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Performance of salt-bridge microbial fuel cell (SB-MFC) with various microorganism cultures on the generation of electricity from tofu wastewater

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ABSTRACT A suitable wastewater treatment system is required due to the high organic compound content in tofu wastewater, which can harm the environment. Biological treatment methods are effective for treating tofu wastewater due to its characteristics. Microbial fuel cells (MFCs) represent one such biological treatment option, effectively removing organic contaminants while generating low-power electricity through bioenergetic reactions. In MFCs, microorganisms are used as biocatalysts to degrade the organic compounds present in wastewater. This study aimed to assess the efficacy of Salt-bridge microbial fuel cells (SB-MFC) using various acclimatized microbe cultures for reducing organic compounds and generating energy from tofu wastewater. Tofu wastewater was sterilized prior to introduction into the reactor. Additional microbes, including the native microbe consortium from tofu wastewater, *Escherichia coli*, *Saccharomycopsis fibuligera*, and a mixed culture of *E. coli* and *S. fibuligera*, were then introduced as biocatalysts. Carbon electrodes were utilized as both the anode and cathode. The results indicate that the mixed culture of *E. coli* and *S. fibuligera* significantly reduced COD and BOD_5 levels, with removal rates of 82.74% and 76.53%, respectively, after 48 h. Furthermore, the culture generated a voltage of 676 mV, a current of 2.53 mA, a power density of 428 mWatt/m^2, and 4.789×10^{-2} kWh of energy. This study contributes to the advancement of SB-MFC by utilizing wastewater and a combination of bacteria and yeast as biocatalysts.

KEYWORDS *Escherichia coli*; Microbial fuel cells; *Saccharomyces fibuligera*; Salt-bridge; Tofu industrial wastewater

1. Introduction

The energy source depletion has become a major issue that must be addressed by researchers and stakeholders. Consumption of fossil fuels remains high despite the scarcity of available resources. Using a variety of technologies, researchers have been attempting to identify alternate sources of energy. At the same time, we face grave environmental issues such as industrial wastewater today (Gude 2016; Naseer et al. 2021; Walter et al. 2022). Utilizing wastewater to produce energy is an excellent strategy, despite several methods having limitations, such as operational cost, field, the use of chemicals in pre-treatment processes, and the byproducts produced during the processes (Rastogi et al. 2021). Microbial fuel cells (MFCs) are biological fuel cell systems with few limitations that can be utilized to treat wastewater and produce energy (Pandey et al. 2016; Li et al. 2018; Rinaldi et al. 2018).

MFCs are one of the fuel cell systems that employ the biological activity of microorganisms that may directly convert substrates to products and generate electrical energy as byproducts (Sekrecka-Belniak and Toczyłowska-Maminska 2018). In addition, biological fuel cells employing a biocatalyst that offers some advantages over metal catalysts for hydrogen fuel cells, such as a variety of biocatalyst alternatives, are superior to metal catalysts, broad substrate range, and easy operational procedures (Pandey et al. 2016; Sekrecka-Belniak and Toczyłowska-Maminska 2018; Mukherjee et al. 2021; Sambavi et al. 2021; Verma and Mishra 2021). MFCs are based on the

catabolic metabolism of microorganisms that degrade the substrate, followed by bioenergetic events indicated by the release of protons (H^+) and electrons from the mitochondria or cytoplasm of the microorganisms into the medium. The electrons that are produced and released into the medium are then transmitted to the anode and drift to the cathode in the form of external resistance through a conductive substance (Cao et al. 2019; Paucar and Sato 2021). It will be reduced by the oxidizer, while the proton diffuses via a proton exchange membrane (PEM) to the cathode chamber proton exchanger and simultaneously forms water with the oxidizer. Intriguingly, the MFCs can utilize a broad spectrum of substrates, such as ethanol, serum albumin, glucose, acetic acid, lactic acid, cysteine, and butyric acid (Pandey et al. 2016; Verma and Mishra 2021). Moreover, MFCs have also been used and developed for bioremediation, wastewater treatment, and bioenergy applications. It is also appropriate for harsh substrates and conditions, including wastewater treatment. More than 80% of organic compounds can be removed from diverse wastewater, including tofu, tapioca, and catering wastewater, according to previous reports (Putra et al. 2018; Permana and Djaenudin 2019; Christwardana et al. 2020; Hadiyanto et al. 2022). Intriguingly, the microbes were able to exploit the high concentration of organic compounds in tofu wastewater and produce low-powered energy during the degradation of organic compounds (Rinaldi et al. 2018; Darwin et al. 2019; Permana and Djaenudin 2019). Therefore, MFC is suited for usage in severe environments, such as waste treatment.

The type and price of PEM, bioconversion efficiency of substrates in wastewater, and power density in MFCs are still problems in the development of MFCs (Walter et al. 2022). Researchers suggested some improvements to MFCs in order to increase their performance, efficiency and operational costs of reactors. One of the approaches is to use a single chamber reactor or to develop a membraneless reactor to avoid the use of expensive PEM (Ahmed et al. 2016; Putra et al. 2018; Zhao et al. 2019; Permana and Djaenudin 2019; Mohd Zaini Makhtar et al. 2021). Another promising strategy is to replace the PEM with a salt-bridge to accommodate the proton transfer (Christwardana et al. 2020; Hadiyanto et al. 2020; Silveira et al. 2020; Mukherjee et al. 2021; Sambavi et al. 2021; Sivakumar 2021). However, the usage of salt-bridge MFC (SB-MFC) is still challenging since MFCs with salt-bridges or high internal resistance may have restricted power generation because most of the electricity generated is lost as heat, which is undesirable for MFCs. Therefore, additional modifications should be considered, such as applying a mixed culture of microorganisms and a solution capable of capturing electrons from the anode.

SB-MFC with various acclimatized microbe cultures was evaluated for the removal of organic compounds and the generation of energy from tofu wastewater. *Escherichia coli*, *Saccharomycopsis fibuligera*, and a combined culture of *E. coli* and *S. fibuligera* were used as biocatalysts of SB-MFC to break down organic compounds and facilitate the production of energy from tofu wastewater. We also used $KMnO_4$ as a catholyte to collect electrons generated by the anode. After 48 h of MFCs, we discovered that the mixed culture consisting of *E. coli* and *S. fibuligera* revealed as the most effective elimination of COD and BOD. In addition, they provided the maximum current, voltage, and power density. The analysis of the remaining $KMnO_4$ concentration also revealed that more electrons were produced in the anode when a mixed culture was used as a biocatalyst, as evidenced by the formation of manganese oxide precipitate (MnO_4) and the decrease in the total Mn concentration of the solution. These findings lead to the development of inexpensive MFCs for removing organic compounds from wastewater and producing low-powered energy from wastewater.

2. Materials and Methods

2.1. Materials

Tofu wastewater sample was acquired from a tofu company in Kabupaten Bandung Barat. Potato dextrose agar (PDA) (Himedia), Granulated agar, peptone, yeast extract (Difco). ammonium sulfate, dipotassium hydrogen phosphate, glucose, potassium dihydrogen phosphate, and potassium permanganate (Merck). The purchased chemicals were utilized in their supplied form. *Saccharomycopsis fibuligera* R64 and *Escherichia coli* (Chemistry Department, Universitas Padjadjaran) were applied as biocatalysts in this study.

2.2. Instrumentation

We constructed a double chamber reactor consisting of the MFCs system. The reactor was composed of cylindrical plastic chambers (1 L) connected by PVC tubing. The reactor was assembled identical to Figure 1. The sterilized-tofu wastewater containing microorganism culture or tofu wastewater containing native microorganisms was poured into the anode compartment, while potassium permanganate ($KMnO_4$) with optimized concentration was added to the cathode compartment. Carbon was utilized as the electrode and positioned in the anode and cathode with dimensions of 4 cm by 10 cm.

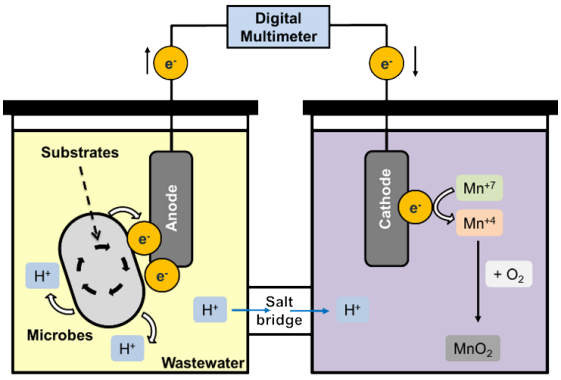

FIGURE 1 Schematic illustration of the membraneless MFC reactor.

2.3. Methods

2.3.1 Cultures of microorganisms

Escherichia coli was inoculated on an agar slope of sterile Luria-Bertani (LB) (1.0% (w/v) of peptone, 0.5% of yeast extract, 1.0% of sodium chloride, and 1.5% of agar), whereas *S. fibuligera* was grown on sterile PDA media (39 g/L). All ingredients were dissolved in distilled water and sterilized for 15 min at 121 °C and 15 PSI in a Hirayama HL36 AE autoclave. *S. fibuligera* or *E. coli* was employed to streak the agar slopes, which were subsequently incubated at 30 or 37 °C for 48 h, depending on the organism. After that, slopes were routinely subcultured every six months and maintained at 4 °C.

2.3.2 Growth medium

Both *S. fibuligera* and *E. coli* were grown in sterilized yeast extract peptone dextrose (YEPD) medium; 1.0% (w/v) of peptone, 0.5% of yeast extract, 0.3% of potassium dihydrogen phosphate, 0.3% of ammonium sulphate, and 2.0% of glucose. *S. fibuligera* and *E. coli* were isolated from a single slope agar colony and inoculated into sterilized 100 mL of YEPD broth in 500 mL Erlenmeyer flask, which was then shaken at 30 °C at 150 rpm for 16–18 h in a shaking incubator (B. Braun Biotech International, Certomat BS-1). The mixed culture of *S. fibuligera* and *E. coli* was prepared by mixing the culture with a 1:1 volume ratio which means 50 mL for each culture to make 100 mL of the total volume of culture. The concentration of both microorganism cultures was fixed at 10^6 CFU/mL (optimized concentration). The growth curve experiment was performed with the same procedures with a longer incubation time which was for 48 h. The growth of *S. fibuligera*, *E. coli* and mixed culture of them was monitored by measuring the optical density at 600 nm (OD_{600}) every 4 h with a spectrophotometer (Hitachi). The measured absorbance was then used to make a growth curve, which will be used to determine the incubation time for the SB-MFC experiments. Before the microorganism cultures were used and grown in tofu wastewater, we did step-wise acclimatization of the cultures by growing them in the mixture of YEPD medium and tofu wastewater. The cultures of microorganisms were gradually acclimated to the conditions of wastewater by varying the ratio of YEPD medium to wastewater: 100:0, 75:25, 50:50, 25:75, and 0:100%. In every acclimatization step, 100 mL of culture was used to inoculate the next mixture of YEPD and wastewater. The final culture of acclimatized microorganisms grown in 100% wastewater was then used as the final culture for SB-MFC experiments and stock culture.

2.3.3 Preparation and characterization of tofu wastewater

The tofu wastewater was provided by a tofu company in Bandung, West Java, Indonesia. Wastewater was then stored in a sterile 5 L Jerry can. Shortly after being refrigerated to 4 °C, a small volume of sample was used for initial characterization. The results of the characterization of tofu wastewater are shown in Table 1. Tofu wastewater is highly concentrated in terms of chemical oxygen demand (COD), biological oxygen demand (BOD_5), and pH. The analyzed parameters were following the Regulation of the Minister of the Environment of the Republic of Indonesia No. 51 about the Standard Quality of Liquid Waste for Industrial Activities.

One litter of the wastewater was separated and will be used for the SB-MFC experiment using the native microorganism consortium. While the remaining 4 L of wastewater was then transferred to a 2 L Erlenmeyer flask (filled with 1 L wastewater) and sterilized for 15 min at 121 °C and 15 PSI in an autoclave. Sterilized wastewater was then used for the SB-MFC experiments using *S. fibuligera*, *E. coli*, and a mixed culture.

TABLE 1 Characteristics of tofu wastewater used in this study.

Parameters	Unit	Results	Standard of Quality*
BOD_5	mg/L	8580	150
COD	mg/L	11590	300
pH	-	3.78 – 4.71	6.0 to 9.0

*Standard of Quality, Ministry of Environment of Republic of Indonesia No. KEP-51/MENLH/10/1995.

2.3.4 Preparation of the SB-MFC experiments

The SB-MFC experiments were initiated by preparing the solution in anode and cathode. In the anode compartment of the SB-MFC reactor, 100 mL of the starting culture was introduced to 900 mL of sterilized tofu wastewater to adjust the total volume to 1,000 mL. The microorganism concentration for each batch of MFCs operations was fixed at 10^6 CFU/mL. For the SB-MFC experiments using the native microorganism consortium, the separated 1 L wastewater, which contained native microorganism consortium, was directly transferred to the anode compartment of SB-MFC reactor, which was sterilized by 70% alcohol to avoid the contaminants. After both compartments of SB-MFC reactor filled with the solution, the carbon electrode was then applied to the compartments. The carbon electrodes were sterilized using 70% alcohol. The SB-MFC reactor was then connected with a digital multimeter (Sanwa 510a PC link) for the current and voltage measurement.

2.3.5 Methods for analysis

In a batch mode, the MFCs experiment was carried out. Based on the growth curve of microorganisms, the MFC process' timing was chosen. Using a digital multimeter, the current and voltage were examined every 4 h (Sanwa 510a PC link). Both before and after the reaction, the COD and BOD were examined. No additional nutrition or microbes were supplied during the reaction. The measurements and analyses for BOD_5, COD, and pH were done

following SNI 6989.72: 2009, SNI 6989.73: 2009, and SNI 06.6989.11: 2004, respectively.

2.3.6 Electrical profile measurements and calculations

MFCs is a closed system and a fixed load of 1 kΩ completed the circuit. Equation 1; calculates the power generation based on the voltage (V) and current (I) measurements recorded by a multimeter, and Equation 2; calculates the power density (Pd). Equation 3; calculates the energy.

$$P\ (mW) = V\ (mV) \times I\ (mA) \quad (1)$$

$$Pd\ (mW/cm^2) = \frac{P\ (mW)}{A\ Anode\ surface\ area\ (cm^2)} \quad (2)$$

$$E\ (kJ) = P\ (mW) \times t\ (seconds) \quad (3)$$

Energy can be converted to kWh (1 joule = 2.7778×10^{-7} kWh).

3. Results and Discussion

3.1. Removal of chemical oxygen demand (COD)

We assessed the effectiveness of the SB-MFCs with various microorganism cultures for removing COD. The COD removal before and after the MFC for 48 h is shown in Table 2. Only 27.52% of the COD in wastewater removed by native microorganisms consortium. On the other hand, the removal percentages for the acclimatized microorganisms cultures of *E. coli*, *S. fibuligera*, and the combination of them were better, which were 73.25%, 78.43%, and 82.74%, respectively. When we employed acclimatized microorganisms in our earlier research, these results were consistent (Permana and Djaenudin 2019). Suggesting that the acclimatization is one of the crucial factors to optimize the activity of microorganisms in unusual culture conditions such as wastewater. Both microorganisms usually require high nutrients in their growing medium, however, they also can growing in medium with minimum nutrients by step-wise acclimatization.

Recent papers on using SB-MFC in wastewater treatment reported high COD removal, reaching 90% for municipal (1.75 mg/L of COD) and carbohydrate-rich synthetic wastewater (3,532 mg/L of COD) wastewater. However, the reaction was performed for 6 to 15 days. In addition, the removal of COD after two days was approximately 80%, which significantly differs from our findings. In addition, they also modified the electrodes and utilized a different wiring system. Thus, without modifying the electrodes or prolonging the incubation duration, our COD removal findings are comparable with their results.

3.2. Removal of biochemical oxygen demand (BOD_5)

BOD_5 is the concentration of organic compounds in wastewater. In addition, it indicated the amount of oxygen necessary for the degradation of organic molecules in a sample via the metabolic pathway. Table 3 displays the results of BOD_5 analysis before and after the MFC reaction for 48 h.

Table 3 demonstrates that all microbes utilized in this study contributed to the reduction of BOD_5 in the tofu wastewater. Due to the activity of microorganisms, the BOD_5 concentration was reduced. It appears that bacteria stimulated the breakdown of organic substances in tofu wastewater. The consortium of native bacteria in tofu wastewater had the lowest BOD_5 removal effectiveness, at just 26.44%. The high concentration of organic compounds may not have been entirely digested by the indigenous bacteria. Whereas the additional of acclimatized *E. coli*, *S. cerevisiae* and the mixture of them to the tofu wastewater resulted in higher BOD_5 removal efficiency, which were 69.11%, 73.19%, and 76.53%, respectively. It suggests that in most cases the additional exogenous microorganisms can increase the BOD_5 removal efficiency and the substrate consumption in MFCs. In addition, one of the advantages of mixed culture is that it increases substrate consumption and degradation because the microorganisms work synergistically. However, it is still unclear which microorganis culture was more dominant than others because we added the microorganism culture at the same concentration of $\sim 10^6$ CFU/mL. However, based on the removal percentage of BOD_5 of *S. fibuligera*, we evaluated that it might be playing a slightly better performance in the biodegradation of organic substances in the tofu wastewater. However, because this is the first report on the use of *S. fibuligera* as a biocatalyst for MFC reactor, thus, further studies are required to support our claim. Although the final BOD_5 concentration after the MFCs has not met the standard of quality (150 mg/L) on all experimental variations, however, the results are still promising and open for further development and modifications.

3.3. Electrical profile

The relationship between the current and voltage of an MFC and the growth curve of microorganisms is predom-

TABLE 2 The COD removal of tofu wastewater after 48 h.

Time (hours)	Native microbes consortium (mg/L)	Microorganisms (mg/L)			
		E. coli	*S. fibuligera*	Mixture	Standard of quality*
0		11590			
48	8400	3100	2500	2000	300
Removal efficiency (%)	27.52	73.25	78.43	82.74	

*Standard of Quality, Ministry of Environment of Republic of Indonesia No. KEP-51/MENLH/10/1995.

TABLE 3 The BOD removal of tofu wastewater after 48 h.

Time (hours)	Native microbes consortium (mg/L)	Microorganisms (mg/L)			
		E. coli	*S. fibuligera*	Mixture	Standard of quality*
0		8580			150
48	6311	2650	2300	2013	
Removal efficiency (%)	26.44	69.11	73.19	76.53	

*Standard of Quality, Ministry of Environment of Republic of Indonesia No. KEP-51/MENLH/10/1995.

inant. Since MFCs were a system dependent on the activity of microbes, it seemed reasonable. Figure 2a depicts the growth curve of microorganisms in wastewater. The cultures of microorganisms were gradually acclimated to the conditions of wastewater by varying the ratio of YEPD medium to wastewater: 100:0, 75:25, 50:50, 25:75, and 0:100%. As indicated in Figure 2a, the growth curve of microbe cultures demonstrated a similar trend, with minor variances in reported results. Additionally, the growth curve of the microbe cultures must correlate with their ability to oxidize substrates in tofu wastewater.

During the 48 h MFC reaction, the current and voltage profiles were measured every four hours (Figure 2b and 2c). Due to the development of microorganisms, which enter their death phase after 36 h, the MFC reaction was conducted for just 48 hours. In the first 28 h, the combined *E. coli* and *S. fibuligera* cultures generated the best results, with a high current and voltage of 2.53 mA and 676 mV, respectively. This result implies that the bacteria have already acclimated to the harsh circumstances of the tofu wastewater and have accomplished an effective substrate conversion in order to create more electrons via complex bioenergetic processes along their metabolic pathway. On the other hand, the current and potential of the single cultures of *E. coli* and *S. fibuligera* were found to be lower than those of the mixed culture. Surprisingly, *S. fibuligera* demonstrated a stronger electrical current than *E. coli*. Possible correlation with their capacity for the breakdown of organic compounds in tofu wastewater, as evidenced by the percentage of COD and BOD_5 elimination. Although the reactor has a constant Ohmic resistance, the potential of *E. coli* and *S. fibuligera* were only slightly different.

One of the advantages of implementing mixed cultures of microorganisms is that they may break down organic wastewater substrates in collaboration. It can also accelerate substrate degradation. Curiously, mixed bacteria cultures also enable pathogens to select the simplest and shortest thermodynamic path or process, resulting in faster substrate oxidation. However, because the bacterium continued to grow for the first 28 h, it is likely that the colony's biofilm increased and caused the current and voltage to drop. In addition, prolonged reaction periods might de-

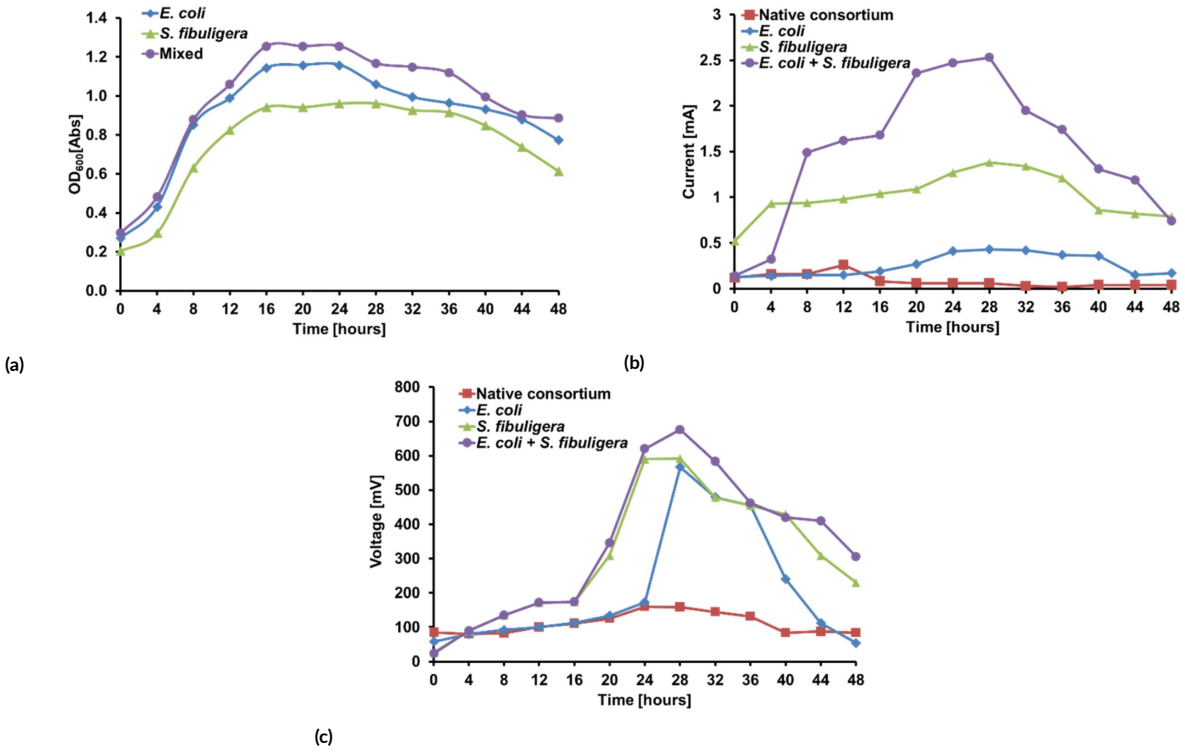

FIGURE 2 Growth curve, current, and potential profile observed from SB-MFC with various microorganism cultures. (a) growth curve of various microorganism cultures. (b) current profile of various microorganism cultures. (c) potential profile of various microorganism cultures.

crease current and voltage since the substrate has been depleted and the microorganisms have entered their death phase.

3.4. Power density and energy

Using the previously mentioned equations 1, 2, and 3, We evaluated the amount of energy produced by various microorganism cultures. Figure 3 illustrates the energy generated. The metabolic rate of the microorganisms in the SB-MFC system affects the amount of electrical energy generated. Due to the employment of different microorganisms, the electrical power produced will vary. Compared to other variations, the mixed culture of *E. coli* and *S. fibuligera* showed higher current and voltage, as calculated from the data of the various treatments. Thus, the value of the power density, which was 428 mWatt/cm^2, was superior to all others. However, a consortium of indigenous microorganisms generated just 2 mWatt/cm^2 of power density. In addition, the energy produced by the mixed culture was 4.789×10^{-2} kWh, while the energy produced by the native microorganism consortium was 2.304×10^{-4} kWh. Based on these results, the performance of the combined culture of bacteria and yeast in the breakdown of organic compounds in tofu wastewater and the generation of electricity is both intriguing and promising. The yeast, *S. fibuligera*, might contribute more than *E. coli*, because a single culture of *S. fibuligera* performed better than *E. coli* in the degradation of organic compounds and electricity generation. Some researchers also reported the potential yeast as a biocatalyst of MFCs, which has shown excellent activity in electricity generation (Islam et al. 2018; Włodarczyk and Włodarczyk 2020; Christwardana et al. 2021; Sayed et al. 2021; Verma and Mishra 2021). SB-MFC added value to wastewater treatment by producing electricity, which can be measured with a digital multimeter, in addition to reducing organic matter.

3.5. Reduction of manganese ions

We applied potassium permanganate as a catholyte solution in the cathode to capture the electron produced and migrated from the anode. Theoretically, Mn^{7+} ions in the solution will be reduced to Mn^{4+}, which is able to react with oxygen in the water to form MnO_4 and can be observed as brown precipitates in the cathode. A further reduction to Mn^{2+} possibly is unlikely due to the amount of electrons produced in the anode and the required energy for the reduction reaction. Table 4 shows the analysis of the remaining Mn concentration in catholyte after the MFCs. As can be seen in Table 4, most of Mn was reduced from the initial concentration 50.00 ppm to 8.30, 6.27, and 5.70 ppm when *E. coli*, *S. fibuligera*, and mixed of *E. coli*, and *S. fibuligera*, respectively, applied as biocatalyst of SB-MFC. It suggests that only a few Mn ions remain in the solution because most Mn ions have already reacted with oxygen to form MnO_4 precipitates. Figure 4 shows the changes in the color of the catholyte solution ($KMnO_4$) before (Figure 4a) and after (Figure 4b) SB-MFC, which indicates the formation of MnO_4 precipitates in the catholyte (right chamber). This change indicates that the reduction reaction of Mn^{7+} ions in the catholyte happened due to the presence of electrons transferred from the anode. However, it was not clear the degree or rate of reduction reaction of Mn^{7+} ions because we only analysed the total Mn concentration before and after the SB-MFC.

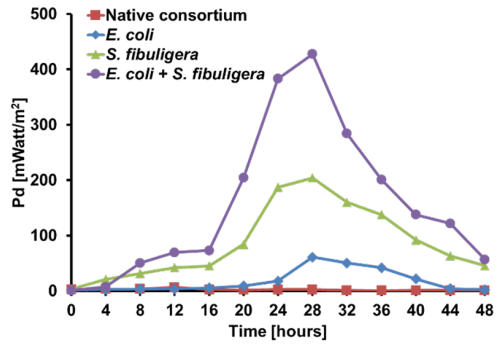

FIGURE 3 Power density of produced in SB-MFC with various microorganism cultures as biocatalyst for 48 hours. The power density was calculated by using equation 3.

TABLE 4 Results of analysis of total manganese (Mn) concentration in the catholyte solution before and after the SB-MFC. The analysis was performed using flame AAS. Mn standard was used to prepare the standard curve of Mn before the sample measurements.

Microorganism	Initial concentration (ppm)	Final concentration (ppm)
E. coli	50.00	8.30
S. fibuligera	50.00	6.27
Mixed	50.00	5.70

(a)

(b)

FIGURE 4 The changes of color of catholyte solution before and after SB-MFC. (a) before. (b) after SB-MFC. MnO_4 precipitates in the catholyte were observed at the bottom of catholyte (right chamber) after SB-MFC.

4. Conclusions

This work evaluated the efficacy of a salt-bridge microbial fuel cell (SB-MFC) using various microbe cultures as biocatalysts in the removal of organic contaminants from tofu wastewater, and the generation of energy was evaluated. The mixed culture of *E. coli* and *S. fibuligera* showed the highest removal efficiency of COD and BOD_5. The mixed culture also showed excellent and promising performance in the electricity generation, which is indicated by the high current and potential profile, power density and energy. This study contributes to the advancement of SB-MFC by utilizing the wastewater and combination of bacteria and yeast as biocatalysts.

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Authors' contributions

DP: Conceptualization, methodology, validation, visualization, data curation, formal analysis, visualization, writing of the original draft, reviewing, and editing. HEP: Conceptualization, methodology, data curation, formal analysis, visualization, writing, reviewing, and editing. OR: Data curation, formal analysis, writing, reviewing, and editing. ME: Data curation, formal analysis, writing, reviewing, and editing. D: Conceptualization, methodology, validation, writing, reviewing, and editing. All authors wrote the manuscript. All authors contributed to the discussion of the paper and approved the final manuscript.

Competing interests

We confirm that there is no conflict of interest in this paper.

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