

A novel application of simple submersible yeast-based microbial fuel cells as dissolved oxygen sensors in environmental waters

Marcelinus Christwardana^{a,*}, Linda Aliffia Yoshi^a, Indraprasta Setyonadi^a,
 Mohammad Rizqi Maulana^a, Ahmad Fudholi^{b,c,**}

^a Department of Chemical Engineering, Institut Teknologi Indonesia, Jl. Raya Puspipetek Serpong, South Tangerang, Banten, 15320, Indonesia

^b Solar Energy Research Institute, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia

^c Research Centre for Electrical Power and Mechatronics, Indonesian Institute of Sciences (LIPI), Bandung, Indonesia

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ABSTRACT

In this study, yeast microbial fuel cells (MFCs) were established as biosensors for in-situ monitoring of dissolved oxygen (DO) levels in environmental waters, with yeast and glucose substrates acting as biocatalyst and fuel, respectively. Diverse environmental factors, such as temperature, pH and conductivity, were considered. The sensor performance was first tested with distilled water with different DO levels ranging from 0 mg/L to 8 mg/L and an external resistance of 1000 Ω . The relationship between DO and current density was non-linear (exponential). This MFC capability was further explored under different environmental conditions (pH, temperature and conductivity), and the current density produced was within the range of 0.14–34.88 mA/m², which increased with elevated DO concentration. The resulting regression was $y = 1.3051e^{0.3548x}$, with a regression coefficient (R^2) = 0.71, indicating that the MFC-based DO meter was susceptible to interference. When used in environmental water samples, DO measurements using MFC resulted in errors ranging from 6.25 % to 15.15 % when compared with commercial DO meters. The simple yeast-based MFC sensors demonstrate promising prospects for future monitoring in a variety of areas, including developing countries and remote locations.

1. Introduction

To date, the world is faced with rapid population growth and economic development, especially in developing and underdeveloped countries. This condition contributes to the increase in the amount of pollutants generated in the ecosystem [1], including the aquatic ecosystem, because environmental quality degradation affects ecology and human health. However, quality maintenance necessitates the control of certain parameters, including chemical oxygen demand (COD), biochemical oxygen demand (BOD) and dissolved oxygen (DO), performed with the development of existing technology [2]. Conversely, wastewater is assumed to contain a number of organic materials to be converted into energy through the concept of waste-to-energy transformation [3].

Oxygen is a vital component in aquatic ecosystems owing to its involvement in various processes, e.g. biological, chemical and biochemical. Furthermore, DO is one of the critical parameters often

used to determine water quality, alongside COD and BOD measurements [4–6]. In addition, valuable information is provided regarding the biological process of organic matter decomposition in aquatic environments [7]. Oxygen further serves as an important indicator of microbial metabolism in wastewater treatment processes during biochemical reactions [8,9].

Several methods, such chemical, physical and electrochemical methods, have been conducted to measure DO levels in water obtained from the aquatic environment [10,11]. Specifically, the electrochemical method is highly considered because of several advantages, including (i) the simple equipment and (ii) high sensitivity [12–14]. Despite these benefits, the method features several disadvantages, including the relatively expensive electrode and membrane materials, long period of measurement, easy interference of electrodes, and unsuitability for long-term use in in-situ monitoring. In addition, problems ensue in instances when most commercial DO sensors use an external power source such as batteries. However, the use of batteries is often not

* Corresponding author.

** Corresponding author at: Solar Energy Research Institute, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia.

E-mail addresses: marcelinus@iti.ac.id (M. Christwardana), a.fudholi@ukm.edu.my (A. Fudholi).

environmentally friendly given their difficult recycling. Batteries also increase the operation and maintenance costs following long-term sensor use in monitoring activities. Cost-effective and efficient forms are major concerns in recent research and aimed at reducing the cost of equipment construction, which increase the importance of energy in development.

Microbial fuel cells (MFCs) are an attractive technology in the production of renewable energy and wastewater management. As a bio-electrochemical device, they utilise microorganisms as biocatalysts to convert chemical energy contained in organic and non-organic compounds into electrical energy [15]. MFCs usually consist of a cation exchange membrane placed in between the anodic and cathodic sides, where the produced protons pass from the anode to the cathode. In addition, both electrodes are connected by external electrical circuits, which allow electron migration through the circuit [16]. The use of MFCs in power plants and environmental control is summarised to a biosensor, which detects chemical contents in water. MFCs can be possibly operated for long periods of up to five years without any form of maintenance due to their high intrinsic stability and reliability [16]. Furthermore, MFC-based biosensors can be used in the measurement of DO in water samples, as adopted in several developing countries. This utility is obtained through the technology that applies the oxygen reduction reaction (ORR) occurring at the cathode. The amount of oxygen present affects the ORR and subsequently changes the cathode potential, which affects the resulting voltage and current. According to Logan [17], the cathodic potential (E_{cat}) value is influenced by three main factors, including temperature, pH and O_2 concentration, and it can be obtained using the following equation:

$$E_{cat} = E_{cat}^0 - \frac{RT}{nF} \ln \frac{1}{[O_2]^{0.5} [H^+]^2} \quad (1)$$

where E_{cat} is the standard adjusted potential of the cathode, E_{cat}^0 is the standard potential of the cathode, $R = 8.31447$ J/mol-K is the gas constant, T is temperature (K), n is the number of electrons transferred, and $F = 96500$ C/mol is Faraday's constant.

Despite being one of the promising measurement methods, the studies on MFC as a DO sensor, especially those using monoculture of microorganisms as biocatalyst, are limited. The related research began with [18,19], who established the relationship between DO and electricity generation in MFCs. Zhang and Angeladaki [20] also investigated the characteristics of submersible MFCs that utilise domestic wastewater as fuel on the anode side, whereas Saba et al. [21] examined the addition of mixed culture biofilms to measure DO levels. Conversely, numerous related studies are available, although none specifically used MFCs as DO sensors. Based on our experiences, Song et al. [22] performed a similar work to this study, but they used sediments as anolytes. Other studies employed mixed cultures originating from wastewater, which raised the minimal anxiety, considering the difference in the contents and types of mixed cultures present in every wastewater, thus subsequently affecting the validity of DO sensor readings. In addition, monocultured microorganisms serve as an alternative for MFC-based types to ensure accuracy, especially in remote areas with limited facilities.

Saccharomyces cerevisiae can be possibly used as an alternative biocatalyst in MFC-based DO biosensors given its advantages, such as availability, easy culture and incubation and resistance to extreme weather [23]. Moreover, yeast produces a stable current output when used in yeast-based biofuel cells [24]. Yeast can produce protons and electrons from metabolic processes using sugar-based substrates, which is advantageous given that sugar is present in most parts of the country. In addition, the application of yeast baker as a biocatalyst is expected to obtain valid results despite the relatively slow yeast electron transfer rate [25,26]. In general, the electron transfer mechanism in microorganisms, including yeast, can occur by direct means, such as with the participation of external membrane cytochromes, or indirect procedures, including endogenous or exogenous mediators as electron shuttle

[27,28]. Based on the advantages and disadvantages of yeasts as MFC biocatalyst, they have promising use in DO-based MFC biosensors.

Contemporarily, the Internet of Things (IoT) has been identified as an incredible advancement. This yeast MFC-based biosensor has potential for further utility by adding engineering and control, which are obtainable through Arduino or wireless technology. Hence, the IoT has a high possibility of compatibility with the operational environment, which expands the application of yeast-based MFC biosensors for targets of low-power sensors, including salinity, pH and conductivity. Meanwhile, sensitivity, response time and detection limits have been tagged as crucial and characterised by an upsurge in the demand for their development, alongside the development of MFC technology.

In this study, a single-chamber submersible yeast MFC was tested as a DO biosensor, including its compatibility for the in-situ monitoring of various environmental waters. Glucose water and carbon felt were respectively used as fuel and electrodes, and the relatively low price of the main components was aimed at the development of a simple and compact system that can be used anywhere and everywhere. The effects of various operating parameters, including external resistance, pH, temperature and conductivity, on the DO biosensor performance were also investigated. The current density was correlated with the DO level checked with commercial sensors to obtain an equation that was subsequently used to determine the DO level of various environmental water samples. The development of submersible yeast MFC-based biosensors is expected to have enormous effects, especially in developing countries. Although the electron transfer at the anode largely affected the rate of oxygen oxidation reaction at the cathode, the electron transfer from yeast to anode, which occurred on the anodic side, will not be discussed in this study. We limited this study only to the effect of the cathode-side operating conditions on the performance of the MFC yeast-based DO meter.

2. Materials and method

2.1. Environmental water sample

Four samples of environmental waters were obtained from sources located in Indonesia, whereas seawater was obtained from Java Sea in North Jakarta. The lake, river and tap water samples were obtained from Situ Gintung, Cisadane River and the area around the Institute of Technology Indonesia, South Tangerang, respectively. The volume of ambient water collected was approximately 5 L per sample. The water samples were collected 4 h prior to use in this experiment. No pre-treatment was conducted for all samples prior to the DO analysis, and the samples were placed in a room with the temperature ranging from 25 °C to 27 °C.

2.2. Construction and operation of yeast-based MFC as a DO biosensor

Three identical cubic MFC reactors were made from acrylic and consisted of one anode chamber with an active volume of 28 mL. The treated Nafion 117 (treated with 3% w/w H_2O_2 , 0.5 M H_2SO_4 and distilled water (DW)) was used as a membrane separator, which was placed at the end of the anode chamber with the cathode facing outward, leading to a direct interaction with the water samples. Both electrodes were made of carbon felt with a projected active area of 7 cm² and no pre-treatment, whereas the anode chamber was filled with a broth solution consisting of 14 mg/mL yeast (Lessafre, Marcq-en-Baroeul, France) as a biocatalyst. Furthermore, yeast extract peptone dextrose medium, consisting of 14 mg/mL glucose (Merck, Darmstadt, Germany), 5 mg/mL yeast extract (Merck, Darmstadt, Germany) and 2.5 mg/mL peptone (Himedia, Mumbai, India), was used as fuel [29,30]. In this condition, yeast extract also played a role as a mediator [31,32]. The anode and cathode were connected to a resistor of 1000 Ω and multi-metre UNI-T UT61E (Dongguan, China) for voltage reading, and the current value was calculated by dividing the resulting voltage with the

applied resistance ($I = V/R$), and current density was determined by dividing the current with the projected cathode area ($J = I/A$) [33]. The MFC was incubated for two weeks to enrich the biofilms on the anode side, during which the broth solution was replaced every three days. After two weeks, a stable voltage and current was obtained, indicating the reactor's readiness for use in DO sensor testing.

The test period required the submersion of the MFC reactor in a 1.5 L container in batch mode operation (Fig. 1). Furthermore, DW water was fed into the container and used to increase the sensor performance, which was validated with real environmental water samples. DW has a pH of 7, a temperature of 25 °C and a conductivity of 50 S/cm. During testing, the air gas containing 21 % oxygen was used to control the DO levels in the container, whereas nitrogen gas created low DO levels and near-anaerobic conditions. The readings from the commercial sensor Lutron WA-2015 (Taipei, Taiwan) were used for comparison (Fig. 1), whereas the variation in external resistance was tested by connecting the MFC reactor to a resistor box with various resistance values of 100, 1000, 10,000 and 100,000 Ω and operated at room temperature (27 ± 2 °C) on each sample for 30 min. Conversely, pH variations (5, 7 and 11) were tested by adjusting the pH of DW with 1 M HCl or NaOH solution. Then, the sensor was tested under various conductivities. The values obtained were adjusted using 0.1 M phosphate buffer saline (PBS) solution to 50, 1230 and 1590 $\mu\text{S}/\text{cm}$, and temperature variations of 21 °C, 27 °C and 33 °C were selected as conditions for DO sensor testing, at which all experiments were performed in duplicates. Given that the MFC reactor was made of acrylic with a thickness of 1 cm, the temperature variations in the catholyte caused no effect on the yeast activity in the anode chamber. Thus, the effect of catholyte temperature on yeast activity in the anolyte can be neglected.

3. Result and discussion

3.1. Current density response to different DO levels in DW

Fig. 2 shows the dynamic response of the sensor to various DO concentrations ranging from 0.2 mg/mL to 8.12 mg/mL. In addition, a gradual increase in the current density was observed with the increase in DO, where a steady state requiring a response time of less than 30 min was reached at each elevated level. This finding indicates a relatively good sensor sensitivity. The correlation between current density and DO concentration was non-linear (exponential) with the R^2 value of 0.96. The R^2 result in the non-linear equation is higher than that of the linear equation [34,35], which means that yeast-based MFCs have unique characteristics in correlation with the DO levels, which is different from MFC-based DO biosensors with sediment or activated sludge on the anode side [20]. Based on yeast MFCs, the sensor also produced good reproducibility and data robustness, resulting in accurate values for

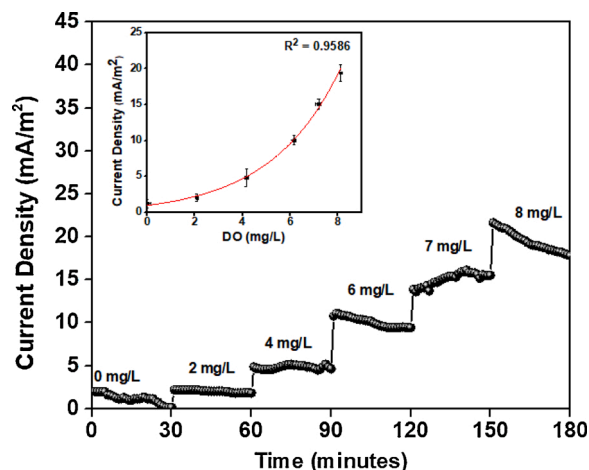


Fig. 2. Current density at various DO levels, where the exponential line is inserted between both parameters. The tests required the use of DW as catholyte at a temperature of 27 °C, pH of 7 and conductivity of 50 $\mu\text{S}/\text{cm}$, whereas external resistance was set at 1000 Ω .

future application of environmental water samples. Considering that the experiments were performed at relatively stable pH and temperature, from Eq. (1), the E_{cat} value decreased with reduced oxygen concentration on the cathode side. Hence, a lesser DO value led to a shift in E_{cat} towards smaller values. This effect also reduced the E_{cell} value, given the stability of E_{an} and the influence of a small E_{cell} on the decrease in current density. Hence, the results obtained in this work followed the basic theory of electrochemistry. Fig. 2 also explains that the lower limit in DO measurement was close to 0 mg/L. The value cannot be absolute 0 mg/L although N_2 gas was injected intensively due to the limit detection of the device. For the upper limit, DO measurements were limited to between 8–9 mg/L considering that this value is the maximum solubility of O_2 in water at 1 atm pressure and a temperature of 25 °C [36].

The above results indicate the potential for yeast MFC-based DO sensor to be cheap and sustainable based on several advantages, including the following: (i) absence of external power sources, (ii) use of plain carbon felt, which is considerably cheaper than expensive noble metals, as electrodes without any modification, (iii) worldwide availability of yeast, (iv) the use of electrical current density as a response, which enhances the ease of assessment of real-time measurements and online monitoring; (v) the possible in situ application of yeast-based MFC DO sensors without any extra construction, resulting from submersible configuration. According to Tao et al. [36], DO is a key factor in

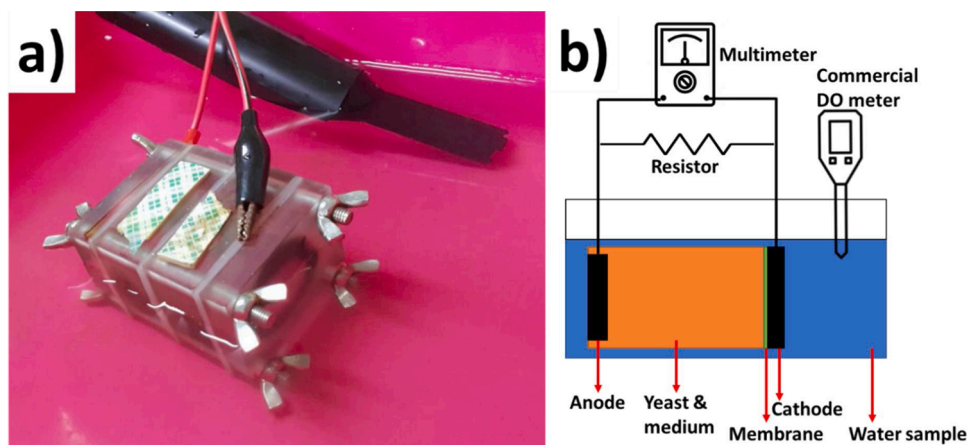


Fig. 1. a) Photograph and b) schematic of yeast MFCs as DO biosensors.

electricity generation, in which a low concentration in cathode liquor is sufficient for the electron acceptor.

3.2. Effect of applied external resistance on the current density response

Ohm's law previously explained the possibility of resistance affecting the cell current density and voltage. Therefore, several tests were performed with values ranging from $100\ \Omega$ to $100000\ \Omega$ to explore the effect of external resistance on the sensor performance. Fig. 3 shows the existence of a non-linear relationship between the current density and DO concentration detected for all tested external resistances, with determination coefficient values between 0.93 and 0.99. This condition follows Ohm's law, which states voltage and current density are directly and indirectly proportional to external resistance, respectively. According to Fu et al. [37], changes in the concentration of DO become a critical limiting factor at low levels of oxygen, whereas external resistance serves as the primary limiting factor for the generation of current with a high amount of DO at the cathode. This condition indicates the indirect influence of external resistance on sensor performance and hence the possibility to confirm the essential role in sensor sensitivity and stability. However, various issues need to be considered before making a suitable choice; the use of a relatively high external resistance reduces the effect of oxygen consumption by the cathode with a prolonged monitoring period [20]. Based on the aforementioned considerations, $1000\ \Omega$ was applied in this study.

3.3. Effect of environmental parameters on the current density response

Zhao et al. [37] explained the effect of pH, temperature, conductivity and type of electron acceptor on the electrocatalytic ORR at the cathode. Based on yeast MFCs, the individual effects on the performance of DO sensors were investigated.

3.3.1. Effect of pH

Fig. 4 shows the performance of DO sensors at various pH. A non-linear relationship was established between the current density and DO concentration, whereas the determination coefficient was the range of 0.96 to 0.98. Specifically, the resulting current density was the highest at the lowest pH of 5 compared with pH 7 and 11. This finding resulted from the increase in efficiency of oxygen reduction at the cathode, which was achieved by increasing the catholyte acidity [38, 39]. According to Erable et al. [40], a low pH ensures the availability of protons for ORR, which is largely used to alleviate the limitations observed during transportation. This condition also compensates for the

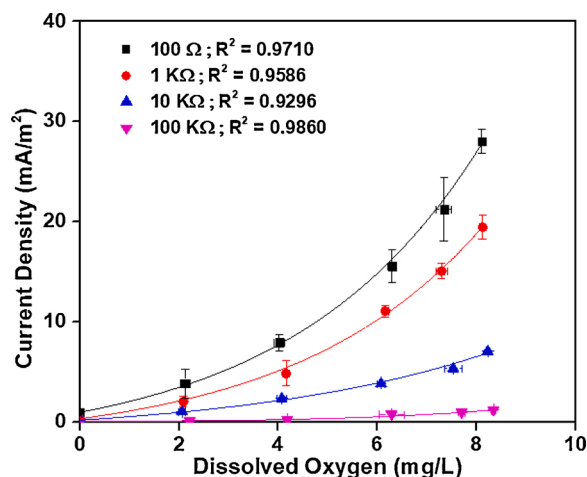


Fig. 3. Effect of various external resistance on DO measurement, in which DW was used as a catholyte (temperature: $27\ ^\circ\text{C}$, pH 7 and conductivity: $50\ \mu\text{S}/\text{cm}$). Error bars show the standard deviation.

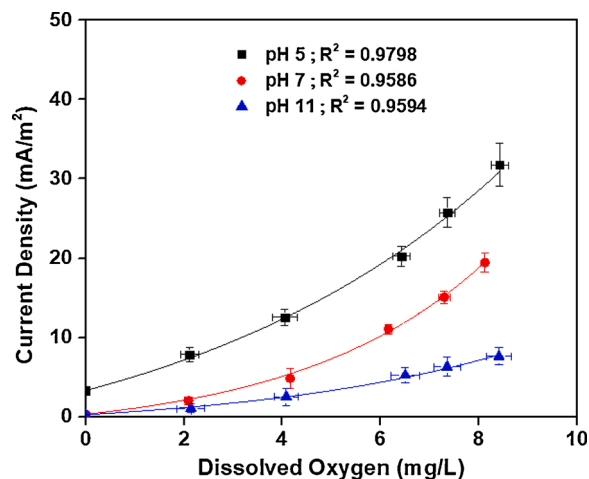


Fig. 4. a) Effect of various pH on DO measurement, in which DW was used as catholyte (temperature: $27\ ^\circ\text{C}$, conductivity: $50\ \mu\text{S}/\text{cm}$ and external resistance: $1000\ \Omega$). Error bars show the standard deviation.

ohmic loss instigated by the membrane separator, whereas the increase in acidity affects the amount of H^+ , which affects the E_{cat} and causes it to shift towards higher values. In consideration of the relatively stable resistance, operating temperature and E_{an} , the E_{cell} value increased at high acidity and current density. Therefore, the partial pressure of O_2 on the catholyte is believed to affect the voltage and output current density, although the slope of voltage on the anode and cathode sides under open circuit voltage (OCV) condition is theoretically $-59\ \text{mV}/\text{pH}$ [41]. The decrease in voltage across the various pH was in the range of $-0.33\ \text{mV}/\text{pH}$ to $-1.59\ \text{mV}/\text{pH}$ or smaller than the theoretical value because the measurement was carried out in a closed-circuit voltage (CCV) condition.

3.3.2. Effect of temperature

The non-linear relationship between the current density and DO concentration was examined at different temperatures ($21\ ^\circ\text{C}$, $27\ ^\circ\text{C}$ and $33\ ^\circ\text{C}$; Fig. 5). Several points that were evaluated included the minimal significant differences between the current density and DO levels. The findings showed the capability of yeast MFC-based DO sensors to work properly within several deviations and a wide temperature range. Second, despite the absence of any significant variation, a specific pattern occurred, showing an increase in the current density at elevated

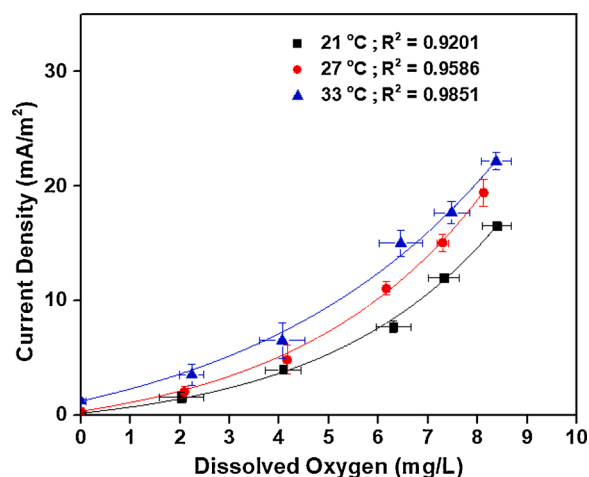


Fig. 5. Effects of various temperatures on DO measurement, in which DW was used as a catholyte (pH 7, conductivity: $50\ \mu\text{S}/\text{cm}$; external resistance: $1000\ \Omega$). Error bars show the standard deviation.

temperatures and the generation of a determination coefficient around 0.92 to 0.99. Thus, the temperature can possibly accelerate the ORR kinetic rate on the cathode, facilitating the movement of E_{cat} value toward a positive direction and subsequently increasing the current density. Therefore, the temperature played a vital role in the kinetics of ORR and resistance. Li et al. [42] reported the capability of temperature values greater than 37 °C to decrease the cathode potential. Surprisingly, the opposite is the case if the temperature value is added to Eq. (1), given the stable conditions of E_{an} , pH and conductivity values in each DO and OCV state. Furthermore, an increase in catholyte temperature led to a decline in E_{cell} , indicating a decrease in the current density.

3.3.3. Effect of conductivity

Conductivity, which is closely related to ohmic loss, is one of the factors influencing the performance of a yeast-based MFC as a DO sensor, [43], where one side is cathodic and is assumed to affect the efficiency of ORR. Similar to previous parameters, the current density obtained showed a non-linear positive relationship with the DO concentrations in various conductivities (Fig. 6), featuring determination coefficients between 0.96 to 0.97. This phenomenon is similar with the occurrence in pH variation, which is characterised by the presence of numerous ion contents in the catholyte. In addition, the ohmic loss in MFC performance often occurs on the anode and cathode sides, and this event is attenuated by increasing the electrolyte conductivity [37,44]. Conversely, the addition of PBS containing ions in the solutions of H^+ , Na^+ , Cl^- , PO_4^{3-} , and K^+ was possible because the formation required a mixture of NaCl, KCl, $NaHPO_4$, and KH_2PO_4 [45]. This condition is in accordance with the review conducted by Guo et al. [46], which showed that PBS can enhance the solution conductivity and buffer capacity. Therefore, an increase in concentration elevated the rate of proton transfer on the cathodic side, which hastened the rate of ORR [44,47]. The increase in proton proportion resulted from a decline in catholyte resistance.

3.4. Application of yeast-based MFC DO sensor on environmental water samples

The equation to be used as a basis for calculating the DO level for environmental water samples must be determined and was done three times. Therefore, the current densities generated from various previous parameters (DO, pH, temperature and conductivity) were combined to create a non-linear relationship and obtain its coefficient. The data consisted of a series of values extracted from the previous section's measurements. Fig. 7a shows the relationship between E_{cell} and

DO concentration in a combination of parameters under the CCV state, which resulted in the equation $y = 1.3051e^{0.3548x}$, with the R^2 value of 0.71. This result deviates from the ideal condition due to the complexities between environmental parameters and current density. When the equation was compared with the baseline (Fig. 7b), the resulting non-linear graphs showed no significant difference. Thus, such deviation caused no serious problem in the application of the equation in environmental water samples. However, a high reproducibility, which was evidenced by the relatively low standard deviation, and reliable results were observed at each point.

Yeast-based MFC sensors were applied in the measurement of DO levels of river, sea, lake and tap water samples, followed by validation using commercial DO meters. Table 1 shows the results. Tap water presented the lowest pH, whereas the sample sourced from the lake displayed the highest values. Conversely, the temperature recorded for each water sample approximated 27 °C, with sea and tap water having the highest and lowest conductivities, respectively. The results of current density obtained from measurements using yeast MFC were then converted to DO values using an equation based on Fig. 7. These outcomes were consistent with the values obtained with commercial sensors and characterised by a relatively insignificant difference between both methods and a percentage error within the range of 6.25 %–15.15 %. Therefore, the MFC-based yeast-based DO sensor was confirmed to have worked properly, although more calibration was needed for the environmental factors.

Tap water possesses a low pH, which theoretically produces a high current density. However, this feature was offset by low conductivity, which reduced the current density to the results measured using MFC ($5.03 \pm 0.15 \text{ mA/m}^2$). The river water was characterised by a relatively near-neutral pH, with a relatively high conductivity. The DO measurements were conducted using the MFC, producing a current density of $11.36 \pm 0.38 \text{ mA/m}^2$. The output was due to the presence of specific inhibitors present in the river water, including wastewater, whereas aquatic biota in the form of phytoplankton were identified in samples sourced from the lake. These phytoplankton can produce and increase the level of oxygen in the aquatic environment. Despite these manifestations, lake water demonstrated a relatively high pH, leading to a low current density at about $8.86 \pm 0.89 \text{ mA/m}^2$. The seawater used was sourced from shallow seas to limit the conductivity level ($236.3 \pm 0.20 \text{ } \mu\text{S/cm}$), although the values observed eventually became higher than those in other samples. This condition affected the proton transfer rate and ORR at the cathode, leading to the generation of a large current density through MFC ($14.57 \pm 0.52 \text{ mA/m}^2$). Furthermore, phytoplankton also produced oxygen in seawater samples and subsequently increased its content.

The capability of yeast-based MFCs as DO biosensors must be determined. For this reason, the comparison of DO concentration measurements from tap water using commercial DO meters and yeast-based MFC was carried out for 30 days. Fig. 8 shows that the gap in DO level measurement using a commercial DO meter and yeast-based MFC exhibited no significant difference. This finding shows that DO measurement using a MFC base is still reasonable, in accordance with the above explanation, and the deviation percentage is in accordance with the data in Table 1. This procedure can be developed in the future to reduce the existing gap by adding certain electronic circuits as support.

3.5. Future prospect of yeast-based MFC biosensor

MFC as biosensors have been studied comprehensively over the past decade; they are characterised by numerous advantages in environmental water-quality detection, including DO, BOD or COD. These applications are performed with real-time monitoring, subsequently enhancing the ease of control of active water conditions [20]. Moreover, MFCs are assumed to serve as a power source [48], which is used at various organic concentrations and confers an effect on the device performance. This procedure can be possibly conducted with different sizes

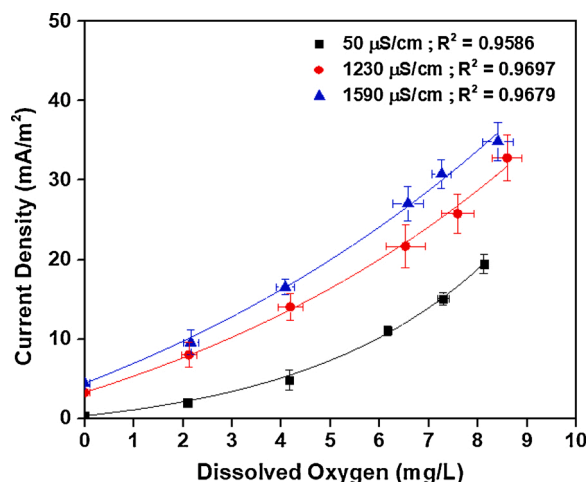


Fig. 6. Effects of various catholyte conductivity levels on DO measurement, in which DW was used as a catholyte at a temperature of 27 °C, pH 7 and an external resistance of 1000 Ω . Error bars show the standard deviation.

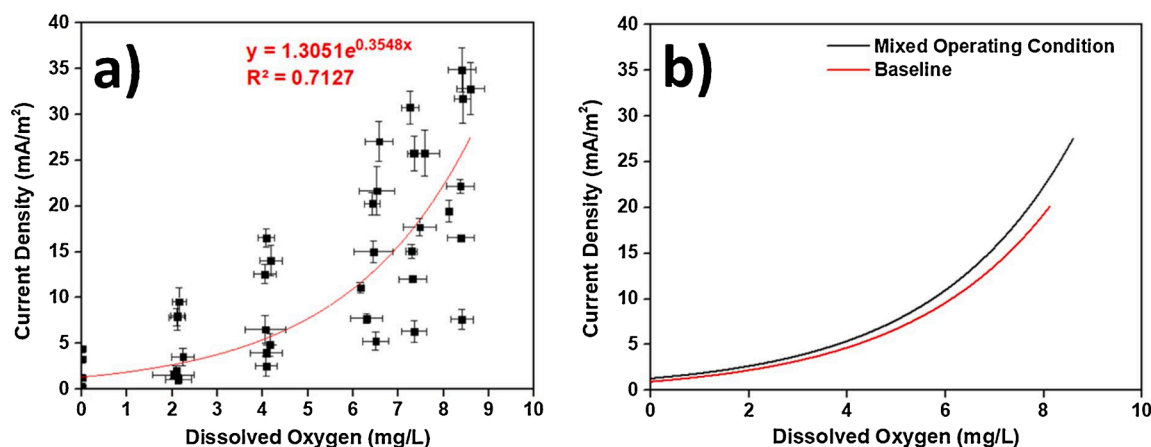


Fig. 7. a) Non-linear relationship line between the current density and DO concentration under various complex parameters (pH, temperature and conductivity). b) Comparison with the DW sample as the baseline (pH 7, temperature: 27 °C and conductivity: 50 μ S/cm). Error bars show the standard deviation.

Table 1

Yeast-based MFC sensors in the measurement of DO levels of river, sea, lake and tap water samples. $\pm$ indicates the standard deviation.

Sample	pH	Temperature (°C)	Conductivity (μ S/cm)	Dissolved Oxygen (mg/L)		
				Yeast MFC	Commercial DO meter	Deviations (%)
River water	6.29 $\pm$ 0.47	27.00 $\pm$ 0.10	154.20 $\pm$ 1.60	6.00 $\pm$ 0.10	6.40 $\pm$ 0.60	6.25
Seawater	8.06 $\pm$ 0.19	25.00 $\pm$ 0.00	236.30 $\pm$ 0.20	6.80 $\pm$ 0.10	6.10 $\pm$ 0.20	11.48
Lake water	8.63 $\pm$ 0.22	26.80 $\pm$ 0.10	195.80 $\pm$ 3.20	5.40 $\pm$ 0.30	5.70 $\pm$ 0.70	7.02
Tap water	6.09 $\pm$ 0.13	26.80 $\pm$ 0.00	57.40 $\pm$ 2.50	3.80 $\pm$ 0.10	3.30 $\pm$ 0.60	15.15

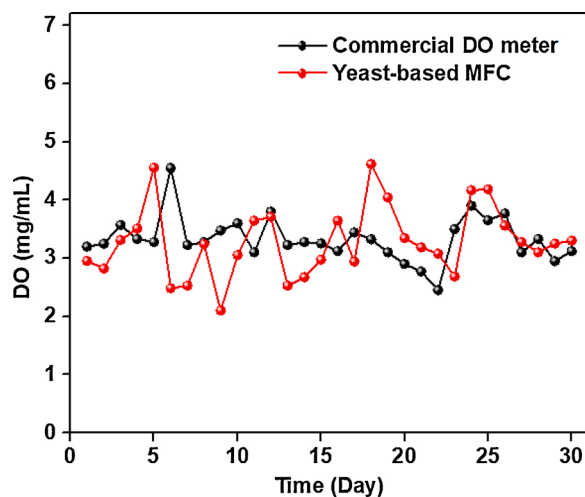


Fig. 8. Long-term DO level measurement in tap water sample using a commercial DO meter and yeast-based MFCs.

and depths of water [16], given that the biosensor quickly responds to changes in the amount of active compounds present in the environment during extreme conditions. Furthermore, an investigation related to the reliability and overall performance of MFC-based biosensor is required, especially for the evaluation of complex aquatic environments. The development process in the laboratory demands accurate examination of the sensor performance to correct possible deviations in the output. Conversely, biofilms at the anode, flow regime and other factors have been associated with the production of electricity, which changes quickly over time [49].

MFC-based DO biosensors are a promising technology, although several limiting challenges, which include low selectivity and expensive operational costs, exist [50]. Selection of improved and cheaper

electrode materials and the advancement of membranes significantly influence futuristic developments. The low-cost and independent sensors alongside real-time online monitoring capabilities are desired in this era due to the rapid growth in the IoT within the community. Furthermore, several MFC-based sensors are expected to spread through the population or industry in the forthcoming years given that a breakthrough in the future demands the capability to detect toxic compounds in certain substances in the environment and the provision of a unique 'signal'. Conversely, various technological developments, including mutagenesis and DNA engineering, possibly support the creation of strong electrogenic microorganisms to increase the MFC efficiency. In addition, these fabricated DO biosensors are tested on sedimented water conditions to monitor the performance quality [22]. Further research and development are therefore expected to support applications on an industrial scale.

4. Conclusion

Based on the results and discussion, yeast-based MFC biosensors were successfully developed. These biosensors can be possibly used to monitor DO concentrations in various environmental waters with high accuracy and within a short period of time. The measurement results showed a non-linear (exponential) function of the cell voltage obtained from the activity at the anode and cathode with DO concentration. In addition, external resistance affected cell voltage, which also played a major role in the sensor sensitivity. A high external resistance was expected to affect the ORR process. The aquatic environmental conditions, in the form of pH, temperature and conductivity, affected the monitoring performance, and the results on various operating conditions obtained an exponential line of $y = 1.3051e^{0.3548x}$, with a regression coefficient (R^2) of 0.71. Therefore, the application of environmental samples consisting of river, reservoir, sea and tap water showed a percentage error within a range of 6.25 %–15.15 %, which indicates the suitability of using MFC-based yeast biosensor in measurements. Therefore, this device provides a useful alternative for in-situ

monitoring in a variety of environmental waters in developed and developing countries and remote areas.

CRedit authorship contribution statement

All the authors have equal contribution in preparation of the manuscript. **Marcelinus Christwardana**: have original idea, conceptualization and methodology. **Linda Aliffia Yoshi** and **Setyonadi**: did the data analysis and validation. **Ahmad Fudholi**: did organization of the manuscript including language corrections and formal analysis. **Ahmad Fudholi**: contributed in substantial revision, editing, review and improvement of the first draft of the manuscript.

Declaration of Competing Interest

The authors declare no conflicts of interests.

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