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The Lipid Content of The Culture Microalgae Using Media of Tapioca Liquid Waste

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Abstract

Microalga *Navicula* sp., *Spirulina platensis*, and *Chlorella* vulgaris have the prospect of being a source of biofuel producers. Rapid cell growth, coupled with the ability to produce large lipids and less pollution, can be used as an alternative to biofuel development. Microalgae cultivation can utilize tapioca liquid waste. Addition of NaCl to regulate salinity, so optimum for microalgae growth. In addition it can reduce toxins by binding to dissolved cyanide acid present in the waste. This study aims to determine the effect of NaCl concentration on tapioca liquid waste on growth and lipid microalgae content. This study used an experimental method with a complete random factorial design. The first factor tested three species of microalgae. The second factor tested seven NaCl concentrations on tapioca liquid waste media. The results showed that the concentration of NaCl 35% in tapioca liquid waste culture media capable of producing biomass of C. *vulgaris* cells with the highest lipid content. The NaCl concentration capable of producing the highest microalgae biomass from the study can be developed to design more effective and efficient tapioca industrial waste treatment without damaging the environment but more productive, as a biofuel producer.

How to Cite

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INTRODUCTION

Microalgae is a vegetable material that is not less important to be explored as a potential source of renewable energy. Microalgae have the prospect of being an alternative source of biofuel substitutes that can be produced as renewable resources. According to incorporated scientists in South Australia's Research and Development Institute (SARDI), oil content in microalgae is greater than jatropha, rapeseed, coconut and palm kernels. The oil content can reach more than 40% (Mata *et al.*, 2010; Oilgae, 2016)primarily produced from food crops and mostly oil seeds are limited in their ability to achieve targets for biofuel production, climate change mitigation and economic growth. These concerns have increased the interest in developing second generation biofuels produced from non-food feedstocks such as microalgae, which potentially offer greatest opportunities in the longer term. This paper reviews the current status of microalgae use for biodiesel production, including their cultivation, harvesting, and processing. The microalgae species most used for biodiesel production are presented and their main advantages described in comparison with other available biodiesel feedstocks. The various aspects associated with the design of microalgae production units are described, giving an overview of the current state of development of algae cultivation systems (photo-bioreactors and open ponds. Elsey *et al.* (2007), states that oil content in microalgae can be detected by screening using Nile red. Hallenbeck (2012), Nile red when reacting with lipids contained in microalga cells will change the red ligand to yellow or red.

The lipid content is highly dependent on the microalgae species and the environmental conditions at which it grows. Microalgae *Navicula* sp., *Spirulina platensis*, and *Chlorella vulgaris* have prospects as sources of biofuel producers that can be produced on an ongoing basis. Rapid cell growth, coupled with the ability to produce very large lipids, and with little pollution can serve as an alternative to biofuels compared to petroleum fuels (Mata *et al.*, 2010)primarily produced from food crops and mostly oil seeds are limited in their ability to achieve targets for biofuel production, climate change mitigation and economic growth. These concerns have increased the interest in developing second generation biofuels produced from non-food feedstocks such as microalgae, which potentially offer greatest opportunities in the longer term. This paper reviews the current status of microalgae use for biodiesel production, including their cultivation, harvesting, and processing. The microalgae species most used for biodiesel production are presented and their main advantages described in comparison with other available biodiesel feedstocks. The various aspects associated with the design of microalgae production units are described, giving an overview of the current state of development of algae cultivation systems (photo-bioreactors and open ponds.

The results of the study Christiani *et al.* (2014); Christiani *et al.* (2015); and Christiani *et al.* (2016) *Navicula, Spirulina*, and *Chlorella* microalgae were able to grow as benthic in rivers affected by tapioca liquid waste. Microalgae have the good ability as the waste absorber so that that waste can be used as culture medium. Tapioca liquid waste as a microalgae culture medium needs to be diluted and decomposed first to become an optimal growth medium.

The addition of NaCl to the culture medium will make the medium more efficient, in addition to increasing the salinity of the media, also serves to antifungal and bind cyanide acid. The addition of NaCl might bind dissolved cyanide acid present in tapioca liquid waste by converting it to NaCN which is a high alkaline (Ardhianto *et al.*, 2013) and (Riyanti & Lukitowati, 2010). The alkaline media conditions can facilitate microalgae utilizing nutrients in waste (Juneja *et al.*, 2013)lipids.

The tapioca liquid waste of agroindustry is one of the largest in Indonesia, rich in nutrients as a medium for microalgae growth (Kabinawa & Agustin, 2005). Tapioca industry produces only tapioca of 20-30% of processed cassava weight, the rest of the sector provides solid and liquid waste (Robby *et al.*, 2013; Eze *et al.*, 2018). Tapioca liquid waste contains many organic ingredients such as carbohydrates (0.29%), protein (0.16%), crude fiber (0.04%), fat (0.22%), and water content (99.75%) (Juneja *et al.*, 2013)lipids.

Tapioca liquid waste can be utilized as microalgae culture medium. Culture media needs to be increased in salinity to suit the growth of microalgae. The addition of NaCl aims to optimize the salinity conditions suitable for microalgae growth. Also used for chlorination of media, so it can reduce the number of bacteria contained in tapioca liquid waste. Research Asthary *et al.* (2016)25%, 50%, 75% and 100% were used as media to grow microalgae S. platensis. During cultivation,the medium pH and biomass production were analyzed, while proximate analysis were also done after havesting. Results showed that S. platensis microalgae grown in 100% wastewater medium yielded the highest biomass among all

other treatments at 4-days of cultivation, about 25% higher than that in control medium. The biomass produced contains about 60% protein which is nearly equal to that reported from other countries .Keywords : microalgae, Spirulina platensis, effluent, paper industry\u00a0\u00a-0ABSTRAKKapasitas produksi industri kertas di Indonesia diperkirakan akan terus meningkat menyebabkan peningkatan air limbah yang dihasilkan. Air limbah industri kertas yang telah diolah pada Instalasi Pengolahan Air Limbah (IPAL), chlorination is one way to sterilize liquid waste, the safer chlorine used is NaCl. Based on the above description, a study was conducted to determine the effect of NaCl concentration on tapioca liquid waste against cell biomass and microalgae lipid levels of *Navicula* sp., *S. platensis*, and *C. vulgaris* on laboratory scale culture, so it can be known the NaCl concentration capable of producing microalgae cell biomass with the highest lipid levels.

Microalgae species that can grow optimally and produce the high lipid content in tapioca liquid waste culture media can be developed to design the tapioca industry as a more efficient biofuel producer of renewable energy. Processing the liquid tapioca waste using microalgae indicator can reduce the pollutants.

METHODS

The research method used was an experimental method using Completely Random Design, factorial pattern. The first factor has tested species of microalgae, namely *Navicula* sp., *S. platensis*, *C. vulgaris*. The second factor is NaCl concentrations in waste media, that are 20; 22.5; 25; 27.5; 30; 32.5; and 35%. The independent variables were concentration level of NaCl of tapioca liquid waste media and microalgae species, while the dependent variable was microalgae density. Supported parameters were the content of N, P, light, pH, and brightness (Abdel-Raouf *et al.*, 2012; Hallenbeck, 2012).

Tools preparation

All equipment were sterilized by soaking in water plus 40 ppm chlorine of 1 ml. 1 L^{-1} of water for 24 hours. Next, the culture bottle was dried and covered with aluminum foil (Isnansetyo & Kurniastuti, 1995).

Preparation of Starter Solution of M-Bio Fertilizer

The preparation of M-Bio fertilizer starter solution using 1000 ml of distilled water mixed with 10 ml of M-Bio and 20 g of sugar in culture bottle, then sealed with aluminum foil and wrapper. After that, the container is wrapped in black plastic and silenced for 2 x 24 hours (Christiani & Hidayah, 2011).

Waste Media Making with Different Levels of NaCl Concentration

Before being used as a culture medium, tapioca liquid waste was aerated and given 5 ml of M-Bio, then incubated for one (Prihantini *et al.*, 2005; Ikhlas *et al.*, 2014). Furthermore, tapioca liquid waste was made according to the research treatment, which was with the concentration level of NaCl of 20, 22.5, 25, 27.5, 30, 32.5, and 35%, each treatment needed as much as 800 ml.

Implementation of Microalgae Culture on Tapioca Liquid Waste Media

Microalgae seeds were taken using dropper drops, then the initial density was calculated by dripping into a Sedgewick rafter and observed using a microscope. Culture bottles are placed on a culture shelf, aerated, and lighting with two TL 40 Watt bulbs. Density formula according to Isnansetyo & Kurniastuti (1995):

$$N_1 = Jbp \times n = \frac{1000 \times n}{Lbp} = \frac{1000 \times n}{3.14 \times r^2}$$

Information :
N_1 = initial density or abundance of dispersed microalgae (cell/ml)
Jbp = field distance of view
LBP = area of view
n = average number of microalgae
1 = number of microalgae
r = 0,5 diameter

Lipid Content Measurement

In the determination of lipid levels by the Mojonnier method, 100 ml of microalgae samples were introduced into Mojonnier tubes, then 15 ml of ethanol and 15 ml of ammonium hydroxide were added, and extracted with a mixture of 15 ml of diethyl ether and 15 ml of petroleum ether (1: 1). The extraction result was then being evaporated and dissolved in an oven at a temperature of 100 ° C until a constant weight was obtained. The extra weight is expressed as the lipid weight in the material (Widjaja, 2010; Indarto, 2008). The collected data of lipid content was recorded and made a histogram.

Analysis Method

The density data were analyzed using variance analysis with 95% and 99% confidence level to know the effect of the addition of NaCl and

microalgae species to grow. The significant result then continued with HSD-test to see the difference between the treatments (Christensen, 2013).

RESULTS AND DISCUSSION

The results of cell biomass observation at the peak of the microalgae population of *Chlorella* sp., *Spirulina* sp., and *Navicula* sp. in culture with tapioca liquid waste media ranged from an average of 13.67 $g.L^{-1}$ to 54 $g.L^{-1}$ (Figure 1). According to Hallenbeck (2012), the nutrient content of the media (tapioca liquid waste) is significant for the growth of microalgae. N elements play a role in the formation of amino acid compounds and chlorophyll, P elements play a role in the structure of ATP, DNA, and phospholipids in cells, while Cl and Mg help the process of photosynthesis.

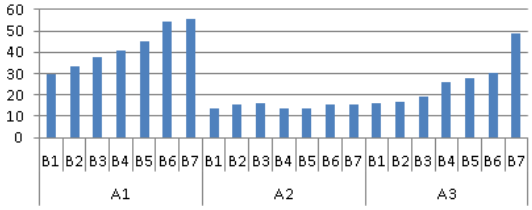

Figure 1. Histogram of microalgae cell biomass on tapioca liquid waste media with the addition of NaCl in various concentration

Description: A1 = *Chlorella*, A2 = *Spirulina* A3 = *Navicula* B1 = NaCl Concentration 20% B2 = NaCl Concentration 22.5% B3 = NaCl Concentration 25% B4 = NaCl Concentration 27,5% B5 = NaCl Concentration 30% B6 = NaCl Concentration 32,5% B7 = NaCl concentration 35%

Chlorella, Spirulina, and *Navicula* microalgae cell biomass were different in tapioca liquid waste with the addition of various concentrations of NaCl. Rini (2008) and Chritiani & Hidayah (2011) stated that an appropriate media would accelerate the different adaptation periods in microalgae. According to Chilmawati & Suminto (2008), differences in adaptation can occur due to differences in the concentration of the culture medium with the fluid in the microalgae cell body. Moreover, the microalgae adaptation process is influenced by differences in the ability of the microalga to absorb nutrients from the given culture medium.

After achieving the adaptation stage, microalgae had been able to use nutrients in the medium so that the growth can be in the optimal or exponential phase. The content of nutrients at the beginning of culture was still high and utilized by microalga-microalgae to do the growth process. An increase in the number of cells will stop at the peak of of the population. At the peak of the population the need for nutrients to be greater, while the nutrient content in the media decreased because of no addition of nutrients to the media. Moreover, there was also a competition for the place of life because there were more the number of cells in a fixed volume. This causes the death of the individual and at the same time decreases the number of cells that grow, so that after experiencing the growth peak, the number of cells decreased, so that the biomass of the cell must be harvested immediately. According Pandhal *et al.* (2017) appropriate harvesting of microalgae based on the growth pattern. Harvesting should be done when microalgae reach peak population. Harvesting is too fast or has not reached the peak of the population, the remaining nutrients are still large enough to harm the predator organism. Harvesting after the peak of the population then there is a lot of death and quality decreased. Good harvesting is tailored to the intended use. Uses of microalga vary, some are direct as a natural feed, as seeds, or can also be dried or stored in a freezer.

The results of variance analysis showed that there was a significant interaction between microalgae species and NaCl addition to microalgae cell biomass ($P<0.05$). microalgae will utilize tapioca liquid waste as a nutrient for growth. *Chlorella* is able to grow faster until it reaches the peak of the population followed by *Navicula* and the slowest of *Spirulina*. Differences in internal properties of microalgae supported by the addition of proper NaCl concentrations will maximize the growth of microalgae cells. The addition of NaCl also has a very real effect on changing media conditions. Giving NaCl is a way of chlorinating to sterilize tapioca liquid waste from other competitor microarganism. microalgae will utilize the liquid waste medium tapioca with maximum as a growth medium (Juneja *et al.*, 2013)lipids.

Addition of NaCl has a very real effect on changes in media salinity conditions that affect the growth optimization. NaCl administration is a safe means of chlorination to sterilize tapioca wastewater. According to Markou *et al.* (2012), chlorination is one way of sterilization of liquid waste, in which case the safe chlorination used is NaCl. NaCl will suppress other microorganism contamination, so it will maximize microalgae in utilizing tapioca liquid waste media as the growth medium. Based on research by Ardhianto *et al.* (2013), NaCl can bind dissolved cyanide acid by forming it into NaCN which is a strong base.

The presence of a strong support will accelerate the acidic wastes into alkaline conditions. The salty conditions make it easier for microalgae to take advantage of nutrients in waste (Juneja *et al.*, 2013)lipids. The results of variance analysis showed that there was a significant interaction between microalgae species and NaCl addition to microalgae cell biomass ($P < 0.05$). *Chlorella* is able to grow faster until it reaches the peak of the population followed by *Navicula* and the slowest of *Spirulina*. Differences in internal microalgae properties supported by the addition of proper NaCl concentrations will maximize the growth of microalgae cells. Addition of NaCl has a very real effect on the changing conditions of the media thus affecting growth. Giving NaCl is a safe chlorination way to sterilize tapioca liquid waste to maximize microalgae by utilizing tapioca liquid waste media as a growth medium (Allwayzy *et al.*, 2010).

Chlorella is a microalga that grows on a wide range of salinity, making it adaptable and able to grow faster. The microalga life cycle is also very short. According to Isnansetyo & Kurniastuty (1995), *Chlorella*'s life cycle is only 2-3 days. It grows at salinity of 0-35 ‰ (optimum 10-20 ‰) and at optimum temperature of 25-30 °C. Asexual reproduction is by cell division or by autospore. *Navicula* is a second microalga that can grow faster than *Spirulina* because this microalga can use organic materials for their life needs. *Navicula*'s life cycle is 3-4 days. Suitable temperatures for microalgae life range from 25-35˚C. Amalah *et al.* (2018) stated that the optimum temperature for life of *Navicula* sp. ranged from 28-30˚C, the optimum salinity reaches from 25-30 ppt. Cheunbarn & Peerapornpisal (2010) and Lodi *et al.* (2003), *Spirulina'*s growth is the slowest compared to *Chlorella* and *Navicula* because it requires a higher salinity to grow optimally. It lives in terrestrial, freshwater, brackish water and seawater which tends to be alkaline with optimum pH of 7.2 to 9.5 (resistant to pH 11). It can hold at high salt level up to 85 ‰, optimum temperature range 25-35 °C. Reproduction by splitting. Besides, the life cycle is also longer 5-6 days (Syaichurrozi & Jayanudin, 2016). The results of the study by Christiani & Hidayah. (2011) showed the peak of the population occurring at 6 days after inoculation in *Spirulina* cultured with a medium fertilized water weed extract. Markou *et al.* (2012) have conducted a study on the cultivation of *S. platensis* in olive oil waste with the addition of sodium hypochlorite (NaOCl). Liquid olive oil waste is an organic waste containing antibacterial ingredients, i.e. phenol compounds. The content of phenol compounds in liquid olive waste causes the biodegradation of this waste to run slowly. The addition of sodium hypochlorite serves as a degradation of phenol compounds. After sodium hipolkorit was added to olive oil waste, it can be used as growth media of *S. platensis*. The case is not much different from the problem of tapioca liquid waste containing cyanide acid.

In the preparation of tapioca, cyanide or CN was eliminated by raw material washing process, starching and separating the cyanide acid (HCN) which was finally discharged as waste. Therefore, tapioca industry needs a lot of water in its processing, for 1 ton of cassava into tapioca that produces about 4,000-6,000 liters of liquid waste (Robby *et al.*, 2012; Harahap *et al.*, 2013). Tapioca liquid waste also contains inorganic materials such as cyanide acid or HCN. In general, tapioca industry uses high cyanide cassava because it has large tuber and contains more starch (Purawisastra & Heru, 2004). Cyanide acid is a weak acid in water and can be readily hydrolyzed in contact with oxygen (Riyanti & Setyoningrum, 2010).

HSD-test results showed that *Chlorella* cultured with the addition of NaCl with different concentrations would be different from *Spirulina* and *Navicula*. *Chlorella* cultured with the addition of NaCl with a concentration of 37.5% yielded the highest cell biomass, unlike *Chlorella* microalgae with 35% NaCl addition, but unlike *Chlorella* cultured on 30% NaCl addition nd so on with *Spirulina* and *Navicula* on different NaCl. Lowest cell biomass is *Spirulina* microalgae with 22,5% NaCl addition. Suminto (2009) stated that the growth of microalga is influenced by the nutrient contained in its medium. Microalgae require macro and micronutrients. The macronutrient elements are the necessary elements needed in large quantities, including: N, P, K, S, Na, Si and Ca, while micronutrients are the essential elements needed in small amounts, Fe, Zn, Cu, Mg, Mo, Co, and Bo. However, the nutrients that are necessary for photosynthesis are C, H, O, N and P. The most essential nutrient needed by microalgae are C 56.3%; N 8.6% and P 1.2% (Juneja *et al.*, 2013) lipids. Carbon is the most significant element required of microalgae in photosynthesis and cell growth (Syaichurrozi & Jayanudin, 2016; Hadi, 2015). Nitrogen is a major component of protein formation that is needed for cell multiplication (Widianingsih et el., 2013; Daefi *et al.*, 2017). Phosphorus is a necessary element in the metabolism of microalgae cells (Amalah *et al.*, 2018). Nutrients in the culture medium will decrease along with the increasing number of cells and the

concentration of biomass (Göksan *et al.*, 2007).

In addition to nutrients, the growth of microalgae is also influenced by the condition of chemical physics of the medium, ie, light intensity, temperature, pH, and salinity (Chrismadha *et al.*, 2006). The results of initial and final light intensity measurements ranged from 75.7 to 77.8 lux. (Abdel-Raouf *et al.*, 2012) the optimal light intensity required in microalgae culture is above 3.8 lux. Light affects the process of photosynthesis of microalgae. The range of light intensity at the time of the study can still support growth. The less light energy resulted in the microalgae photosynthesis rate is slow, and the intensity of light is too high resulting in photoinhibition (Hasanudin, 2010; Suminto, 2009).

Salinity is the soluble concentration of salt in water. Christiani *et al.* (2015; Zebek (2007), salinity is related to the osmotic pressure of microalgae cells and is directly related to the absorption of nutrients for metabolism. Microalga able to live in the range of salinities freshwater to the sea that ranged from 0 to 70‰ and the optimum salinity for growth is 25 to 35‰ . In the treatment without the addition of NaCl, there was a competition between microalgae and the decomposing bacteria, while in the treatment with the addition of NaCl, the decomposing bacteria in the tapioca liquid waste was died because they could not stand the salinity stress. This is in line with Sari *et al.* (2012) that cultivates *S. platensis* in palm oil waste in the absence of NaCl addition ; it is known that there is competition in utilizing nutrients contained in the waste with the decomposing bacteria.

Tapioca liquid waste media is a source of nitrogen and phosphorus which have a role in the productivity of lipids. This is thought to have something to do with the process of lipid biosynthesis as occurs in microalgae cells. Lipid biosynthesis in microalgae requires acetyl-CoA as the lipid formation. If excess glucose synthesis, it will be converted into lipid compounds as energy reserves (Elsey *et al.*, 2007). As protein and lipid levels continue to rise, proteins are broken down into amino acids, while lipids are broken into fatty acids to be energized or stored in lipid form (Ermavitalini *et al.*, 2017).

The lipid content of each treatment was seen on the histogram (Figure 2). NaCl concentration of 35% in tapioca liquid waste culture media was able to produce the highest *Chlorella vulgaris* microalgae cell biomass with 43% lipid content, followed by NaCl concentration of 32.5%. Followed by *Spirulina* and *Navicula* microalgae with different concentrations.

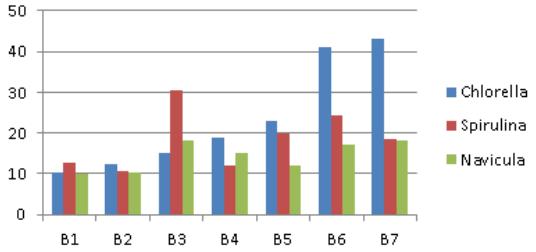

Figure 2. Histogram of microalgae lipid content cultured on tapioca liquid waste media with added NaCl concentration with different concentration

Information: B1 = NaCl Concentration 20% B2 = NaCl Concentration 22.5% B3 = NaCl Concentration 25% B4 = NaCl Concentration 27,5% B5 = NaCl Concentration 30% B6 = NaCl Concentration 32,5% B7 = NaCl concentration 35%

The higher the density of the microalgae, the higher the lipid content. Schenk & Thomas (2008); Sari *et al.* (2012) and Scragg *et al.* (2003) stated that lipid content is affected by the frequency or abundance of cells. *Chlorella vulgaris* cell density is high, then the upper levels of lipid produced. The higher the added NaCl concentration also, the higher the yielding lipid. The lipid content produced at each treatment was different because the process of extracting the lipid microalgae in each procedure was done by using a sample of wet weight. Ermavitalini *et al.* (2017), the highest biomass and lipid content are found in 10 Gy irradiated microalgae are 0.833 grams biomass and 41 % lipid content. Harahap *et al.*, 2013) stated that thesamples of microalgae that still contain the water element therein will produce fewer lipids than the example of heavy dry microalgae when lipid extraction is performed. If lipid extraction was performed using the dry weight of 25 ml of the microalgae sample, the resulting dry mass was too small to observe, since the importance of the dry weight of each microalgae was only about 10% of the wet weight. Nutrients contained in the culture medium also affect the increase in lipid content in microalgae cells (Umdu *et al.*, 2009; Widjaja, 2009; Carrero *et al.*, 2011).

The formation of lipids from proteins involves a series of processes that lead to the formation of acetyl-CoA and then into the flow of lipid biosynthesis. Proteins will be broken down into amino acids-amino acids. Amino acids-amino acids undergoing metabolic pathways through pyruvate are alanine, cysteine, glycine, and threonine. Chisti (2007) and Chisti (2008), stated that a metabolic relationship between carbohydrates,

proteins, and fats, i.e., acetyl-CoA competition, which is a precursor to various biosynthetic pathways such as lipids, proteins, and carbohydrates. Musdalifah *et al.* (2013); Patil *et al.* (2008) and Song *et al.* (2008), stated that microalgae cell lipid biosynthesis is initiated by condensation of glycerol with three fatty acid molecules with the aid of lipase enzyme catalyst. Glycerol derived from α-glycerophosphate removed by phosphate groups by phosphorylation reaction, the formation of fat acids requires some acetyl-CoA, two pairs of electrons (2 NADPH), and ATP energy. NADPH may be available from the pentose phosphate respiration pathway, and ATP from pyruvate glycolysis which is the original compound of acetyl-CoA.

The NaCl concentration capable of producing the highest microalgae biomass from the study can be developed to design more effective and efficient tapioca industrial waste treatment without damaging the environment but more productive, as a biofuel producer. Microalgae species that can grow optimally in a tapioca liquid waste culture medium and contain high lipids can be developed to design tapioca industrial waste processing, a biofuel renewable energy producer. Tapioca wastewater treatment using microalgae for biofuel products will be more efficient without damaging the environment and more productive. *Navicula* sp., *S. platensis*, and *C. vulgaris* are also useful as feed because the cell walls are composed of complex sugars, making them easily digested by fish. The study from Simanjuntak et al, (2016) showed a feed-intensive stimulation cycle with *S. platensis* supplementation to promote growth, hematology, and fish body composition (i.e. *Osphronemus gouramy*).

CONCLUSION

NaCl content in tapioca liquid waste can produce cell biomass and lipid content of microorganisms *Navicula* sp., *Spirulina platensis*, and *Chlorella vulgaris.* NaCl concentration 35% in liquid tapioca culture media medium capable of producing biomass microalgae cells *C. vulgaris* highest with lipid content.

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