Harvesting of Dunaliella salina using electrocoagulation with helix electrode and producing HHO gas as promising Hydrogen fuel

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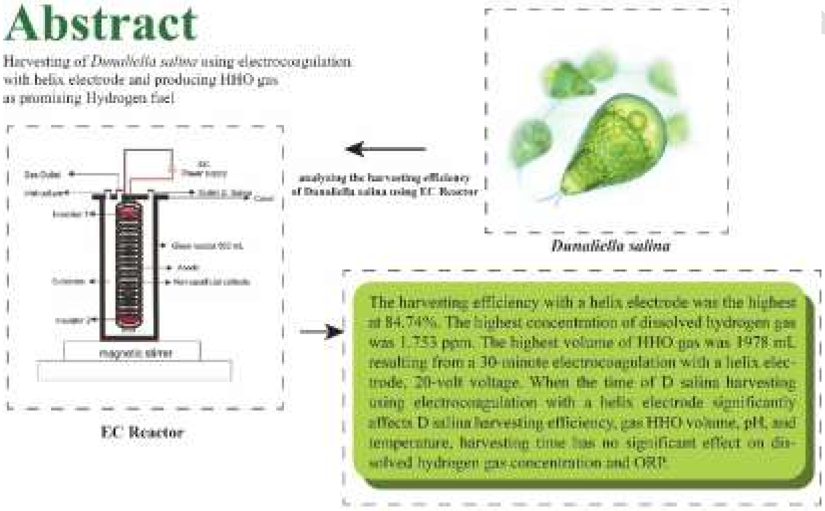
GRAPHICAL ABSTRACT



Abstract

Harvesting of *Dunaliella salina* using electrocoagulation with helix electrode and producing HHO gas as promising Hydrogen fuel

The harvesting efficiency with a helix electrode was the highest at 84.74%. The highest concentration of dissolved hydrogen gas was 1.753 ppm. The highest volume of HHO gas was 1978 mL resulting from a 30-minute electrocoagulation with a helix electrode, 20-volt voltage. When the time of D salina harvesting using electrocoagulation with a helix electrode significantly affects D salina harvesting efficiency, gas HHO volume, pH, and temperature, harvesting time has no significant effect on dissolved hydrogen gas concentration and ORP.

ABSTRACT

Biofuels are the only sustainable alternatives. Microalgae, effective biomass sources, can serve as biofuels, while hydrogen gas, produced from renewable sources, is a non-polluting energy source. Electrocoagulation (EC) with a helical electrode and HHO gas (used as hydrogen fuel) was used to analyze the harvesting efficiency of *Dunaliella salina*. Harvesting was performed using an acrylic EC reactor on a batch scale. Helical 304 stainless steel (cathode) and solid, cylindrical Fe (anode) were used at 20 V and electrolysis times of 1, 5, 10, 15, 20, 25, and 30 min. The highest harvesting efficiency was 84.74%, while the highest concentration of dissolved hydrogen gas was 1.753 ppm. The highest HHO gas volume (1,978 mL) occurred after 30 min of EC. Nanoscale glovaries formed on the electrode through heterogeneous nucleation at the solid–liquid interface. The nanobubbles grew, and their buoyancy forces released gas from the cathode for 30 min, producing 1,978 mL of HHO. The HHO gas at the convex electrode removed coagulated *D. salina* flocs through electroflotation. Harvesting time significantly affected harvesting efficiency, HHO gas volume, pH, and temperature but did not significantly affect the concentration of dissolved hydrogen gas and ORP.

Keywords: *Dunaliella salina*, electrocoagulation, helical, HHO gas, ORP

INTRODUCTION

Fossil-fuel utilization contributes the most to greenhouse gas release into the troposphere. Currently, research initiatives are aimed at finding sustainable fossil-fuel alternatives that can be commercialized to meet the market demand [1]. Biofuels obtained from renewable sources are the only sustainable alternatives for the global economy. Microalgae have shown tremendous potential as sources of biomass that can serve as biofuels [2]. Microalgal cultivation requires manageable land that produces high amounts of biomass [3]. Green energy sources reduce the dependence on imported fossil fuels, reduce GHG emissions, and realize decarbonization in the energy sector [4]. Once microalgae grow, they must be harvested and processed into a form that can be used for specific applications. However, the harvesting and processing steps are generally complicated and expensive. Researchers are currently developing more efficient and cost-effective methods of harvesting microalgae.

Electrocoagulation (EC) is a microalgal harvesting method that must be explored owing to its ease of operation, rapid process, adaptability, environmentally friendliness, and *low footprint* [5]. Moreover, EC can save up to 89% of energy compared with centrifugation alone [6]. It combines water electrolysis and coagulation. Metal ions released from the electrode (e.g., Fe^{3+}) combine with hydroxyl ions to form metal hydroxides or polyhydroxides, such as $Fe(OH)_3$, which functions as a coagulant. The voltage, initial microalgae concentration, and electrolysis time are essential operating parameters during EC [7, 8]. Most studies on EC used *Chlorella* as the harvested microalgae [9-11]. In addition, several other freshwater and marine microalgae, including *Botryococcus* [12], *Desmodesmus* [13], *Microcystis [7],* and *Nannochloropsis* [14], have been harvested using EC. However, the microalgae species *Dunaliella salina* has received little attention [15].

Hydrogen gas (H_2) is generated during EC. It lifts the floc to the culture surface, a process known as electroflotation, and can be removed by skimming. The oxygen produced at the anode forms hydrogen peroxide, an intermediate that helps to oxidize non-toxic and toxic species [16]. Microbubbles (O_2 and H_2) resulting from water electrolysis are highly recommended for further investigations [5]. The combination of O_2 and H_2 through electrolysis is known as HHO gas, hydroxy gas, oxy-hydrogen, or Brown’s gas [17, 18]. Further investigation is required to produce HHO gas as a promising hydrogen fuel when harvesting *D. salina* using electrocoagulation with a helical electrode.

This study aimed to analyze the harvesting efficiency of *D. salina* using EC with a helical electrode and to produce HHO gas as hydrogen fuel. *D. salina* harvesting was performed on a batch scale using an acrylic EC reactor. Helical 304 stainless steel was used as the cathode, and solid, cylindrical Fe was used as the anode. The voltage was set to 20 V, and the electrolysis time varied between 1, 5, 10, 15, 20, 25, and 30 min.

METHODOLOGY

The experiment in this study was conducted using a laboratory scale. *D. salina* cultures were obtained from UgoPlankton, Jepara Regency and cultured at UPT Laboratory, C-BIORE Diponegoro University, Semarang, Indonesia, and Environmental Laboratory, Faculty of Engineering, Diponegoro University. The experiment was conducted twice to obtain highly accurate data.

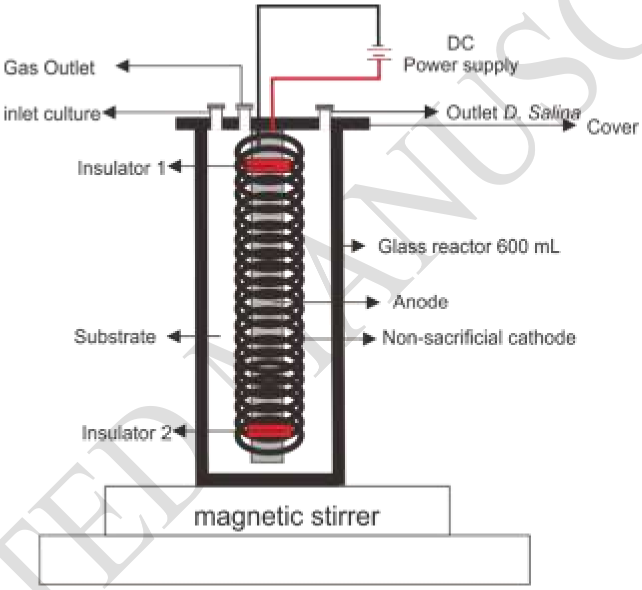


Figure 1. EC reactor: cylindrical acrylic reactor with a total volume of 1,500 mL..

A cylindrical acrylic reactor was used (Figure 1). The helical electrode used was 304 stainless steel with 18–20% chromium, 8–10.5% nickel, 0.08% carbon, 2% manganese, silicon, phosphorus, and molybdenum. Similar to nickel, manganese helps preserve the austenitic phase in steel. *D. salina* was harvested by EC in batch mode at room temperature using an EC reactor containing 1,500 mL of *D. salina* culture. The EC reactor was tubular with a cathode and anode. The anode was spiral stainless steel with a dimension of 7 cm and a length of 10 cm. The cathode was solid, cylinder-shaped Fe with a 2.5-cm diameter and 10-cm length. Moreover, the cathode was coated with a circular insulator and then inserted into the anode, the two being 2.5 cm apart, so that the anode and cathode did not stick together. The EC reactor was tightly sealed using polyethylene equipped with 0.5-cm holes for the cathode, anode, gas emission, inlet, and D. *salina* culture outlet. The holes were closed using silicone glue (Dextone) so that no air or liquid escaped. The experiment was conducted at a constant voltage of 20 V. EC systems were operated at various EC times (1–30 min). A 220-V AC source was converted to a DC source using an AC–DC adapter (Sunshine 30V 5A, P-3005D; China).

D. *salina* was stirred at 400 rpm during EC using a stirrer (DLAB MS-PA LED Digital Magnetic Stirrer; USA). Stirring was stopped for 30 min before sampling. Thus, the flocs were allowed to settle at the bottom of the reactor. The upper phase is hereafter referred to as the supernatant, whereas the lower phase is referred to as the concentrate. Samples were collected from the supernatant (2 cm from the surface) to determine the temperature, pH, concentration of dissolved H_2, and ORP. The combination of O_2 and H_2, HHO or Brown's gas, through electrolysis was channeled to an inverted graduated cylinder containing water to measure its volume. This measurement method is the direct-method test developed by the US Bureau of Mines for general mine safety applications [19, 20].

To ascertain the amount of *D. salina* that could be harvested using EC spirals, the *D. salina* harvesting efficiency (%) was calculated. The harvesting efficiency was calculated based on the method of Papazi et al. [19], as shown in Equation (1)

$$Harvesting\ efficiency\ (\%) = \left(\frac{OD_0 - OD_1}{OD_0}\right) x\ 100\% \text{.....................} (1)$$

where harvesting efficiency is the amount of *D. salina* that can be harvested (%), OD_o is the initial optical density of *D. salina*, and OD_1 is the optical density of *D. salina* after harvesting. Two sets of tests were performed to determine the dissolved hydrogen content, pH, ORP, and optical density. The accuracy of the hydrogen meters was within ten ppb. A pH meter (Trans Instrument, Singapore) was used to measure the pH of the samples, and an ORP meter (China) was used to assess the ORP of the samples. A visible spectrophotometer (GENESYS 150, Thermo Scientific, USA) was used to measure optical density. Each experiment was performed twice to ensure accuracy. Origin was used to create graphs and for statistical analysis.

Results and Discussion

Dunaliella salina harvest efficiency

D. salina biomass was harvested to separate it from culture media using low energy at the time of thickening or drying. The EC approach can increase harvesting efficiency [21]. Studies have investigated how to attain high harvesting efficiencies to achieve maximum and sustainable yields.

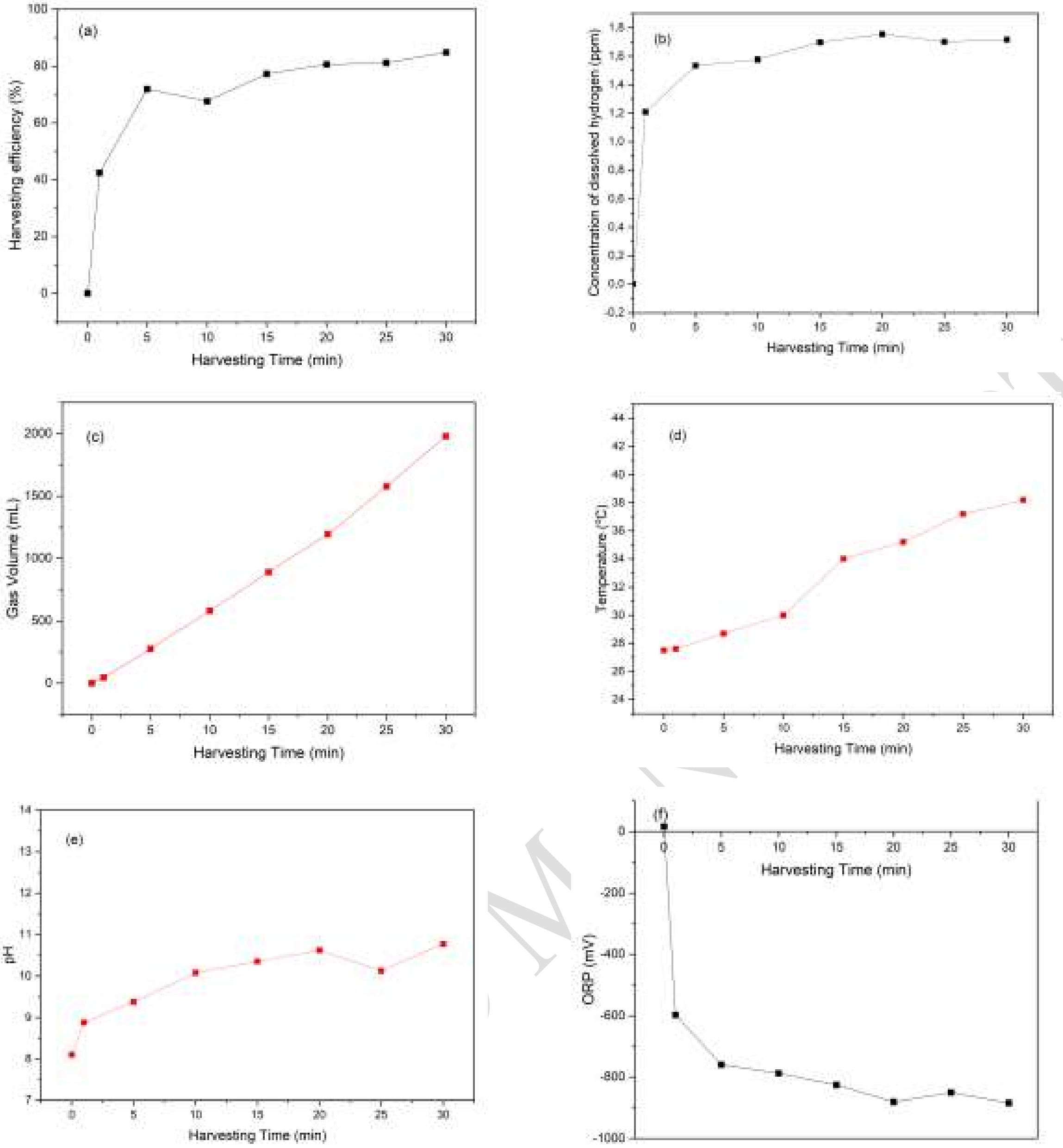


Figure 2 Graphs of (a) *D. salina* harvesting efficiency, (b) dissolved hydrogen gas concentration, (c) volume of HHO gas used as hydrogen fuel, (d) temperature, (e) pH, and (f) oxidation–reduction potential (ORP) when *D. salina* was harvested by EC with a helical electrode for 30 min.

Figure 2(a) shows that there was a sharp increase in harvesting efficiency from 42.37% to 71.95% between 1 and 5 min, respectively. From 10 to 30 min, the harvesting efficiency increased slightly. The highest harvesting efficiency (84.74%) was reached after 30 min of EC, while the lowest harvesting efficiency (42.37%) was reached after 1 min of EC. The effect of electrolysis time on *D. salina* harvesting efficiency was analyzed using Origin. An ANOVA test was performed using a regression model, with a p-value (usually called the independent variable) smaller than the predetermined significance level ($\alpha = 0.05$) being considered significant. The ANOVA produced a p-value of 0.02724. This value indicated that harvesting time significantly affected the *D. salina* harvesting efficiency. The longer the electrocoagulation time, the more Fe^{3+} ions are released by the anode. Fe^{3+} ions will react with hydroxyl ions to form poly-hydroxides, which function as coagulants in the *D salina* harvesting

This finding was also reported by Mahmood, Kim [22], who stated that the harvesting efficiency for *D. salina* reached <85% when using an EC system with a rectangular Fe anode, an operating time of 3 h, and a precipitation time of 4 h. The electrode had an area of 24 cm^2 and a *D. salina* concentration of 0.5 g L^{-1}. A study used two rectangular electrodes with a surface area of 24 cm^2 and an optimal electrolysis time of 20 min to harvest *D. salina* [23]. A maximum microalgal harvesting efficiency of 98.06% was achieved using the rectangular aluminum electrodes at a stirring speed of 222 rpm.

EC is based on electrochemical phenomena in which oxidation, or the removal of electrons, occurs at the cathode. In contrast, reduction, or the addition of electrons, occurs at the anode surface [24]. Coagulants are produced in situ during EC; thus, EC does not require additional chemicals [25]. Metal ions released from the anode (Fe^{3+}) combine with hydroxyl ions to form metal hydroxides or polyhydroxides, such as $Fe\ (OH)_3$, which functions as a coagulant, according to Equation (1). Notably, $Fe2^+$ ions are the primary ions released by the Fe electrode, even at high voltages [26]. According to Equation (2), Fe^{2+} is very slowly oxidized by oxygen in acidic media, whereas Fe^{2+} can be rapidly converted to $Fe(OH)_{2(s)}$ in neutral or alkaline media and further oxidized to $Fe(OH)_{3(s)}$ according to Equations (3) and (4) [27]. At the Fe anode, side reactions also occur due to gas production, according to Equation (5). Side reactions may also occur under specific conditions during EC. For example, a high voltage can produce $O_{2(g)}$ on the anode surface [28], according to Equation (5).

Reaction at anode:

$$Fe(s) \rightarrow Fe^{2+} + 2e^- \quad (1)$$

$$Fe^{2+} + O_{2(aq)} + 2H_2O \rightarrow Fe^{3+} + 4OH^- \quad (2)$$

$$Fe^{2+} + 2OH \rightarrow Fe(OH)_{2(s)} \quad (3)$$

$$Fe(OH)_{2(s)} + O_{2(aq)} + 2H_2O \rightarrow 4Fe(OH)_{3(s)} \quad (4)$$

Side reactions at the anode:

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

Microalgal harvesting during EC is mainly associated with three phenomena: adsorption, coagulation, and flotation. Coagulants are produced in the EC chamber owing to the reaction at the anode [Equation (1)–(5)]; meanwhile, H_2 forms at the cathode [Equation (6)–(7)]. The resulting coagulant forms flocs surrounded by metal hydroxides, which are efficient adsorbents.

The volume of HHO gas as Hydrogen fuel

H_2 is a non-polluting energy source because it is produced from renewable sources, and it does not cause global warming [29]. Previous studies primarily defined dissolved hydrogen as H_2 dissolved in water. During electrolysis, H_2 undergoes bubble formation, growth, and detachment [30]. The concentration of dissolved hydrogen in high-temperature water can affect the primary water stress corrosion cracking susceptibility of nickel-based alloys, Alloy 600, Alloy 82, and Alloy 182 [31]. Molecular hydrogen-dissolved alkaline electrolyzed water has various physiological activities, such as antioxidative activity [31].

Figure 2(b) shows that the concentration of dissolved H_2 increased slightly, from 1.209 ppm to 1.535 ppm between 1 and 5 min, respectively. The highest concentration of dissolved H_2 was 1.753 ppm, resulting from 30 min of EC. Meanwhile, the lowest dissolved H_2 concentration (1.209 ppm) was reached after 1 min of EC process. The results of the ANOVA variance analysis produce a p-value of 0.06951. This value shows that the harvesting time has no significant effect on the concentration of dissolved hydrogen gas. ANOVA test shows that harvesting time has no significant influence on dissolved hydrogen gas. The ANOVA test informs that the longer the harvester, the concentration of dissolved hydrogen gas is not significantly affected. Although hydrogen gas is produced continuously at the cathode according to equations (6) and (7), it has a very low solubility in 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) [32]. This means that for every million molecules of water, only about 1.6-1.8 hydrogen molecules are dissolved in water. As the harvesting time increases, dissolved hydrogen gas will be saturated, then the gas will evaporate from the solvent (water) and flow through the gas outlet hose.

During electrolysis, the concentration of dissolved gases near the electrode increases and reaches saturation, and depending on the degree of saturation, it is most likely associated with the surface structure of the electrode [30].

The volume of HHO gas as Hydrogen fuel

Microbubbles resulting from water electrolysis are highly recommended for further investigations [5]. The third research objective was to analyze the volume of HHO gas as hydrogen fuel produced during *D. salina* harvesting using EC with helical electrodes. The combination of O_2 and H_2 through electrolysis is known as HHO gas, hydroxy gas, oxy-hydrogen, or Brown's gas [17, 18]. An advantage of EC is that it is more efficient at harvesting microalgae and producing HHO gas, which can be used as fuel gas.

Figure 2(c) shows that the volume of HHO gas between 1 and 30 min increased linearly with the time of harvesting. The highest volume of HHO gas was 1,978 mL, resulting from 30 min of EC at 20 V. The lowest volume of HHO gas was 46 mL, which was reached after 1 min of EC. The

ANOVA produced a p-value of 1.66829E-8. This value showed that harvesting time significantly affected the volume of HHO gas used as hydrogen fuel. The ANOVA test informs that the longer the harvesting causes a significant increase in the concentration of HHO gas. During 30 minutes of D Salina harvesting, HHO gas is produced continuously at the cathode according to the equation (6) dan (7). HHO gas is evaporate from the solvent, then flows through the outlet hose.

Most researchers have focused on the production of H_2 from liquid-waste EC processes. For example, a study on tartrazine degradation using EC produced 0.88 ml of H_2 at an optimum voltage of 15 V. This test was conducted over 4 hours using an aluminum plate as the anode and a stainless-steel plate as the cathode [33].

H_2 is produced at the cathode in acidic media [Equation (6)] and neutral or alkaline media [Equation (7)].

Reaction of the cathode under acidic conditions:

$$4H^{+}{}_{aq} + 2e^{-} \rightarrow H\frac{n}{2}{}_{2\,(g)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(6)$$

Reaction of the cathode under neutral or alkaline conditions:

$${}_{nH2O}{+}ne^{-}\frac{n}{2}{\rightarrow}{}_{H2(g)}nOH\text{-}aq \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(7)$$

Chen, Wiedenroth [34] revealed that nanobubbles form on electrodes through heterogeneous nucleation at the solid–liquid interface. After nucleation, the nanobubbles grow, and the buoyancy forces of the growing bubbles release gas from the cathode surface. This process continues until numerous bubbles are generated [30].

The HHO gas at the convex electrode formed during the study helped remove coagulated *D. salina* flocs through electroflotation [35]. This finding is supported by another study [35], which found that EC produced bubbles that help lift flocs to the culture surface.

Temperature and pH

Kobya, Omwene [36] stated that the pH of a solution affects electrode dissolution, the zeta potential of colloidal particles, the distribution of Fe^{3+} ions, and the solubility of amorphous hydroxides. The pH of a solution also affects the ratio of positive to negative ions present in the solution. This ratio plays a vital role in neutralizing negatively charged cell surfaces, which causes cells to clump together and bond. Lucakova, Branyikova [8] confirmed that ideal floc production occurred at a pH between 3 and 7.

Figure 2(e) shows that the initial pH of the *D. salina* culture was 8.10. The pH increased from 8.88 to 9.38 between 1 and 5 min, respectively. The highest pH (10.77) was reached after 30 min of EC. In this study, the lowest pH (8.88) was reached after 1 min of EC. The pH of the cultures increased over time owing to the continuous production of OH^- ions at the cathode, and the final pH of the study tended to stabilize at 10. This pH value was also obtained by Hashim et al., who reported that the final effluent EC results in a stable pH of 10 [37]. The ANOVA produced a p-value of 0.00512. This value indicates that the time of harvest has a significant effect on the pH value at the 0.05 level, the slope is significantly different from zero. During the 30-minute D salina harvesting process, OH- ions are produced continuously. The longer the electrolysis process is carried out, the more OH^- ions are produced, so the pH increases [33]. When OH^- ions produced at the cathode have not reacted with Fe^{2+} and the formation of Fe $(OH)_{3(s)}$. Hence pH solution increases rapidly near the anode [38]. pH increased solution can accelerate the oxidation of Fe^{2+} and result in higher harvesting efficiency[39].

The solution significantly affected the speciation distribution of Fe^{2+}/Fe^{3+} during EC. An increase in temperature facilitates the mass transfer of Fe^{2+} from the anode surface to the solution and increases the hydrolysis rate of Fe^{2+} to $Fe(OH)_{2(aq)}$ [27]. Figure 2(d) shows that the temperature after 1 to 30 min tended to increase. The ANOVA produced a p-value of 3.41229E-6. This value indicated that harvesting time had a significant effect on temperature. The highest temperature (38.2°C) was reached after 30 min of EC, while the lowest temperature (27.6°C) was reached after 1 min of EC.

Oxydation Reduction Potential (ORP)

ORP measures how easily a substance can be oxidized or reduced. It is the potential (voltage) at which oxidation occurs at the anode (positive) and reduction occurs at the cathode (negative) of an electrochemical cell [40]. Compounds or solutions with an ORP less than zero typically have powerful antioxidative effects because they can prevent or significantly decrease the degree of oxidation.

Figure 2(f) shows that the ORP after 1 to 30 min tended to decrease. Specifically, the initial ORP was 17 mV, after which it decreased significantly to -597 mV after 1 min. At 5 and 10 min, it was -760 mV and -788 mV, respectively. The highest ORP (-597 mV) was reached after 1 min of EC, and the lowest ORP (-884 mV) was reached after 30 min of EC. The ANOVA produced a p-value of 0.05893. This value indicated that harvesting time had a nonsignificant effect on ORP. This result was also obtained by Al-Shannag et al., who indicated that the ORP decreased owing to Fe ions being dissolved. In this case, the presence of H_2 resulted in a negative ORP value for the solution, indicating that the solution had significant antioxidative potential [41]. H_2 can release electrons and engage in reduction reactions, and it is a highly effective reducing agent.

Conclusion

This study had two main objectives: to analyze the harvesting efficiency of *D. salina* using EC with a helical electrode and to produce HHO gas as hydrogen fuel. The harvesting of *D. salina* in the study was associated with three phenomena: adsorption, coagulation, and flotation. The highest harvesting efficiency was 84.74%, while the highest concentration of dissolved H_2 was 1.753 ppm. Hydrogen's solubility in water was very low, approximately 1.6–1.8 ppm at STP. The highest volume of HHO gas was 1,978 mL, which was reached after 30 min of EC at 20 V. The *D. salina* harvesting time after EC significantly affected the harvesting efficiency, HHO gas volume, pH, and temperature, but it had no significant effect on the concentration of dissolved H_2 and ORP. In future research, the flame test should also be performed for the HHO gas produced during the harvesting of *D. salina*.

Acknowledgment

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Reference

1. Biswal, T., K.P. Shadangi, and P.K. Sarangi, *Technologies of Microalgae Biomass Cultivation for Bio-Fuel Production: Challenges and Benefits.* Biorefinery Production of Fuels and Platform Chemicals, 2023: p. 147-178.
2. Biswal, T., K.P. Shadangi, and P.K. Sarangi, *Algae Biofuel Production Techniques: Recent Advancements.* Biorefinery Production of Fuels and Platform Chemicals, 2023: p. 131-146.
3. Banerjee, S., et al., *Microalgal pandora for potent bioenergy production: A way forward?* Fuel, 2023. **333**: p. 126253.
4. Salam, M.A., M.A.A. Shaikh, and K. Ahmed, *Green hydrogen based power generation prospect for sustainable development of Bangladesh using PEMFC and hydrogen gas turbine.* Energy Reports, 2023. **9**: p. 3406-3416.
5. Das, P.P., M. Sharma, and M.K. Purkait, *Recent progress on electrocoagulation process for wastewater treatment: A review.* Separation and Purification Technology, 2022. **292**: p. 121058.
6. Fayad, N., et al., *Harvesting of microalgae Chlorella vulgaris using electro-coagulation-flocculation in the batch mode.* Algal research, 2017. **25**: p. 1-11.
7. Meetiyagoda, T.A.O.K. and T. Fujino, *Optimization of electrocoagulation–flotation treatment with an aluminum anode to enhance Microcystis aeruginosa cell removal efficiency.* Journal of Water Process Engineering, 2022. **48**: p. 102871.
8. Lucakova, S., et al., *Electrocoagulation reduces harvesting costs for microalgae.* Bioresource Technology, 2021. **323**: p. 124606.
9. Castellanos-Estupinan, M.A., et al., *Design of an electroflotation system for the concentration and harvesting of freshwater microalgae.* Chemical Engineering Transactions, 2018. **64**.
10. Lal, A. and D. Das, *Biomass production and identification of suitable harvesting technique for Chlorella sp. MJ 11/11 and Synechocystis PCC 6803.* 3 Biotech, 2016. **6**: p. 1-10.

11. Rahmani, A., et al., *Hydrogen recovery from the photovoltaic electroflocculation-flotation process for harvesting Chlorella pyrenoidosa microalgae.* International Journal of hydrogen energy, 2017. **42**(31): p. 19591-19596.
12. Xu, L., et al., *Development of an efficient electroflocculation technology integrated with dispersed-air flotation for harvesting microalgae.* Journal of Chemical Technology & Biotechnology, 2010. **85**(11): p. 1504-1507.
13. Montaño Saavedra, M.D., et al., *Growth of Desmodesmus subspicatus green microalgae and nutrient removal from sugarcane vinasse clarified by electrocoagulation using aluminum or iron electrodes.* Dyna, 2019. **86**(211): p. 225-232.
14. Matos, C.T., et al., *Nannochloropsis sp. biomass recovery by Electro-Coagulation for biodiesel and pigment production.* Bioresource technology, 2013. **134**: p. 219-226.
15. Liu, Q., et al., *Energy-producing electro-flocculation for harvest of Dunaliella salina.* Bioresource technology, 2017. **241**: p. 1022-1026.
16. Boinpally, S., et al., *A state-of-the-art review of the electrocoagulation technology for wastewater treatment.* Water Cycle, 2023.
17. Sudrajat, A., et al., *Pengaruh Penambahan Gas HHO pada Mesin Bensin Terhadap Emisi dan Konsumsi Bahan Bakar.* Jurnal Ilmiah Giga, 2020. **23**(1): p. 8-19.
18. Subramanian, B. and S. Ismail, *Production and use of HHO gas in IC engines.* International Journal of Hydrogen Energy, 2018. **43**(14): p. 7140-7154.
19. Diamond, W., et al., *The modified direct method: A solution for Obtaining accurate coal desorption Measurements.* 2001.
20. Papazi, A., P. Makridis, and P. Divanach, *Harvesting Chlorella minutissima using cell coagulants.* Journal of applied Phycology, 2010. **22**(3): p. 349-355.
21. Barros, A.I., et al., *Harvesting techniques applied to microalgae: a review.* Renewable and sustainable energy reviews, 2015. **41**: p. 1489-1500.
22. Mahmood, A., J.H. Kim, and J.-W. Park, *Application of metal-air fuel cell electrocoagulation for the harvesting of Nannochloropsis salina marine microalgae.* Renewable Energy, 2021. **171**: p. 1224-1235.
23. Maleki, M.-g., M. Almassi, and N. Nasirian, *Optimization of Microalgae Harvesting and Separation Process by Electrical Coagulation in Biodiesel Production Cycle Using Response Surface Methodology.* Journal of Agricultural Engineering Soil Science and Agricultural Mechanization,(Scientific Journal of Agriculture), 2020. **43**(3): p. 425-440.
24. Syam Babu, D., et al., *Industrial wastewater treatment by electrocoagulation process.* Separation Science and Technology, 2020. **55**(17): p. 3195-3227.
25. Martínez-Huitle, C.A. and E. Brillas, *Decontamination of wastewaters containing synthetic organic dyes by electrochemical methods: a general review.* Applied Catalysis B: Environmental, 2009. **87**(3-4): p. 105-145.
26. Dubrawski, K.L., et al., *Production and transformation of mixed-valent nanoparticles generated by Fe (0) electrocoagulation.* Environmental science & technology, 2015. **49**(4): p. 2171-2179.
27. Hu, Q., et al., *Recent advances in phosphate removal from municipal wastewater by electrocoagulation process: A review.* Separation and Purification Technology, 2022: p. 122944.
28. Ingelsson, M., N. Yasri, and E.P. Roberts, *Electrode passivation, faradaic efficiency, and performance enhancement strategies in electrocoagulation—a review.* Water Research, 2020. **187**: p. 116433.
29. Bockris, J.O.M., *The hydrogen economy: Its history.* International Journal of Hydrogen Energy, 2013. **38**(6): p. 2579-2588.
30. Ando, K., Y. Uchimoto, and T. Nakajima, *Concentration profile of dissolved gas during hydrogen gas evolution: an optical approach.* Chemical Communications, 2020. **56**(92): p. 14483-14486.

31. Kim, T., et al., *Effects of dissolved hydrogen on the crack-initiation and oxidation behavior of nickel-based alloys in high-temperature water.* Corrosion Science, 2016. **106**: p. 260-270.
32. Institute, M.H. *Concentration and Solubility of H2*. 2023 [cited 2023; Available from: https://molecularhydrogeninstitute.org/concentration-and-solubility-of-h2/.
33. Slamet, S. and R. Kurniawan. *Degradation of tartrazine and hydrogen production simultaneously with combination of photocatalysis-electrocoagulation*. in *AIP Conference Proceedings*. 2018. AIP Publishing.
34. Chen, Q., et al., *Electrochemical nucleation of stable N2 nanobubbles at Pt nanoelectrodes.* Journal of the American Chemical Society, 2015. **137**(37): p. 12064-12069.
35. Apshankar, K.R. and S. Goel, *Review and analysis of defluoridation of drinking water by electrocoagulation.* Journal of Water Supply: Research and Technology—AQUA, 2018. **67**(4): p. 297-316.
36. Kobya, M., et al., *Phosphorous removal from anaerobically digested municipal sludge centrate by an electrocoagulation reactor using metal (Al, Fe and Al-Fe) scrap anodes.* Process Safety and Environmental Protection, 2021. **152**: p. 188-200.
37. Hashim, K.S., et al., *Electrocoagulation as a green technology for phosphate removal from River water.* Separation and Purification Technology, 2019. **210**: p. 135-144.
38. Lakshmanan, D., D.A. Clifford, and G. Samanta, *Ferrous and ferric ion generation during iron electrocoagulation.* Environmental science & technology, 2009. **43**(10): p. 3853-3859.
39. Holt, P.K., et al., *A quantitative comparison between chemical dosing and electrocoagulation.* Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2002. **211**(2-3): p. 233-248.
40. Suslow, T.V., *Oxidation-reduction potential (ORP) for water disinfection monitoring, control, and documentation.* 2004.
41. Podyacheva, E., et al., *Syngas Instead of Hydrogen Gas as a Reducing Agent─ A Strategy To Improve the Selectivity and Efficiency of Organometallic Catalysts.* ACS Catalysis, 2022. **12**(9): p. 5145-5154.