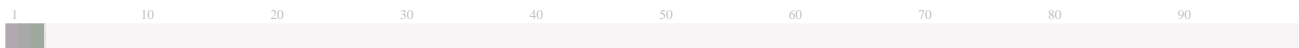


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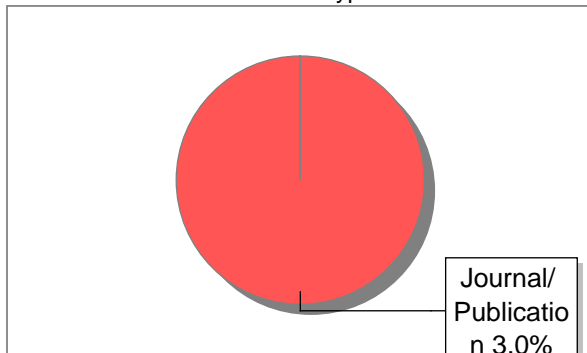
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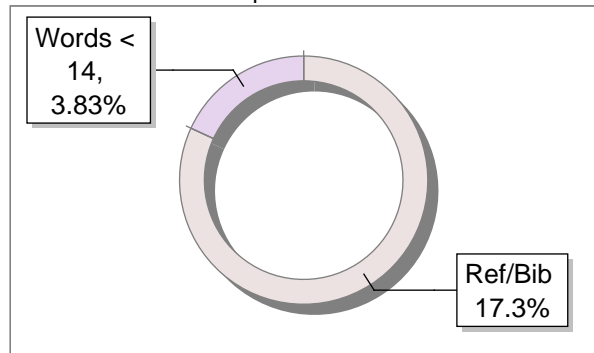
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Treating The Tofu Wastewater (TWW) using a Green Technology of Microbial Fuel Cell (MFC) System

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Abstract

Tofu is one of the favorable food products that is produced from soybeans. It can be easily produced, contains high nutrition, is cheap, and its raw material is abundant. Unfortunately, a lot of wastewaters are generated during the tofu production process. Generally, Tofu Wastewater (TWW) contains high organic pollutants which can reduce the water quality when it is directly discharged into the river. This work tries to reduce the organic pollution levels in TWW by using a green technology of Microbial Fuel Cell (MFC) system. The efficiency of TWW treatment was determined based on the reduction of Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) levels, decrement of Total Suspended Solid (TSS) and pH changes. In addition, the amount of the electrical generated by MFC during the TWW treatment was investigated as a value added. The results showed that maximum COD and BOD removals were obtained $60.200 \pm 2\%$ and $61.500 \pm 7.600\%$, respectively. Whereas, the decrement of TSS was observed at $42.700 \pm 1.600\%$. Moreover, the electrical generation involves the current density the power and of MFC were obtained $389.900 \pm 23 \text{ mA/m}^2$ and $110.800 \pm 9.100 \text{ mW/m}^2$, respectively. These results indicate the MFC could be used to treat the TWW and generate the electrical power at the same time.

Keywords

TWW Treatment, MFC, Organic Pollutants, Current Generation

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1. INTRODUCTION

Indonesia is a developing country and most of its population are middle classes and below. Their living is more supported by farming, buying and selling activities in the traditional market, and/or home industry sectors. Among these sectors, the tofu industry is interesting to investigate because it has high economic value, is one of the most favorable industries and is an inexpensive industry. Unfortunately, the tofu industry has negative impacts on the environment due to its wastewater. Tofu industries may generate a lot of wastewaters (28% v/w) during the process (Sayow et al., 2020). Although Tofu Wastewater (TWW) is organic waste degradable, it may cause contamination in rivers and smell bad if it is directly dumped into the river without the treatment processes. Therefore, the TWW must be treated to reduce these pollutants before it is dumped into the environment.

Based on some research, the TWW contains rich organic matter such as carbohydrate, protein, and organic acids. In TWW, the carbohydrate such as sucrose, stachyose, fructose, glucose and raffinose were obtained around of 34.400% (w/w total carbohydrate), 21.300% (w/w), 12.300% (w/w), 7%

(w/w) and 5.6% (w/w), respectively (Wang et al., 2020; Corzo-Martínez et al., 2016). In addition, TWW containing of total nitrogen (190-950 mg/L N₂), phosphor (45.600 mg/L total P), and metals (i.e., Na, Cu, Zn, Mg, Mn, Co, Fe and Ca) (Qiu et al., 2019).

In general, wastewater such as Palm Oil Mill Effluent (POME) (Satar et al., 2017), Chess Whey Wastewater (CWW) (Carvalho et al., 2013; Rosa et al., 2014), Brewery Wastewater (BWW) (Seluy and Isla, 2014), were treated by using conventional technology such as a fermentation system (Faisal et al., 2016; Qiu et al., 2019). Although the fermentation systems are easy to handle and relatively cheap, but their efficiencies (based on the chemical oxygen demand, COD removal) were obtained around of 64.700-70.500% (Faisal et al., 2016; Qiu et al., 2019; Wang et al., 2020). So far, the highest COD removal in TWW by using the MFC system was obtained around 49.300% (Dewi et al., 2020). These results were relatively low compared to the Microbial Fuel Cell (MFC) efficiency which were more than 70% of COD removal (Satar et al., 2018; Faisal et al., 2016). In addition, the other research reports the TWW can also

be treated by using MFC systems to generate the electrical power at where the anode of MFC systems were enriched with single culture (i.e., *Staphylococcus saprophyticus* and *Aeromonas caviae*) (Putra et al., 2012). As described above, the MFC has a double function for reducing pollutant levels and generating electrical power. Therefore, these facts encourage the authors to treat the TWW by using one of the new green technologies of the MFC system using mixed culture. This work is aimed to treat the TWW using the MFC system at where the anode has been enriched with mixed-culture of anaerobic sludge of TWW.

In addition to the high efficiency, easy to operate, relatively inexpensive, the MFC systems have an additional advantage, namely generating the electrical power. Some parameters such as COD removal, Biological Oxygen Demand (BOD) removal, pH change and Total Suspended Solid (TSS) were investigated to evaluate the efficiency of TWW treatment. In addition, the electrical generation include current density and power generation were measured to investigate the MFC performance.

2. EXPERIMENTAL SECTION

2.1 Materials

Acrylic blocks, graphite foam (100% of Carbon, C), Cation Exchange Membrane (CEM, CMI 7000s) were obtained from Fuel Cell Institute Laboratory Universiti Kebangsaan Malaysia (UKM). Chemicals such as sodium acetate (CH_3COONa , 99%), ammonium chloride (NH_4Cl , quality for analysis), potassium dihydrogen phosphate (KH_2PO_4 , quality for analysis), and potassium hydrogen phosphate (K_2HPO_4 , quality for analysis) were purchased from online shops. Whereas, Deionized Water (DW) was purchased from a chemical shop in the special regency of Yogyakarta.

Whereas, instruments such as spectrophotometer (Thermo scientific) were used to analyze the Chemical Oxygen Demand (COD). The pH tester (HI98103, Hanna Instruments) was used to identify the initial and final pH of TWW. The current generation of the MFC system was measured by using a digital multimeter (Landeks 9250).

2.2 Methods

2.2.1 Microbial Fuel Cell (MFC) Design

The schematic of the dual chamber MFC system was presented in Figure 1(a). The MFC system was fabricated using acrylic blocks with size of 10 width, length 5 and 10 height. The anode and cathode active volume were 50 mL respectively. The anode and cathode chamber were separated by using a Cation Exchange Membrane (CEM, CMI 7000s). The anode and cathode materials were fabricated from graphite foam. Before MFC was operated, both anode and cathode materials were treated by soaking in 99% ethanol overnight and then washed by using deionized water. The Figure 1(b) and Figure 1(c) show the treated electrodes and CEM separator. Lastly, both anode and cathode were

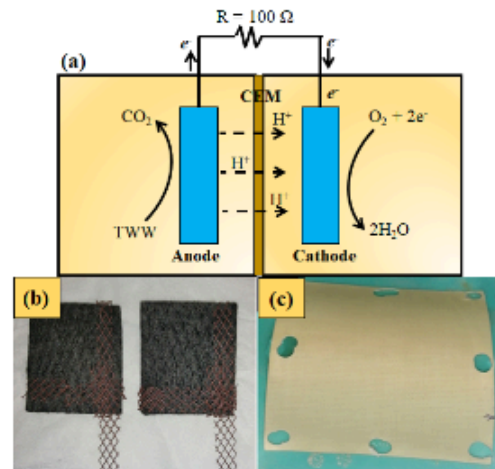


Figure 1. The Schematic of MFC System (a), Photograph of Electrodes (b) and CEM Separator during Treatment Process (c)

dried in a vacuum oven overnight at 80°C . The aim of electrode treatment is to remove the physical and biological impurities (Satar et al., 2018). In this work, the cathode was prepared without the chemical catalyst and only used oxygen as a natural catalyst.

2.2.2 TWW Pre-Treatments

TWW sample was collected from the tofu home industry pond at Barepan Margoagung Sayegan Sleman Yogyakarta, Daerah Istimewa Yogyakarta Indonesia. The pH, COD, BOD, and TSS were measured by using pH meter, the American Public Health Analysis (APHA) methods (Soto et al., 2021), titration and pro-weight filter methods, respectively. The characteristics of TWW were presented in Table 1. Due to the low pH may inhibit the microorganism activities, so the Phosphate Buffer Solution (PBS) was added into TWW to adjust pH in neutral (pH 6-7). The Phosphate Buffer Solution (PBS) consists (all in g/L); 4.580 K_2HPO_4 and 2.450 KH_2PO_4 , 0.310 NH_4Cl , and 0.130 NaCl (Wang et al., 2020).

Table 1. TWW Sample Characteristics Before Treatment

Parameters	Units	Average Values
Chemical Oxygen Demand (COD)	mg/L	1000 ± 20
Biological Oxygen Demand (BOD)	mg/L	325 ± 2.600
Total Suspended Solid (TSS)	mg/L	114.700 ± 16.500
The pH _{initial}	-	7.200 ± 0.100

2.2.3 MFC Setup and Operation

The MFC systems were operated in batch mode condition. The schematic MFC was shown in Figure 2 Both anode and cathode were connected with titanium wire to transfer



Figure 2. Photograph of MFC Setup and Operations Connected with a Multimeter Digital

the generated current from anode to the cathode. The anode was priority enriched with Electroactive Bacteria (EAB) using anaerobic TWW sludge. During the anode enrichment process, 1 g/L acetate was used as substrate in the anode compartment while the Phosphate Buffer Solution (PBS) in the cathode. To enhance the current generation, the cathode compartment was continuously aerated by using an aquarium air pump (AA-99). The generated currents were measured by using a multimeter digital (Landeks 9250) with interval of 2 h. Once the enrichment process was reached, the acetate was then replaced with the fresh TWW sample. TWW was characterized based on the COD, BOD, TSS and pH before and after MFC operations. All experiments were performed in triplicate and reported in average and deviation standard values.

2.2.4 Efficiency of TWW Treatment

Some parameters such as COD, BOD, and TSS of TWW before and after MFC run were carefully measured by using suitable equipment. In this work, percentage of COD, BOD, and TSS were used to assume the efficiency of TWW treatment. All parameters were tested based on the National Standard of Indonesian (SNI); SNI 6982.15: 2019 water and wastewater part 2 for COD analysis, SNI 6982.72: 2009 wastewater part 72 for BOD analysis, SNI 6982.14: 2009 water and wastewater part 14 for DO analysis, SNI 6989.3: 2019 water and waste water part 3 for TSS analysis. The national standard for the wastewater quality of soy processing was regulated by The Minister of Environment and Forestry of The Republic of Indonesia No. 5 of 2014 as presented in Table 2 (Kambuaya, 2014a).

2.2.5 MFC Performance Monitoring; Coulombic Efficiency

The Coulombic Efficiency (CE) can be defined as the fraction of electrons recovered as currents divided by the starting organic matter (Logan et al., 2006). Therefore, when the current generated is integrated along with operation time, then the total coulomb from the system will be obtained. From this, the CE can be determined by using the Equation (1).

$$CE = \frac{M \int_0^t Idt}{F \cdot b \cdot V_{an} \cdot \Delta COD} \quad (1)$$

Where the M is molecular weight of oxygen (32 g/mol), $\int Idt$ is the integrated currents over operation time of the system (A.s), F is Faraday's constant (96485 C/mol), b is number of electrons exchanged per mol oxygen, ΔCOD is the change of chemical oxygen demand (mg/L), and V_{an} is anolyte volume (mL).

3. RESULTS AND DISCUSSION

3.1 COD Reduction and Current Generation During EAB Enrichment Stage

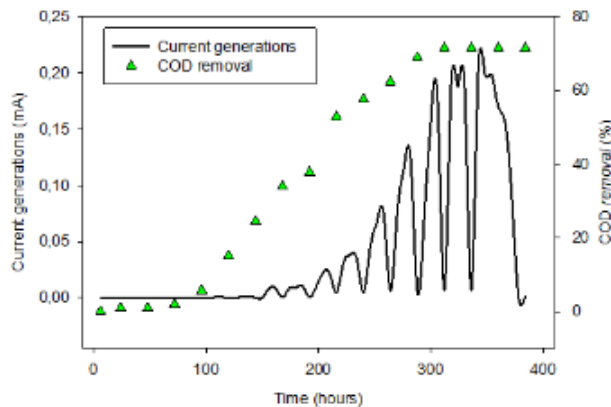
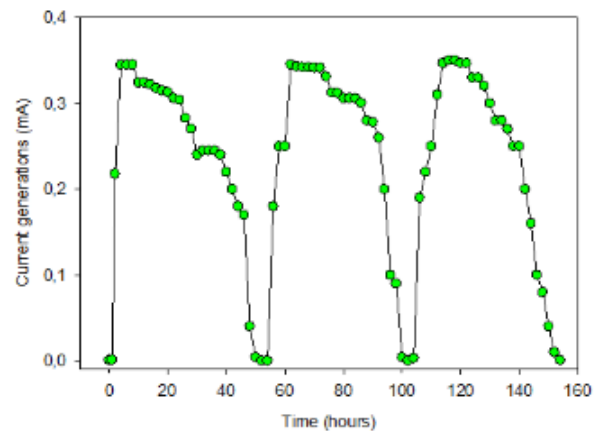
As reported in our previous work (unpublished), the enrichment process is a procedure to provide and enrich the biofilm structure at the anode. The anodic biofilm acts as a biocatalyst to convert the chemical energy composed in electron donors to electrical energy (current). The good enrichment process will produce high current generation (Pham et al., 2008), as well as high treatment efficiency. Based on Figure 3, the enrichment process was performed in the anode MFC for two weeks (342 h). The maximum current generation was observed 0.190 mA using 1 g/L acetate as substrate. Whereas, the removal of COD and BOD were obtained 71.400% and 50% respectively. Based on these facts, the enrichment process was then stopped and was followed with the TWW treatment using MFC. The enrichment process has been successfully reached when the current generations were constant for three consecutive tests.

3.2 TWW Treatment and Current Generation After Steady State Condition

Once the enrichment process of Electroactive Bacteria (EAB) was reached, the acetate substrate was then replaced with TWW. Around 2 h after feeding with TWW in the anode compartment, the current generation gradually started to increase from 0.001 mA to 0.350 mA (Figure 4). The generated currents from the MFC system were quite constant for three subsequent cycles at each of two days duration (± 48 h). At these stages, the maximum of current density and COD removal were obtained in the average of 382.900 ± 23.100 mA/m² and $60.200 \pm 2\%$, respectively. Principally, the COD removal has a positive correlation with the current generation from the MFC system (Zinadini et al., 2017).

Table 2. The Selected National Standards for The Quality of Soy Processing Wastewater in Indonesia (Kambuaya, 2014a)

Parameters	Unit	Soybean Processing		
		Soy Sauce	Tofu	Tempe
pH		6–9	6–9	6–9
TSS	mg/L	100	200	100
COD	mg/L	300	300	300
BOD	mg/L	250	150	150
Maximum Quantity (Volume)	(m ³ /ton)	10	20	10

**Figure 3.** The Current Generation and COD Removal (%) during Enrichment Stage**Figure 4.** Current Generation of MFC using TWW as Substrate

So far, the performances of MFC to remove the COD in the real wastewater were observed in the range of 70-90% (Malekmohammadi and Mirbagheri, 2021). However, this work shows the COD removal was quite lower than that selected literature, based on Table 3. The COD of TWW treatment was reduced from the 1000 mg/L to 400 mg/L for 48 h of MFC operation. This result shows the COD removal of TWW was slightly lower compared to the COD removal during the enrichment process (71.400%). This fact might be due to the organic matter compositions of TWW being more complex compared to the single substrate (sodium acetate) used at the enrichment process as reported in our previous work (unpublished work) that. In addition, the presence of toxic components in TWW might also cause the lower COD removal (Stein et al., 2012).

3.3 COD Removal and Current Generation as Indicators of MFC Efficiency

It is known that the COD is one of the important parameters to represent the level of organic pollutants in wastewater. When the COD can be consumed by Electroactive Bacteria (EAB) in high concentration, so the amount of generated current and power are also high (He et al., 2021). Principally,

organic pollutants are converted by EAB into electrons and protons and are then transferred into the cathode. Subsequently, electrons and protons with the presence of oxygen are converted into water Equation (3) (Logan et al., 2006). As described in Equation (4), the condition of electrolyte in anode (anolyte) is quite acidic due to the excess of protons. The acidic condition of anolyte is indicated by reduction of pH. In this work, the COD was decreased from 1000 mg/L to 400 mg/L (corresponding to 60% of COD removal). At this stage, the pH change was obtained from 7.200 to 6.500. In addition, the current and power densities during the COD reduction were observed $389.900 \pm 23 \text{ mA/m}^2$ and $110.800 \pm 9.100 \text{ mW/m}^2$.

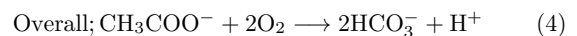
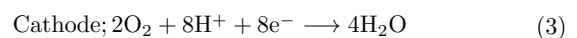
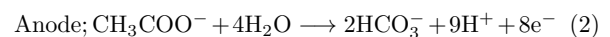


Table 3. Summary of COD Removal, BOD Removal, TSS, pH Changes, The Current Density, Power Density for TWW After Treatment and Selected References

Parameter	Units	This Work	Other Reports
COD Removal	%	60 ± 2	28-49.300 (Putra et al., 2012; Hermawan et al., 2014)
BOD Removal	%	61.500 ± 7.600	22.900-28.900 (Hermawan et al., 2014)
TSS	%	42.700 ± 1.600	47.400 (Ruhmawati et al., 2017)
The pH _{final}	-	6.500 ± 1	7.320 (Faisal et al., 2016)
Current Density (<i>J</i>)	mA/m ²	382.900 ± 23	-
Power Density, (<i>P</i>)	mW/m ²	110.800 ± 9.100	2.870x10 ⁴ (Dewi et al., 2020)
CE	(%)	0.006 ± 0.001	-

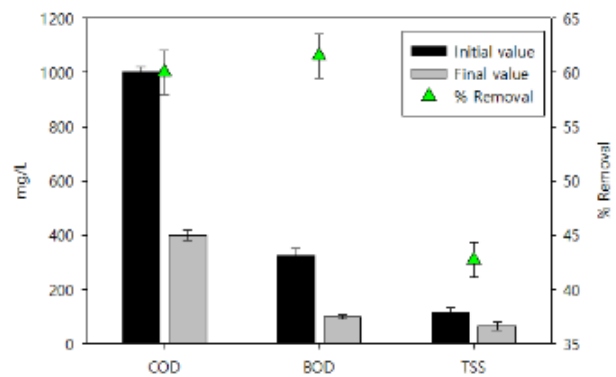
As shown in Equation (1) above, the COD change also affects the Coulombic Efficiency (CE). Although the COD removal was relatively high but the current generation was quite low, consequently the CE was low. The low current generation might be due to the ohmic and or activation losses phenomena in the MFC system. Flow of electrons from anode to cathode is very associated with the presence of resistance, interconnections and type of electrolytes (anolyte and catholyte). In addition, the activation losses are extremely increased at the low currents. As shown in Table 1, the CE was observed in a very low percentage of 0.006%. Principally, the low CE can be anticipated by reducing the distance of anode and cathode, applying catalyst on electrodes, increasing the electrode surface area and operating temperature (Logan et al., 2006).

As presented above, the current generation was directly associated with the COD removal. This fact indicates that the amount of generated current can be used as an indicator to predict the EAB activities in the MFC system. In addition to the cathode catalyst and ohmic loss, the efficiency of the MFC system was also determined by EAB activities in the anode. Therefore, the enrichment process in anode of EAB must be performed before the system was operated in real experiment.

3.4 BOD and TSS Removals

In addition to the COD, both BOD and TSS are also important parameters to assess the quality of wastewater. Based on the regulation of Environment and Forestry of the Republic of Indonesia No.5 of 2014 that the maximum value of BOD for wastewater must not exceed the quality standard of 150 mg/L before it is removed to the environment (Royani et al., 2021). From Figure 5, the BOD values before and after treatment were decreased from 325 mg/L to 125 mg/L (corresponding to 61.500% of BOD removal). This fact indicates the quality of TWW after treatment meets the standard.

Furthermore, the TSS value is generally used to investigate the presence of organic or inorganic pollutants in wastewater. The TSS is a crucial parameter in identifying the water quality associated with the clarity level. The

**Figure 5.** The Typical of COD, BOD and TSS Changes before and after Treatment

high number of solids present in water, the high TSS value, thus the less clear the water will be. The effects of the high TSS value raise a negative impact to the ecosystem due to the low dissolved oxygen, consequently inhibit the organism growth (Amerian et al., 2019). This work shows the TSS was reduced from 114.700 mg/L to 65.700 mg/L (corresponding to 42.700% TSS removal). The maximum value of TSS in TWW before and after treatments were lower than the quality standard (200 mg/mL), therefore, it can be safely removed into the environment.

3.5 pH Change

The pH is one of the important parameters that must be evaluated before and after TWW treatment. Wastewater quality is determined based on pH value in which the higher pH of 9 or lower pH of 6 indicates poor quality of wastewater (Kambuaya, 2014b). The pH of TWW before and after treatment were presented in Table 1 and Table 3, respectively. The pH of TWW was changed from 7.2 to 6.5 for two days of MFC operation. The change of pH might be due to the presence of Volatile Fatty Acids (VFAs) as a result of the organic matter consumed by EAB. As reported by Owusu-

Agyeman et al. (2020) that the yield of VFAs such as acetic, valeric and caproic were generally increased after treatment. Therefore, the pH of TWW was decreased after treatment.

4. CONCLUSIONS

The treatment of TWW was successfully performed by using a dual chamber MFC system for 154 h. The results show the highest removal of COD, BOD and TSS were observed $60 \pm 2\%$, $61.500 \pm 7.600\%$ and $42.700 \pm 1.600\%$, respectively. Whereas, the average of current density, power density and CE were obtained $382.900 \pm 23 \text{ mA/m}^2$, $110.800 \pm 9.100 \text{ mW/m}^2$ and 0.006% , respectively. Based on these results, the MFC system could be considered as one of good approaches to reduce the organic pollutant levels in TWW and to generate the currents at the same time.

5. ACKNOWLEDGEMENT

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