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## Performance of Single Chamber Microbial Fuel Cell (SCMFC) for biological treatment of tofu wastewater

To cite this article: D Permana and Djaenudin 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **277** 012008

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# Performance of Single Chamber Microbial Fuel Cell (SCMFC) for biological treatment of tofu wastewater

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**Abstract.** The wastewater of tofu industries consists of organic compounds and in turn, may affect the environment; therefore, a proper wastewater treatment system is needed. Based on its characteristics, biological treatment is a good method to treat tofu wastewater. One of the biological treatment methods that can be used is Microbial Fuel Cell (MFC), which can reduce the pollutant and at the same time generating low-power electricity. This system utilizes microorganisms as a biocatalyst to degrade organic compounds in the wastewater. This study aimed to examine the performance of Single Chamber MFC (SCMFC) to decrease biochemical oxygen demand (BOD<sub>5</sub>) and chemical oxygen demand (COD) of the tofu wastewater, as well as to generate electricity. Tofu wastewater was sterilized then filled into the reactor. Microbes that either have been acclimatized or not acclimatized were then added. Bacteria that were used were one of the three consortiums of native microbes of tofu wastewater, namely *Escherichia coli*, *Saccharomycopsis fibuligera*, and mixed culture of *E. coli* and *S. fibuligera*. Carbon (C) was used as both anode and cathode. We found that the acclimatized mixed culture of *E. coli* and *S. fibuligera* showed high BOD<sub>5</sub>, COD removal after 48 hours at 76.57 and 77.22 %, respectively. It also generated 5.49 mA of current, 757 mV of voltage, and the electrical energy produced was  $9.216 \times 10^{-5}$  kWh. The results suggest that using mixed microorganisms is one of the strategies to improve the electricity generation of MFC. The scale-up of the volume, selection of microorganism cultures, and immobilization could be other strategies for further studies.

## 1. Introduction

Tofu industry is one of the common industries in Indonesia. It produces wastes, both solid and liquid, but liquid waste has a higher impact on the environment rather than solid wastes. The liquid waste contains a high concentration of organic substances such as proteins, carbohydrates, and fats, which is harmful to the environment and causes terrible odor and a decrease of dissolved oxygen [1]. Some methods can be used to reduce organic substances in tofu wastewater. The physical-chemical techniques are usually used, for example, coagulation utilizing some coagulant such as ferric chloride, ferric sulphate, and aluminum sulfate [1,2]. However, the implementation of that method in Indonesia faces some obstacles i.e. the complexity of processes, high amount of coagulant, limited area for treatment, and high electricity cost for aeration [1,2]. On the other hand, the abundance of food industry wastewater and biomass in Indonesia would become sources for alternative renewable energy if utilized using proper technology.

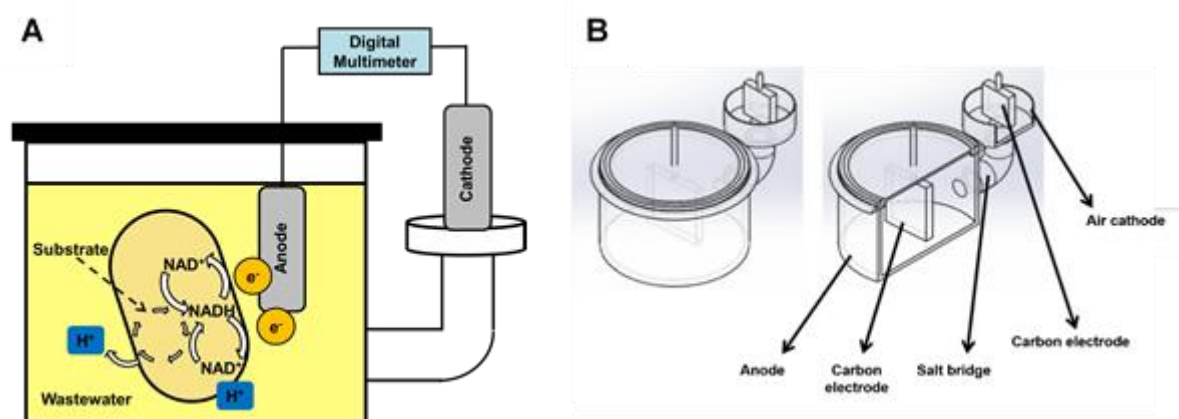
One technology to utilize the wastewater and biomass is biological fuel cell (biofuel cell), which is a technology to convert biochemical sources into electricity [3–6]. Biofuel cell uses cheaper and environment-friendly biocatalyst compared with the metal catalyst of the fuel cell [7]. Microbial fuel



cell (MFC) is one of the biofuel cells which is effective and efficient because it uses living microorganisms as a biocatalyst. The substrates of MFC cover a broad range, including glucose, acetic acid, butyric acid, lactic acid, ethanol, cysteine, and serum albumin [8]. MFC is also suitable for the extreme condition in the wastewater treatment [3,9–12]. It had been used for the treatment of wastes, for example, domestic waste. Our previous reports show that MFC is able to utilize and remove organic substances in wastewater up to 80% [3,4,6]. A high concentration of organic substances in tofu industrial wastewater can be utilized by microorganisms in MFC as energy sources to produce electricity and at the same time reduce the concentration of organic materials [4,6,9,10].

The modifications of MFC were proposed by researchers to improve its performance. The efficiency and power density of MFC are influenced by factors such as electron transfer from microbes to electrodes, species, the concentration of microorganisms, proton exchange membranes (PEM), and electron mediators [5,13–16]. One of the promising MFC modifications is the use of the salt bridge to substitute the expensive PEM.

Previous studies in MFC mostly report the use of dual chamber reactors, which have disadvantages such as bigger reactors and the use of catholyte as electron acceptor [17,18]. The use of mixed culture and small reactors (single chamber) can be alternatives to improve MFC [19–22]. In this context, we proposed the use of single chamber microbial fuel cell (SCMFC) with various microorganism cultures to utilize tofu wastewater and produce electricity.



**Figure 1.** Schematic illustration (A) and design of reactor (B) of this study.

## 2. Material and methods

### 2.1. Materials

Microorganism cultures used were *Escherichia coli* and *Saccharomycopsis fibuligera* R64 (Biochemistry Laboratory, Chemistry Department, Universitas Padjadjaran). Tofu industrial wastewater was obtained from a tofu company in Lembang, West of Bandung, Indonesia. The chemicals used were yeast extract (Difco), peptone (Difco), ammonium sulfate (Merck), potassium dihydrogen phosphate (Merck), dipotassium hydrogen phosphate (Merck), granulated agar (Difco), and Potato Dextrose Agar (PDA) (Himedia). The purchased chemicals were used as received.

### 2.2. Instrumentation

In this study, based on our previous reports we proposed a compact and affordable design of the SCMFC reactor that consisted of an anode chamber and an air cathode. The reactor was made from plastic material and PVC pipes. The maximum volume capacity of the reactor was 1 L. The SCMFC reactor was assembled as illustrated in Figure 1. The anode chamber was filled with tofu wastewater, and carbon electrode (C) was used and placed in the anode and cathode with 4 cm of width  $\times$  10 cm of weight. Carbon was used as an electrode because it was low-cost, inert, and commonly used in MFC.

## 2.3. Methods

**2.3.1. Microorganisms culture.** *E. coli* culture was grown on agar slope of sterile Luria-Bertani (LB) containing (in % w/v): 0.5 of yeast extract, 0.5 of peptone, 0.3 of ammonium sulfate, 0.3 of potassium dihydrogen sulfate, and 1.5 of agar. The agar slopes were streaked with *E. coli* and incubated at 37°C for 48 hours (Mettler Toledo). Slopes were stored at 4°C and sub-cultured every six months. *S. fibuligera* culture was grown on sterile Potato Dextrose Agar (PDA) (39 g/L).

**2.3.2. Growth medium.** The growth medium used for *E. coli* and *S. fibuligera* was yeast extract peptone dextrose (YEPD) medium, which consisted of (in % w/v): 0.5 of yeast extract, 0.5 of peptone, 0.3 of ammonium sulfate, 0.3 of potassium dihydrogen phosphate, and 2.0 of glucose. All ingredients were weighed and dissolved in distilled water and sterilized using an autoclave (Hirayama HL36 AE) at 121°C, 15 psi for 15 minutes. One single colony of *E. coli* or *S. fibuligera* was taken from slope agar, inoculated into new sterile 100 mL YEPD broth in 500 mL Erlenmeyer flask, then incubated and shaken at 30°C, 150 rpm for 18 hours using a shaking incubator (B. Braun Biotech International, Certomat BS-1). The ratio of Erlenmeyer size to culture volume was 4:1 to maintain the availability of dissolved oxygen. The starter culture was then transferred into tofu wastewater medium in the SCMFC reactor. The concentration of microorganisms added was  $\sim 10^6$  CFU/mL and the volume of starter culture was 10 % (v/v) from the total volume of 1000 mL. The volume of tofu wastewater was 900 mL.

**2.3.3. Wastewater preparation.** Wastewater was obtained from a tofu company in Lembang, West of Bandung. Wastewater was stored in a 5 L sterile jerry can. Sterilization was done with the addition of 70% alcohol into the jerry can, shaken well and removed before the wastewater was loaded. The sample was placed in a refrigerator (4°C) before use and initial characterization. One litre of the wastewater was directly used as the inoculum of a consortium of native microbes, while another 4 L of wastewater was sterilized by using an autoclave at 121°C, 15 psi for 15 minutes.

**2.3.4. The characteristics of tofu wastewater.** The characteristics of tofu wastewater from a tofu company in Lembang, West of Bandung is shown in Table 1. The tofu wastewater had a high concentration of BOD<sub>5</sub> and COD and a low pH. All of the parameters exceeded the quality standard of liquid waste for industrial activities mentioned in the Regulation of the Minister of Environment of the Republic of Indonesia, No. 51 the Year 1995 about Quality Standard of Liquid Waste for Industrial Operations. Further processing of pH, BOD<sub>5</sub>, and COD was mandatory according to the quality standard. The parameters measured were pH, BOD<sub>5</sub>, and COD because they are general parameters for investigating the efficiency of the wastewater treatment and essential in the determination of the level of pollution of water.

**Table 1.** Characteristics of tofu wastewater used in this study.

Parameter	Unit	Result	Quality standard <sup>a</sup>
<b>BOD<sub>5</sub></b>	mg/L	8700	150
<b>COD</b>	mg/L	11880	300
<b>pH</b>	-	3.78 – 4.71	6.0 – 9.0

<sup>a</sup> Quality standard, Minister of Environment of Republic of Indonesia No. KEP-51/MENLH/10/1995.

**2.3.5. Methods for analysis.** The MFC experiment was conducted in a batch mode for 48 h to study the performance of the system and the voltage. The time of the MFC process was decided based on the growth curve of the microorganisms. The voltage, current, and pH were measured every 4 h using a digital multimeter (Sanwa 510a PC link). Sampling was conducted at the initial and end of the reaction to determine the COD and BOD<sub>5</sub>. The BOD<sub>5</sub>, COD, and pH measurement and analysis referred to SNI

6989.72: 2009, SNI 6989.73: 2009, SNI 06.6989.11: 2004, respectively. During the reaction, no additional microorganisms and nutrition were added.

**2.3.6. Power density and energy.** The MFC was a closed system. The circuit had a fixed load of 1 K $\Omega$ . Both voltage and current were measured by a digital multimeter (Model Sanwa CD800a). The power generation was calculated using equation (1) based on the data of potential and current obtained from the multimeter, while power density was calculated using equation (2) [13,14]. The energy was calculated using equation (3). Energy can be converted to kWh (1 joule = 2.7778 x 10<sup>-7</sup> kWh).

$$P = V \times I \quad \dots\dots\dots (1)$$

$$Pd = P \div A \quad \dots\dots\dots (2)$$

$$E = P \times t \quad \dots\dots\dots (3)$$

P = Power (mW)

V = Potential (mV)

I = Current (mA)

Pd = Power density (mW/cm<sup>2</sup>)

A = Anode surface area (cm<sup>2</sup>)

E = Energy (kJ)

T = Time of fermentation (second)

### 3. Results and discussions

#### 3.1. Biochemical oxygen demand (BOD<sub>5</sub>) removal

The SCMFC is one of the wastewater treatment systems that depend on the activity of microorganisms to degrade organic compounds in wastewater. The BOD<sub>5</sub> represents the content of organic compounds of the wastewater. The BOD<sub>5</sub> declared the amount of oxygen that is required for biochemical degradation of organic compounds of wastewater. The measurement result of BOD<sub>5</sub> before and after processing through SCMFC for 48 hours for each variation of the microbes can be seen in Table 2.

**Table 2.** BOD<sub>5</sub> removal from tofu wastewater using various microorganism cultures.

Time (hour)	Native microbes consortium (mg/L)	Unacclimated microorganisms (mg/L)			Acclimatized microorganisms (mg/L)			Quality standard <sup>a</sup>
		SF	EC	Mixture	SF	EC	Mixture	
0	8700	8700	8700	8700	8700	8700	8700	150
48	6186	2600	3100	2300	2232	2697	2038	
Removal efficiency (%)	28.90	70.11	64.36	73.56	74.35	69.00	76.57	

SF: *S. fibuligera*

EC: *E. coli*

<sup>a</sup> Quality standard, Ministry of Environment of Republic of Indonesia No. KEP-51/MENLH/10/1995.

Table 2 shows that all microorganisms that were used in this study could reduce the BOD<sub>5</sub> of tofu wastewater. The concentration of BOD<sub>5</sub> decreased due to the activity of microorganisms. It is suggested that microorganisms catalyzed the process of decomposition of organic matters of tofu wastewater. The lowest efficiency of BOD<sub>5</sub> removal was shown by the native consortium of microorganisms of tofu wastewater, which was only 28.90%. It could be caused by the high concentration of organic substances that digested hardly by the native microorganism. However, tofu wastewater with additional microbes (either with or without acclimatization) and mixed culture resulted in higher efficiency of BOD<sub>5</sub> removal. The efficiencies of BOD<sub>5</sub> removal of acclimatized microorganism cultures of *E. coli*, *S. fibuligera*, and mixtures were 74.35, 69.0, and 76.57%, respectively. They were also higher compared

to the unacclimatized microorganisms cultures. One of the benefits of the use of mixed culture microbes is they could perform better degradation because the microorganisms worked synergistically. The final concentration of BOD<sub>5</sub> after SCMFC treatment on all variations had not met the quality standard (150 mg/L), because of the microbe concentration was low. The bacteria added for each species on each of the variations was  $\sim 10^6$  CFU/mL. The concentration was not enough to degrade organic compounds with quite a high concentration of the tofu wastewater [4].

**Table 3.** COD removal from tofu wastewater using various microorganism cultures.

Time (hour)	Native microbes consortium (mg/L)	Unacclimated microorganisms (mg/L)			Acclimatized microorganisms (mg/L)			Quality standard <sup>a</sup>
		SF	EC	Mixture	SF	EC	Mixture	
0	11880	11880	11880	11880	11880	11880	11880	300
48	8316	3420	4180	3040	2952	3504	2706	
Removal efficiency (%)	30.00	71.20	64.80	74.40	75.15	70.50	77.22	

SF: *S. fibuligera*

EC: *E. coli*

<sup>a</sup> Quality standard, Ministry of Environment of Republic of Indonesia No. KEP-51/MENLH/10/1995.

### 3.2. Chemical oxygen demand (COD) removal

The COD concentration of tofu wastewater was also determined. Table 3 shows the value of the COD concentration and removal efficiency before and after processing through SCMFC for 48 hours. The COD removal efficiency from tofu wastewater by native microbes was only 30%. However, the SCMFC with unacclimatized *E. coli*, *S. fibuligera*, and both combination resulted in average COD removal efficiencies of 64.80, 71.20, and 74.40%, respectively. The acclimatized *E. coli*, *S. fibuligera*, and both combination resulted in better removal efficiencies of 70.50, 75.15, and 77.22%, respectively. The acclimatized microbes that were already adapted to the condition of wastewater could oxidize organic compounds more effectively compared to the unacclimated microorganism cultures [5].

Recent reports on the use of SCMFC in wastewater treatment reported that the COD removal was high and reached 90% for other types of wastewater such as municipal (1755 mg/L of COD) and carbohydrate-rich synthetic wastewater (3532 mg/L of COD). However, they also prolonged the reaction from 6-15 days [23,24]. Furthermore, they also modified the electrodes and used special wires. The removal of COD after 2 days was shown to be approximately 80% and only slightly different from our result. Thus, our result in COD removal was comparable even though we did not modify the electrodes and prolong the incubation time.

### 3.3. Evaluation of the wastewater pH

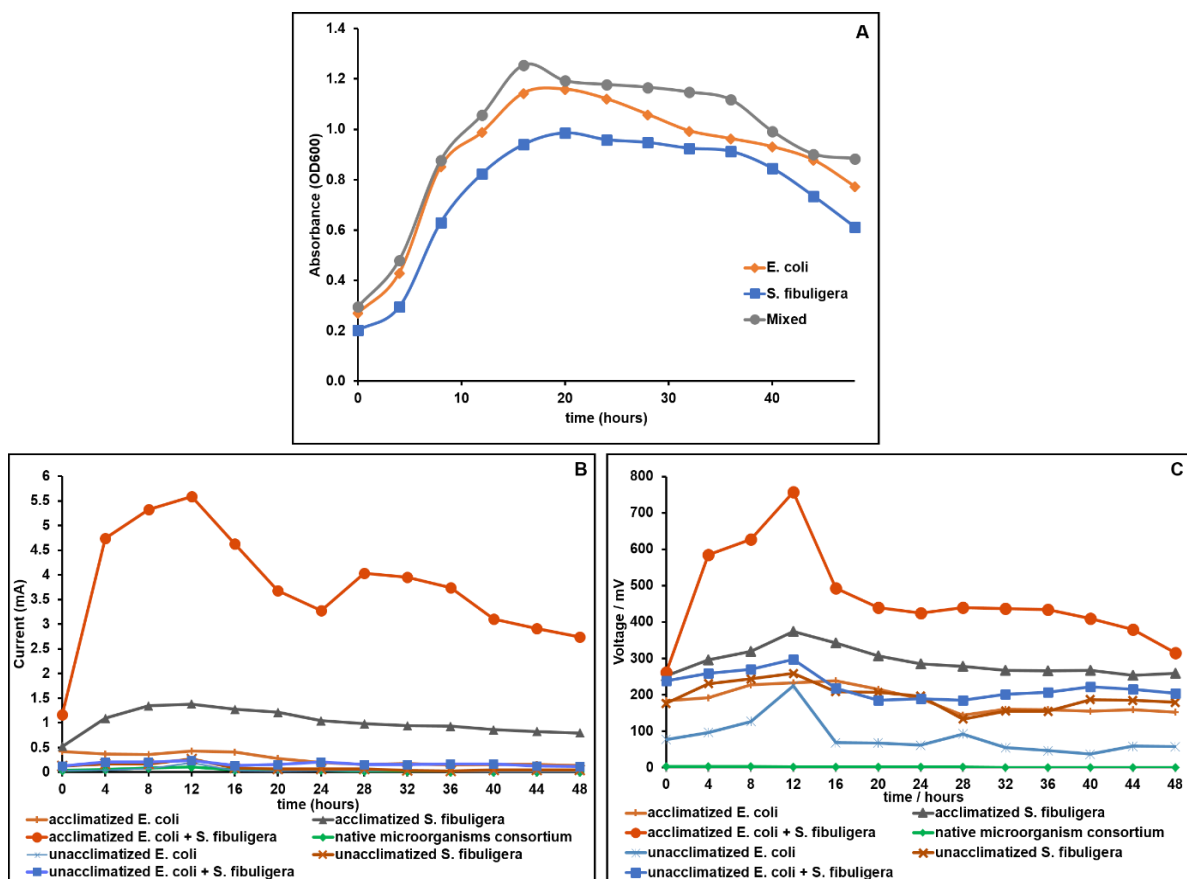
Next, we measured the pH of tofu wastewater to observe the performance of SCMFC to increase the pH of the wastewater. We used tofu wastewater in similar condition as received. We did not adjust the pH in order to study the ability of the microorganisms to adapt to the extreme condition. The measurement of the pH was related to their function in controlling the growth of microorganisms because the activity of the microorganisms was pH-dependent (Table 4). The increases in the reaction time were followed by a rise of pH. It was because the process of the degradation of organic substances (carbohydrates, proteins, and fat) by the microbes would produce protons and electrons. The protons then would move into the cathode through the salt bridge. An increased pH level of the waste was associated with the flow of protons from the anode to the air cathode that reduced the number of protons of the wastewater. Therefore, the increases in the tofu wastewater pH at the end of the process of SCMFC were low due to the limited transfer of protons, since we only used a salt bridge and not a proton exchange membrane (PEM). The advantages of the salt bridge are the low cost and applicability to the large reactor [5].

**Table 4.** The changes of pH of tofu wastewater before and after MFC process.

	Microorganisms	Initial pH	Final pH
<b>Without acclimatization</b>	Native microbes consortium		3.94
	<i>E. coli</i>		4.26
	<i>S. fibuligera</i>		4.80
	Mixture	3.78	4.00
<b>With acclimatization</b>	<i>E. coli</i>		4.79
	<i>S. fibuligera</i>		4.80
	Mixture		4.75

### 3.4. Current and voltage profile

The current and voltage profile are commonly correlated to the growth curve of the microorganisms. Figure 2A shows the growth curve of the microorganisms in the wastewater medium. Microorganisms were acclimatized to the conditions of wastewater slowly by changing the percentage of growth medium and wastewater; 100:0, 75:25, 50:50, 25:75, and 0:100%. The growth curve of microorganism cultures was showed the same trend and only slight differences were observed. Furthermore, the growth curve of the microorganism cultures must be correlated to its activity to oxidize the substrates in tofu wastewater.



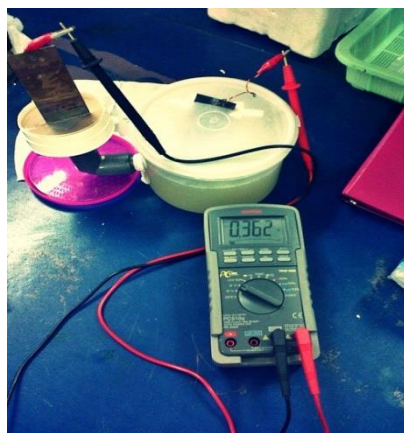
**Figure 2.** The growth curve of microorganisms and electricity profile of MFC. Growth curve of microorganisms in 48 hours (A), current (B), and voltage profile (C) of SCMFC of tofu wastewater with various microorganism cultures for 48 hours.

The measurement of current and voltage profile was conducted every 4 h during the process of SCMFC for 48 hours (Figure 2B and 2C). The SCMFC was finished only for 48 h, because of after that our microorganisms have entered the death phase. The acclimatized mixed culture of *E. coli* and *S. fibuligera* showed the best results with high current and voltage were 5.49 mA and 757 mV, respectively, at the first 16 hours. This result was because the microbes were adapted to the extreme condition of the wastewater and performed an excellent metabolism to generate more electrons from substrate conversion through complex bioenergetics reaction [5,9].

One of the advantages of the use of a mixed culture of microorganisms was they worked synergistically to degrade the organic substrates of wastewater. It also could increase the rate of substrate degradation. Interestingly, the mixed culture of microorganisms also allowed the microbes to find the easiest and shortest thermodynamic path or process resulting in faster oxidation of substrates [5]. However, because of the microorganisms still growing until the first 28 h, the biofilm of microorganism probably formed and caused the lower current and voltage [25]. In addition, the longer reaction time might also cause lower current and voltage because of the substrate was limited and the microorganisms started entering the death phase.

**Table 5.** The generated energy from tofu wastewater by using SCMFC.

Microorganisms		Average of voltage (V)	Average of current (A)	Energy (kWh)
<b>Without acclimatization</b>	Native microbes consortium	$1.06 \times 10^{-3}$	$3.22 \times 10^{-5}$	$1.643 \times 10^{-9}$
	<i>E. coli</i>	$8.17 \times 10^{-2}$	$3.92 \times 10^{-5}$	$1.536 \times 10^{-7}$
	<i>S. fibuligera</i>	$1.93 \times 10^{-1}$	$8.69 \times 10^{-5}$	$8.016 \times 10^{-7}$
	Mixture	$2.22 \times 10^{-1}$	$1.60 \times 10^{-4}$	$1.703 \times 10^{-6}$
<b>With acclimatization</b>	<i>E. coli</i>	$1.85 \times 10^{-1}$	$2.57 \times 10^{-4}$	$2.280 \times 10^{-6}$
	<i>S. fibuligera</i>	$2.89 \times 10^{-1}$	$1.06 \times 10^{-3}$	$1.468 \times 10^{-5}$
	Mixture	$4.80 \times 10^{-1}$	$4.02 \times 10^{-3}$	$9.216 \times 10^{-5}$



**Figure 3.** The measurement of voltage from a reactor during the MFC process for 48 hours by using a digital multimeter. The display of the digital multimeter shows the value of the measured voltage at a specific time.

### 3.5. Produced energy

Finally, we calculated the energy that was produced by various microorganism cultures by using equation 1, 2, and 3. The energy generated is shown in Table 5. The amount of electrical energy in the system SCMFC was influenced by the metabolic rate of the microbes. Because of the use of different microbes, different electrical power was produced. The acclimatized *E. coli* and *S. fibuligera* showed a higher current and voltage compared to other variations. The value of the energy that was produced by



the acclimatized mixed culture of *E. coli* and *S. fibuligera* was  $9.216 \times 10^{-5}$  kWh. However, the energy that was generated by a consortium of native microorganism was only  $1.643 \times 10^{-9}$  kWh. The acclimatized mixed microorganisms showed a promising power generation compared to unacclimatized microorganisms which are recommended for further development. The mixed culture of bacteria and yeast showed good combination so far. SCMFC gave the additional value of wastewater treatment, not only to reduce organic matters but also to generate electricity which can be monitored by using a digital multimeter (Figure 3) [5].

#### 4. Conclusions

The performance of SCMFC in tofu wastewater treatment was reasonable. The best BOD<sub>5</sub> and COD removal was shown by the acclimatized mixed culture of *E. coli* and *S. fibuligera*, a combination of a bacteria and yeast of 50% and 50%, respectively. The electricity generated by the operation of SCMFC was  $9.216 \times 10^{-5}$  kWh with 5.49 mA of current and 757 mV of voltage. For future development, we propose our design of membraneless reactors that can be manufactured using low-cost materials such as plastic container and cheaper salt bridge materials.

#### Acknowledgement

This study was supported by a research grant from the Research Unit for Clean Technology, Indonesian Institute of Sciences (LIPI). We thank Mahyar Ependi, Oman Rohman, and Rida Afiatika for the technical support.

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