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HYDROLYSIS OF FOOD WASTE AND PRODUCTION OF BIOELECTRICITY USING SINGLE CHAMBER MICROBIAL FUEL CELL FOR THE SUSTAINABLE FUTURE

SIMPLISTE DUSABE, SRI RACHMANIA JULIASTUTI* AND RADEN DARMAWAN

Chemical Engineering Department, Institut Teknologi Sepuluh Nopember, Campus ITS, Keputih-Sukolilo, 60111 Surabaya, Indonesia.

**Corresponding author: juliaz30@chem-eng.its.ac.id*
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Abstract: The complex organic-rich nature and nutrients of food waste (FW) can be converted into bioelectricity. This research investigated the hydrolysis of FW by three types of microorganisms, the produced electricity and the removed pollutants using a Single Chamber Microbial Fuel Cell (SC-MFC). FW was first diluted with water at different ratios (w/v) of 2:1, 1:1 and 1:2 and blended, then, hydrolysed by *Aspergillus aculeatus, Aspergillus oryzae,* and *Candida rugosa*. DNS reagent was used to analyse glucose production. The hydrolysate was mixed with Sidoarjo mud since it contains electricigen bacteria and fed to SC-MFC for direct electricity generation. In addition, *Shewanella oneidensis MR-1* was used in this study as it is one of the electrogenic bacteria with high productivity. Experiments revealed that the highest glucose reading obtained was 11.36 g/L by mixing all the fungi with a concentration ratio of (w/v) 2:1. The highest power density of 8515.35 mW/m^2, 78.38% of Biochemical Oxygen Demand (BOD_5) removal and 84.87% of Chemical Oxygen Demand (COD) removal was also obtained by mixing all the fungi with a concentration ratio (w/v) of 2:1. Therefore, this study indicates that fungal hydrolysis may be used as an alternative pretreatment to conventional FW utilisation in MFC for bioelectricity production.

Keywords: Food waste, fungal hydrolysis, microbial fuel cell, bioelectricity.
Abbreviations: Single Chamber Microbial Fuel Cell (SC-MFC), food waste (FW).

Introduction

Energy is the basis of sustainable development. Currently, most human activities are supported by fossil fuels (Ucal & Xydis, 2020). This indirectly causes fossil fuels to become a primary human need. Energy demands will continue to increase every year while the availability of fossil fuels is limited. In addition, the ongoing use of fossil fuels is not sustainable due to greenhouse gas emissions generated by the use of said fossil fuels as a power source (Dominic *et al*., 2019). Waste management and replacing non-renewable fossil fuels with something more sustainable are universal issues affecting communities and the environment. Poorly managed waste contaminates the environment, and endangers human and animal health (Munir *et al*., 2021). So, an adequately managed waste that produces renewable energy for a sustainable future is essential.

The rising population and global economic development have caused food waste generation to increase dramatically. The Food and Agriculture Organization (FAO) data from the United Nations showed that more than one-third of food is wasted worldwide (Chinie, 2020). In most countries, food waste is taken to a landfill or incinerated. However, different researchers criticise these methods for their negative side effects. For example, land availability limitations, toxins, leachate, and greenhouse gases are the main problems for landfills. On the other hand, high energy costs and hazardous gases are associated with incineration (Istrate *et al*., 2021). Although Anaerobic Digestion (AD) of food waste has received attention as a long-term solution for food waste management for biogas production (Yin *et al*., 2016), different researchers revealed that this method has many problems. Firstly large sludge, due to the inefficient and slow microbial pathways.

Secondly, AD has a long solid retention time and complex process configuration. In addition, there is a low conversion efficiency of methane to electricity (Khan *et al*., 2017). All of the methods above do not offer a solution for food waste management. There is a need for technology to remove the organic pollutants in food waste while producing renewable energy since there is a growing demand for energy. Microbial Fuel Cells (MFCs) seem to be a new technology to produce value-added products from FW and reduce environmental impacts.

Recently, researchers reported MFCs can directly convert chemical energy stored in organic substances to electricity through catalytic reactions of exoelectrogenic bacteria (Adekunle *et al*., 2019; Gul *et al*., 2021). MFC has attracted significant research interest due to its potential for generating energy in an environmentally sustainable manner. In MFCs, the biodegradation of organic matter and electron transfer efficiency determine the generation of bioelectric energy (Pepè Sciarria *et al*., 2019). The exoelectrogenic bacteria are essential in MFC to remove pollutants while producing electrical energy (*James et al*., 2020). Researchers revealed that the mixture of exoelectrogenic bacteria might boost electricity production (Gul *et al*., 2021). In this research, we used *Shewanella oneidensis MR-1*, an exoelectrogenic bacteria with high productivity (Bai *et al*., 2021) and Sidoarjo mud containing bacteria that might play a key role in MFC electron transfer (Darmawan *et al*., 2017). Sidoarjo mud flow is due to the mud volcano eruption in May 2016 in Sidoarjo, East Java, Indonesia. It consists of 70% solids and 30% water, with a 32%-40% salinity, pH 6.6-7, Cation Exchange Capacity (CEC) 3.89-35.42 (meq/100g), and total organic carbon 54.75%-55.47% (Purnomo & Rachmadiarti, 2018).

The performance of MFC in producing electrical energy is not only influenced by the types of exoelectrogenic bacteria used. There are other several factors such as the types of contaminants degraded (Kumar *et al*., 2019), types of the electrode (Jung & Pandit, 2018; Zhou *et al*., 2020), the types of configuration (Al Lawati *et al*., 2019) and the operating conditions of the MFC, including but not limited to pH, temperature, salinity, and shear stress (Gul *et al*., 2021). In this research, we used food waste from a restaurant. The FW from the restaurant contained high organic compounds such as starch, lipids, and proteins (Li *et al*., 2019) that might be used as a carbon source in MFC.

However, pretreatment is required to use food waste as a substrate to produce value-added compounds (Ma *et al*., 2017a; Rajesh Banu *et al*., 2020). Different pretreatment methods are physical (Ariunbaatar *et al*., 2014), mechanical (Shanthi *et al*., 2018; 2019), physicochemical (Kavitha *et al*., 2017), chemical (Kannah *et al*., 2018), biological (Pleissner *et al*., 2014) or their combination was developed to enhance the hydrolysis of organic compounds in FW into simple monomers. In this research, we used biological pretreatment because it is eco-friendly and no undesirable byproducts are formed (Ma *et al*., 2017b; Zhang *et al*., 2020). Researchers revealed that a single enzyme treatment is less efficient than the treatment with a mixed enzyme (Kim *et al*., 2006). However, commercial enzymes are not cheap and exist in a single variety (Khulbe & Mugesh, 2019; Dudutienė *et al*., 2020). It was revealed that fungi rich in hydrolytic enzymes could significantly enhance the hydrolysis of food waste (Ma *et al*., 2017a). Therefore, fungi rich in hydrolytic enzymes (Moon & Song, 2011; Pleissner *et al*., 2014), namely *Aspergillus aculeatus, Aspergillus oryzae* and *Candida rugosa* at their log phases were used in this work. At log phases, the number of cells increased exponentially (Maier & Pepper, 2015) and is the healthiest (Division & Curve, 2021).

On the other hand, electrode materials play a significant role in MFC performance. Different materials may be used to make a perfect anode for MFCs, which require a large surface area to enhance the electron transfer rate. Anodic materials are also necessary because they help microbes to oxidise organic substrates by increasing their metabolic rate (Srivastava *et al*., 2015). Since microorganisms significantly impact the power density produced in MFC, the appropriate anode materials must be selected. Carbon-based materials such as carbon cloth, felt, paper, brushes, and mesh are used as an electrode in MFC due to their cost effectiveness, good electron transfer kinetics, high chemical and mechanical stability, with high conductivity (Yaqoob *et al*., 2020). In this research, the authors used carbon cloth twill A 220 since it has several advantages. These include enormous porosity value, chemical and thermal stability, higher specific area, and mechanical strength with high electrical conductivity (Do *et al*., 2018; Yaqoob & Rodríguez-Couto, 2020). Moreover, a Single Chamber Microbial Fuel Cell (SC-MFC) was used in this study. The electrodes are placed in the same compartments in SC-MFC configurations. They are both in a simple anode compartment with no definitive cathode compartment and no proton exchange membranes. This configuration has advantages such as low cost for construction, no need for membranes, low internal resistance, high power output, and ease of construction (Logroño *et al*., 2017).

Previous researchers concentrated on eliminating organic contaminants in FW rather than extracting energy from them. Therefore, this study aimed to produce electricity from the organic compound in food waste by integrating hydrolysis and Microbial Fuel Cells (MFCs). After the blended FW had been separated into solids and liquids, hydrolytic fungi hydrolysed the liquid. Then, the hydrolysate was mixed with exoelectrogenic bacteria (bacteria in Sidoarjo mud and *Shewanella oneidensis MR-1*) and fed to SC-MFC for energy production. In addition, pollutant removal (COD and BOD_5) was discussed. The hydrolysis of food waste was investigated regarding maximal releases of glucose when only *A. aculeatus*, only *A. oryzae* ws, and only *Candida rugosa* were used and the mixture of all three fungi.

Materials and Methods

Material

Materials used in this study are fungi (*A. aculeatus, A. oryzae* and *Candida rugosa*), food waste, Sidoarjo mud, *Shewanella oneidensis MR-1*, titanium wire, carbon cloth twill A 220 and 3,5-dinitro salicylic acid (DNS). The fungi were purchased at Universitas Airlangga – Laboratory of Clinical Microbiology whereas FW was collected at Rumah Makan Asia Timur restaurant near Institut Teknologi Sepuluh Nopember (ITS). Sidoarjo mud was collected at a depth of between 25 cm and 40 cm below the outer surface of the ground at coordinates 7^{o}31’45.6 “S and 112^{o}42’43.6” E in Porong, Sidoarjo, East Java. Titanium wire was bought at Nilaco (Tokyo, Japan) and carbon cloth twill A 220 was purchased at Toko Ngagel Jaya Kimia. On the other hand, all the chemicals used in this research are from Merck (Darmstadt, Germany).

The MFCs Reactor

A single chamber microbial fuel cell (SC-MFC) made in PET bottles with a batch system was used in this study. The bottle has 8 cm and 20 cm in width and length, respectively. Carbon cloth was used as an electrode. The cathode was placed on the mud’s surface for direct contact with oxygen since oxygen contributes to higher power production (Chen *et al*., 2015), while the anode was set at 5 cm from the bottom of the bottle. The electrode working surface area is 22.8 cm^2. As shown in Figure 1, the external circuit was connected to the cathode and anode by Titanium wire, with an external resistance of 1 kΩ soldered to the Printed Circuit Board (PCB). The digital multimeter monitored the voltage output and electrical current.

Media Preparation and Sterilisation

In 1,000 ml of distilled water, 39 g of Potato Dextrose Agar (PDA) medium was dissolved and heated till boiling and homogenous. The medium was then autoclaved at 12°C for 15 minutes at 2 atm to sterilise it. Then, 10% of tartaric acid was used to adjust the pH to 5.5.

Microorganism Growth

A spore of *A. aculeatus, A. oryzae,* and *Candida rugosa* was grown aseptically on a PDA fresh medium. The incubation was carried out at 35°C. Each fungus's growth phase was monitored regularly to find the optimum time for each one. A hemocytometer was used to count the culture media cells (Absher, 1973). The process was repeated three times to get the average value of the cell growth number. The specific growth rate (μ) for each fungus at a particular time was calculated using Equation 1 below (Yarmush & Pedersen, 1995; Levenspiel, 1999).

$$\mu = \frac{lnx - lnx_o}{t - t_o} \quad (1)$$

X and X_0 are cell concentrations at time *t* and *t* = 0, respectively.

Food Waste Hydrolysis

A FW with a moisture content value of 61.17% was collected at Rumah Makan Asia Timur restaurant, located near ITS, and stored in a fridge at 2°C before its use. Then, diluted at different concentration ratios (w/v) 1:2, 1:1 and 2:1 as shown in Table 1, and immediately blended with a kitchen blender. The simple separation method was used to separate solid and liquid residues. The hydrolytic microorganisms at their log phases then hydrolyse the liquid residue within 48 hours. FW from restaurants contains a variety of large macromolecules (Li *et al*., 2019), so, a single enzyme should not be effective for glucose production. The authors used three hydrolytic microorganisms at their log phases to hydrolyse FW 10^{11} cells for only *A. aculeatus,* only *A. oryzae,* only *Candida rugosa*, and the mixture of the three fungi to hydrolyse FW.

Glucose Analysis

The DNS method was used to analyse the produced glucose after hydrolysis as described by Miller (Miller G.L., 1959). The spectrophotometer at 540 nm wavelength was used to measure the absorbance. The different known concentrations of glucose solution were used to make the standard glucose curve by plotting glucose concentration against absorbance.

MFC Experiment

In this study, 500 g of the substrate's total weight and 500 g of Sidoarjo mud were mixed. The 12 variables were analysed, as seen in Table 1. 10^{11} cells of *Shewanella oneidensis MR-1* at its log phases were added to each variable. The carbon cloth twill A 220 was cut into 5 cm x 2 cm with a 2 mm width and sewn with titanium wire. The titanium wire for the anode was 30 cm in length, while the titanium wire for the cathode was only 15 cm. Before the SC-MFC operated, the carbon cloth was dipped in a 1 M NaCl solution for its activation, then, the substrate mixture was placed in the MFC chamber, as seen in Figure 1. The anode was placed in the substrate and pressed down to remove the excess air bubbles. It was set 5 cm from the bottom of the chamber, while the cathode was placed on the mud's surface.

The MFC chamber was connected to an external electrical circuit and observed for 18 days using a batch reactor in which no additional substrate was added during the observation period. A digital multimeter was used to analyse the electrical current and voltage produced every day. The power density can be calculated as shown in Equation 2, where V is voltage (V), I is current (mA), and A is electrode area (m^2) (Raad *et al*., 2020). The Biochemical Oxygen Demand (BOD_5) analysis was carried out every three days, referring to SNI 6989.72: 2009, while COD analysis was carried out on the first and last days of observation, referring to SNI 6989.2: 2009. The percentage of BOD_5 and COD removal can be calculated using Equation 3.

$$power\ density\ (mW/m^2) = \frac{I\ (mA)\ x\ V\ (volt)}{A\ (m^2)} \quad (2)$$

$$\%\ Removal = \frac{Initial - Outlet}{Initial}\ x\ 100\% \quad (3)$$

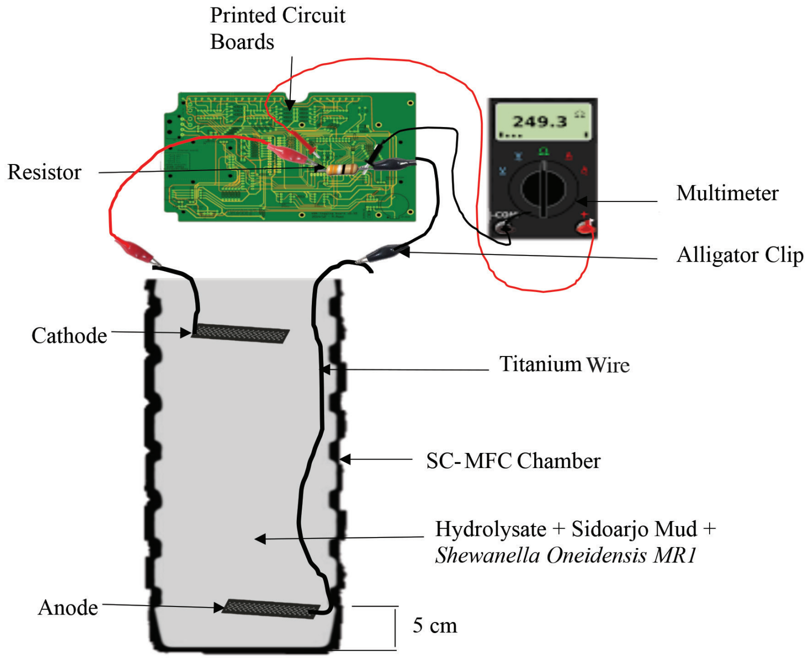

Figure 1: Single Chamber Microbial Fuel Cell (SC-MFC) diagram

Table 1: Sample code and different variations

Code	Substrate Ratio	Microorganism Used	Food Waste (gram)	Water (ml)
w/v 1:2 O	1:2	*A. oryzae*	167	333
w/v 1:2 A	1:2	*A. aculeatus*	167	333
w/v 1:2 C	1:2	*Candida rugosa*	167	333
w/v 1:2 M	1:2	*Mixture of all*	167	333
w/v 1:1 O	1:1	*A. oryzae*	250	250
w/v 1:1 A	1:1	*A. aculeatus*	250	250
w/v 1:1 C	1:1	*Candida rugosa*	250	250
w/v 1:1 M	1:1	*Mixture of all*	250	250
w/v 2:1 O	2:1	*A. oryzae*	333	167
w/v 2:1 A	2:1	*A. aculeatus*	333	167
w/v 2:1 C	2:1	*Candida rugosa*	333	167
w/v 2:1 M	2:1	*Mixture of all*	333	167

Results and Discussion

Microbial Growth Curve

A PDA medium was used to grow all the hydrolytic fungi. The inoculation size was 1.5×10^8 cells/ml, 2×10^8 cells/ml, and 2.5×10^8 cells/ml for *A. aculeatus, A. oryzae,* and *Candida rugosa*, respectively. Despite having identical culture conditions, the growth curves of *A. aculeatus, A. oryzae,* and *Candida rugosa* were different, as seen in Figure 2. They have different specific growth rates. The specific growth rate (μ) between 5 and 10 days for *A.*

aculeatus, A. oryzae, and *Candida rugosa* were calculated using Equation 1 and were 0.404/day, 0.411/day, and -0.083/day, respectively. The higher the specific growth rate, the more microbes hydrolyse organic substrate, resulting in a higher conversion of organic matter into glucose, as seen in Figure 3. Glucose produced by FW hydrolysed by *Candida rugosa* with a low specific growth rate was low. It was noticed that there was a considerable increase in the number of cells at the log phase (Maier & Pepper, 2015). The log phase starts on the 9th day and the optimum time was on the 13th day, 15th day and the optimum time was on the 26th day, 4th day and the optimum time was on the 7th day, 6th day and the optimum time was on 10th day for *A. oryzae, A. aculeatus, Candida rugosa,* and the mixture of all the three fungi, respectively.

Food Waste Hydrolysis and Glucose Production

The code w/v 2:1 M, the food waste concentration ratio 2:1 hydrolysed by the mixture of three fungi, produces the highest glucose, which is 11.362 g/L, as seen in Figure 3. In the concentration ratio of 2:1, the quantity of starch converted to glucose is high compared with other ratios since the amount of FW used was high. Therefore, by increasing the food waste ratio, the glucose content increases. The food waste ratio should be increased to produce more glucose. On the other hand, the mixture of three fungi produced a high amount of reducing sugar since the mixed enzyme pretreatment has a better reduction efficiency than a single enzyme treatment (Kim *et al*., 2006; Moon & Song, 2011).

Power Density

MFC removes pollutants while simultaneously producing electricity from wastewater (Slate *et al*., 2019). MFC works by oxidising the organic substrate into smaller molecules using the microorganism, and producing electrons and protons H^+. Then, the produced electrons are transported to the cathode through an external electrical circuit and converted into electrical energy while the protons migrate into the cathode surface. The electrons and protons react to reduce oxygen and produce water in the

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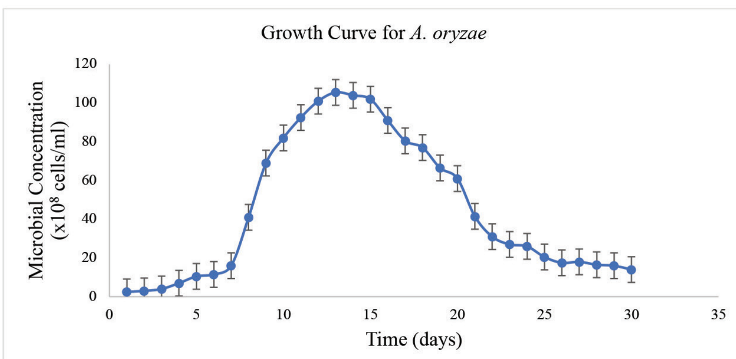

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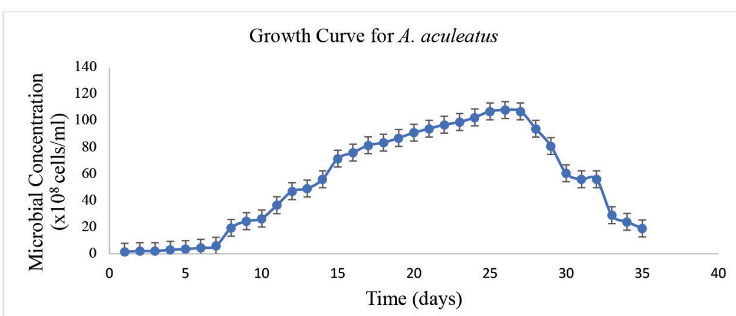

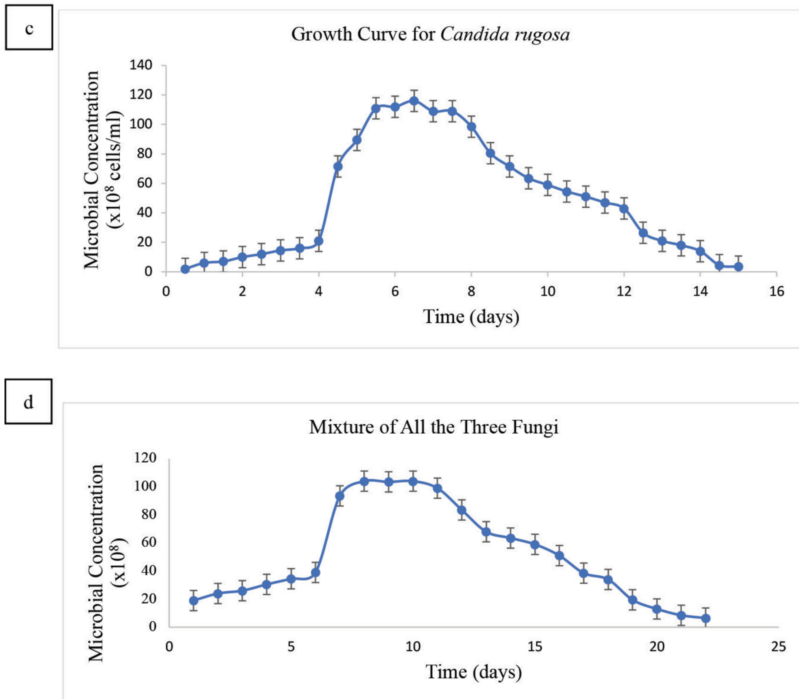

Figure 2: Growth curves of (a) *A. oryzae*, (b) *A. aculeatus*, (c) *Candida rugosa*, and (d) mixture of all the three fungi on a PDA medium

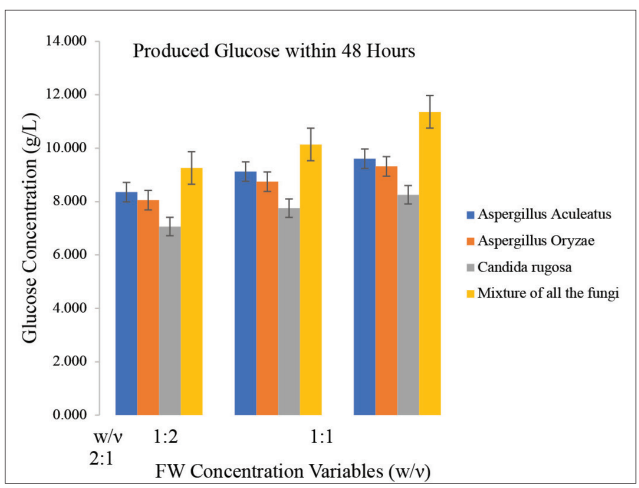

Figure 3: The effect of hydrolytic microorganisms on glucose production from food waste within 48 hours

cathode chamber. The protons and electrons are consumed in the cathode by reducing the soluble electron acceptors such as oxygen to form water. In brief, the reactions that occur in MFC are as shown in Equations 4 and 5 (Dutta & Kundu, 2018):

$$\text{anode: Organic substrate} \rightarrow H^+ + e^- + \text{oxidised substrate} \quad \textbf{(4)}$$

$$\text{cathode: } 4H^+ + 4e^- + O_2 \rightarrow 2H_2O \quad \textbf{(5)}$$

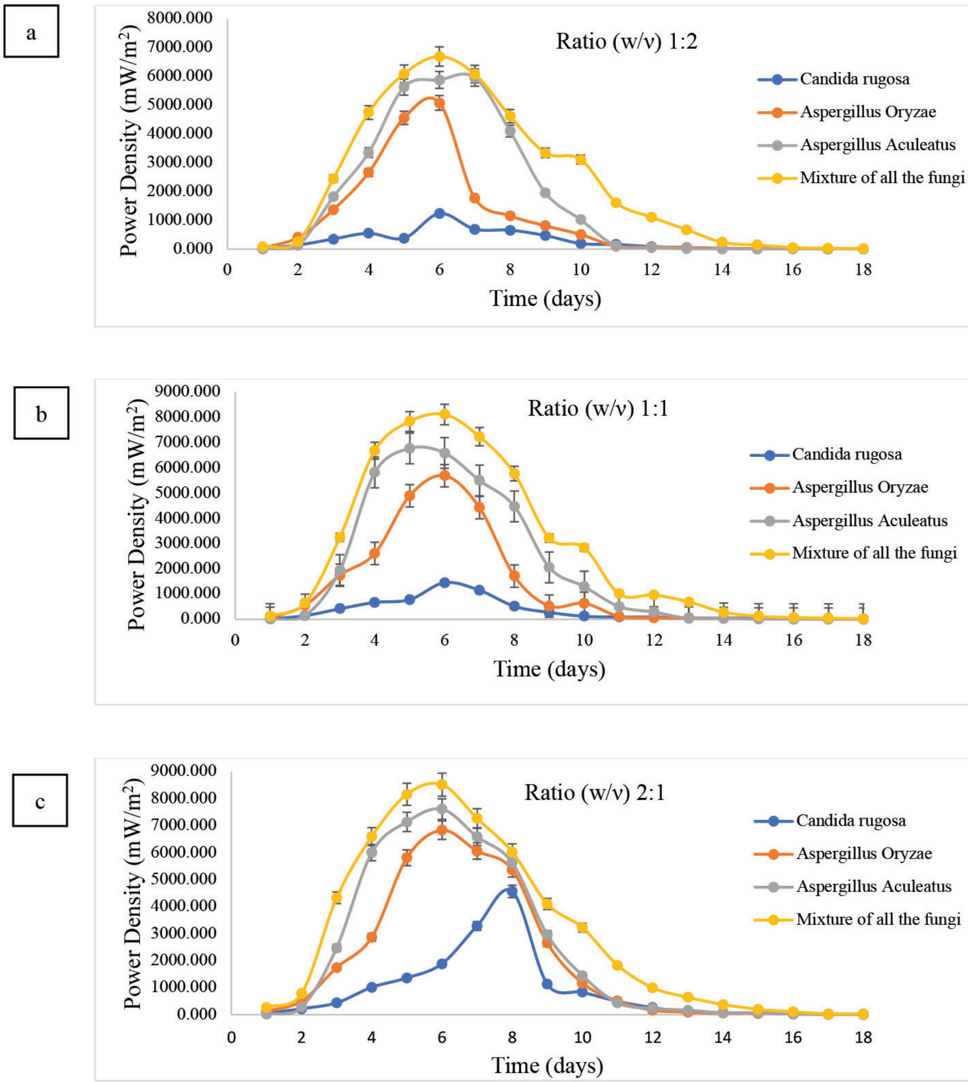

Figure 4: Power density produced using SC-MFC on different FW concentration ratios hydrolysed by fungi rich in hydrolytic enzymes. (a) represents ratio (w/v) 1:2, (b) represents the ratio (w/v) 1:1, and (c) represents the ratio (w/v) 2:1

The power density generated in the first days was low due to the adaptation of bacteria to the new environment, as shown in Figure 5. The power density increased until the 7th day and declined for the rest of the days due to the reduced substrate (glucose) since our system is batch. Logan reported that the decreasing power density in MFC is mainly caused by the low availability of oxidised compounds, resulting in a decreased bacterial population growth (Logan, 2009). The highest power density was produced where is a mixture of hydrolytic microorganisms. The mixed enzyme pretreatment produces a high amount of glucose compared with a single microorganism (Kim *et al*., 2006), resulting in increased power density. In MFC, electricity is produced due to the conversion of chemical energy into a biodegradable organic substances (Silveira *et al*., 2020). The food waste concentration ratio of 2:1 hydrolysed by the mixture of three fungi (w/v 2:1 M) achieved the highest power density of 8515.351 mW/m^2 on the 7th day of MFC operation. In the concentration ratio of w/v 2:1, the quantity of starch converted to glucose was too high, resulting in the highest power density when compared with other ratios.

The Growth of Microbial Population in MFC

The microbial growth depends on substrate concentration, pH, and temperature (Hwang *et al*., 2019). The highest number of microbial population (34.5x10^9 cells/ml), as seen in Figure 5 was achieved by variable w/v 2:1 M. This is due to the most elevated organic substrate

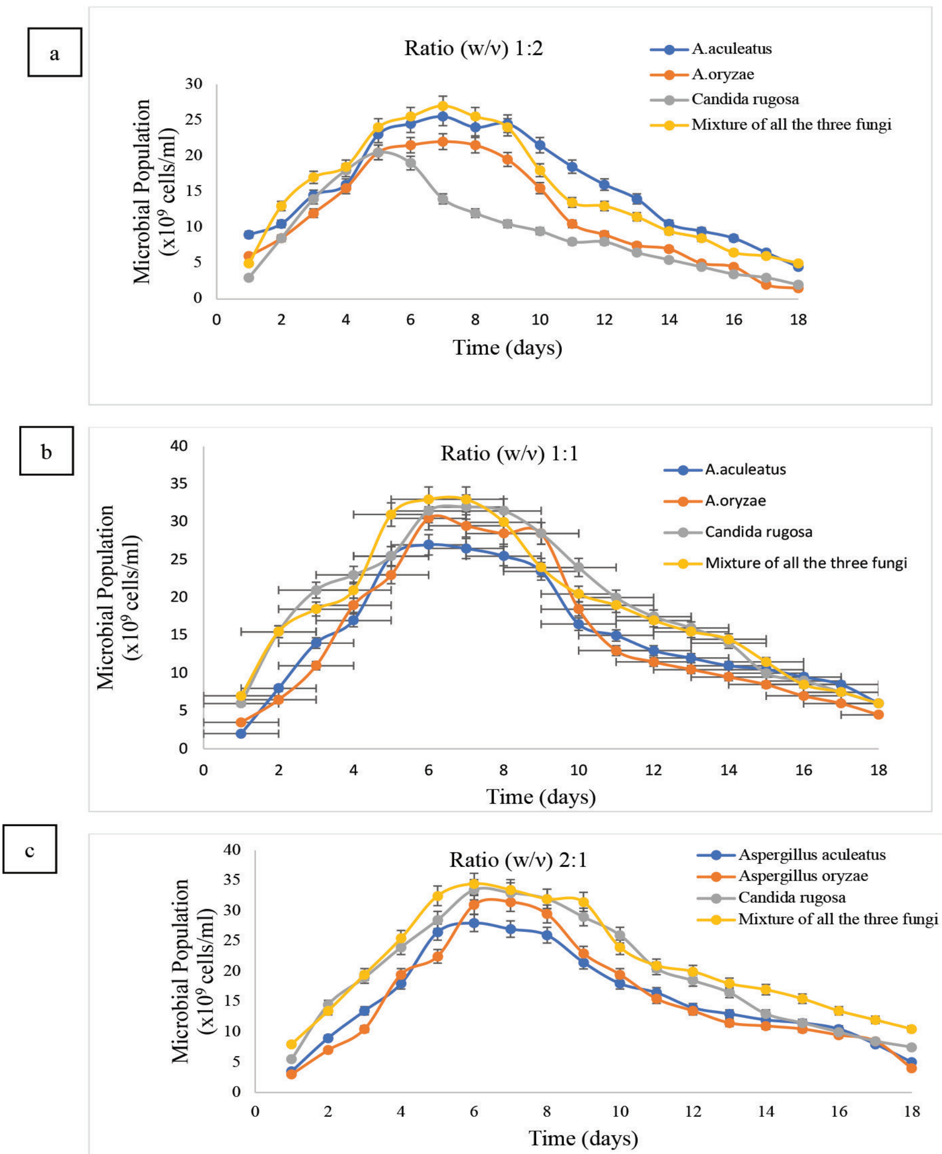

Figure 5: Microbial population growth in MFC on different FW concentration ratios hydrolysed by fungi rich in hydrolytic enzymes. (a) represents ratio (w/v) 1:2, (b) represents the ratio (w/v) 1:1, and (c) represents the ratio (w/v) 2:1

present in the ratio (w/v) 2:1 compared with other ratios. Nuryana reported that the increase in power density in MFC is directly proportional to the exoelectrogenic bacteria that transports electrons to the anode (Nuryana *et al*., 2020). In this research, looking at Figures 4 and 5, there is a steady increase in microbial growth and power density until the 6th to 7th day of operation. Therefore, it can be concluded that the bacteria that grew in our reactor were mainly exoelectrogenic bacteria. From the 7th day to the rest, the decline of bacteria shows that they have entered the death phase. It was attributed to the low availability of organic compounds since our system was batch.

BOD_5 and COD

Gul (2021) revealed that the decrease of BOD_5 in MFC indicates that active bacteria oxidise the large molecules into smaller molecules, while producing energy (Gul *et al*., 2021). Figure 6 and Table 2 show that w/v 2:1 M achieved 78.378% of BOD_5 removal on the 18th day of MFC operation. The highest pollutant removal in w/v 2:1 M compared with other variables due to its higher initial organic content and higher microbial concentration, as seen in Figure 5. Figures 4 to 6 show the highest pollutant removal and power density production, occurs where the microbial population is highest. So, the authors assume that the bacteria in the reactor in the present study are mostly electrogenic.

COD determines how much oxygen is necessary to oxidise organic compounds using a strong chemical oxidant (Hina *et al*., 2021), indicating water pollution (Lokman *et al*., 2021). This study achieved the highest COD removal of 84.874% by w/v 2:1 M on the 18th day of MFC operation. This highest COD removal is

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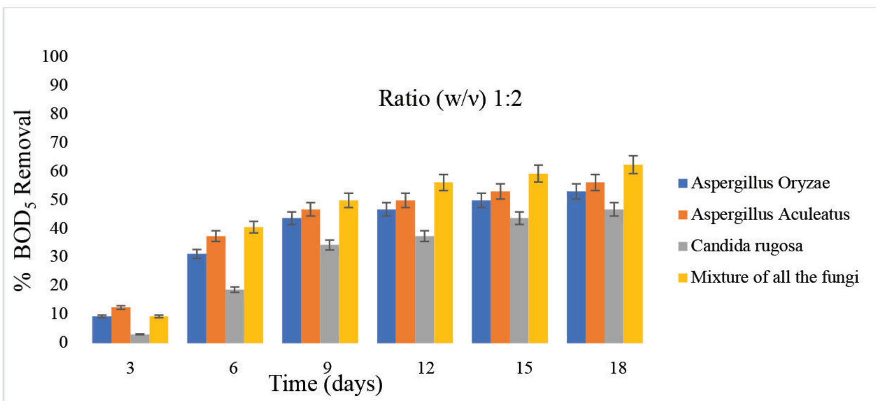

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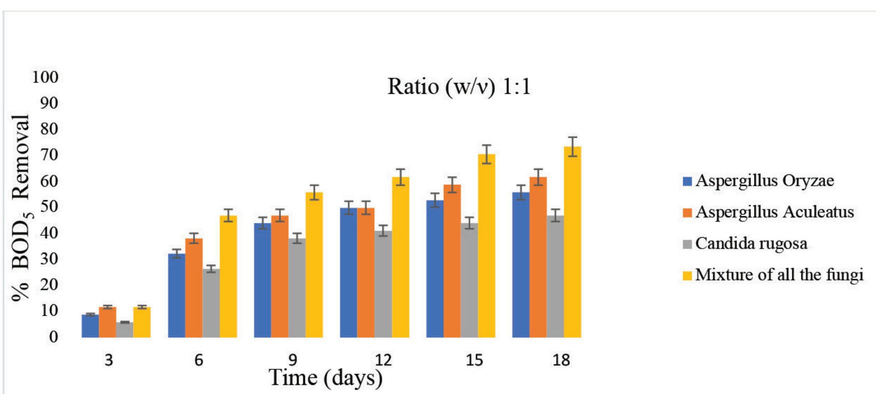

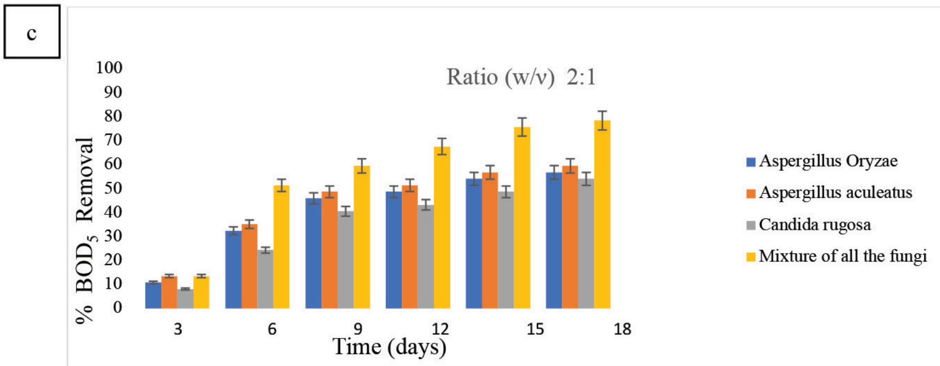

Figure 6: BOD_5 removal in MFC using different FW concentration ratios hydrolysed by fungi rich in hydrolytic enzymes. (a) represents ratio (w/v) 1:2, (b) represents the ratio (w/v) 1:1, and (c) represents the ratio (w/v) 2:1

achieved in the w/v 2:1 M variable due to the higher bacteria concentration, as seen in Figure 5, which oxidises glucose to simple molecules.

Microbial activity influences pollutant removal. On the other hand, microbial activity is influenced by the microbial concentration and substrate concentration (Hwang *et al*., 2019). As seen in Figures 6 and 7, the most effective removal of BOD_5 and COD occurs on the 6th to 7th day of MFC operation; this was directly proportional to the highest microbe population present in our reactor, as seen in Figure 5. From the 7th to the 18th day, there has been a continuous decrease in microbial population numbers due to the low availability of organic compounds as a substrate since our reactor was batch.

Conclusion

This research proposed an innovative and environmentally friendly method for food waste management. The electric energy was produced from the organic compound in food waste by integrating hydrolysis and microbial fuel cells.

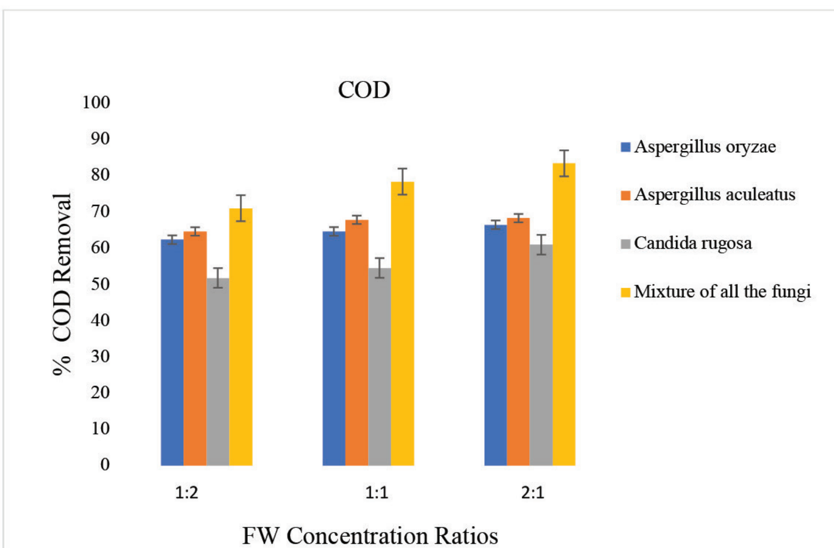

Figure 7: COD removal in MFC using different FW concentration ratios hydrolysed by fungi rich in hydrolytic enzymes

Table 2: Different variables on MFC performance for power density production and pollutant removal

Sample Code	BOD_5 Removal		COD Removal		Power Density Max	
	(%)	Standard Deviation (±)	(%)	Standard Deviation (±)	(mW/m^2)	Standard Deviation (±)
w/v 1:2 O	53.13	0.855269	62.39	0.943063	5079.386	0.82481
w/v 1:2 A	56.25	0.822168	64.68	0.867164	5957.456	1.510304
w/v 1:2 C	46.88	0.754805	51.83	1.069445	1236.842	0.993378
w/v 1:2 M	62.50	0.881074	71.10	0.880124	6686.842	1.369318
w/v 1:1 O	55.88	0.479175	65.78	1.071585	5696.053	1.579733
w/v 1:1 A	61.76	1.57152	68.89	0.552631	6779.386	1.578019
w/v 1:1 C	47.06	0.926685	56.00	1.163014	1152.632	1.753843
w/v 1:1 M	73.53	0.790894	79.11	0.882912	8123.684	1.63316
w/v 2:1 O	56.76	1.136222	69.33	0.884403	6828.947	1.849078
w/v 2:1 A	59.46	1.854294	71.01	0.860953	7609.649	1.015788
w/v 2:1 C	54.05	0.937653	64.29	0.624872	4566.228	1.507857
w/v 2:1 M	78.38	1.406383	84.87	0.799925	8515.351	1.143926

A. oryzae, A. aculeatus, and *Candida rugosa* greatly enhance food waste hydrolysis towards glucose production. The result showed that the mixture of the three microorganisms produced a high amount of glucose, leading to higher power density production in MFC and higher pollutant removal. Therefore, the current finding indicates that MFC may be used as a better solution for food waste management.

Acknowledgements

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