JEE Journal of Ecological Engineering
Volume 21, Issue 2, February 2020, pages 80–88
https://doi.org/10.12911/22998993/116344

Received: 2019.12.10
Revised: 2019.12.23
Accepted: 2020.01.07
Available online: 2020.01.25

Reduction of Microalgae by Copper Ion in Impressed Current Anti Fouling System for Biofouling Prevention in Saline Environment

Herman Pratikno[1*], Harmin Sulistiyaning Titah[2], and Handayanu[1]

[1] Department of Ocean Engineering, Faculty of Marine Technology, Institut Teknologi Sepuluh Nopember, Keputih, Sukolilo, 60111 Surabaya, Indonesia

[2] Department of Environmental Engineering, Faculty of Civil, Environmental and Geo Engineering, Institut Teknologi Sepuluh Nopember, Keputih, Sukolilo, 60111 Surabaya, Indonesia

* Corresponding author's e-mail: hermankelautan@gmail.com

ABSTRACT

The biofouling causes corrosion in marine environment, also known as the biological corrosion. The biological corrosion occurs in the metal material on coastal buildings, offshore buildings, port buildings and shipboard. One method to prevent the biological corrosion is ICAF (Impressed Current Anti-Fouling). The study on the microalgae that cause biofouling was conducted in laboratory scale using a simple ICAF system. The variables were the operating time of the simple ICAF system, the strength of the electric current and the species of microalgae. The determination of cell number of microalgae was conducted using a Neubauer improved Hemocytomete method, while determination of the concentration of Cu ion was conducted using Atomic Absorption Spectrophotometry (AAS). The aim of the research was to determine of microalgae, *Isochrysis galbana* and *Botryococcus* sp, population reduction using ICAF system. On the basis of the results, the highest population reduction occurred in *Isochrysis galbana* and *Botryococcus* sp reaching 77.5% and 50%, respectively. The highest concentration of Cu that was produced during the operation of the simple ICAF system reached 4.08 ± mg/L. In conclusion, ion Cu that was produced during the operation of the simple ICAF system can reduce the cell number of *Isochrysis galbana* and *Botryococcus* sp.

Keywords : biocorrosion, biofouling, impressed current anti-fouling, Cu ion, microalgae

INTRODUCTION

The marine building technology consists of coastal buildings, offshore buildings and port / pier buildings. One of the offshore marine structures is a production platform with complete equipment including faucets, tanks, cooling devices, heaters, generators, pumps. The sea water is the efficient mean used on board and coastal/ offshore buildings for the release of this heat (Allal et al., 2017).The cooling system can use the sea water; it is known as the sea water cooling system. However, the sea water cooling system has a deficiency. The quality of sea water that be used can affect the process in the cooling system.

Some of microorganisms such as bacteria, diatoms, microalgae were responsible for microfouling, meanwhile the adhesion of larger organisms such as algae, mussels and barnacles can cause macrofouling (Cao et al., 2011). The sea water pollution can cause increasing in the population of some microorganisms. The microorganisms that cause microbiofouling also grow well in a contaminated environment. On the basis of the study by Azam and Malfatti (2007), 1 µL of surface seawater may consist of 10,000 viruses; 1,000 bacteria; 110 cyanobacteria; 10 eukaryotic algae and 10 protists that can affect the adhesion to produce the biofouling. Diatoms can attach directly to clean surfaces such as stainless steel and glass just after a few hours of immersion (Cooksey and Wigglesworth-Cooksey, 1995). The biofouling, known as biological corrosion, causes corrosion in the marine environment. Biological

corrosion occurs in the metal material. One method to prevent the biological corrosion is ICAF (Impressed Current Anti-Fouling). The ICAF is a new corrosion preventing system that use a low electric current. The ICAF can cause the decay of some corrosive organisms. However, the effectiveness of this tool in inhibiting various types of bio-fouling has not been proven. The research used a simple power electric system and two materials as cathodes and cathode for determining the effectiveness of the system. The ion from anodes can be produced continuously to reduce the population of microorganisms. The designed ICAF system reactor uses direct current (DC), the cathode uses steel material commonly used in marine buildings, as well as a copper (Cu) plate as an anode.

On the basis of the previous study, a reduction in the population of *Pseudomonas fluorescens* was 98.5%. at 0.1 A electric current and 3 min of operating time (Pratikno and Titah, 2016). The reduction in the population of *Pseudomonas fluorescens* occurred due to the presence of the Cu ions that were released from the anode into the saline solution in the ICAF system. The Cu ions have the ability to kill microorganisms, including the *Pseudomonas fluorescens*. The Cu toxicity of the *Pseudomonas fluorescens* was indicated by the LC50 value of 16.8 mg/L (Botsford, 1998). Meanwhile, the percentage reduction in the population of *Vibrio algynoliticus* and *Escherichia coli* reached 99.4% and 90% (Pratikno et al., 2019a; Pratikno and Titah, 2017). The concentration of the Cu ion during the operation of the ICAF system reached 17.85 ± 0.01 mg/L to 20.9 ± 0.03 mg/L. It indicated that a simple ICAF system can reduce the population of *Pseudomonas fluorescens, Vibrio algynoliticus* and *Escherichia coli* with different variations of operating time and electric currents. According to Pratikno et al. (2019b), the operation of ICAF on the *Chrorella vulgaris* algae showed that the algae population could be reduced to 99.98% at a current of 1 A, a duration of 10 min and a concentration of Cu (17.9 ± 0.07 mg/L). Meanwhile, the smallest decreasing reached 97.57% at electric current of 0.3 A, the duration of 5 min and the concentration of Cu (15.52 ± 0.25 mg/L). This showed that the Cu ions that formed during the operation of the ICAF system can reduce the population of *Chlorella vulgaris*.

Isochrysis galbana was a species of Haptophyta with a species of the *Isochrysis* genus. This microalgae species was an extraordinary food for shellfish larvae (Anonymous, 2016). On the other hand, *Isochrysis galbana* can cause biofouling. The other species of microalgae that can cause biofouling include *Platymonas subcordiformis* and *Navicula*, *Barnacle nauplii* (Dongdong et al., 2018). *Botryococcus* sp was a green pyramid-shaped planktonic microalgae that was potentially very important in the field of biotechnology. The colonies that were united by a lipid biofilm matrix can be found in lakes and oligotrophic estuaries of tropical or tropical climates, and it can bloom when the dissolved inorganic phosphorus levels increase. This species was famous for its ability to produce high amounts of hydrocarbons, especially oil in the form of Triterpenes, which were usually around 30–40% of their dry weight (Metzger and Largeau, 2005). *Botryococcus* sp has a relatively thick cell wall that was accumulated from the previous cellular divisions and making the extraction of cytoplasmic components rather difficult when compared to other green algae species. The majority of the useful hydrocarbon oil was outside the cell of *Botryococcus* sp (Wolf et al., 1985). *Botryococcus* sp has the ability to form biofilms (Zhang et al., 2018). The purpose of the research was to determine of reduction of the microalgae i.e. *Isochrysis galbana* and *Botryococcus* sp during the operation of a simple ICAF system.

MATERIALS AND METHODS

Preparation of cathode and anode

Steel material was applied in the field of marine buildings or ships. This material was be used as cathode with size of 15 x 15 x 1 cm. Copper (Cu) was applied as anode with similar cathode dimension.

Propagation of microalgae

The microalgae species of *Isochrysis galbana* and *Botryococcus sp* were taken from *Balai Perikanan Budidaya Air Payau* (BPBAP) in Probolinggo. The propagation of *Isochrysis galbana* and *Botryococcus sp* was carried out using sterile artificial saline water. The salinity of sterile artificial saline water was 35‰, thus it was comparable with the salinity of the original seawater. The growth of *Isochrysis galbana* and

Botryococcus sp was conducted at laboratory of Environmental Remediation in ITS campus. The intensity of light was 6000 to 8000 Lux during the propagation process and the duration was around 20 days. The ratio of sterile artificial saline water, Walne media and vitamin for *Botryococcus sp* growing were 700 mL : 1 mL : 1 mL). Afterwards, the 30% (v/v) of *Botryococcus sp* stock were placed in that media. Meanwhile, the growth media for *Isochrysis galbana* were 700 mL of sterile artificial saline water, 1 mL of diatom fertilizer, 1 mL of vitamin, 1 mL of silicate, and 300 mL of *Isochrysis galbana* stock. All propagation reactors were stirred using a small mixer to homogenize the culture of microalgae during the growth process. Figure 1 described the propagation of microalgae.

Operation of simple ICAF system

The design of the simple ICAF system was carried out based the earlier research (Pratikno et al. 2019a,b; Pratikno and Titah, 2017, Pratikno and Titah, 2016). The operation of the simple ICAF system was conducted using a plastic reactor with the dimensions of 18 x 10 x 13 cm. The distance of cathode and anode was 10 cm. The volume of sterile artificial saline water was 1500 mL and the culture addition of *Isochrysis galbana*amounted to 5%. The similar stage was applied for reduction of *Botryococcus sp*. Microalgae of *Botryococcus sp* culture was added in 1500 mL of sterile artificial saline water with 35‰ of salinity. Figure 2(a) described the operation of the simple ICAF system in the *Isochrysis galbana culture* which was carried out with variations in electrical wear and test time. Figure 2 (b) showed the operation of the simple ICAF system on the *Botryococcus sp* reduction.

The operation of ICAF system was carried out on the time variation (5, 7 and 10 min) and electrical current (0.1, 0.3, and 0.5 Ampere) based on the earlier study for bacteria. The determination of the number of cells of *Isochrysis galbana* and *Botryococcus sp* was conducted using a Neubauer improved Hemocytomete method based on Perez (2006). The concentration of the Cu ions was analysed using Atomic Absorption Spectrophotometer (AAS).

Determination of parameters

A Neubauer improved Hemocytometer was calculated based on magnification microscope of 100x. The equation was used to measure the number of cells in a predetermined room.

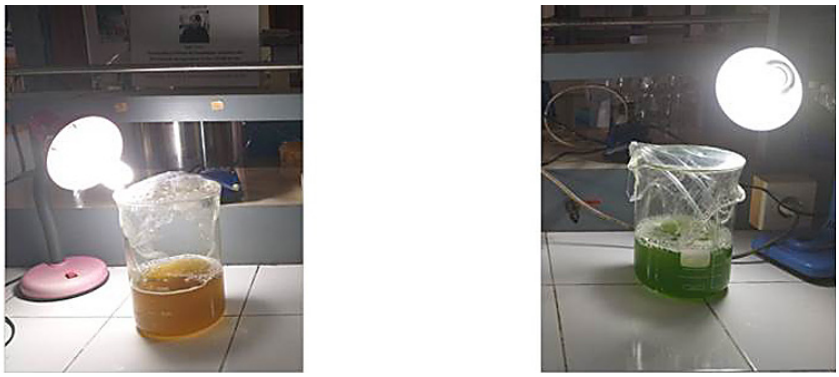

Figure 1. (a) Propagation of *Isochrysis galbana* and (b) *Botryococcus sp* culture

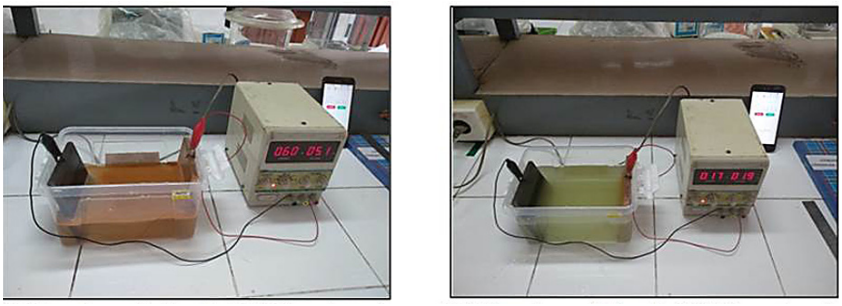

Figure 2. Operation of the simple ICAF system on *Isochrysis galbana* and *Botryococcus sp* reduction

$$\text{Average cells} = \frac{\text{cell visible}}{5 \text{ squares}} \quad (1)$$

$$\text{Dillution Factor} = \frac{\text{Final volume after added diluent}}{\text{Diluted inoculum volume}} \quad (2)$$

$$\text{Cell density}\left(\text{cell/mL}\right) = \text{Average cells x Dillution Factor x } 10^4 \quad (3)$$

The method to determine the Cu concentration was similar with the previous study (Pratikno et al. 2019a,b; Pratikno and Titah, 2017, Pratikno and Titah, 2016). The saline water samples were taken from the simple ICAF system reactor. Then, all samples were filtered using paper filter and analyzed using Atomic Absorption Spectrophotometer (AAS) model Z-2000 Series Hitachi (Japan) at Laboratory of Energy, LPPM ITS.

RESULTS AND DISCUSSION

Population reduction of *Isochrysis galbana*

Figure 3 showed the growth rate of *Isochrysis galbana* on sterile artificial seawater media with salinity of 35‰. This media artificial seawater media with salinity of 35‰ was added with media for diatoms, silicate and vitamins. According to Alkhamis and Qin (2013), *Isochrysis galbana* can live in a wide range of salinity, i.e. 10‰ to 60‰, but the optimal growth was reached at 35‰ of salinity. Other studies reported that *Isochrysis galbana* can live at the salinity of 5‰ to 60‰ (Kaplan et al., 1986).

Liang and Utting (1980) reported that the optimal salinity in artificial seawater for the growth of *Isochrysis galbana* was in the range of 15–25 ‰. Another study by Fabregas et al., (1984) reported that there was a close relationship between salinity and nutrients for the growth of *Isochrysis galbana* and the optimum growth of *Isochrysis galbana* occurred at the salinity of 15–35‰. The growth of *Isochrysis galbana* could be slow due to the increase in the salinity concentrations, occurring at 31–36‰ (Liang and Utting, 1980). Aeration was carried out during the microalgae culturing process. The effects did not known between oxygen concentration, and the specific growth rate of microalgae with the addition of dissolved oxygen.

On the basis of the growth rate curve of *Isochrysis galbana*, the middle of log phase was reached at the age of 8 days. The age of *Isochrysis galbana* used in simple ICAF system was 8 days, according to the *Isochrysis galbana* growth rate curve.

Figure 4 showed the temperature during the simple ICAF system process on *Isochrysis galbana.* The temperature during testing was around 27 °C. According to Kaplan et al. (1986), *Isochrysis galbana* can grow well at temperatures above 19°C. Another factor besides micronutrients and vitamins that affects the algae growth is pH . Figure 5 shows the pH during the simple ICAF system process in *Isochrysis galbana*. The pH value during the test amounted to 8. The *Isochrysis galbana's* response to pH variations was investigated (Grima et al., 1992). Grima et al., (1992) reported that the growth of *Isochrysis galbana* would be stunted if the pH was below 8. The growth of *Isochrysis galbana* also started to be stunted if the pH was below 9.

Figure 6 showed the decreasing in the number of *Isochrysis galbana* cells during the operation of a simple ICAF system. The higher the value of the electric current and the longer the

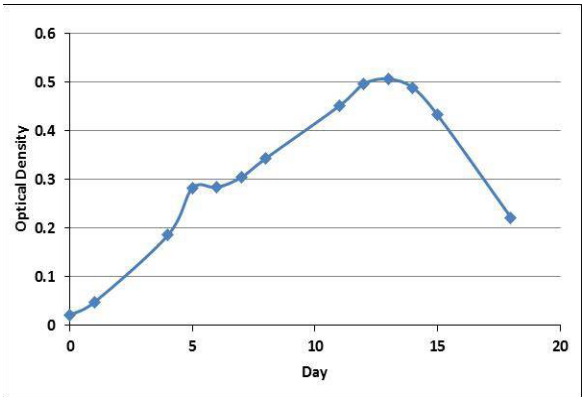

Figure 3. Growth rate curve of *Isochrysis galbana*

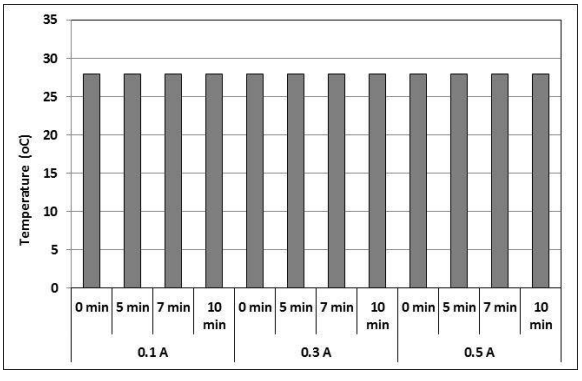

Figure 4. Temperature during the operation of the simple ICAF system

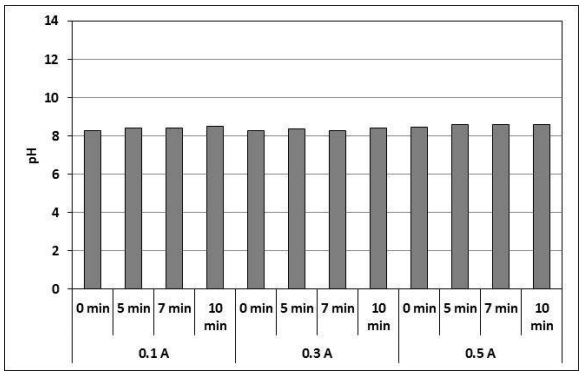

Figure 5. pH during the operation of the simple ICAF system

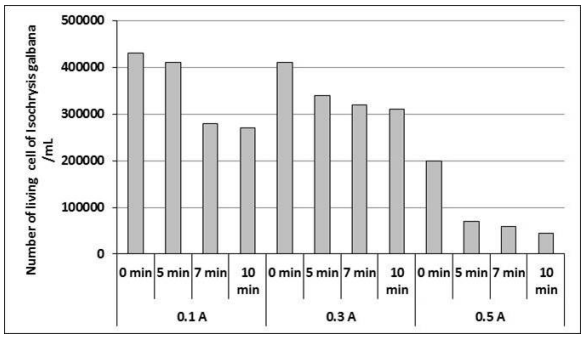

Figure 6. Number of cells of *Isochrysis galbana* during the operation of the simple ICAF system

time, the greater the decreasing of *Isochrysis galbana* number cells. The percentage decrease in the number of *Isochrysis galbana* cells reached 77.5% (Figure 7).

According to Foulkes (2000), the ions of Zn, Cu, Ni, Co, Mo, and Cr are essential trace elements for life. However, high concentrations of Cu will be toxic to organisms such as *Isochrysis galbana*. According to Liu et al., (2011), the effective concentration (EC50) value of Cu in *Isochrysis galbana* was 31.4 µmol/L equivalent to 7.9 mg/L. According to Miazek et al. (2015), *Isochrysis galbana* has a 50% of growth inhibition on the Cu concentration of 0.01–0.018 mg/L with a time of 72 h. The toxic effects of some heavy metals in *Isochrysis galbana* were as follows: Cu> Pb> Cd (Liu et al., 2011), so that *Isochrysis galbana* can be used as an indicator for toxicological bioassays.

Figure 8 showed the concentration of the Cu ions during operation of a simple ICAF system on *Isochrysis galbana*. The highest Cu ion concentration reached 2.5 ± 0.08 mg/L at the highest electric current (0.5 A) and a operation time of 10 min.

Population reduction of *Botryococcus sp*

Figure 9 showed the growth rate curve of *Botryococcus* sp in artificial seawater at the salinity of 35‰. The growth of Botryococcus sp reached a half of the log phase at 8 or 9 days. Gani et al., (2017) reported that the middle of exponential phase of *Botryococcus* sp was 12 days. On the basis of these data, *Botryococcus* sp with a 9 day age was chosen for use in the operation of a simple ICAF system with a variety of electric currents and operating times.

Figure 10 showed the temperature during the simple ICAF system process in *Botryococcus* sp. The temperature during the testing was 27–28°C. Lupi et al. (1991) reported that *Botryococcus braunii* cannot grow at temperature above 32°C, and there were no previous studies which investigated the effect of temperatures below 18°C for the growth of *Botryococcus braunii*. According to Lee et al., (2015), the growth of *Botryococcus braunii* is optimum at a temperature of 25°C. Figure 11 described the pH value during the simple ICAF system process in *Botryococcus* sp. The pH value during the test was 8, or it was a normal pH.

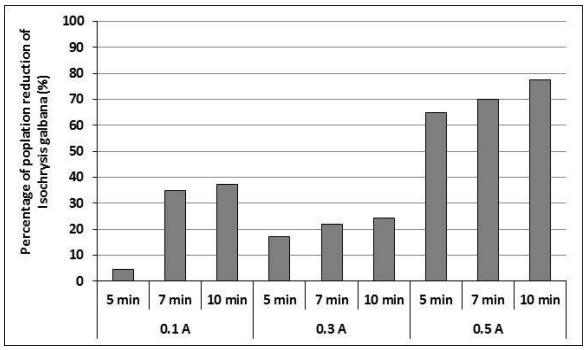

Figure 7. Reduction of *Isochrysis galbana* cells during the operation of the simple ICAF system

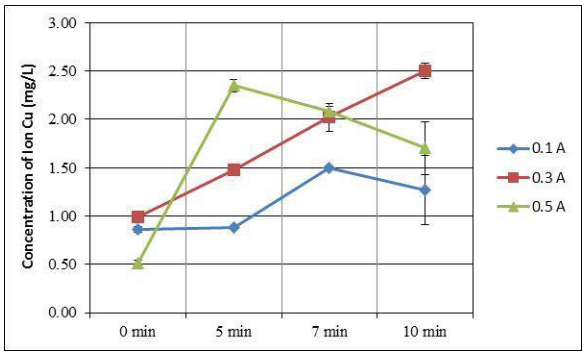

Figure 8. Concentration of Cu during the operation of the simple ICAF system

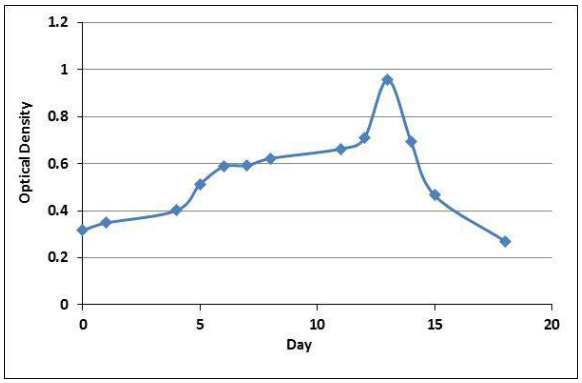

Figure 9. Growth of *Botryococcus* sp Curve

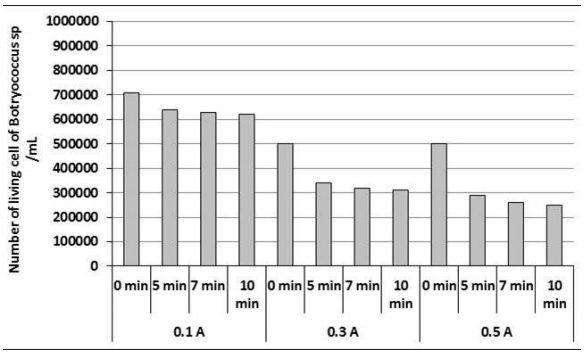

Figure 12. Number of *Botryococcus* sp cells during the operation of the simple ICAF system

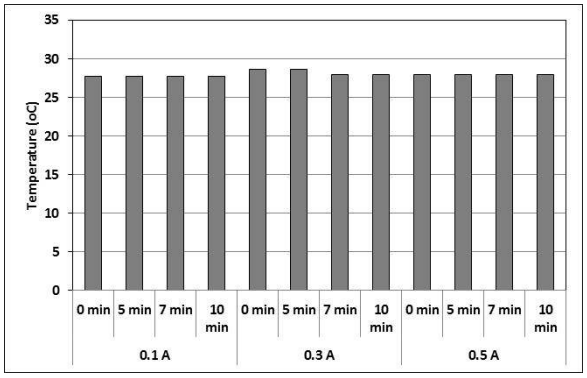

Figure 10. Temperature on *Botryococcus* sp during the operation of the simple ICAF system

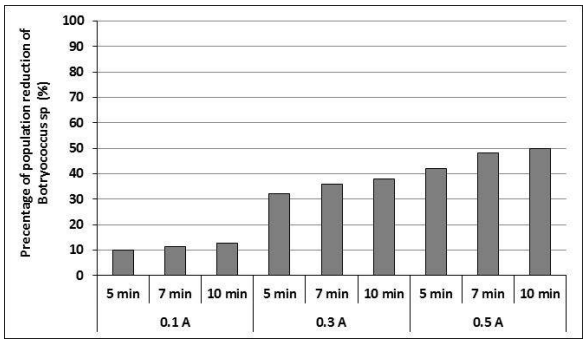

Figure 13. Reduction in the cell number of *Botryococcus* sp during the operation of the simple ICAF system

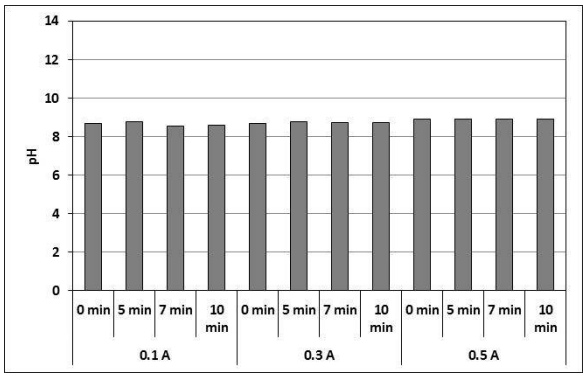

Figure 11. pH on *Botryococcus* sp during the operation of the simple ICAF system

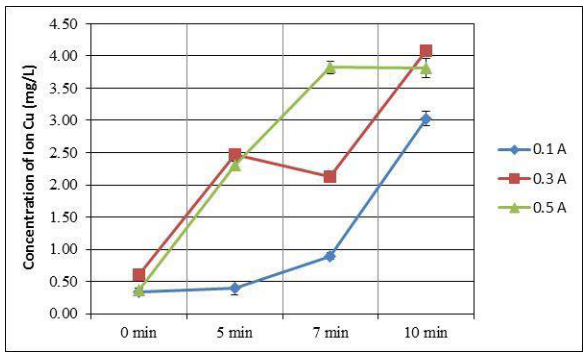

Figure 14. Concentration of Cu during the operation of thr simple ICAF system on *Botryococcus* sp

Botryococcus microorganisms showed a variety of pH adaptability (Dayananda et al., 2017).

The population of Botryococcus sp in the operation of a simple ICAF system was shown in Figure 12. On the basis of this graph, the greater the value of the electric current and the longer the operation time, the lower the *Botryococcus* sp population. Figure 13 showed the percentage reduction in *Botryococcus* sp during the operation of the simple ICAF system. The highest percentage reduction in *Botryococcus* sp population reached 50% in an electric current of 0.5 A and 10 min of operating time.

However, high concentrations of Cu can be toxic to *Botryococcus* sp. Some heavy metals i.e. Cu, Ni and Fe were metals that were usually observed to have toxic properties on algae, if the concentration of those heavy metals were above the trace element concentration limit. Cu was one of the most toxic metals (Juneja et al. 2013).

Toxic metals can inhibit carbon fixation and delay the absorption of nutrients in algae (Rai and Mallick, 1993). Figure 14 showed the concentration of the Cu ions during the operation of the simple ICAF system on *Botryococcus* sp. The highest Cu ion concentration reached 4.08 ± 0.01 mg/L.

CONCLUSIONS

The highest decrease in the cell number of *Isochrysis galbana* and *Botryococcus* sp reached 77.5% and 50% at the highest electric current (0.5 A) and a operation time of 10 min, respectively. The highest concentration of Cu reached 4.08 ± mg/L. In conclusion, the Cu ion that was produced during the operation of the simple ICAF system can reduce the cell number of *Isochrysis galbana* and *Botryococcus* sp.

Acknowledgements

The author would like to thank Kemenristek-DIKTI (Ministry of Research, Technology and Higher Education, Republic of Indonesia) Perguruan through scheme of Penelitian Dasar Unggulan Perguruan Tinggi – second year research, PTUPT 2019 for funding this research with No of contract, 5/E1/KP.PTNBH/2019.

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