

# Potency of Yeast – Microalgae *Spirulina* Collaboration in Microalgae-Microbial Fuel Cells for Cafeteria Wastewater Treatment

Sylvia Anggraeni Motto<sup>a,b</sup>, Marcelinus Christwardana<sup>c,\*</sup>, Hadiyanto<sup>a,b,\*\*</sup>

<sup>a</sup>Department of Chemical Engineering, Faculty of Engineering, Diponegoro University, Jl. Prof. Soedarto SH, Tembalang, Semarang 50275, Indonesia

<sup>b</sup>Center of Biomass and Renewable Energy (C-BIORE), Diponegoro University, Jl. Prof. Soedarto SH, Tembalang, Semarang 50275, Indonesia

<sup>c</sup>Department of Chemical Engineering, Institut Teknologi Indonesia, Jl. Raya Puspitek Serpong, South Tangerang 15320, Indonesia

\*Corresponding author: marcelinus@iti.ac.id (M. Christwardana)

\*\*Corresponding author: hadiyanto@live.undip.ac.id (Hadiyanto)

**Abstract.** The effect of yeast *Saccharomyces cerevisiae* combined with *Spirulina platensis* in microalgae-microbial fuel cells (MMFCs) on the treatment of cafeteria wastewater to generate electricity and produce microalgae biomass was investigated. A treated cafeteria wastewater which has Chemical Oxygen Demand (COD) of 33,500 mg·L<sup>-1</sup> was used as the substrate and compared with commercial sugar as the control sample. During the process, the substrate was changed partially after 5 days and resulted power density of 73.7±4.57 mW·m<sup>-2</sup>. The COD of cafeteria wastewater removed by 30.15% from its initial concentration and then decreased by 40.82% after substrate partially replaced. Increasing optical density of microalgae in the cathode side from 0.6 to 1.37 could improve the oxygen reduction reaction which useful for electricity production. The result showed that cafeteria wastewater has feasibility as the substrate of yeast – *Spirulina* MMFCs to gain bioelectricity which can be used as an alternative electricity source for developing countries, for example, Indonesia.

## 1. Introduction

Technology for generating renewable energy is continuously developed, closely related to the increasing energy needs, reduced fossil fuels, and environmental problems. Indonesia as a developing country cannot be separated from the problem. To address these problems, the development of effective, efficient, and environmentally friendly clean technologies become one of the important issues.

One of the suitable green technologies developed in Indonesia is Microbial Fuel Cells (MFCs). Microbial fuel cell is an environmentally friendly technology that converts chemical energy into electrical energy by utilizing microbes as biocatalysts [1-3]. Microalgae-Microbial Fuel Cell (MMFC) is one of the MFCs systems that collaborate microbes and microalgae. In this system, microbes on the anode side will degrade the biochemical of the substrate to produce electrons and protons, while microalgae on the cathode side as photosynthetic microorganisms have a role to convert solar energy and produce oxygen through photosynthetic reactions which are later used as protons and electron acceptors in the process oxygen reduction reaction (ORR) [4,5]. Microalgae biomass on the cathode side



contains high levels of protein and carbohydrates that can be utilized as alternative food products for humans, as concentrates for animal feed or fertilizer for plants [6].

Organic wastewater is a good source of nutrients that can be utilized by MMFC as a microbial substrate [7]. One of the organic waste that can be utilized as MMFC substrate is cafeteria wastewater. Cafeteria wastewater is very easy to obtain, especially in Indonesia as the second largest cafeteria waste producer (300 kg per person per year) after Saudi Arabia (427 kg per person per year) [8]. Cafeteria wastewater contains many biochemicals that can be utilized by microbes in MMFC to generate electricity. Biochemical is mostly derived from the remaining rice that contains many carbohydrates, which can be degraded into simple sugars by microbes. The rest of the meat, fruits, and vegetables also contain lots of protein and fat, in which the carbon, nitrogen, and phosphate content can be a substrate for microbial growth, so it can generate electricity maximally.

Yeast *Saccharomyces cerevisiae* is one of the most effective microbes used as a biocatalyst that can produce electricity [9]. Yeast is an anaerobic facultative microbe which means it can grow in an aerobic or anaerobic environment, making it easy to be cultivated. Relatively cheap price and commercially produced in Indonesia, make added value to the utilization of yeast as MMFC biocatalyst. Although it has several advantages, but yeast has limits in converting substrate to electrical. To overcome these problems, several ways have been done such as electrode development or MFC system development.

In MMFC, microalgae can be used on both anode or cathode sides. However, the utilization of microalgae on the cathode side is more appropriate, considering that microalgae can produce oxygen for ORR process. One of the microalgae that is easily cultivated is *S. platensis*. *Spirulina* has good resistance to pH changes or contamination which can also grow quickly in freshwater or brackish water and in tropical climate such as in Indonesia.

Some previous studies about cafeteria wastewater treatment using MFC have been conducted by researchers [10-14]. But, most of them used mixed culture bacteria from activated sludge which has the potential to pollute the environment after being used in the treatment process. This is certainly not favoured because can damage environment ecosystems, and moreover, environmentally friendly processes are preferred. The use of environmentally friendly microbes such as yeast as an electroactive microbe for cafeteria wastewater treatment have never been done. Meanwhile, most of researchers used open air cathode [10,13,14] or electrons acceptor solution [11,12] in their cathode side.

In this study, baker's yeast from *S. cerevisiae* would be used as an anode biocatalyst to degrade cafeteria waste to produce electricity, while *S. platensis* would be cultivated in the cathode chamber as an oxygen supply in the MMFC reaction. As our intellectual novelty, environmental friendly baker's yeast used to replace the use of activated sludge as electroactive microbes, while microalgae used as oxygen producer for electrochemical process, which that combination never been done before by other researchers. Salt bridge is used as ion exchange to replace the role of membrane which has price relative more expensive than salt bridge. Electrochemical characteristics, COD reduction of cafeteria waste, and microalgae biomass growth were investigated in this work.

## 2. Materials and Methods

### 2.1. Materials

Commercial baker's yeast *S. cerevisiae* and commercial sugar (sucrose) were purchased from local market in Semarang, Indonesia. While microalgae *S. platensis* was obtained from PT Nealgae Indonesia Makmur (Sukoharjo, Indonesia). Modified Bangladesh no. 3 nutrients [15] which consisting of NaCl,  $(\text{NH}_2)_2\text{CO}$ ,  $\text{Ca}(\text{H}_2\text{PO}_4)$ ,  $\text{P}_2\text{O}_5 \cdot \text{K}_2\text{O}$ , and  $\text{NaHCO}_3$  used as microalgae medium during pre-cultivation in laboratory environment for 2 weeks before used in MMFC.

### 2.2. Cafeteria wastes

The cafeteria waste used as the substrate in the MMFC was collected from the student cafeteria at Diponegoro University, Semarang, Indonesia. The food waste mainly comprises boiled rice, vegetables,

fruit, tea, cooked meat and bones, tofu, and tempe. Then the food waste was crushed in an electrical blender PHILLIPS HR2056 (Amsterdam, Netherlands) for two minutes. After that, cafeteria food waste was filtered to remove the coarse material and avoiding the clogging problem. The characteristic of treated cafeteria wastewater is shown in Table 1.

**Table 1.** Simple characteristic of treated cafeteria wastewater.

Parameter	Value <sup>a</sup>	Unit
COD	33500±2100	mg·L <sup>-1</sup>
TDS	156±24	mg·L <sup>-1</sup>
Electroconductivity	2058±124	μS·cm
pH	3.03±0.4	-
Temperature	28.6±1.3	°C
Colour	Brownish white	-

<sup>a</sup>Value±standard deviation based on triplicate measurements

### 2.3. MMFC configuration

A double-chamber MMFC with a working volume of 600 mL for each chamber was constructed using glass as shown in Figure 1. The spacing between the anode and cathode placed on opposite sides was 10 cm. The anode and cathode were made of carbon rods without any pre-treatment with the surface area of 13.56 cm<sup>2</sup> each electrode. Salt bridge which made following reference [16], was placed between anode and cathode chamber to change the role of membrane as protons migration path and separator. The anode chamber of MMFC was inoculated with 14 mg·mL<sup>-1</sup> of yeast *S. cerevisiae* and the prepared cafeteria waste was directly used as the substrate, while *S. platensis* with optical density (OD) of 0.6 was used as cathode biocatalyst to increase Oxygen Reduction Reaction (ORR). Sugar with concentration 14 mg·mL<sup>-1</sup> used as substrate and acted as control variable. Anode part was operated at anaerobic condition while cathode part was in aerobic condition. Furthermore, pH of electrolyte solution (yeast + substrate) in anode part was adjusted at 6. Air bubbles was fed on cathode part for microalgae agitation purpose. A cool white lamp with temperature colour was 6000 K was placed 15 cm near cathode side as artificial sunlight with dark/light cycle was 12:12. The small tube was installed from anode side to cathode side for CO<sub>2</sub> gas movement. The resistor with value of 1000 Ω was connected across the anode and cathode as external resistance. The MMFC was operated in fed-batch mode at room temperature for 8 days. The 200 mL of MMFC solution in anode chamber was changed with the fresh substrate after 5 days of experiment to maintain yeast sustainability as biocatalyst [17,18].



**Figure 1.** Photograph of MMFC configuration.

## 2.4. Analysis

**2.4.1. Electrochemical analysis.** An analog multimeter KRISBOW KW06-229 (Jakarta, Indonesia) was used to measure the output voltage and current across the external resistance and was monitored every 24 hours. The current density was determined by dividing the current with the electrode surface area, while power density was calculated by multiplying between current density and resulted voltage.

**2.4.2. COD analysis.** COD of cafeteria wastewater at day-0, day-5, and day-8 were measured based on the dichromate solution reduction under specified conditions by using Hanna Instrument HI-83099, HI-839800, and HI-93754B-25 (Rhode Island, USA).

**2.4.3. Algae biomass analysis.** OD of microalgae *S. platensis* was measured at day-0 and day-8 by using a spectrophotometer Optima SP-300 (Tokyo, Japan) with wavelength of 678 nm which related with peak of chlorophyll-a [19].

## 3. Results and Discussion

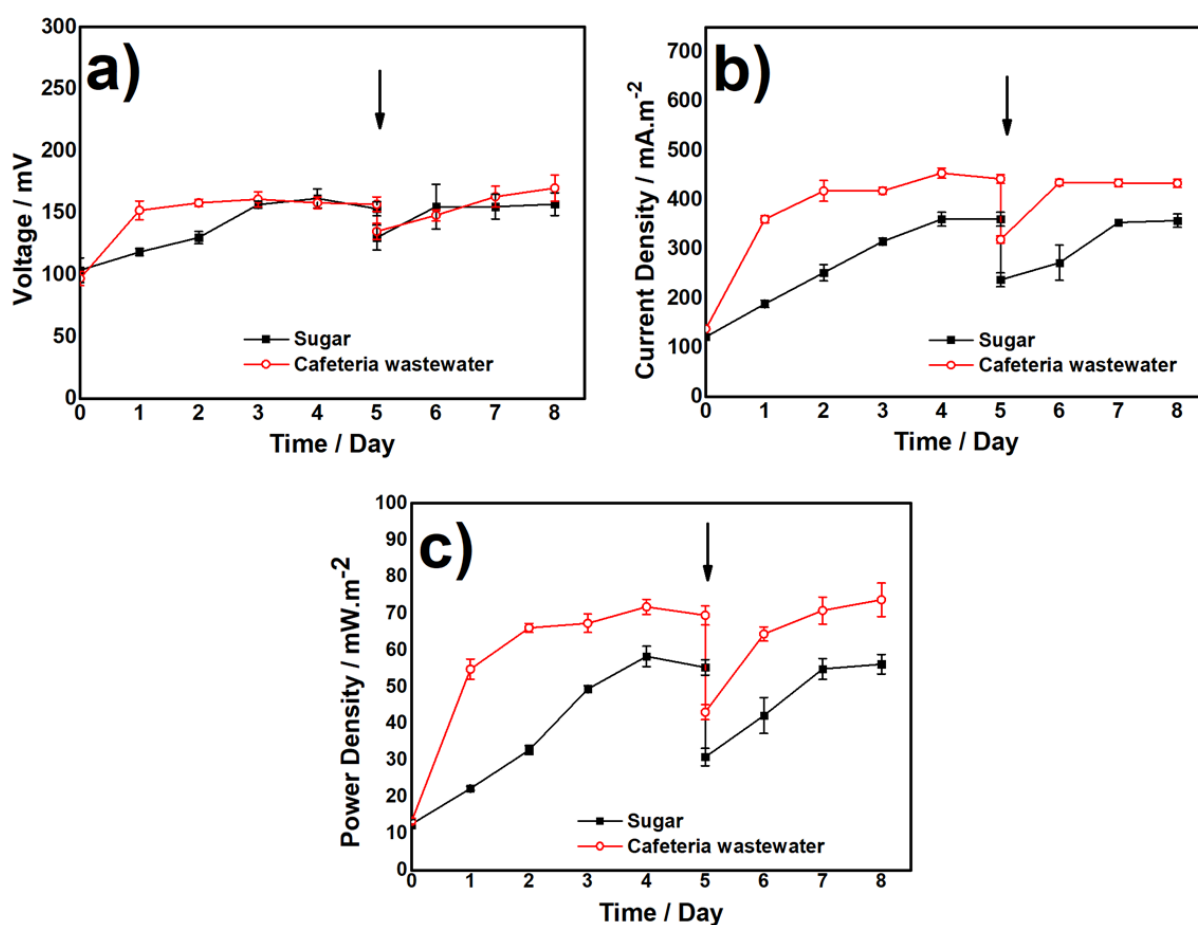
### 3.1. Bioelectricity production

Bioelectricity of MMFC adopting cafeteria wastewater as substrate is shown in Figure 2a-c. From Figure 2a, the close circuit voltage of MMFC adopting commercial sugar had more or less similar value compared to cafeteria wastewater as substrate with the value was around 150 mV. MMFC which utilized sugar had slower increasing voltage to reach their steady-state voltage compared to MMFC which utilized cafeteria wastewater. MMFC which utilized sugar could reach the steady-state condition in day-3 while MMFC which utilized cafeteria wastewater reached that condition in day-1. The voltage decreased in day-5 when 200 mL of anode solution was replaced by fresh substrate. The changing chemical content in anolyte affect to value of anode potential ( $E_{\text{anode}}$ ) which can also change the value of voltage between anode and cathode [20].

The resulted current density is shown in Figure 2b. From that figure, there three things can be noticed. First, the value of current density from MMFC which utilized cafeteria wastewater at steady-state condition was higher compared to sugar as substrate with the current density value at steady-state condition were 450 and 360 mA.m<sup>-2</sup>, respectively. Sugar which consist of sucrose chain could be

hydrolyzed acidically into simple form sugar [21] before consumed by yeast to grow and generate electricity. While in cafeteria wastewater not only contained disaccharides, but also polysaccharides from vegetables, tofu, and tempe which could be hydrolyzed acidically and resulted more simple monosaccharides compared to sugar hydrolysis. Second, the MMFC utilized sugar as substrate needed longer to time to reach steady-state condition with the required time was 4 days. On the other hand, the MMFC which utilized cafeteria wastewater need 2 days to reach steady-state current density. It means, cafeteria wastewater more effectively utilized by yeast compared to sugar since there were any other biochemicals contained in cafeteria wastewater which increased current generation. Third, similar to phenomena in voltage generation, current density decreased when some amount of anolyte was replaced with fresh substrate. When some anolyte was changed, some live or dead yeast cells wasted and affect to current density generation. At the same time, fresh substrate would have consumed by remain live yeast cell to grow and produce electricity. For that reason, the current density getting increase slowly after replaced with the fresh one.

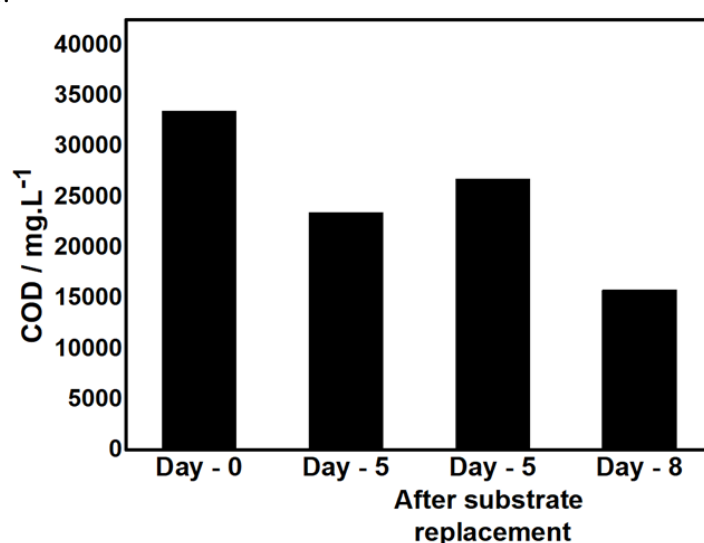
Figure 2c shows the resulted power density which produced by the MMFC system. The highest value of resulted power density by MMFC adopting sugar and cafeteria wastewater were  $58.3 \pm 2.75$  and  $73.7 \pm 4.57 \text{ mW.m}^{-2}$ , respectively. It is clear that utilization of cafeteria wastewater as substrate by yeast give the big impact in bioelectricity production, shown by generating higher power density. Yeast consumed organic compounds inside cafeteria wastewater to do Citric Acid Cycle (Krebs cycle). Krebs cycle activated the redox reaction of NAD/NADH and also FAD/FADH inside the cytochrome at the same time. The activation of NAD/NADH and FAD/FADH resulted 24 protons and electrons respectively and go to endogenous mediator through electrons transport chain. Protons and electrons carried by endogenous mediator into surface of electrode by diffusing the cell membrane [22].



**Figure 2.** (a) Close circuit voltage, (b) current density, and (c) power density of MMFC adopting sugar and cafeteria wastewater as substrate. Arrow indicates the substrate replacement.

### 3.2. COD removal

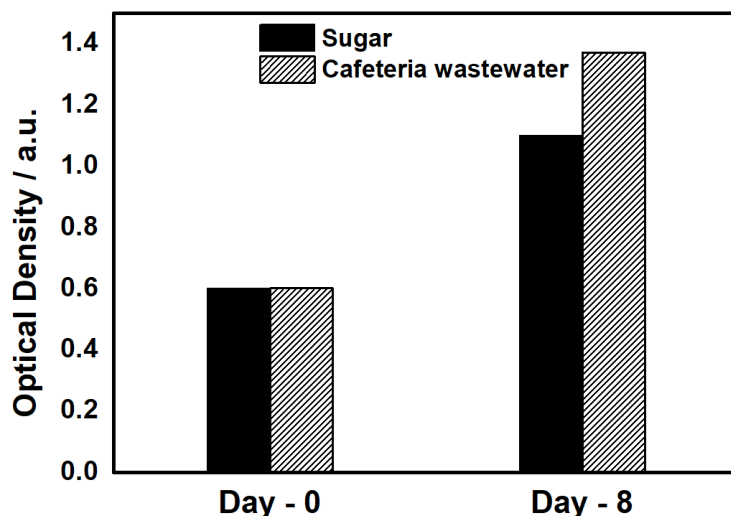
Figure 3a presented the COD removal from cafeteria wastewater by yeast in MMFC system. From the experiment, there are two noticeable things. First, the COD of cafeteria wastewater decreased 30.1% from 33,500 to 23,400  $\text{mg}\cdot\text{L}^{-1}$  in first 5 days. Second, the COD of solution a little bit increased from 23,400 to 26,700  $\text{mg}\cdot\text{L}^{-1}$  after fresh substrate solution was replaced partially, and gradually decreased 40.8% from 26,700 to 15,800 in next 3 days. This means that yeast consumed the biochemical inside the cafeteria wastewater such as carbon, nitrogen, and phosphate to produce protons and electrons [23]. that MMFCs proposed in this work for COD reduction purpose is an effective method to treat the cafeteria wastewater.



**Figure 3.** (a) COD of cafeteria wastewater as substrate during MMFC process.

### 3.3. Algae biomass production

One factor which effects on MMFC performance is characteristic of cathode biocatalyst. As increase in the number of microalgae in cathode side, the resulted  $\text{O}_2$  for ORR also increase. It caused increasing electrons acceptance in cathode side and affect to performance of MMFC. From Figure 4a, the final OD of *S. platensis* in MMFC which adopting sugar as substrate was lower compared to MMFC which adopting cafeteria wastewater with the value was 1.1 and 1.37, respectively. In MMFC which utilizing cafeteria wastewater,  $\text{CO}_2$  production was higher compared to another substrate. High  $\text{CO}_2$  produced from fermentation process by yeast and native anaerobic microbes was then transferred to cathode side for microalgae growth and resulted in higher OD. Higher number of microalgae made the number of oxygen for ORR was higher.



**Figure 4.** Optical density of microalgae *S. platensis* as cathode biocatalyst of MMFC.

#### 4. Conclusions

Bioelectricity generation was achieved with efficient biodegradation of organic matters in MMFC system. High power density ( $73.7 \pm 4.57 \text{ mW.m}^{-2}$ ) was produced when cafeteria wastewater was acted as substrate. During the process, the biochemicals inside the cafeteria wastewater which represented by COD could be degraded effectively by yeast *S. cerevisiae* compared to sugar. COD removal (30.15%) was achieved in MMFC adopting cafeteria wastewater as substrate in first 5 days, while COD removal was achieved 40.82% in next 3 days after some substrate was replaced. Oligosaccharides and disaccharides in cafeteria wastewater hydrolyzed acidically by fermentation process into simple form of sugar which can be consumed by yeast compared to sugar which only consist of disaccharides form. The biomass of microalgae *S. platensis* increased as increase in optical density from 0.6 to 1.37 after consumed high  $\text{CO}_2$  which produced by fermentation of cafeteria wastewater on anode side. With large number of microalgae biomass in cathode side, ORR which occurred increased so that it can improve the performance of MMFC system.

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