Impressed Current Anti Fouling (ICAF) to Reduce Population of *Chlorella Vulgaris* Cause Bio Corrosion on AH36 Steel in Marine Environment

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Abstract. Corrosion can cause damage to steel. One of the main causes of corrosion is biofouling. The *Impressed Current Anti Fouling* (ICAF) method is one way to prevent the microfouling. The purpose of the study was to calculate reduction of *Chlorella Vulgaris* population using a simple ICAF system. The simple ICAF reactor was operated with variation of electric current (0.3, 0.5 and 1 A) and duration time (5, 7 and 10 min). Steel of AH36 has a role as a cathode, meanwhile pure copper (Cu) was an anode. The cell number of *Chlorella Vulgaris* was determined using haemacytometer method. The concentration of Cu was determined using *Atomic Absorption Spectrophotometers* (AAS). Based on the results, the simple ICAF system showed the decreasing of *Chlorella Vulgaris* cell number with the highest percentage of 99.98% at electrical current of 1 A, duration time of 10 min and concentration of Cu (17.9 ± 0.07 mg/L). Meanwhile, the lowest of the cell number reduction was 97.57% at electrical current of 0.3 A, duration time of 5 min and concentration of Cu (15.52 ± 0.25 mg/L). In conclusion, ion Cu that was produced during operation simple ICAF system can reduce *Chlorella Vulgaris* population.

Keywords: **Anode; biofouling; cathode; duration time; electrical current; microalga; simple ICAF system.**

1 Introduction

Nowadays, the development of various technologies, especially in the field of maritime both onshore and offshore increase rapidly. The increasing demand for energy availability makes the demand for maritime natural resources increase too, such as oil and natural gas industries. However, the use of technology in the offshore field also has some shortcomings and constraints. The polemic problems are the incidence of damage to parts that were caused by corrosion of seawater. The rate of corrosion that occurs in the marine environment is relatively very fast. This condition occurred due to the sharing of substances dissolved in seawater which were capable of dissolving other substances. For example, such as dissolved gases, organic compounds of living organisms, and inorganic salts have a greater concentration than other liquids so that can cause corrosion [1]. The construction of steel that placed at marine environment as piles can also be corroded due to the presence of microbes that can increase oxygen concentration [2].

Many causes of corrosion, one of the main causes of corrosion in materials was bio-fouling. Bio-fouling was an accumulation of undesirable biological matter and occurred on the surface of a material. This condition was caused either by macroorganism (macrofouling) and the result of biofilm production which was usually caused by several microorganisms such as sulphate reducing bacteria, sulfur-sulphide oxidizing bacteria, manganese iron oxide bacteria, fungi, microalgae, and protozoa [3]. The existence of biofouling especially in the hull was very detrimental because it can increase the ship resistance that culminates in the high fuel usage, maintenance cost, and the process of parameter interference [4]. Bio-fouling can also occur at onshore and offshore building.

Based on our earlier research, ICAF system have ability to decrease the number of cells of bacteria *Pseudomonas fluorescens* with reached 98.5% - 99.9% [5]. The percentages of population reduction on *Vibrio alginolyticus* were 87.3% - 99.4%. The measured concentration of Cu^{2+} ions during the operation of the simple ICAF system reached 4.3 mg/L to 18.3 mg/L [6].

The purpose of the study was to calculate reduction of microalgae (*Chlorella Vulgaris*) population using a simple ICAF system with high-strength low-alloy (HSLA) AH36 steel was as a cathode and copper was as an anode. Copper was chosen due to it was known as an inhibitor for biofouling [7].

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2 Materials and Methods

2.1. Materials Preparation

The material of HSLA AH36 steel was be used as cathode with size 15 x 15 x 1 cm. HSLA material was applied in the field of marine buildings or ships. The metal of Cuprum (Cu) was applied as anode with similar dimension of cathode i.e. 15 x 15 x 1 cm.

2.2 Simple ICAF System Reactor

The simple ICAF system used the DC current. The reason to use the DC current because of the anode and cathode supply must be differentiated between positive and negative current sources. The design of simple ICAF system was conducted based on our earlier research [5,6]. Figure 1 showed the running of simple ICAF system on *Chlorella Vulgaris* reduction.

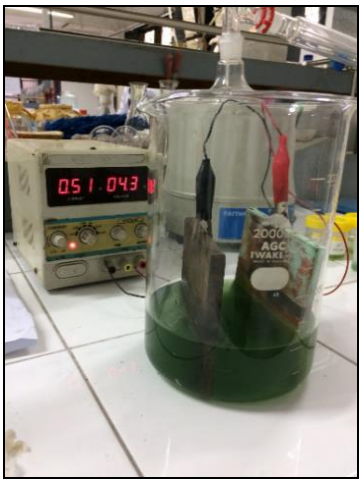

Fig. 1. The running of simple ICAF system.

2.3 Microalgae Preparation

Chlorella Vulgaris was taken from *Balai Perikanan Budidaya Air Payau* (BPBAP) in Probolinggo. After that, the growth stage of *Chlorella Vulgaris* was conducted using filtered seawater with salinity of 35‰ at laboratory of Environmental Remediation in ITS campus. The flux of light during growing process was 6000 – 8000 Lux and the duration was 10 days [8]. The sterile of filtered seawater was mixed with vitamins (ratio water: vitamin = 1 L: 1 mL) and it was aerated using small aerator to agitate the culture of *Chlorella Vulgaris*.

2.4 Running of Simple ICAF System Reactor

The operation of the ICAF system were carried out on the time variation (5, 7 and 10 min) and electrical current (0.1, 0.3, and 0.5 Ampere) based on earlier our study for bacteria. The main parameter was the population of *Chlorella Vulgaris* at initial time and after operation of ICAF system. The determination of number of cells of *Chlorella Vulgaris* was conducted using a neubauer improved Hemocytomete method based on Perez [9]. The ion Cu concentration was analyzed using Atomic Absorption Spectrophotometer (AAS).

2.5 Determination of Parameters

A neubauer improved Hemocytometer was observed using magnification microscope of 100x. The formulas were used to calculate the number of cells that begin by counting the number of cells in a predetermined room.

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

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

$$\text{Cell density}\,(\text{cell/mL}) = \text{Average cells x Dillutio n Factor x } 10^4 \quad (3)$$

The method determine of Cu concentration was similar to our previous study [5,6]. All samples were taken from the saline solution during simple ICAF system was operating. After that, all samples were filtered using paper filter and were analyzed using Atomic Absorption Spectrophotometer (AAS) model Z-2000 Series Hitachi (Japan) at Laboratory of Energy, LPPM ITS. The calculation of cell number reduction percentage was conducted following this formula.

$$\text{Percentage of cell number reduction} = \left(\frac{N1 - N2}{N1}\right)x100\% \quad (4)$$

With explanation,
N1 = initial cell number
N2 = cell number after t time

2.6 Statistical Analysis

The experimental data of Cu concentration during operation of simple ICAF system reactor were subjected to an analysis of variance (ANOVA) using SPSS Statistics for Windows version 21.0 (SPSS, Inc., Chicago, IL). Statistical significance was defined as $p < 0.05$.

3 Results and Discussion

Figure 2 depicted the growth curve of *Chlorella Vulgaris*. Based on this graph, the end of exponential phase for difference of *Chlorella Vulgaris* density was on day 4. The half of exponential phase showed the number cell around 60 x 10^5 cells/mL. The monitoring parameters during *Chlorella Vulgaris* growth were also measured. Figure 3 showed the temperature during *Chlorella Vulgaris* growth. The temperature reached 30 -

32.5 ºC. According to [10,11,12], the optimal temperature for *Chlorella Vulgaris* growth was around 30°C. The maximum biomass productivity reached in this condition. Another research reported that optimum temperature for growing occurred between 30-35 ° C after 7 days of culture [13]. pH during *Chlorella Vulgaris* growth reached 6.8 - 8.5. Based on Khalil et al. (2010) [14], *Chlorella Vulgaris* can grow in a large range of pH (4-10) and most biomass productivity occurred in the alkaline condition (pH = 9 and 10).

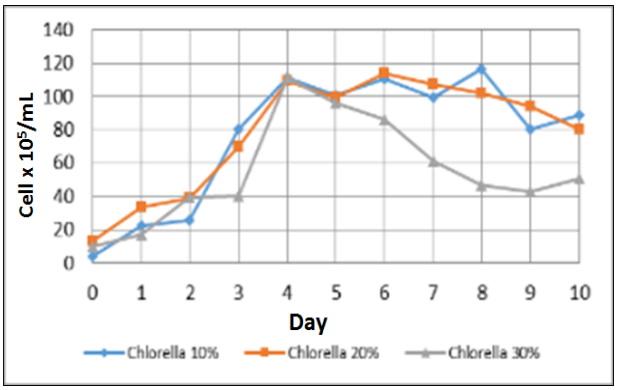

Fig. 2. *Chlorella Vulgaris* Growth Curve

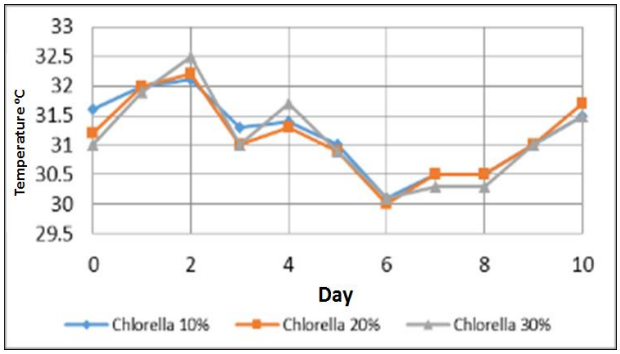

Fig. 3. The Temperature During *Chlorella Vulgaris* Growth

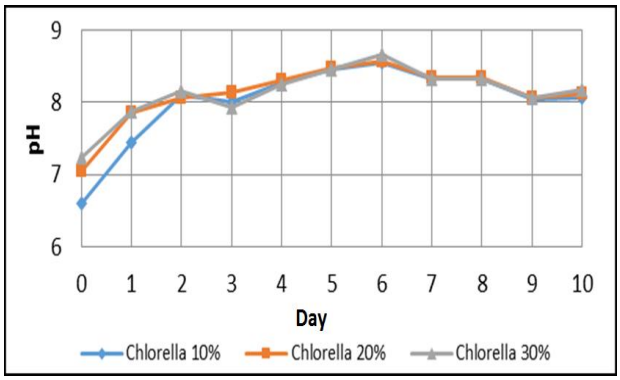

Fig. 4. The pH During *Chlorella Vulgaris* Growth

Table 1 showed the number of cells of *Chlorella Vulgaris* living cells before and after running of simple ICAF system. Based on Table 1, the decreasing of number living cells occurred at all electrical current variation. The electric current increased so that the dead cell of *Chlorella Vulgaris* increased. The trend was as well as in the time variation. The percentages of number cell of *Chlorella Vulgaris* reduction was calculated using equation (4). Based on Figure 4, the value range of reduction percentages were 97% - 99%. The highest of percentage of number cell of *Chlorella Vulgaris* reduction reached 99.98% at electrical current of 1 A and time of 10 min. However, the other variation of electrical current also showed the high of reduction percentages. Based on our previous study (Pratikno and Titah, 2018) [5], the range of *Pseudomonas fluorescens* population decreasing was 98.5% to 99.9% and it reached 87.3% - 99.4% for *Vibrio alginolyticus* population reduction [6].

Table 1. Number of *Chorella Vulgaris* Living Cells

Electrical Current	Number of Living Cells x 10^3			
	0 min	5 min	7 min	10 min
0.3 A	8550	208	24	10
0.5 A	8550	168	20	8
1 A	8550	14	6	2

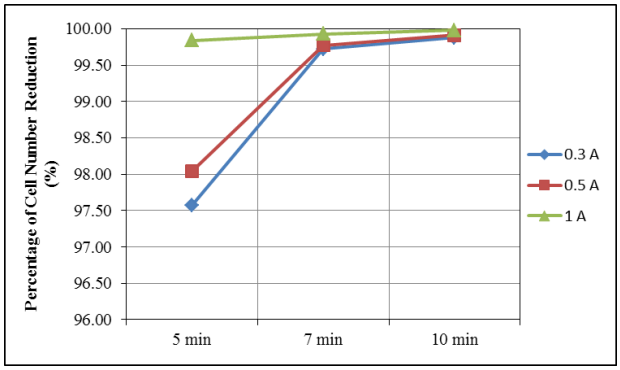

Fig. 5. The percentages of *Chlorella Vulgaris* Population during Simple ICAF System Operation

Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mo^{2+}, and perhaps Cr^{4+}, $^{6+}$ are essential in trace amounts for some living systems, but at higher concentration, they may become very toxic [15]. Low concentration of Cu^{2+} promoted the growth of some algae, but higher concentrations retarded the growth, caused reduction in cell division [16,17,18], and inhibited the synthesis of chlorophylls in *Chlorella Vulgaris* [19]. It may bind to chloroplast membranes and other cell protein causing degradation of chlorophylls molecules [20].

Regaldo et al. (2013) [21] reported that the exposure of Cu can reduce population of *Chlorella Vulgaris* up to 81.97% and 92.53% with variation time of exposure. HuiLing et al. (2012) [22] reported that 5 µmol/L or similar with 1.16 mg/L treatments of Cu, Cr, Zn, Cd and Pb can inhibit the growth of *Chlorella Vulgaris* significantly, and the effect became weaker with an increase in exposure duration. According to Kondzior and Butarewicz (2018) [23] reported that the Cu concentration of 0.15 mg/L after 7 days of incubation can reduce chlorophyll a by 63%, chlorophyll b by 58% and carotenoids by 60% on *Chlorella Vulgaris* species. The concentration of ion Cu during operation of simple ICAF system was shown in Figure 6. The measured concentration of Cu ions during the operation of the

simple ICAF reached 15.52 ± 0.25 mg/L to 20.80 ± 0.17 mg/L. Based on the Figure, the concentration of Cu ion increased with time increasing. It indicated that the increasing ion Cu production affected with the length of duration time and electrical current.

Based on statistical analysis using ANOVA, the difference of Cu ion concentration between electrical current and time variation was no significant ($p > 0.05$). It is similar with of number cells reduction percentages. It also showed no significant ($p > 0.05$) due to the percentages showed high reduction at all variation.

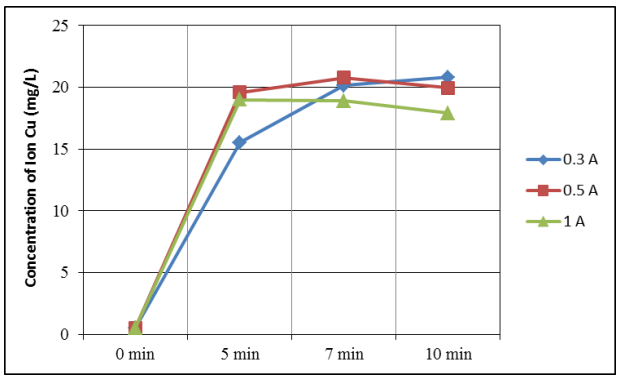

Fig. 6. The Concentration of Cu during Simple ICAF System Operation

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