from stillage in food waste ethanol fermentation (Ma *et al.* 2018). The treated water can be used as process water to produce biofuel.

There are several technological approaches used to manage the wastewater produced after converting food waste to biofuel, but improper utilization of these methods releases pollutants into the air, soil, and water (Pimentel *et al.* 2004, 2007). Furthermore, global energy crises have prompted the exploration of alternative energy resources such as biofuel (Gírio *et al.* 2010). Food waste management with environmentally friendly impacts and futuristic visions has attracted researchers.

Recently, an integrated system was introduced to address the problems of wastewater provision for biofuel production. For example, ethanol-methane coupling fermentation system from cassava yields 317 g of ethanol and 68.7 g of methane per kg of cassava (Zi *et al.* 2013). The study of the characteristics of ethanol fermentation with a recycled mixture of stillage and anaerobic effluent from cassava showed that it reduced the fermentation time by 40% and the ethanol production rate was increased (Yang *et al.* 2016a). In addition, the recycling of stillage as diluted water instead of fresh water for ethanol production showed a 13 to 47% reduction in water consumption and reduced the stillage volume discharge (Shojaosadati *et al.* 2015). However, stillage strength rose with an increase in recycling time and after five cycles the by-product, including LA and salt accumulation, prolonged the fermentation time from 24 h to 96 h (Ma *et al.* 2016). Another study showed that the recycling of 10, 20, and 30% of thin stillage did not reduce the bioethanol production efficiency from triticale (Małgorzata *et al.* 2011). Recycling biological processed water for ethanol production offers not only the benefit of reduced fresh water consumption but also utilized part of the organic content and provided a closed-circuit circulation process. Although studies have been conducted for ethanol fermentation from food waste, there is little information about the influence of recycling process water on ethanol production performance, particularly from FW.

Wastewater under three types of technology, which were methane fermentation, electrodialysis, and microbial fuel cell, were used as process water for saccharification process in bioethanol production from food waste. The influence of these wastewaters on ethanol productivity was investigated and corresponding key factors governing ethanol production efficiency were identified.

EXPERIMENTAL

Material Preparation

The food waste (FW) used in this study was collected from a canteen at the University of Science and Technology, Beijing, China. First, unfermented substances including bones, plastics, and paper wastes were manually removed. The FW was ground to small pieces (< 2 mm). Thereafter, the FW slurry was sealed in labeled plastic bags and stored at -20 °C for subsequent tests. Before ethanol fermentation, the composition of FW was analyzed as listed in Table 1. The glucoamylase used for FW hydrolyzed was provided by the Beijing Dong Hua Qiang Sheng Biochemical Technology Company (Beijing, China). Dry yeast for fermentation was obtained from Anqui Company, China, and was stored at 4 °C.

Table 1. Food Waste Composition

Parameter				Biomass Composition Elements (%)		Metal Elements (%)			
						Heavy Metal		Light Metal	
pH	5.26	Reducing sugar	13.65	C	53.68	Cr	8.76×10^{-3}	Na	16.077
TS%	17.22	Total sugar	60.23	H	5.01	Co	1.10×10^{-4}	K	4.261
VS%	7.73	Starch	46.12	N	2.54	Cu	6.5×10^{-3}	Mg	0.616
SS%	14.64	Protein	15.56	O	37.9	Zn	2.45×10^{-2}	Ca	3.025
VSS%	4.5	Lipid	18.06	-	-	Ni	2.50×10^{-3}	Al	0.217
DS%	2.58	Cellulose	2.26	-	-	Mn	9.35×10^{-3}	Fe	0.141
TCOD g/L	104.5	-	-	-	-	Pb	4.75×10^{-4}	P	2.264
SCOD g/L	68.4	-	-	-	-	Cd	2.42×10^{-4}	S	1.697

Collection and Preparation of Waste Water

The stillage for food waste ethanol fermentation was obtained from three different laboratory-scale units, namely an anaerobic digester, a microbial fuel cell (MFC) device, and electrodialysis with a bipolar membrane (EDBM). The stillage was obtained as described in a previous study (Ma *et al.* 2016). After five recirculation cycles in food waste ethanol fermentation, the diluted stillage was used for bioelectricity production using a MFC device as described in previous research (Ma *et al.* 2018). After electricity steady state was reached, the concurrent water remaining was collected (FWM). Another technology used stillage for EDBM to remove salts and recovery lactic acid, from which water was collected (FWE). The methane water was prepared using an ethanol pre-fermentation method of food waste reported by Yu *et al.* (2018). The stillage was used as substrate during the AD process under mesophilic conditions (35 °C) in laboratory bench-scale. After the reactor had worked for more than half a year, wastewater (FWS) was collected and used for ethanol fermentation without any treatment. The characteristics of the different processes are listed in Table 2.

Set-up of Ethanol Fermentation Batch Experimental

To investigate the influence and stability of different processes of water on ethanol production from food waste, fermentation processes were conducted using tap water (FWW), methane effluent (FWS), MFC liquid (FWM), and electrodialysis with bipolar membrane water (FEW) in four reactors under the same conditions at a constant temperature of 37 ℃ for 60 h.

Table 2. Characteristics of Process Water Based - FW Biological Treatment

Parameter	Unit	FWS	FEW	FWM
pH	/	7.6-7.0	6.5-6.3	6.82-6.7
TS	%	4.94 ± 0.4	1.7 ± 0.3	0.6 ± 0.14
VS	%	2.75 ± 0.5	1.4 ± 0.5	0.3 ± 0.23
TCOD	g/L	84.2 ± 12.5	161 ± 9.2	32 ± 10.5
SCOD	g/L	14.8 ± 7.2	146 ± 5.3	11 ± 6.45
Ethanol	g/L	ND	ND	0.006 ± 0.001
Lactic acid	g/L	4.46 ± 2.6	20 ± 1.5	3.22 ± 2.05
Formic acid	g/L	ND	ND	0.002 ± 0.001
Acetic acid	g/L	1.1 ± 2.1	5.4 ± 0.9	ND
Propionic acid	g/L	1.08 ± 0.7	1.2 ± 0.6	ND
Isobutyric acid	g/L	0.55 ± 0.5	0.22 ± 0.4	ND
Butyric acid	g/L	0.6 ± 0.2	0.65 ± 0.7	0.29 ± 0.15
Isovaleric acid	g/L	0.78 ± 0.6	0.96 ± 0.32	0.18 ± 0.06
Valeric acid	g/L	0.8 ± 0.12	0.72 ± 0.45	0.36 ± 0.04
* Notes: ND: means not detected, / : means not applicable				

Enzymatic hydrolysis

FW was milled and mixed with water at a ratio of 2:1. Accordingly, 200 g of FW material were put into four 500 mL beakers, and 100 mL of processed water (FWW, FWS, FWM, and FWE) was added to each of the beakers. Glucoamylase with the dose of 100U/g (Aoboxing Company, China) was added to enhance the hydrolysis process. The beakers were saccharified at 60 ± 1 °C in a water bath (AI BOTE, ZNCL-GS) for 6 h. At the end of saccharification, the broth from each beaker was centrifuged using a large capacity, low temperature, high speed centrifuge (TGL-16M) for 15 min at a rotation rate of 4000 r/min. Finally, the aqueous solution was stored at a temperature of 0 to 4 ℃ for further fermentation.

Fermentation

Fermentation experiments were conducted in four 500 mL flasks holding with a working volume of 400 mL. The aqueous mixture was inoculated with yeast *Saccharomyces cerevisiae* at 10% of the total volume. The flasks were placed in a temperature-controlled incubator at 37 ± 1 ℃ for 60 h. During that time, samples were taken every 12 h, and the corresponding fermentation parameters and the VFAs accumulated were detected.

Analytical Methods

The pH value was measured using a pH meter (Shanghai Bailun Company, Shanghai, China). Chemical oxygen demand (COD), soluble COD, total solids (TS), volatile solids (VS), suspended solids (SS), and volatile suspended solids (VSS) were analyzed following the standard methods of the American Public Health Association

(APHA 2005). The glucose was measured by high performance liquid chromatography (HPLC). The ethanol and VFAs concentrations of the initial and fermented samples of hydrolyzed FW were determined using gas chromatography (Shimadzu GC-2010 plus, Tokyo, Japan) equipped with a flame-ionized detector. The results were quantified and expressed in terms of g/L. Food waste elemental compositions including C, H, O, N, and S were measured using an elemental analyzer (Vario EL III CHNS, Elementar Analysensysteme GmbH, Shanghai, China).

RESULTS AND DISCUSSION

Properties of Process Water and Food Waste

To assess the influence of the recycling of different process water on ethanol fermentation, the ethanol production performance using each type of recycled water as diluted water was compared to the performance of tap water. The characteristics of these recycled waters were detected before fermentation, as shown in Table 2. The pH values for all raw waters ranged from 6.5 to 7.6, which was close to the pH of the tap water (pH 7.42). The total and volatile solids of methane-recycled water were more than that of MFCs and electrodialysis for recycled water. There were initial organic acids contents detected before fermentation started which made these waters prime for ethanol production compared to the minimal content of organic acids found in tap water (Yang *et al.* 2016a). Furthermore, the concentration of lactic acid was higher for recycled water. The composition of food waste depends mainly on people's eating habits. For example, breakfast in China mostly is composed of starches, which can easily convert to glucose via the saccharification process. As shown in Table 1, food waste is rich in starches (46.12%) and has a total sugar content of about (60.23%), which may promote ethanol fermentation. Furthermore, there is a sufficient amount of biomass and metal elements.

Performance of Ethanol Fermentation *via* Methane Water

As shown in Fig. 1b, the maximum ethanol concentration achieved with FWS was 36.7 g/L lower than that of FWW (49.5 g/L) after 48 h of processing. Considering that, the methane fermentation process can be accomplished within four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Zhang *et al.* 2014). First, the polymeric materials were converted into volatile fatty acids (VFAs) by hydrolysis and acidification stages, and then methane-forming bacteria consumed the VFAs and produced methane and carbon dioxide. However, there were remaining acids in the effluent of methane fermentation due to partial consumption. This suggested that methane water components could accelerate ethanol production; however, the rate was still slower than tap water. According to Narendranath *et al.* (2001), the acetic or lactic acid in the effluent could decrease the yeast growth rate and reduce ethanol production. Secondly, the higher initial value of methane water pH (6.87) could promote the VFAs production compared with that of tap water (4.88). The pH of fermentation broth with methane water (Fig. 1a) ranged between 4.92 and 6.87. The optimal pH value of saccharified liquid could range from 4 to 4.5 (Tang *et al.* 2008). When the pH of the fermentation medium is higher than the optimal value, yeast produces acid instead of ethanol (Tahir *et al.* 2010). Therefore, the fermentation media showed increased acid production using methane water. For an integrated wastewater system of ethanol-methane utilized for ethanol production of cassava, results showed that ethanol yield increased by 3% at a pH of 4.2, while it decreased

at a pH of 6.2 (Yang *et al.* 2016b). Furthermore, the utilization of mixed process water (composed of stillage and digestate) in ethanol fermentation revealed that the fermentation rate elevated from 1.07 to 2.01 g/L/h, but ethanol production did not increase (Yang *et al.* 2016b). Another study on the recirculation of distillery waste in ethanol fermentation from FW showed a lesser concentration of ethanol (about 30 g/L) compared to this study, in addition to a decrease in the concentration till, which reached 3 g/L in the 4th cycle of fermentation (Wei *et al.* 2015).

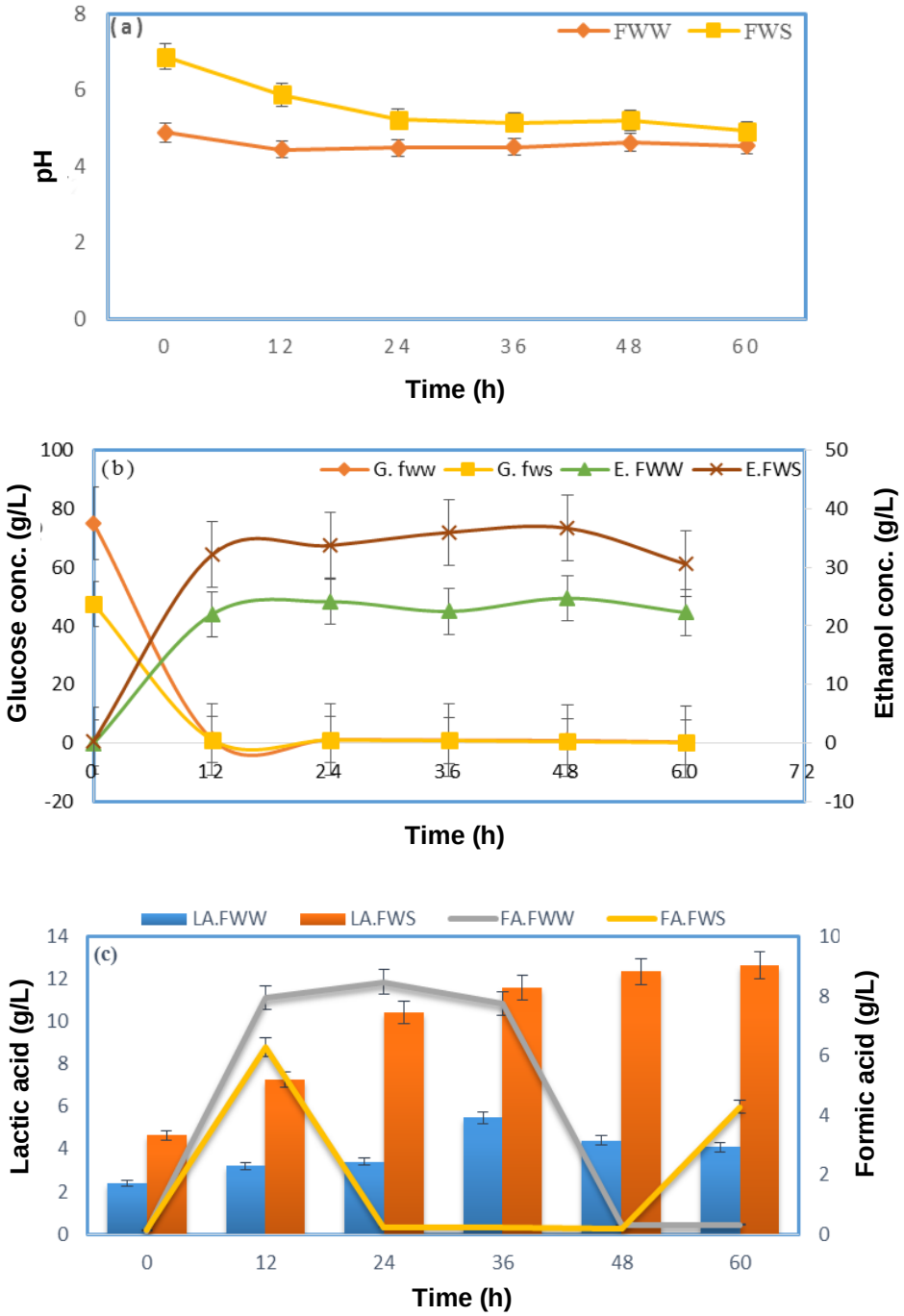

Fig. 1. Ethanol fermentation performance using methane water (FWS) vs. tap water (FWW): (a) pH change, (b) ethanol production and glucose consumed, and (c) lactic and formic acid change

There were various volatile fatty acids produced during ethanol fermentation, such as lactic, acetic, formic, propionic, butyrate, and valeric acids. Lactic and formic acid showed noticeable concentration increases compared with the others. However, lactic acid with methane water represented higher concentrations than with tap water (Fig. 1c). The initial concentration with tap water was 2.38 g/L, which increased to reach 5.47g/L, and then decreased to 4.037 g/L after 60 h of fermentation. Lactic acid concentration with methane water showed significant increases from 4.64 to 12.67g/L (Fig. 1c). Lactic acid (LA) has been reported to be the main inhibitor for ethanol production from food waste, since the high concentration of LA affected yeast growth (Thomas *et al.* 2002). The results shown in Fig. 1c. suggest lactic acid content could not totally inhibit ethanol production, but could stress *Saccharomyces cerevisiae*. The LA concentration of 2-8 g/L exerts stress on yeast that could lead to declined ethanol production (Narendranath *et al.* 2001).

Formic acid is a degradation product of furfural and 5-hydroxymethylfurfural (HMF). Furfural and HMF are formed by the dehydration of pentose and hexose sugars, respectively (Jönsson *et al.* 2013). Figure 1c shows that its content in the fermentation medium with methane water sharply increased to about 6.28 g/L after 12 h of fermentation, then decreased sharply to zero in the following 36 h. After 60 h it again increased sharply to about 4.28 g/L. For comparison, there was stable formic acid production with tap water during ethanol fermentation. The concentration of weak acids production during fermentation depends on the pH value, which is a critical variable during ethanol fermentation (Palmqvist and Hahn-Hägerdal 2000b). The fermentation medium with methane water showed unstable formic acid concentrations, which might be due to the varied pH values, ranging from 4.92 to 6.87 (Fig. 1c). However, maximum ethanol generated was achieved at the lowest formic acid content. Furan components inhibit the decomposition pathways of numerous microorganisms (Luo *et al*. 2002). Formic acid is considered a toxic matter produced in the saccharified broth, and its inhibition effect is attributable to its toxicity to microorganism growth, particularly *Saccharomyces cerevisiae*. The study carried by Qi *et al.* (2017) reported that $CaCO_3$ could serve as a buffer to alleviate the inhibition effect of formic acid by elevating the pH value of the fermentation medium. This result suggested that the lower concentration of ethanol was due to the hampering of formic acid to the yeast. However, the influence of formic acid on yeast still needs to be clarified.

Performance of Ethanol Fermentation *via* Electrodialysis Left Water

The fermentation time was reduced by 50% using electrodialysis water compared with tap water. Ethanol concentration rapidly increased within the first 12 h to 35 g/L using electrodialysis water; the concentration with tap water reached 43 g/L (Fig. 2b). Maximum ethanol production (47 g/L) was achieved using electrodialysis water after 24 h of fermentation, while the maximum ethanol was achieved with tap water (49.5 g/L) after 48 h of fermentation. The ethanol production was nearly completed within 24 h. Although the ethanol concentration was slightly lower than that of tap water, the fermentation time decreased by 50%, which was more suitable for ethanol industry than tap water. Stillage recycled in ethanol production would accumulate numerous kinds of byproducts; however, a previous study on starch-based substrates showed there was no significant influence on ethanol production, even for 75% stillage recirculation (Wojciech 2010).

The pH of the fermentation broth gradually decreased with fermentation time of 60 h from 5.2 to 4.4 (Fig. 2a). Previous research stated that the optimal range of 4.0 to 5.0 for ethanol fermentation provided ethanol efficiency of about 61.9% (Lin *et al.* 2012).

Therefore, the maximum ethanol concentration was achieved at 24 h that had a medium pH value.

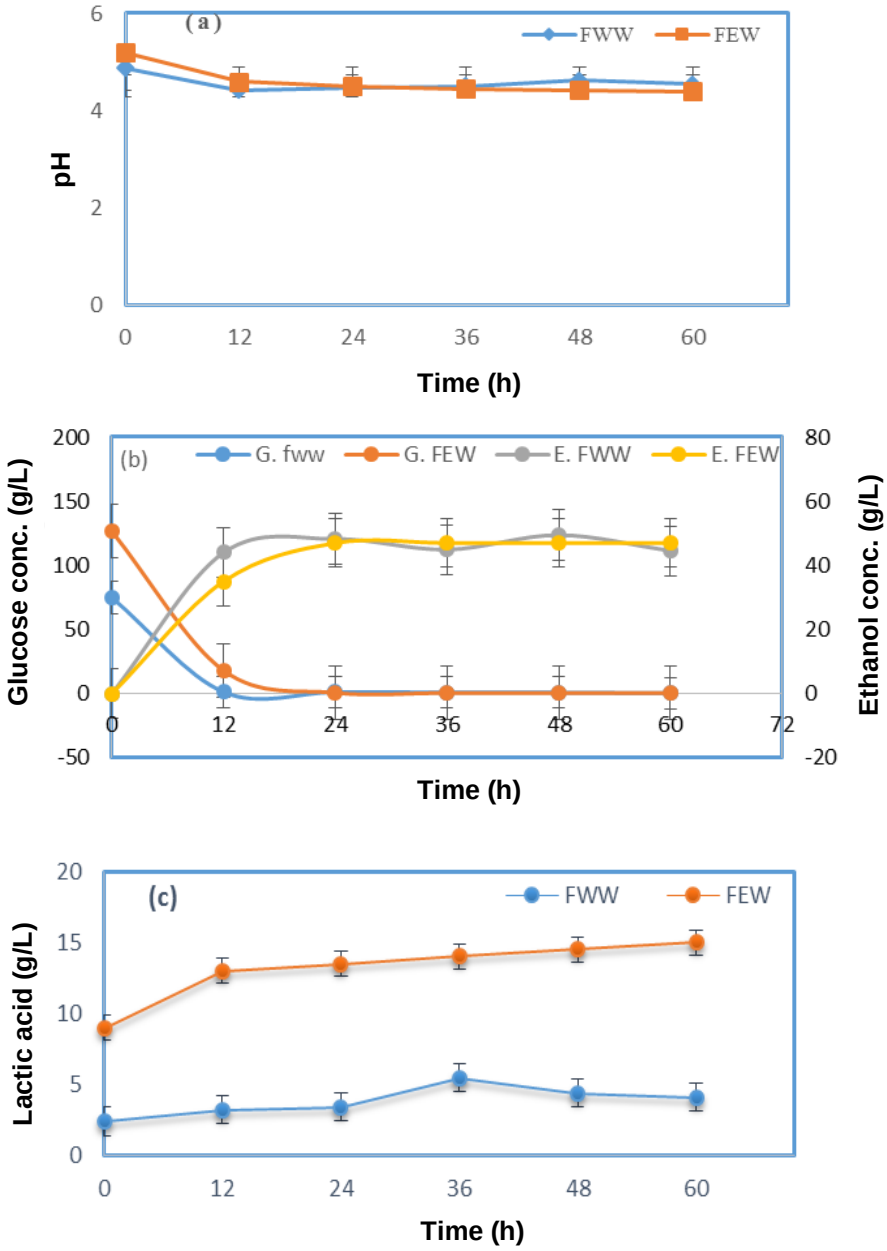

Fig. 2. Ethanol fermentation performance using electrodialysis left water (FEW) *vs*. tap water (FWW): (a) pH change, (b) ethanol production and glucose consumed, and (c) lactic acid change

Lactic acid is produced within fermentation systems due to the carbohydrate metabolism, and it is produced by the action of lactic acid bacteria (Graves *et al.* 2006). Lactic acid in the electrodialysis water before saccharification was 20 g/L, which could increase due to the presence of lactic acid bacteria in food waste. This investigation showed that the initial content of lactic acid in the fermentation broth was 9 g/L. Then it increased to 13 g/L within the first 12 hours, and gradually increased to reach 15 g/L after 60 hours (Fig. 2c). This result revealed that the lactic acid content after 60 h of fermentation was

less than that before the saccharification process. Studies carried out on the effect of lactic acid on ethanol production from corn mashes stated that LA concentrations of at least 40 g/L could severely reduce ethanol production (Graves *et al.* 2006), which was more than in this study.

Another study showed that lactic acid concentration of about 20 g/L does not have inhibitory effects on ethanol fermentation, but at 60 g/L, the fermentation media showed considerable inhibition effect. In conclusion, the accumulation of lactic acid could extend the fermentation time (Ma *et al.* 2016). In this study, the fermentation time was reduced by 24 h, obviously indicating that the lactic acid content did not influence the microorganism growth and metabolism. In addition to lactic acid, acetic, butyric, propionic, and formic acids were produced in the fermentation system at lesser quantities and evaporated in the distillation process.

Performance of Ethanol Fermentation *via* MFC Residual Water

A maximum ethanol result of 46.41 g/L was obtained after 24 h of fermentation using FWM water, which was the most comparable to tap water. Although the maximum ethanol yield with tap water was 49.5 g/L higher than that of MFC water, the fermentation time using MFC water was reduced by 50%; which could affect the overall ethanol production cost. Prolonged fermentation duration could lead to more consumed energy, which is considered the second highest cost in ethanol production (Zi *et al.* 2013). The ethanol content found in the saccharified broth before fermentation using MFC water was 0.07 g/L, and increased rapidly after 12 h to about 44.96 g/L. Whereas there was no ethanol detected before fermentation using tap water, that number increased to about 43.9 g/L after 12 h of fermentation (Fig. 3b). The initial pH value in the fermentation system using FWM water was (6.09), slightly higher than the optimal value, and then it dropped to 4.6 after 12 h of fermentation. Efficient ethanol performed under a pH of 4.62, which is in the optimal range.

The research by Wei *et al.* (2015) on stillage recycling in ethanol production from food waste showed a decreased pH value after fermentation for each cycle until the 3rd, which caused severe acidification of the fermentation media and inhibited ethanol fermentation. In this study, there was no significant impact of pH on ethanol production during 60 h of fermentation and the minimum pH value reported was about 4.6, suitable for microorganism growth (Fig. 3a).

With regard to organic acids effect, lactic acid showed minimal content in the fermentation system, approximately similar to that of tap water, and had no effect on the ethanol fermentation process (Fig. 3c). As shown in Fig. 3d, there was a substantial amount of formic acid accumulated during the fermentation process. The initial fermentation system content using MFC water was 0.56 g/L, rapidly increased to about 10.28 g/L after 12 h of fermentation. Then, the value slightly increased to about 11 g/L after 24 h, and decreased gradually to about 10.48 g/L in the following 36 h. Weak acids have been explored as inhibitors to microbes' cell growth, and accordingly, can be used for food preservation. The influence of formic acid on yeast activity could be related to the pH value because the effect of undissociated acid is a function of pH, so that it increases with pH decreases (Palmqvist and Hahn-Hägerdal 2000b). In short, the current study showed maintaining of pH value in the favoring of ethanol fermentation, which could control inhibition due to organic acids accumulation.

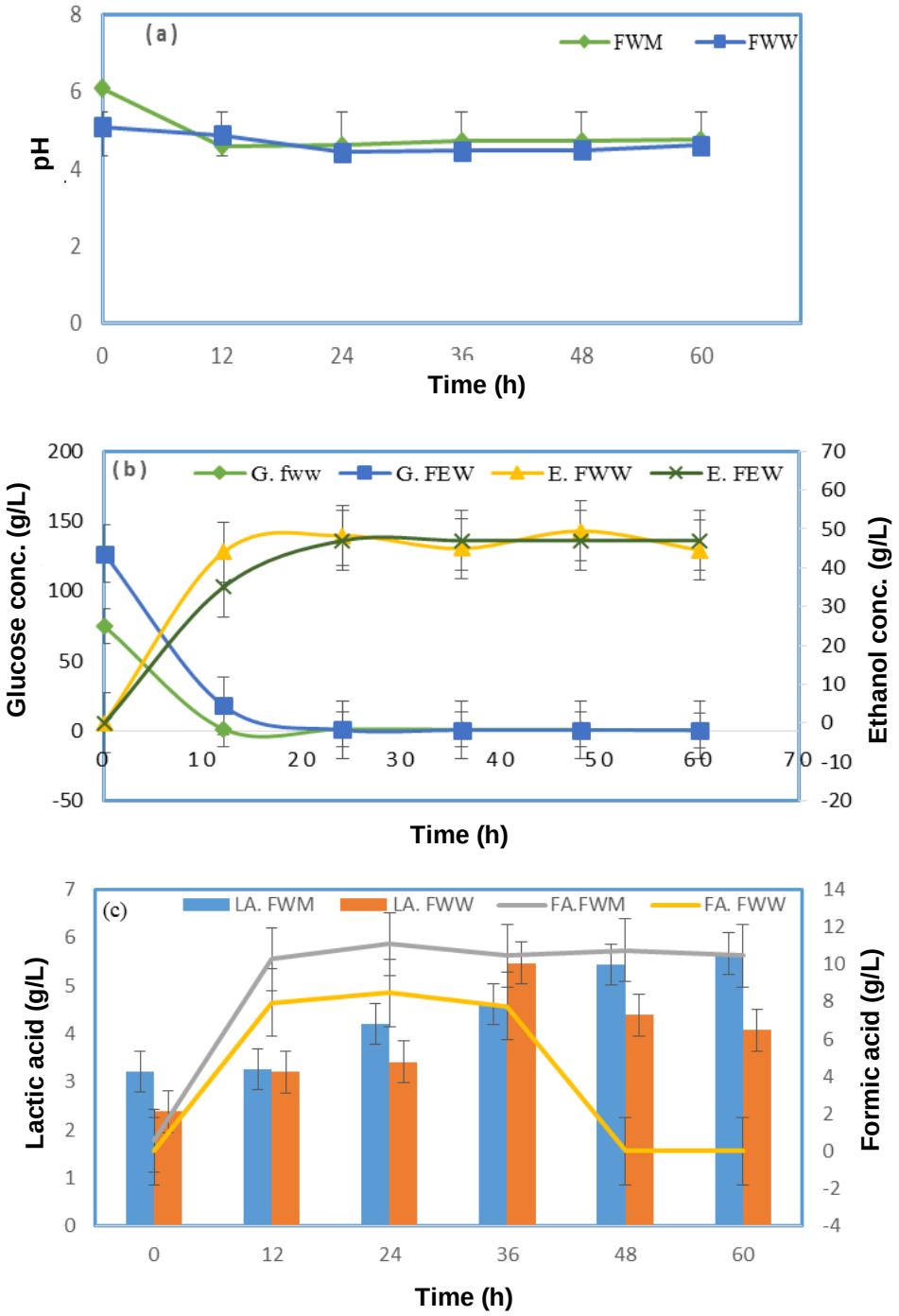

Fig. 3. Ethanol fermentation performance using MFC left water (FWM) *vs*. tap water (FWW). (a) pH change, (b) ethanol production and glucose consumed, and (c) lactic and formic acid change

Potential Inhibition Effects of Volatile Fatty Acids on Ethanol Fermentation

The production of volatile fatty acids within the fermentation system is inevitable due to bacterial contaminants in food waste. When these VFAs are present in significant values, they can have an influence on the ethanol fermentation process. In some cases, they inhibit the fermentation by their toxicity on yeast. Aliphatic acid can inhibit fermentation by either uncoupling the metabolism or accumulating intracellularly in its anionic form

(Baral *et al.* 2014). The two mechanisms are both based on the following principle: (i) Dissociated weak acids cannot diffuse across the plasma membrane, and their inhibition is due to the flows of undissociated acid into the cytosol; and (ii) Dissociation of the weak acid occurs in the cytosol due to the neutral intracellular pH, thus resulting in the decrease of cytosolic pH (Palmqvist and Hahn-Hägerdal 2000b). The influenced concentration of undissociated acids mainly depends on the pH (Qi *et al.* 2017). Ethanol fermentation can be inhibited when undissociated propionic acid is greater than 53.2 mmol/L with a pH value less than 4 (Zhang *et al.* 2012). Another report mentioned that there was inhibition effect on yeast growth due to the presence of lactate, acetate, and propionate (Moon 2010). Table 2 shows that there was VFA content in these waters before they were mixed with FW, and their content varied depending on the different process waters. For methane water, the higher content (6 g/L) was due to acetate, which declined in the fermentation system after 12 h to around 3 g/L, and remained around this value during the 60-hour fermentation. The hampering effect of acetic acid on yeast increased when the pH of the fermentation medium decreased (Graves *et al.* 2006). The lowest pH value within the optimal range of ethanol fermentation was 4 to 4.5. Therefore, acetic acid had no inhibition effects on ethanol fermentation using methane water. The dominant acid detected in fermentation system, including lactic, formic, and acetic, did not completely inhibit ethanol fermentation, but the ethanol concentration was lower than that of tap water. Additionally, the other volatile fatty acids showed insignificant amounts in the fermentation system (Fig. S2). With regard to the MFC water, the higher content of volatile fatty acid was due to acetic acid. The initial content of acetic acid was 7.41 g/L, which decreased rapidly to 2 g/L after 12 h of fermentation and remained around this value during the following 60 h of fermentation. The effect of acetic acid was investigated in corn mash. Content greater than 8 g/L can reduce ethanol production (Graves *et al.* 2006). In this study, the higher content was about 7 g/L lower than the inhibition value, and there was no inhibition due to acetic acid for ethanol fermentation. It should be mentioned that the other VFAs showed insignificant contents in the fermentation system, excluding lactic and formic acid, which have been discussed above (Fig. S3). The bipolar membrane electrodialysis was used to extract the VFAs from recycling stillage, so that there were insignificant initial contents of VFAs in FEW water. In summary, this study revealed that the volatile fatty acids content before and during the ethanol fermentation process had no negative influence on the fermentation process because of stable pH values.

CONCLUSIONS

1. This study established an integrated process for ethanol fermentation from food waste saccharification based on wastewater reuse and zero discharge. Reused wastewater was obtained by different treatment methods. The high-efficient performance showed that recycled water can be potentially used for ethanol fermentation from food waste.
2. Among the three different recycled waters, electrodialysis recycled water supported the best growth of *Saccharomyces cerevisiae*, generating the highest concentration of ethanol (47 g/L). Compared to tap water, the fermentation time was shortened by up to 50% using both microbial fuel cell (MFC) and electrodialysis recycled water.

3. Furthermore, the volatile fatty acids had no inhibition effects on ethanol production as detected in this study. This recycling process can reduce both water and energy consumption, in addition to eliminating wastewater discharge.

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