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*Original Research Article*

# Experimental Investigation of by-Product Hydrogen Gas in the Harvesting Process of *Dunaliella salina* using a Non-Sacrificial Cathode

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## Abstract

Hydrogen gas is considered a pollution-free fuel in the future. However, the EC process using these non-sacrificial electrodes requires further research especially for the production of dissolved hydrogen gas and the efficiency of microalgae harvesting. The purpose of this study was to investigate the concentration of dissolved hydrogen gas produced from the harvesting process of *Dunaliella salina* microalgae species using EC and ORP concentrations including pH, harvesting efficiency due to voltage variations and harvesting time using EC with non-sacrificial electrodes. *D salina* harvesting using EC reactor made of cylindrical borosilicate glass. Spiral-shaped type 304 stainless steel (non-sacrificial) serves as the cathode and solid cylindrically-shaped Fe serves as the anode. The voltage set varies between 16, 18, and 20 Volts, while the electrolysis time is varied between 1.3.5 minutes. The results showed that the highest dissolved hydrogen concentration of 820 ppb (0.820 ppm) produced from the EC process used 18 V for 3 minutes. The volume of gas could not be measured because most of the hydrogen gas was dissolved in the *D salina* culture, so it was not enough to evaporate within 3 minutes. The maximum ORP concentration of -413 mV resulting from the EC process uses 18 V for 3 minutes. When hydrogen gas is present in a solution, it can decrease the ORP value of the solution. At EC time with non-sacrificial electrodes for 5 minutes managed to harvest *D salina* 50.79%; 61.90%; 74.60% at voltages of 16 V, 18V, and 20 V respectively.

**Keywords**: Electrocoagulation; hydrogen gas; non-sacrificial electrode, *Dunaliella salina*

## 1. Introduction

Recent concerns related to global warming have pushed scientists into extensive research on biomass fuels as an alternative to fossil fuels (Phiri et al., 2021). Microalgae have potential in various applications, such as biofuels, food, feed, cosmetics, and pharmaceuticals (Rahul S. et al., 2021). Biomass microalgae is an attractive feedstock for biofuel production due to its high microalgae productivity and high lipid content (Vandamme et al., 2011a). However, microalgae production is not yet economically viable, and one of the main obstacles is the high cost of the microalgae harvesting process, which typically accounts for about 20-30% of the total production cost (Brennan and Owende, 2010). Efficient and low-cost harvesting methods are important research topics to realize large-scale microalgae industrialization (Laraib et al., 2022). Recent advances in microalgae technology have resulted in many efficient harvesting techniques to improve microalgae harvesting. Techniques used to harvest microalgae include centrifugation, coagulation, ultrasonic, pH change, filtration, etc., (Nguyen et al., 2019).

The advantages of the EC process are because it is more efficient to harvest microalgae, does not produce harmful emissions, is environmentally friendly and produces hydrogen gas that can be used as gas fuel or other purposes. Microalgae harvesting using electrocoagulation (EC) produces bubble of hydrogen gas ($H_2$) and oxygen gas ($O_2$). This gas is produced from the electrode while helping to remove coagulated floc through the electro-flotation process (Apshankar and Goel, 2018). Hydrogen gas is considered a pollution-free fuel in the future for several reasons: it contains no carbon, has a maximum energy content per unit mass among all known fuels, and water is the only byproduct produced upon combustion (Singh and Das, 2018).

During the microalgae harvesting process using EC, two electrodes (usually made of iron or aluminum) are submerged in wastewater and an electric current is applied. The current causes the electrode to corrosion, releasing metal ions into the solution. The aluminum content of harvested microalgae biomass is less than 1%, while the concentration of aluminum in the process water is below 2 mg/L (Vandamme et al., 2011b). This leads to contaminated microalgae.

Hydrogen gas will evolve to lift the floc to the surface of the solution, this process is called electro-floatation, and this floc can be removed by skimming. The oxygen produced at the anode forms hydrogen peroxide, which is an intermediate and helps in the oxidation of non-toxic and toxic species (Boinpally et al., 2023). Microbubbles ($O_2$ and $H_2$ gases) resulting from the electrolysis of water are highly recommended for further investigation (Das et al., 2022). The electrodes commonly used in EC are sacrificial metal electrodes. These electrodes are made of metals such as magnesium, iron, zinc, or aluminum that have a lower electrochemical potential than the metal you want to protect. When the two metals are immersed in an electrolyte solution, the more reactive metal (sacrificial metal) oxidizes, releases electrons, and oxidizes to metal ions in solution. During the EC process, the applied current causes the sacrificial metal to dissolve, producing active coagulant compounds (Safwat, 2020). This process results in the sacrificial metal corroding or reducing mass over time.

Previous research utilised aluminium (Al) electrodes in the D-salina harvesting procedure, with a harvesting efficiency of approximately 97-98% (Baierle et al., 2015; Liu et al., 2017). But the final product of biomass contains alluminium that exceeds the values recommended by government regulations. In order to reduce corrosion and reduce electrode mass during the EC process, non-sacrificial electrodes are needed. Non-sacrificial electrodes are electrodes that do not undergo corrosion or mass reduction during electrochemical processes. The use of non-sacrificial electrodes can avoid the problem of metal ion pollution in microalgae products (Ghernaout, 2019). They are often made of metals that are relatively stable and do not easily oxidize or decompose during electrochemical reactions such as Platinum (Pt), Carbon (C) (Ghernaout, 2019), Gold (Au), Silver (Ag), Titanium (Ti), and stainless steel (Ghernaout et al., 2015). Steel stainless electrodes are very environmentally friendly made of metal alloys that are resistant to corrosion. Stainless steel electrodes avoid periodic electrode replacement and do not provide metal contamination to wastewater and harvested biomass (Boinpally et al., 2023). However, the EC process using these non-sacrificial electrodes requires further research, especially for the production of dissolved hydrogen gas and the efficiency of microalgae harvesting.

The purpose of this study was to investigate the concentration of dissolved hydrogen resulting from the *D salina* harvesting process using EC and the concentration of ORP including pH, harvesting efficiency due to voltage variations and duration of *D salina* harvesting time using EC with non-sacrificial electrodes. Due to its protein development, content, and manufacturing capabilities, *D salina* is interested in biotechnology. Industrial cultivation of *D salina* produces β-carotene, which is highly valued by humans. Rapid growth and high salt content distinguish D saline from other microalgae (Castellanos-

Huerta et al., 2022). This research was carried out harvesting *D salina* using an EC reactor equipped with two electrodes, stainless steel as a spiral-shaped non-sacrificial electrode functioning as a cathode and solid cylindrically-shaped Fe functioning as an anode. The cross-sectional area of stainless steel and Fe is 2.52 $m^2$ and 0.088 $m^2$ respectively. The voltage set varies between 16, 18, and 20 Volts, while the electrolysis time is varied between 1.3.5 minutes.

## 2. Method

### 2.1. EC reactor design

The location of this research is in the laboratory of the Centre of Biomass and Renewable Energy Laboratory (C-BIORE) located at Diponegoro University, Semarang, Indonesia (7° 02' 47.9" S, 110° 26' 35.9" E). The EC reactor is made of cylindrical borosilicate glass with a total volume of 600 mL (Figure 1). The purpose of using this reactor material is so that the *D salina* harvesting process using EC can be seen because the reactor is transparent. The reactor is equipped with two electrodes, spiral-shaped type 304 stainless steel serves as the cathode (non-sacrificial electrode) and solid cylindrically-shaped Fe serves as the anode. The cross-sectional area of stainless steel and Fe is 2.52 $m^2$ and 0.088 $m^2$, respectively. Both electrodes are connected to a DC (direct current) electric current source equipped with adjusted voltage and current buttons. The lid of the EC reactor is given a hole to channel the gas formed during the *D salina* harvesting process. The end of the hose is input to the gas volume gauge. The reactor cap is also equipped with inlet holes and *D salina* culture outlets to be harvested. This hole also serves as a sampling location for *D salina* to be analyzed in the laboratory.

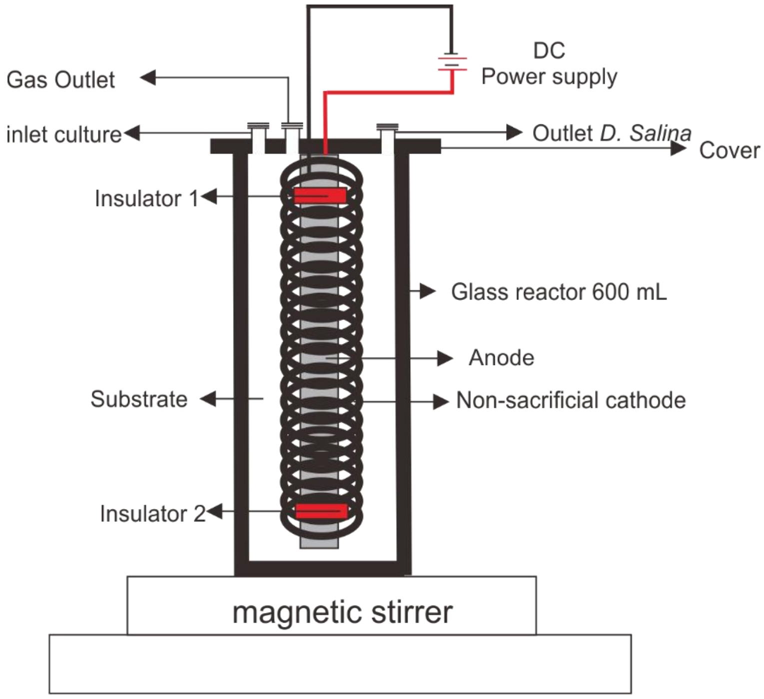


**Figure 1**. The EC reactor scheme is made of cylindrically-shaped borosilicate glass with a total volume of 600 mL

### 2.2. Sample preparation

According to Sugiyono (Sugiyono, 2013), population as a generalized area consisting of objects / subjects that have certain qualities and characteristics determined by researchers to be studied and then drawn conclusions. While the sample is part of the number and characteristics possessed by the population. Based on this understanding, the sample in this study is *D salina* microalgae culture.

Cultivation is done to cultivate or develop *D salina* in a controlled or artificial environment. The controlled environment in this study was lighting using 8-Watt LED lamps with a light intensity of 1500 lux, temperature (° C) to adjust the laboratory room, and air discharge of 3.5L / minute. Researchers used

0.5 liters of *D salina* seeds inserted into Erlenmeyer then added aquadest up to a volume of 1 liter. Researchers added a nutrient mixture of F/2 Guillard+salt mix of 1 mL/L. each. The growth of *D salina* is known by measuring the optical density value at a wavelength of 442 nm using a visible spectrophotometer (Spectroquant, Merck, Germany). When microalgae cultures reach the stationary phase, *D salina* is ready for harvesting due to less microalgae growth and more cell contact. These are ideal conditions for biomass harvesting (de Godos et al., 2011)

## 3. Experimental setup and analysis

A 500 mL *D saline* culture was inserted into the EC reactor in which a 3 cm magnetic stirrer was inserted. The reactor cap is installed and tightened with dextone glue so that no air enters. The stirrer is set at a speed of 400 rpm (DLAB Digital Magnetic Stirrer, USA). The cathode and anode are connected to the adapter as an external power source. The voltage varies between 16, 18, and 20 Volts, while the electrolysis time is varied between 1.3.5 minutes. During the EC process, the volume of gas formed is carefully observed. This measurement method refers to The direct method test was developed for general mine safety applications by the U.S. Bureau of Mines (Diamond et al., 2001) The *D salina* culture samples taken are the initial sample and *D salina* samples after harvesting using EC. Optical density measurements indicate the effectiveness of D. salina harvesting. Liu declares that the use of optical density as a method for measuring microalgal biomass is both accurate and effective (Lu et al., 2017).

The sample is immediately analyzed after sampling. 25 mL of samples were taken to analyze dissolved hydrogen, pH, ORP, and Optical density parameters. The sample is taken from the outlet hole using a water pump. The samples were tested at the laboratory of the Centre of Biomass and Renewable Energy Laboratory (C-BIORE) and the environmental laboratory located at Diponegoro University, Semarang, Indonesia. Tests of dissolved hydrogen, pH, ORP, and Optical density are performed duple. 25 mL of sample is inserted into a beaker glass then a hydrogen meter (YY400, China) is dipped into the sample. Hydrogen meters have an accuracy of ±10ppb. The same sample measured pH value using a pH meter (trans instrument, Singapore), ORP measured using an ORP meter (YY400, China). Optical density is measured using a visible spectrophotometer (Spectroquant, Merck, Germany). All the experiments were performed in duplicate. Analysis graph were done using Origin.

## 4. Result and discussion

### 4.1. Dissolved hydrogen gas

Dissolved hydrogen gas is the amount of hydrogen gas dissolved in a liquid, such as water. The solubility of hydrogen in water is very low, about 1.6-1.8 parts per million (ppm) at standard temperature and pressure (STP) (Molecular Hydrogen Institute, 2023) This means that for every million molecules of water, there are only about 1.6-1.8 molecules of hydrogen dissolved in water. The first objective of this study was to analyze the concentration of dissolved hydrogen produced from the *D salina* harvesting process using EC with non-sacrificial electrodes. Figure 2 (a) present the concentration of dissolved hydrogen gas during the *D salina* harvesting process at voltages of 16, 18, 20 V and electrolysis time of 1, 3, 5 minutes. Based on the results showed that the highest dissolved hydrogen concentration of 820 ppb (0.820 ppm) produced from the EC process used 18 V for 3 minutes, while the lowest dissolved hydrogen concentration of 326 ppb (0.326 ppm) produced from the EC process used 16 V for 1 minute. These results show that the greater the voltage and electrolysis time, the greater the concentration of dissolved hydrogen produced. Higher voltages lead to increased hydrogen gas production in the D-salina harvesting process. However, the relationship is not always linear because the EC process involves water electrolysis, electrolyte concentration, temperature, electrode material, and overall cell design. The initial concentration of hydrogen is 0 ppb. This is likely because the cultivation process of D salina does not produce hydrogen gas. In this study, the volume of the gas could not be measured because most of the hydrogen gas was dissolved in the *D salina culture,* so it was not enough to evaporate within a period of

3 minutes. The expected harvesting process in *D. Salina* is characterized by its rapidity and great efficiency, with the primary objective of reducing operational costs.

Electrolysis of *D salina* culture produces bubbles of oxygen (O2) and hydrogen gas (H2) flowing through the suspension (Dukić and Firak, 2011 & Landels et al., 2019) These bubbles tend to stick to algae cells or swarms which then float to the top of the suspension (Rahmani et al., 2017) With increasing duration of electrolysis and constant current density, the amount of hydrogen gas produced increases. The electrolysis half-reaction following equation (1), (2), and (3) (Li et al., 2022)

Cathode : $2H_2O_{(l)} + 2e^- \rightarrow H_{2(g)} + 2OH^-$ $E^o = -0.83$ V (1)

Anode : $4OH^- \rightarrow O_2 + 2H_2O + 4e^-$ $E^o = +0.4$ V (2)

Net reaction : $2H_2O \rightarrow O_{2\,(g)} + 2H_{2(g)}$ $E^o = -1.23$ V (3)

In this study, *D salina* harvesting used a spiral-shaped stainless-steel cathode (cross-sectional area of 2.52 m2) and a cylindrically-shaped iron anode (cross-sectional area of 0.088 m2). SS cathodes are non-sacrificial electrodes, where SS electrodes do not undergo corrosion or mass reduction during electrochemical processes. The process of reduction of water at the cathode results in the formation of hydrogen gas and hydroxyl ions, as shown in equation (1) with the value Eo = -0.83 V. Electricity of water produces microbubbles (gases O 2 and $H_2$). This is an advantage of the EC process with non-sacrificial electrodes because it is more efficient in harvesting microalgae, environmentally friendly and produces hydrogen gas that can be used as gas fuel or other purposes.

A few studies focus on the production of hydrogen gas from the EC process of liquid waste. The tartrazine degradation process using EC succeeded in producing 0.88 ml of $H_2$ gas at an optimum voltage of 15V with a test time of 4 hours using aluminum plate as anode and stainless steel plate as cathode (Slamet and Kurniawan, 2018).

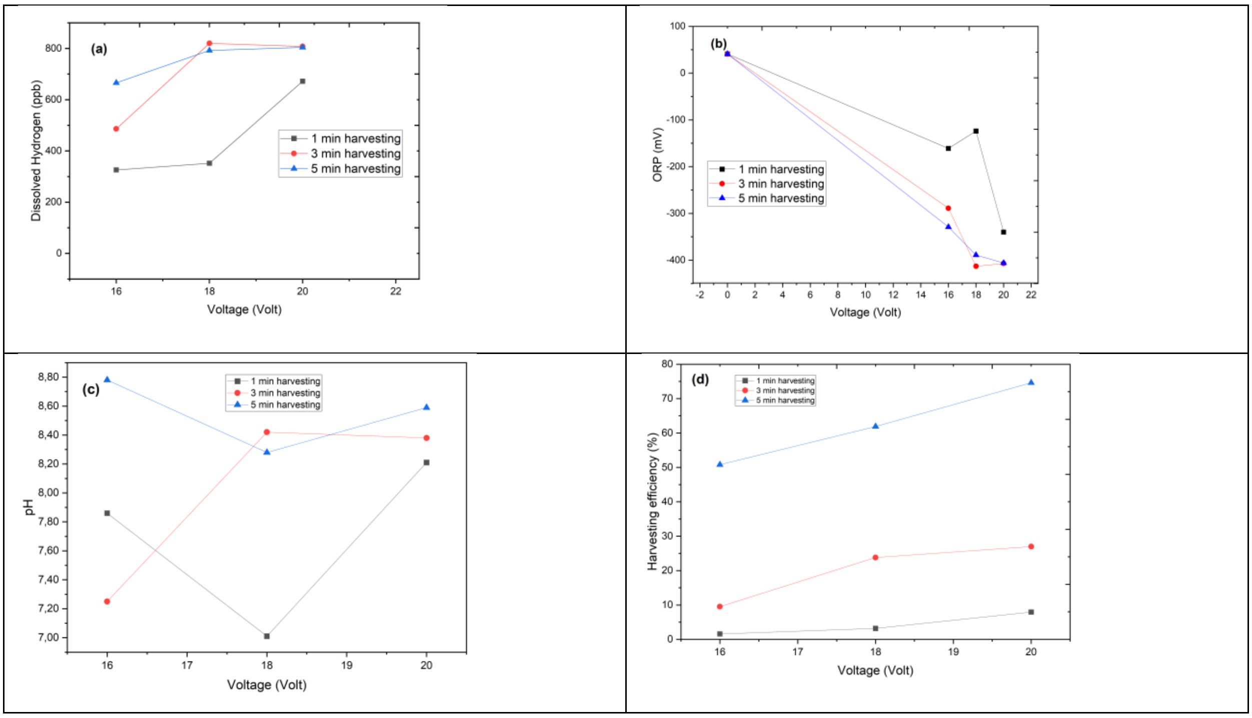


**Figure 2.** Concentration of (a) dissolved hydrogen gas, (b) ORP, (c) pH, and (d) harvesting efficiency during the *D salina* harvesting process at voltages of 16, 18, 20 V and electrolysis times of 1, 3, 5 minutes.

### 4.2. Oxidation reduction potential (ORP)

The second objective of this study was to analyze the concentration of ORP during the *D salina* harvesting process using EC. Figure 2 (b) present ORP concentration during *D salina* harvesting process at voltages of 16, 18, 20 V and electrolysis time of 1, 3, 5 minutes. Redox potential is a measure of a substance's ability to accept or give up electrons in redox reactions. In redox reactions, electron transfer occurs between two chemical species called oxidation and reduction. Redox potential is used to measure how easily a substance can be oxidized or reduced. The oxidation-reduction potential is the potential (voltage) at which oxidation occurs at the anode (positive) and reduction occurs at the cathode (negative) of an electrochemical cell (Suslow, 2004).

Based on the results of this study showed that the initial ORP concentration in *D salina* culture was 41 mV. When *D salina* was harvested using EC, researchers found a decrease in the maximum ORP concentration of -413 mV resulting from the EC process using 18 V for 3 minutes, while the highest ORP concentration of -124 mV resulting from the EC process using 18 V for 1 minute. This result is supported by Al-Shannag et al who stated that ORP decreased due to increased voltage, the solution became more reductive due to the presence of dissolved iron ions (Al-Shannag et al., 2013). Hydrogen gas can act as a powerful reducing agent (Podyacheva et al., 2022), with its ability to release electrons and carry out reduction reactions. In this case, the presence of hydrogen gas can cause the ORP of the solution to be negative, indicating high antioxidant potential (Al-Shannag et al., 2013 & Podyacheva et al., 2022). The process of reduction of water on the cathode results in the formation of hydrogen gas and hydroxyl ions, as shown in equation (1) with the value $E^o$ = -0.83 V.

In this study, the initial ORP concentration in *D salina* culture was positive (+), after electrocoagulation produced negative ORP (-). A negative ORP value indicates the presence of antioxidants or reducing compounds that can reduce oxidation ability in a solution. When the ORP value is negative, it indicates that the solution has a high ability to perform reduction, which is the process of reducing electrons or adding hydrogen. When hydrogen gas is present in a solution, it can affect the ORP value of that solution. Compounds or solutions with negative ORP often have strong antioxidant properties, as they are able to stop or slow oxidation.

### 4.3. pH

Figure 2 (c) shows the pH value of *D salina* culture during the EC process. The initial pH value of 6.65 indicates the culture is close to neutral. The greater the voltage and electrolysis time causes the pH to increase. In the process of harvesting D. salina using EC, a water electrolysis process occurs. Water molecules ($H_2O$) result in hydrogen ions ($H^+$) and hydroxide ions ($OH^-$). Hydroxide ions contribute to increasing the pH of the solution. The maximum pH value of 8.78 resulted from the treatment using 16 V for 5 minutes. A higher pH rise indicates a lower concentration of $H^+$ ions and a higher concentration of hydroxide ions ($OH^-$). This occurs due to redox reactions at the cathode that release ions ($OH^-$). Increased pH provides evidence that cathode activity is more dominant in this EC process (Al-Shannag et al., 2013) At a voltage of 16 V, the pH values of *D saline* cultures were 7.86; 7.25; 8.78 at 1, 3, and 5 minutes of processing, respectively. At a voltage of 18 V, the pH value of D saline culture is 7.01; 8,42; 8.28 each on processing for 1, 3, and 5 minutes. At a voltage of 20 V, the pH value of *D salina* culture is 8.21; 8,38; 8.59 each on processing for 1, 3, and 5 minutes.

pH measurement is important because it has an important impact on EC processes. The ratio of positive and negative ions in a solution is affected by pH. This ratio plays an important role in neutralizing negatively charged cell surface charges causing cells to bind and clump (Lucakova et al., 2021) The formation of floc is best in the range of 3-7. In this study, the optimum pH to form floc when *D salina* was harvested for 1 minute using a voltage of 18 V (pH=7.01) or EC for 3 minutes using a voltage of 16V (pH=7.25).

### 4.4. Harvesting efficiency

Figure 2 (d) shows the percentage efficiency of harvesting *D salina* culture using EC process with non- sacrificial electrode. At EC time for 1 minute managed to harvest *D salina* 1.59%; 3.17%; 7.94% at voltages of 16V, 18V, and 20V respectively. At EC time for 3 minutes managed to harvest *D salina* 9.52%; 23.81%; 26.98% at 16V, 18V, and 20V voltages respectively. At EC time for 5 minutes managed to harvest *D salina* 50.79%; 61.90%; 74.60% at voltages of 16V, 18V, and 20V respectively. Based on this result, it can be concluded that the greater the voltage, the higher the efficiency of harvesting *D salina* culture. The longer the EC process, the higher the harvesting efficiency of *D salina culture*.

The reason behind this improved harvesting efficiency mechanism is due to the chemical properties of the electrodes. Increased concentration of electrode ions that cause floc (Çırak, 2018). In this study, i(Çırak, 2018)on metal (Fe 3 +) released from the anode will combine with hydroxyl ions to form metal-hydroxide or polyhydroxide, such as $Fe(OH)_3$ which functions as a coagulant in the coagulation process. The harvesting process is also influenced by the flotasi process, where micro gas bubbles that help flocate microalgae floc (Boinpally et al., 2023). The *D salina* harvesting method using EC requires a smaller amount of chemicals compared to some other harvesting methods and does not require the addition of additional chemicals.

The efficiency of microalgae conservation in the EC process involves three phenomena, namely adsorption, coagulation, and flotation. Coagulants are produced in the electrocoagulation chamber due to reactions at the anode (Equation (1)), at the same time formed gas H2 cathode (Equation (2)) and hydroxyl ions at the anode (Equation (3)). This resulting coagulant is responsible for the formation of floc surrounded by metal hydroxide, which serves as an efficient adsorbent.

Reaction in anode : $Fe_{(s)} \rightarrow Fe^{3+}{}_{aq} + ne^-$ (4)

$H_2O \rightarrow 4H^+{}_{aq} + O_{2\,(g)} + 4e^-$ (5)

Reaction incathode : $nH_2O_{(l)} + ne^- \rightarrow \frac{n}{2} H_{2\,(g)}\ nOH^-{}_{aq}$ (6)

On the other hand, electrolysis of water produces microbubbles (gases O2 and $H_2$). Microbubbles generated from electrocoagulation are highly recommended for further investigation (Das et al., 2022) The combination of O2 and H2 gases is called HHO gas or Brown's gas according to equation (3) (Sudrajat et al., 2020) The results of this study are supported by previous research. The EC system using rectangular-shaped Fe anodes has a harvesting efficiency of <85% with an operating time of 3 hours and a precipitation time of 4 hours (Mahmood et al., 2021). The electrode used has an area of 24 cm(Mahmood et al., 2021)$^2$ and a concentration of *D. salina* of 0.5 g L-1. Harvesting *D. salina* uses two rectangular electrodes with a surface area of 24 cm2 and the best electrolysis time of 20 minutes (Maleki et al., 2020). A maximum microalga harvesting efficiency of 98.06% was obtained when using rectangular-shaped aluminum electrodes and a stirring speed of 222 rpm. Although the maximum efficiency in the study was (Maleki et al., 2020) 74.60%, the advantage of this study was the short harvesting time (5 minutes) compared to the previous study which took 3 hours (Mahmood et al., 2021), and 20 minutes (Maleki et al., 2020).(Mahmood et al., 2021)(Maleki et al., 2020).

## 5. Conclusion

The purpose of this study was to analyze the efficiency of D salina harvesting, dissolved hydrogen concentration, ORP concentration resulting from the D salina harvesting process using EC with non-sacrificial electrode. Based on the results showed that the highest dissolved hydrogen concentration of 820 ppb (0.820 ppm) produced from the EC process used 18 V for 3 minutes, while the lowest dissolved hydrogen concentration of 326 ppb (0.326 ppm) produced from the EC process used 16 V for 1 minute. These results show that the greater the voltage and electrolysis time, the greater the concentration of dissolved hydrogen produced. In this study, the volume of gas could not be measured because most of the hydrogen gas was dissolved in the D salina culture, so it was not enough to evaporate within 3 minutes. The maximum ORP concentration of -413 mV resulting from the EC process uses 18 V for 3 minutes, while the highest ORP concentration of -124 mV resulting from the EC process uses 18 V for 1 minute. A negative ORP value indicates the presence of antioxidants or reducing compounds that can reduce oxidation ability in a solution. When hydrogen gas is present in a solution, it can decrease the ORP value of the solution. In this study, the optimum pH to form floc when D salina was treated using EC for 1 minute using a voltage of 18 V (pH = 7.01) or EC for 3 minutes using a voltage of 16V (pH = 7.25). At EC time for 5 minutes managed to harvest D salina 50.79%; 61.90%; 74.60% at voltages of 16V, 18V, and 20V respectively.

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