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QUALITY OF *Tetraselmis* sp. CULTURED BY TIGER SHRIMP AQUACULTURE WASTE AS FERTILIZER

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ABSTRACT

Tiger shrimp aquaculture waste still contained amount of nutrients needed by phytoplankton for its growth. Among nutrients was resulted from intensive aquaculture still can be used by phytoplankton. Phytoplankton used nitrates and phosphates for its growth and development. Phytoplankton requires nitrate concentration in 0,9-3,5 mg/ and phosphate in 0,27-5,51 mg/l. Utilization of tiger shrimp aquaculture waste for Tetraselmis sp. quality can be seen using fluorescence microscope with the cell of Tetraselmis sp. had to stained by acridine orange before observed using fluorescence microscope. The concept of this staining when cells of Tetraselmis sp. radiated green colour showed that cells was alive whereas cells radiated orange colour showed that cells was dead or damaged. This matter caused living cells could reducted acridine orange pigment enzymatically until become green radiated cells, instead damaged cells showed orange colour. Research procedures included cultivating Tetraselmis sp. in various dosages waste of tiger shrimp aquaculture as fertilizer. These various dosages are 2 g/l, 4 g/l, 6 g/l and 8 g/l. Culture media for Tetraselmis sp. was saltwater. Sterile saltwater was put into jars about 1 L and later added wastewater to jars. Reading of protein expression helped using imageJ software that showed histogram graphics accompanied by mean. Mean resulted by graphics indicated protein could be expressed. Based on expression of fluorescence protein with 2 g/l wastewater dosage showed that amount of protein could be ecpressed was higher than other wastewater dosage by mean value from ImageJ.

Keywords: Fluorescence Microscope, Image J, Tiger Shrimp Wastewater, Tetraselmis sp. Cells

I. INTRODUCTION

Fisheries production in Indonesia has great potential which from catching and aquaculture. Gross Domestic Product (GDP) successfully reached IDR 177,8 from Ministry of Marine Affairs and Fisheries in year 2015. Global fish needs increasing regulary and to fulfill that not only boosted in fish catch but also aquaculture activities. One of the commodities has large market interest is shrimp, especially shrimp in brackish water (Amalia, 2022).

Tiger shrimp (*Penaeus monodon*) is one of the shrimp varieties has economic value and can be cultured in Indonesia besides vannamei shrimp and until now still be superior commodity both export and local. It causes increasing of shrimp production demand with intensive aquaculture. Intensive aquaculture with high density organisms and much artifial feed will affect enviromental problems such as nutrition enhancement (Evania *et al*., 2018). Nutrition enhancement can contaminate surrounding environment. Contaminated environment has negative impact for living organisms, therefore it requires handling method to reduce wastewater or reuse wastewater. Some studies reported that the phytoplankton can be grown using aquaculture wastewater instead of the expensive synthetic medium often used as it contains all the necessary nutrients required for it growth (Mtaki *et al.,* 2021). Among nutrients in wastewater from intensive aquaculture still can be used by phytoplankton. Phytoplankton used nitrates and phosphates for its growth and

development (Marsela *et al.,* 2021). Phytoplankton requires nitrate concentration in 0,9-3,5 mg/ (Suryadi *et al.*, 2017) and phosphate in 0,27-5,51 mg/l (Rumanti *et al*., 2014). Cell building and as a source of nutrients are the main function of nutrients that phytoplankton required and for phyplankton cultivation needed variety of organic compounds namely macro and micro nutrients (Suwoyo *et al.,* 2020).

Tremendeous efforts have been made to microalgae cultivation in wastewaters over the past few decades (Zhu *et al.,* 2016). Utilization of tiger shrimp aquaculture waste for *Tetraselmis* sp. quality can be seen using fluorescence microscope. Fluorescence microscope is multifunction tool to know about celluler processes. One of the basic requirements is fluorophore shows spectroscopy characteristic and specific sensitivity for cellular parameter changing (Schäferling, 2012). Determining cell viability with observation through fluorescence microscope by staining cells. In research applications and laboratory investigation, acridine orange (AO) is a well known inexpensive fluorescence dye. Furthermore, the AO dye has been mostly used for cell viability observation, cell cycle determination and cell physiology study (Khemthongcharoen, 2011). The fluorescence measurement was carried out with Acridine Orange (AO) as probe. AO has conjugated planar structure and can insert between two adjacent base pairs in a DNA helix to make the fluorescence intensity remarkably increased (Wang and Xu, 2013). Based on that examination, this research was carried out with observation microalgae cell quality through AO cell stained and observed using fluorescence microscope.

II. RESEARCH METHODS

2.1 Time and Place of Research

This research was conducted in November 2015 until July 2016 at Reproduction and Fish Breeding Laboratory and Biosciences Laboratory, Brawijaya University.

2.2 Materials and tools

2.2.1 Material and tools in Cultivation of *Tetraselmis* sp.

Material was needed in cultivation of *Tetraselmis* sp. consist of *Tetraselmis* sp. seeds, tiger shrimp aquaculture waste, Walne fertilizer, salt water, aquadest, vitamin and tissues.

Tools was needed in cultivation of *Tetraselmis* sp. consist of glass jars, measuring cylinders, Erlenmeyer of 500 ml, digital scales, aerators, aeration hoses, airstones, fluorescent lamp, microscope, cover glass, dropper pipette, cottons, haemocytometer, hand tally counter and aluminium foil.

2.2.2 Material and tools in Fluorescent Protein Analysists

Material was needed in Fluorescent Protein Analysists consist of *Tetraselmis* sp. seeds, Acridine orange, glycerol, glacial alcohol acetic acid, and ethanol.

Tools was needed in Fluorescent Protein Analysists consist of Fluorescence Microscope, high pressure mercury lamp HBO 50, glass filters BG 12 dan BG 3, panchromatic fine-grain film NP 15 (15^0DIN) dan coolbox.

2.3 Research methods

Research procedures included cultivating *Tetraselmis* sp. in various dosages waste of tiger shrimp aquaculture as fertilizer. This various dosages are 2 g/l, 4 g/l, 6 g/l and 8 g/l. Culture media for *Tetraselmis* sp. was saltwater. Sterilization was carried out for saltwater before cultivation and cooled down until 24 hours. After 24 hours, sterile saltwater was put into jars about 1 L and later added wastewater to jars.

2.3.1 Experimental design

Experimental design of this research used completely randomized design with three repetitions for each dosage. Quality of *Tetraselmis* sp. was observed by FSX Fluorescence Microscope and analyzed using ImageJ application. Gene expressions approached were observed by value of mean which showed.

2.3.2 Data Interpretation

Data Interpretation in this research was fluorescence analysis using FSX Fluorescence Microscope with acridine orange staining. The concept of this staining when cells of *Tetraselmis* sp. radiated green colour showed that cells was alive whereas cells radiated orange colour showed that cells was dead or damaged. This matter caused living cells could reducted acridine orange pigment enzymatically until become green radiated cells, instead damaged cells showed orange colour (Hadioetomo, 1993).

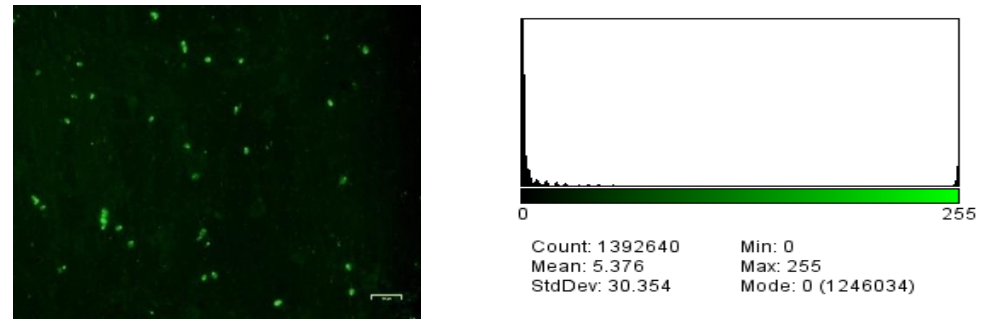
III. RESULTS AND DISCUSSION

3.1. Results

Influences of utilization tiger shrimp wastewater for quality of *Tetraselmis* sp. was observed once in every three days until cultivating in the ninth day. Quality of *Tetraselmis* sp. served in Figure 1. In the Figure 1 showed that the highest mean value was found in 8 g/l dosage of tiger shrimp wastewater. Mean value respectively as followed with 2 g/l dosage showed 5,376, 4 g/l dosage showed 7,940, 6 g/l dosage showed 5,376 and 8 g/l dosage showed 8,325.

Based on Fluorescent Protein, expressions, cultivation of *Tetraselmis* sp. during 6 days served in Figure 2. In the Figure 2 showed that the highest mean value was found in 2 g/l dosage of tiger shrimp wastewater. Mean value respectively as followed with 2 g/l dosage showed 14,333, 4 g/l dosage showed 3,015, 6 g/l dosage showed 11,712 and 8 g/l dosage showed 5,638.

Based on Fluorescent Protein, expressions, cultivation of *Tetraselmis* sp. during 9 days served in Figure 3. In the Figure 3 showed that the highest mean value was found in 4 g/l dosage of tiger shrimp wastewater. Mean value respectively as followed with 2 g/l dosage showed 7,409 4 g/l dosage showed 8,252, 6 g/l dosage showed 0,832 and 8 g/l dosage showed 5,333.



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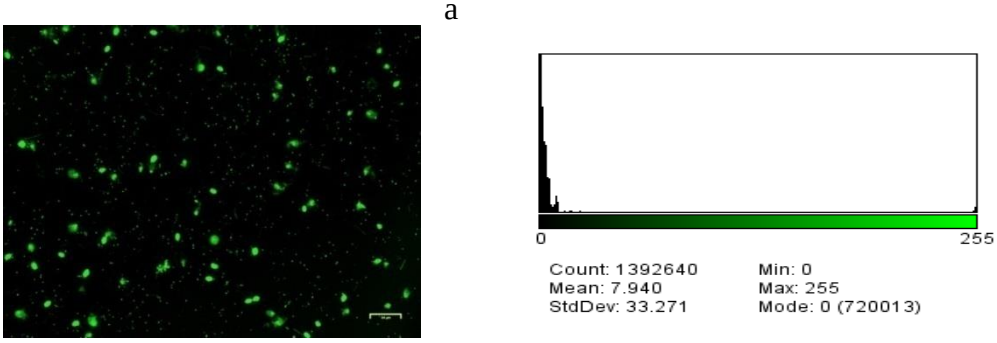

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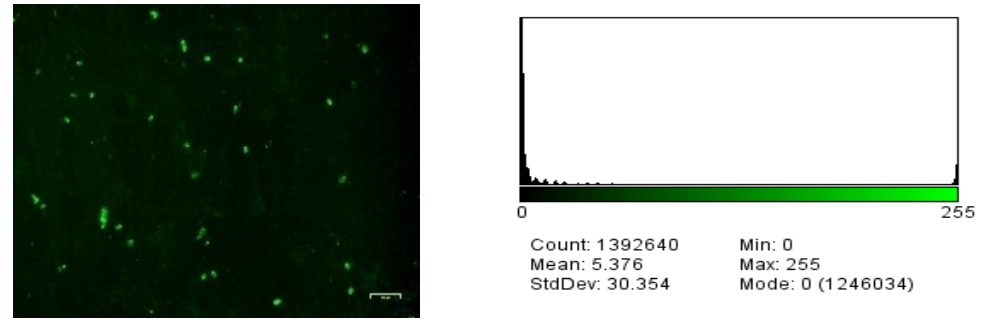

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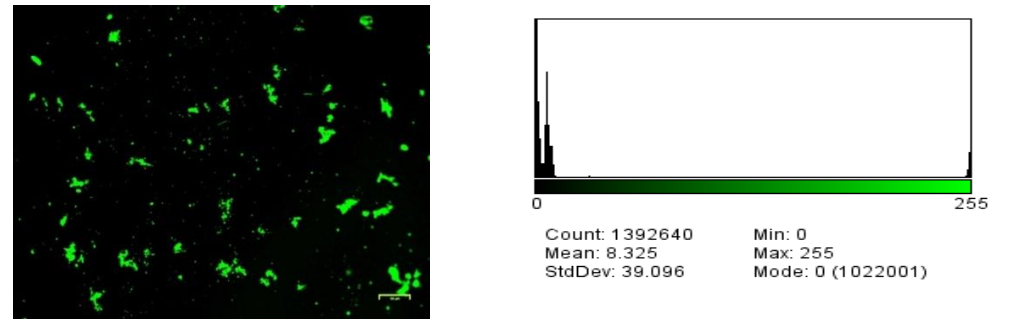

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Figure I. Cultivation of *Tetraselmis* sp. during 3 days. a. Dosage of Tiger Shrimp Wastewater was 2 g/l; b. Dosage of Tiger Shrimp Wastewater was 4 g/l; c. Dosage of Tiger Shrimp Wastewater was 6 g/l; d. Dosage of Tiger Shrimp Wastewater was 8 g/l

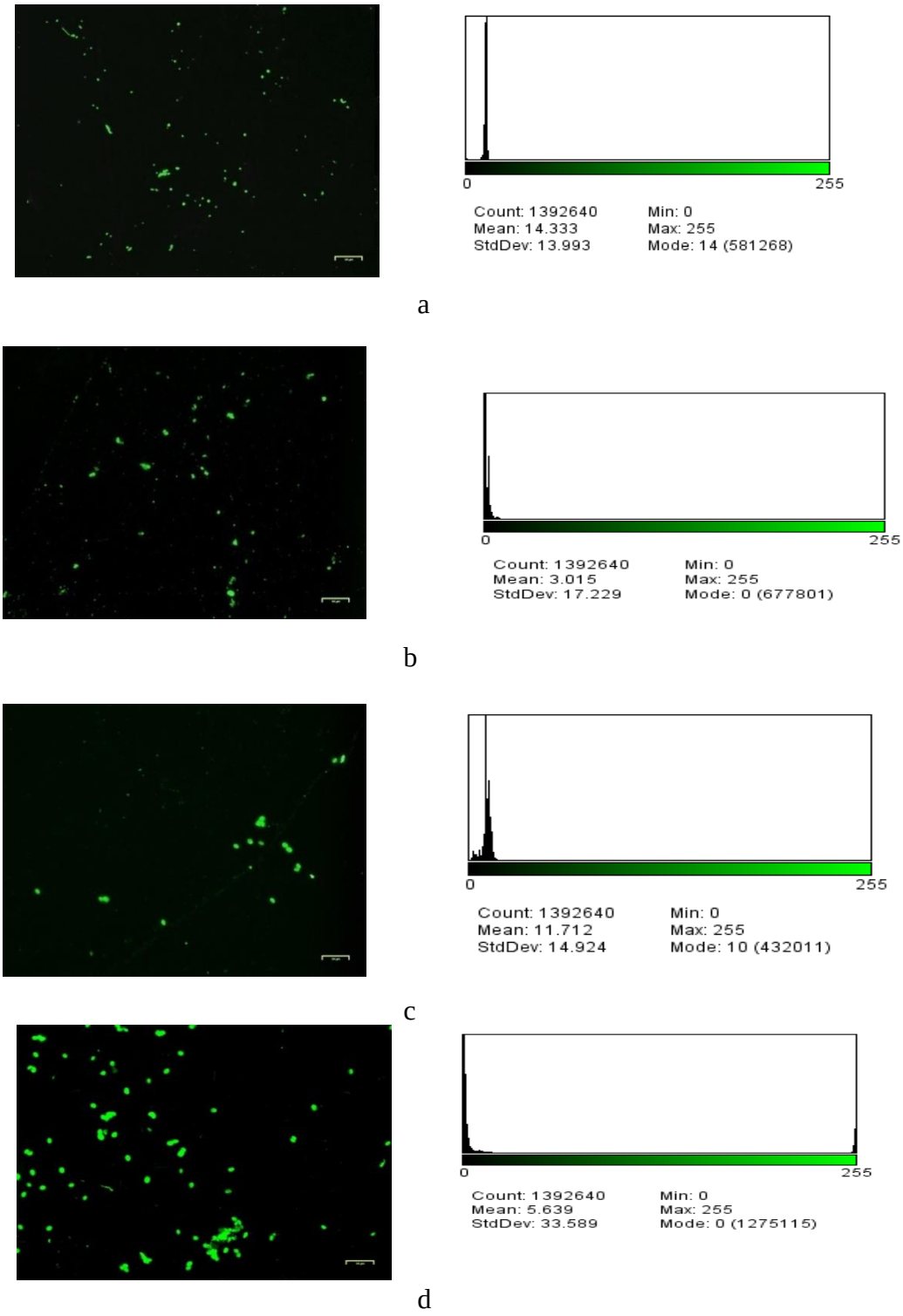
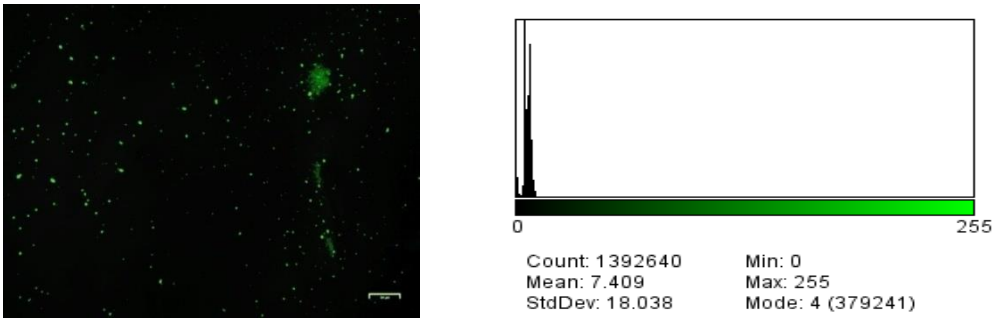


Figure 2. Cultivation of *Tetraselmis* sp. during 6 days. a. Dosage of Tiger Shrimp Wastewater was 2 g/l; b. Dosage of Tiger Shrimp Wastewater was 4 g/l; c. Dosage of Tiger Shrimp Wastewater was 6 g/l; d. Dosage of Tiger Shrimp Wastewater was 8 g/l



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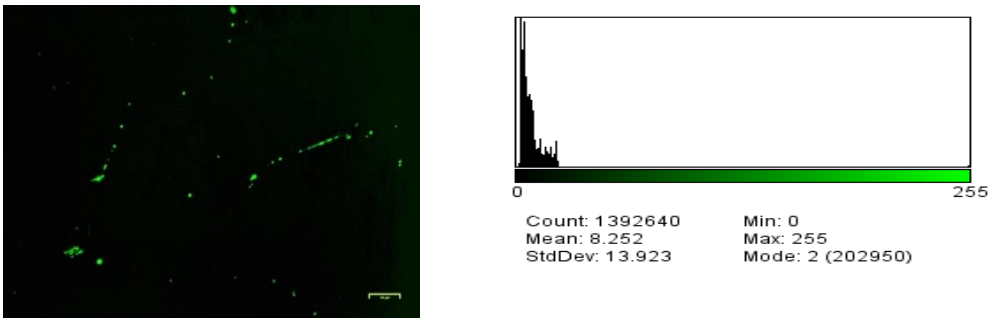

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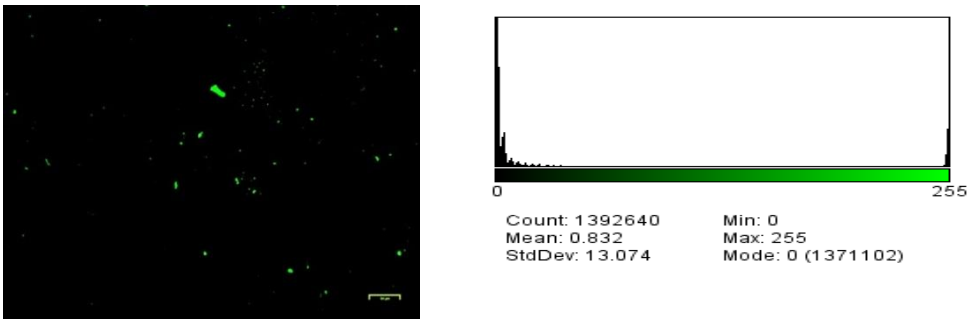

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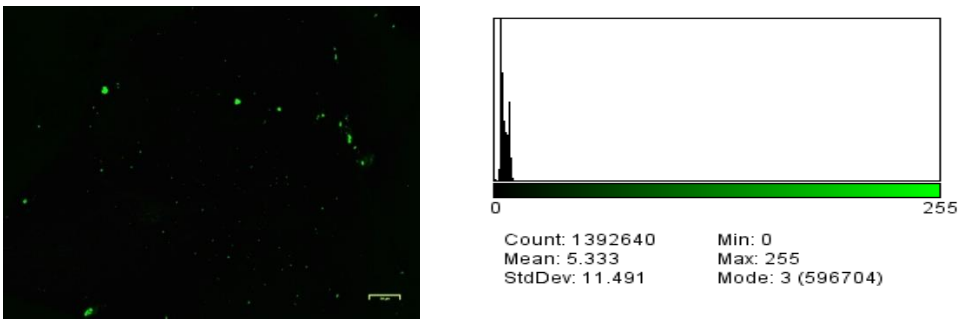

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Figure 3. Cultivation of *Tetraselmis* sp. during 9 days. a. Dosage of Tiger Shrimp Wastewater was 2 g/l; b. Dosage of Tiger Shrimp Wastewater was 4 g/l; c. Dosage of Tiger Shrimp Wastewater was 6 g/l; d. Dosage of Tiger Shrimp Wastewater was 8 g/l

3.2. Discussion

Based on fluorescence expressions in the Figure 1, 2, and 3 showed acridine orange capable to binded with *Tetraselmis* sp. due ionic binding between acridine orange with *Tetraselmis* sp. Acridine orange is one of base fluorescent staining due to cation contained. The cation will be binding with negative ion from cell and binding DNA through insert between DNA's bond (Lv *et al*., 2011). Living cell of phytoplankton capable to reduction acridine orange staining enzymatically causing radiated green colour, whereas damaged or dead cells radiated orange colour (Hadioetomo, 1993). Cells histochemistry staining method of *Tetraselmis* sp. using staining one step method staining is FITC (*Fluorescens isothiocyanate*) method which fluorescence will directly be binding to the protein inside the cell and observing with fluorescence microscope (Fannessia *et al*., 2015). Reading of protein expression helped using image J software that showed histogram graphics accompanied by mean. Mean resulted by graphics indicated protein could be expressed.

Image J is a program Java based which can using all systems of computer operation. ImageJ provides facilities for digital images. One of that ImageJ facilities is how many proteins can be expressed (Ramadhani *et al.*, 2016). Mean value from histogram graphics showed not all proteins could be expressed in various wastewater dosages. The result of this research in Figure 1, 2 and 3, the highest mean value was in treatment with 2 g/l wastewater dosage. It showed that *Tetraselmis* sp. cells with 2 g/l wastewater dosage had more protein than other wastewater dosages. It caused the higher nutrient were absorbed would increase the rate of nutrient absorption that would be resulted in higher cells density, the nutrient became poison and disrupted microalgae cells metabolism (Meritasari *et al.*, 2012).

IV. CONCLUSION

Based on expression of fluorescence protein with 2 g/l wastewater dosage showed that amount of protein could be ecpressed was higher than other wastewater dosage by mean value from ImageJ.

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