

The effect of dilution level of liquid tapioca waste culture medium and concentration of phosphate on the growth of microalgae *Navicula* sp.

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Abstract. Amalah N, Widyartini DS, Christiani, Hidayah HP. 2018. The effect of dilution level of liquid tapioca waste culture medium and concentration of phosphate on the growth of microalgae *Navicula* sp.. *Nusantara Bioscience* 10: 65-69. Liquid tapioca waste can be used as microalgae culture medium due to its nutrient contents that can support the life of microalgae. Liquid tapioca waste contains phosphate nutrient that can influence cell division and fat formation. Liquid tapioca waste is usually still highly concentrated, so it has to be diluted first to allow the light penetrates the microalgae growth medium. The aim of this research was to determine the effect of interaction between dilution level of liquid tapioca waste and concentration of phosphate on the density of *Navicula* sp. This research employed a factorial treatment design laid out in a Completely Randomized Design. The first factor was the dilution level of liquid tapioca liquid waste, consisted of 0%, 10%, 20%, and 30%, and the second factor was the concentration of phosphate comprised of 0 ppm, 10 ppm, 20 ppm, and 30 ppm. ANOVA results indicated a significant interaction between the dilution level of liquid tapioca waste and concentration of phosphate on the density of *Navicula* sp. DMRT post hoc test showed that dilution of liquid tapioca liquid at the level of 20% without phosphate addition was the best treatment that produced the highest *Navicula* sp. density.

Keywords: Dilution, *Navicula* sp., phosphate, tapioca liquid waste

INTRODUCTION

Microalgae are photosynthetic microorganisms that can grow much faster than terrestrial plants and live in harsh conditions due to their unicellular or simple multicellular structure (Mata et al. 2010). These organisms are potential to be used by various industries as food, artificial and natural feed, filter organisms, food supplement, the pharmaceutical industry, and even as alternative energy in the form of *biofuel* (Hermanto et al. 2011). The *biofuel* content in microalgae is exceptionally high; it can reach over 50% of dry biomass. One of the microalgae with high *biofuel* content is *Navicula* sp. living in both saline and freshwater environments. *Navicula* sp. is also present in the water stream contaminated by liquid tapioca waste (Umdu et al. 2008).

In addition to its function to increase the microalgae biomass, utilization of liquid tapioca waste as a culture medium of microalgae means also increase the added value of the waste. The liquid tapioca waste contains suspended organic materials such as protein, fat, carbohydrate as well as other dangerous organic compounds like cyanide, nitrate, ammonia, etc. (Riyanti et al. 2010). In the water stream, the organic waste might be naturally dissolved by microorganism into simpler organic compounds like N, P, Si, C, and K that are important for microalgae growth. However, before being utilized as a microalgae culture medium, the liquid tapioca waste needs to be diluted to allow sunlight penetrates into the medium so that photosynthesis process can occur optimally. Diluting any liquid waste will change the concentration of the liquid and

break some nutrients like phosphate into simpler compounds necessary for microalgae to grow (Ahmad 2012).

The current study aimed to determine the effect of interaction between dilution levels of liquid tapioca waste and phosphate concentration on growth and density of *Navicula* sp. cultured in liquid tapioca waste. Data obtained will be useful in exploring and developing *Navicula* sp. as biofuel raw material by applying this waste as a growing medium to minimize the problem of environmental pollution and increase the added value of the waste.

MATERIALS AND METHODS

This research was carried out in Aquatic Biology Laboratory of Jenderal Soedirman University. Research materials used included *Navicula* sp., Zarrouk growth medium, tapioca liquid waste, K₂HPO₄, M-Bio fertilizer, chlorine 40 ppm, PP indicator, reagents Na₂CO₃ 0,01 N, boric acid 1%, Conway indicator, Devarda alloy, H₂SO₄ 0,05 N, HNO₃, HClO₄, and H₂O, coloring reactor, aquadest, aluminium foil, Whatmann filter paper of W-41, wrapper, black plastics, universal pH paper.

This research was done experimentally employing a Completely Randomized Design in factorial treatments consisted of two factors, i.e., the dilution level of liquid tapioca waste and concentration of phosphate. The dilution level of liquid tapioca waste (A) comprised of : A₀ = 0% (control), A₁ = 10%, A₂ = 20%, and A₃ = 30%. The concentration of phosphate (B) consisted of : B₀ = 0 ppm

(control), $B_1 = 10$ ppm, $B_2 = 20$ ppm, and $B_3 = 30$ ppm. Each treatment was performed in triplicate. The main parameter observed was the density of *Navicula* sp., while the environmental parameters observed were growth medium temperature, pH, light intensity, salinity, released CO_2 , nitrate, and phosphate.

Procedures

Research procedures included preparation of research equipment/tools, production of M-Bio fertilizer as the starter, mass rearing/propagation of *Navicula* sp. in a Zarrouk growth medium (control), preparation of growth medium containing tapioca liquid waste at various qualifying levels as well as phosphate concentrations, and culturing of *Navicula* sp. on the liquid tapioca waste. The M-Bio starter was prepared by mixing 10 ml M-Bio and 20 g sugar into 1000 ml aquadest in a tightly closed cultured-glass bottle and then was covered with aluminum foil and wrapped. The cultured-glass bottle was then covered with black plastic and left for 2 x 24 hours at room temperature.

Five ml stock solution of *Navicula* sp. was then poured into the culture bottle containing 35 ml Zarrouk growth medium and 760 ml aquadest. The cultured bottle was then closed by using an aerator and covered with aluminum foil, incubated for one week at room temperature before counting the culture density. The liquid tapioca waste was added with 5 ml M-Bio, incubated for one week. Then, the liquid tapioca waste was diluted as follows: 0%, 10%, 20%, and 30%. The concentration of phosphate was made of 0 ppm, 10, ppm, 20 ppm, 30 ppm; 800 ml solution each of these treatments was prepared for the following steps for *Navicula* sp.

The *Navicula* sp. starter culture was calculated for initial density using Sedgewickrafter from the first day to the seventh day. The *Navicula* sp. starter was poured into cultured bottles containing liquid tapioca waste, and the bottles were then put on the culture shelf, aerated, and lighted using two 40 Watt lamps. The *Navicula* sp. density was calculated daily by counting the population abundance. The abundance curve was generated based on the daily average density of *Navicula* sp. during the culturing period. Measurements of temperature, pH, light intensity, salinity, and the released CO_2 level were as stated for the environmental parameters of growth medium. Nitrate contents were calculated by distillation method while phosphate contents were measured using a colorimetric method.

Observed variable and data analysis

The primary observed variable in this study was the density of *Navicula* sp. Also, the environmental and the conditions of culture medium such as temperature, pH, light intensity, salinity, levels of released CO_2 , nitrate content, and phosphate content were observed. *Navicula* sp. density data were subjected to ANOVA to see the treatment effect. The Duncan Multiple Range Test was employed to separate the treatment means of both the dilution level of liquid tapioca waste and concentration of phosphate as well as the interaction between the two treatments, and to determine the maximum density of the

Navicula sp. Environmental and culture conditions data were analyzed descriptively.

RESULTS AND DISCUSSION

Results

Obtained data of *Navicula* sp. density was subjected to analysis of variance to see the treatment effect on this observed variable. Results of the study revealed that the interaction between liquid tapioca waste dilution level and phosphate concentration caused no significant effect on *Navicula* sp. density at the first day and the seventh day of cultivation. However, the liquid tapioca waste dilution level and phosphate concentration effect on *Navicula* sp. density was highly significant ($P < 0.01$) at the second day, the fourth day and the fifth day of cultivation, and significant ($P < 0.05$) at the third day and the sixth day of cultivation. The single factor treatment of tapioca waste dilution level alone highly significantly affected ($P < 0.01$) the *Navicula* sp. density at both the first day and the seventh day of cultivation.

The study results showed that the treatment combination of liquid tapioca waste dilution level and phosphate concentration producing the highest density of *Navicula* sp. at the second day was 10% liquid tapioca waste dilution level and phosphate concentration of 20 ppm ($16,974,522$ cell mL^{-1}) while that on the third day was 20% liquid tapioca waste dilution level and phosphate concentration of 0 ppm ($31,029.724$ cell mL^{-1}), at the fourth day was 10% liquid tapioca waste dilution level and phosphate concentration of 20 ppm ($10,721,868$ cell mL^{-1}), and at the fifth and the sixth days was 20% liquid tapioca waste dilution level and phosphate concentration of 20 ppm, with the average density of, respectively, $8,662,420$ cell mL^{-1} and $6,443,737$ cell mL^{-1} . The results also showed that the single factor treatment of liquid tapioca waste dilution level that produced the highest density of the *Navicula* sp. at the first and the seventh days of the study was 20% dilution level of liquid tapioca waste treatment, with an average density of, respectively, 14543.524 cell mL^{-1} and 5520.170 cell mL^{-1} .

The growth of microalgae *Navicula* sp.

Results of this study showed that the growth of *Navicula* sp. on the liquid tapioca waste medium had a better density level than the control treatment grown on the Zarrouk growth medium. On the first day of cultivation, *Navicula* sp. was still adapted to the culture medium. The *Navicula* sp. started an exponential growth rate from the first to the fourth days of cultivation and reached the stationary period and even the lag phase in a more extended cultivation time. The highest density level of *Navicula* sp. was reached on the third day of the study on the 20% liquid tapioca waste dilution level treatment without phosphate addition, with an average density of 31029.724 cell mL^{-1} . *Navicula* sp. faced a gradual decrease in density at the fourth to the sixth days, and it finally ceased on the seventh day of cultivation (Figure 1).

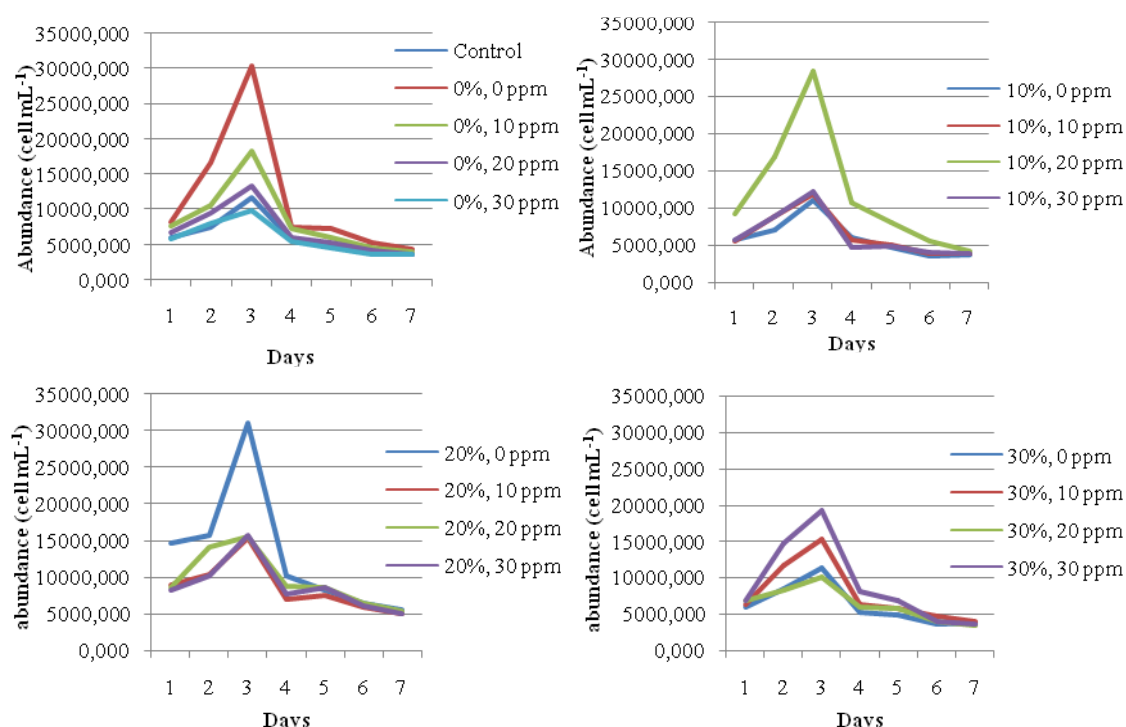


Figure 1. The growth of microalgae *Navicula* sp. within 7 days of observation in a varying dilution rate of tapioca liquid (0%, 10%, 20%, 30%; clockwise) and concentration of phosphate (0 ppm, 10 ppm, 20 ppm, 30 ppm)

Table 1. Measured data of temperature, pH, light intensity, and salinity during the culture period of *Navicula* sp.

Sample	Temperature (°C)		pH		Light intensity (lux)		Salinity (ppt)	
	1st day	7th day	1st day	7th day	1st day	7th day	1st day	7th day
A ₀ B ₀	26-27	31	4-5	8	75.7-77.8	75.7-77.8	30	30
A ₀ B ₁	26-27	30.5	4-5	8	75.7-77.8	75.7-77.8	30	30
A ₀ B ₂	26-27	30	4-5	8	75.7-77.8	75.7-77.8	30	30
A ₀ B ₃	26-27	31	4-5	8	75.7-77.8	75.7-77.8	30	30
A ₁ B ₀	26-27	30.5	4-5	8	75.7-77.8	75.7-77.8	30	30
A ₁ B ₁	26-27	30	4-5	9	75.7-77.8	75.7-77.8	30	30
A ₁ B ₂	26-27	30	4-5	9	75.7-77.8	75.7-77.8	30	30
A ₁ B ₃	26-27	30	4-5	8	75.7-77.8	75.7-77.8	30	30
A ₂ B ₀	26-27	31	4-5	8	75.7-77.8	75.7-77.8	30	30
A ₂ B ₁	26-27	31	4-5	8	75.7-77.8	75.7-77.8	30	30
A ₂ B ₂	26-27	31	4-5	8	75.7-77.8	75.7-77.8	30	30
A ₂ B ₃	26-27	31	4-5	8	75.7-77.8	75.7-77.8	30	30
A ₃ B ₀	26-27	31.5	4-5	8	75.7-77.8	75.7-77.8	30	30
A ₃ B ₁	26-27	31	4-5	8	75.7-77.8	75.7-77.8	30	30
A ₃ B ₂	26-27	31	4-5	7	75.7-77.8	75.7-77.8	30	30
A ₃ B ₃	26-27	30	4-5	8	75.7-77.8	75.7-77.8	30	30

Note: Data of the current study were a favorable rate of the growth of *Navicula* sp.

Table 2. Observed data of freed CO₂, nitrate and phosphate contents

Parameters	Content	
	1st day	7th day
Freed CO ₂	136.4 mg ml ⁻¹	0 mg ml ⁻¹
Nitrate	43.863 ppm	22.622 ppm
Phosphate	33.491 ppm	25.0789-183.888 ppm

Note: Data of the current study were a favorable rate of the growth of *Navicula* sp.

Environmental data

The density of *Navicula* sp. is not only affected by N and P contained in the culture medium but also by physical and chemical factors such as growth medium's temperature, pH, light intensity, salinity, aeration, and freed CO₂. Observed data of environmental conditions during culture period are shown in Table 1 and Table 2.

Discussion

The growth density of *Navicula* sp. under liquid tapioca waste medium was denser than that under the control medium (Zarrouk growth medium) (Figure 1). The denser *Navicula* sp. under liquid tapioca waste growth medium was caused by the better nutrient content of the growth medium to support the growth of *Navicula* sp. Nutrients such as N and P (Table 2) for examples can be well absorbed by the organisms in forms of inorganic nutrients. Before being utilized as a culture medium of *Navicula* sp., the liquid tapioca waste seemed to have been degraded into simpler inorganic compounds by some microorganism contained in the M-Bio fertilizer like *Bacillus*, *Lactobacillus*, yeasts, and *Acetobacter*. Those simpler inorganic compounds are used by *Navicula* sp. for a living (Christiani and Hidayah 2011). *Navicula* sp. could live on the growth medium containing liquid tapioca waste with a short period of adaptation. This adaptation phase was indicated by the slow increase of cell number caused by environmental changes (Gunawan and Muhamat 2015). Cahyaningsih et al. (2005) stated that the lag phase is a phase where the density rate is lower than the rate of death. Microalgae lost its metabolic ability due to the decrease of nutrients availability on its environment, leading to the death of some cells due to lack of food.

The exponential growth of *Navicula* sp. was observed from the first to the fourth days of cultivation and the density reached a peak on the third day (Figure 1). According to Christiani and Hidayah (2011), an increase in density is supported by the availability of nutrient elements required by *Navicula* sp. for cell division process. The increase in cell number indicates the continuous active cell division, so the addition of a very significant amount of cells causes this phase to be known as the exponential phase or phases of the log. The density of *Navicula* sp. was more affected by the dilution level of liquid tapioca waste than the concentration of phosphate, presumably because the total amount of phosphate content in the liquid tapioca waste medium was adequately available for the growth and the life of *Navicula* sp. The additional level of phosphate did not affect the density of *Navicula* sp.

After experiencing a peak density, at the fourth to the sixth days, the density of *Navicula* sp. gradually decreased due to the decrease in the nutrient content of the growth medium (Figure 1). The density of microalgae will decrease since the available nutrient on the growing medium has decreased causing the organisms to compete one to another for food or nutrients (Cahyaningsih et al. 2005). In this situation, therefore, the dilution level of liquid tapioca waste and concentration of phosphate could affect the growth and the life of *Navicula* sp. and so its density level. Dilution level determines the medium concentration, which is strongly related to the ability of light to penetrate into the culture medium. The higher the dilution level the lower the light intensity penetrates the culture medium easily. The light intensity penetrating the culture medium will be used by the organisms to perform photosynthesis process. Ahmad (2012) found that phosphate plays significant roles in the formation of microalgae cell structure as well as cell density. Phosphate takes a role in cell division and fat formation. The

interaction between the dilution level of liquid tapioca waste and concentration of phosphate on the liquid tapioca waste medium would optimize the growth and density of the microalgae. At the seventh day, *Navicula* sp. was death, which is known as the phase of death. The death phase is the phase where the rate of density is less than the rate of death. Microalgae lost its metabolic ability due to the decrease of nutrients availability on its environment, leading to the death of some cells due to food shortage (Christiani and Hidayah 2011).

The effect of liquid tapioca waste dilution level treatment on the density of *Navicula* sp. was more significant than that of the concentration of phosphate. This might be probably due to the total amount of phosphate content in the liquid tapioca waste medium was adequately available for the growth and live of *Navicula* sp. The additional level of phosphate then did not much affect the density of *Navicula* sp. The dilution level of liquid tapioca waste changed total light intensity penetrating into the culture medium. The high dilution levels must be supported by fast-growing cells, whose illumination requirements can only be met at low biomass concentrations, whereas low dilution levels caused the growth of culture to be severely restricted by self-shading of *Navicula* sp. In such a situation, if the irradiance supplied to the system is increased at a given dilution level, the light availability inside the culture increases (Bezerra et al. 2011). Light intensity that was able to penetrate the liquid waste affected the photosynthesis processes as well as total amount of nutrients to be absorbed by a microorganism, which then further affected their density. The higher density rate, the lower amount of nutrients available in the growth medium. Gunawan and Muhamat (2015) found that the growth rate of microalgae at culture media was positively correlated with the nutrient content of the media.

The dilution level of liquid tapioca waste might affect microorganism absorption capability on particular nutrient compounds, which then affected the density of the microorganism. The higher the dilution levels, the lower the nutrient content in the medium. However, an extremely low dilution level may result in only a minimum number of nutrients that can be absorbed by the microalgae. The dilution level of 20% is the most optimum level for growing the *Navicula* sp. on the liquid tapioca waste medium since, at this dilution level, the total amount of nutrients available in the growth medium are adequate for their life and produced the highest density.

The density of *Navicula* sp. was affected by the availability of N and P contained in the culture medium (Table 2). Nitrogen is needed by the microorganism for protein synthesis, chlorophyll formation, nucleic acids (DNA and RNA) synthesis, and saturated fats synthesis. The floral organisms could only absorb nitrogen in the form of nitrate (NO_3), which then is further processed into protein as their energy source. Phosphate is one of those important nutrient compounds required in a large amount to support the growth of microalgae. Phosphate is important for microalgae to form structural cell components and so their cell density. Phosphate plays a role in cell division and/or fat formation. Phosphate also has a role in the

photosynthesis processes, where microalgae utilized it in the form of orthophosphate (Ahmad 2012). Microalgae require phosphate in the concentration of 0.018-0.09 ppm with a maximum level of 8.90-17.80 ppm. The best phosphate concentration for the growth of microalgae is more than 0.20 ppm (Surogi and Iwnosky 2002). According to Hastuti (2007), *Navicula* sp. could grow optimally on a culture medium supplied with the phosphate concentration of 20.37-185.23 ppm.

The density level of *Navicula* sp. was also affected by physical and chemical factors such as the temperature and pH of the growth medium, light intensity, salinity, aeration, and freed CO₂. All these mentioned factors were observed in the present study, except aeration (Table 1). Hastuti (2007) stated that the *Navicula* sp. grow optimally in a temperature range of 28-30°C. The temperature of culture medium will affect chemical processes of the organisms where at a temperature less than 10°C the density level of microalgae decreases, but a temperature of over 35°C, these organisms may likely be dead. pH affects the density level of *Navicula* sp. directly. At the extreme levels of both high and low pH, the microalgae cell divisions could be disrupted due to disruption of metabolic activities. Prihantini et al. (2005) reported that microalgae prefer to live on pH range of 4.5-9.3, but *Navicula* sp. needs acidity level range from 8.2-8.7 to grow optimally (Hastuti 2007). Light intensity affects the microalgae photosynthesis processes; the lower level the light intensity the slower the photosynthesis would be, which then affect the density (Prihantini et al. 2005). Microalgae photosynthesis can also be enhanced as the nutrient concentration increases with high light intensity; it can become weak when cell density increases with low light intensity (Kumar et al. 2010). According to Surogi and Iwnosky (2002), the optimum light intensity for the growth of microalgae is 3.8 lux.

Salinity is calculated as total or concentration of dissolved ions in the water stated in per mil (ppt or part per thousand). Changes in the salinity of water often affect the growth, metabolism, and photosynthesis of phytoplankton. The composition of intracellular lipid of microalgae was reported to change in response to environmental salinity. Salt might have a direct effect on processes involved in electron transport and/or photophosphorylation and result in a decrease in the quantum efficiency of photosynthesis (Kirrolia et al. 2011). Furthermore, Hastuti (2007) reported that the optimum salinity level for growth of *Navicula* sp. ranged from 25-30 ppt. The freed CO₂ on liquid tapioca waste was used maximally by the *Navicula* sp. to grow to allow maximum cell density. The freed CO₂ plays an important role in the microalgae photosynthesis processes. With the help of water and sunlight, carbon dioxide plays a role on the photosynthesis processes in producing energy that is going to be used by the microorganisms to support their biosynthesis process, cell density and cell production, mobility, and reproduction. During photosynthesis, microalgae use CO₂ as a carbon source to grow and reproduce. Microalgae cells contain approximately 50% carbon, of which 1.8 kg CO₂ can be fixed to produce 1 kg of microalgae biomass. The CO₂ fixation efficiency for

microalgae is about 10–50 times higher than terrestrial plants (Chisti 2007).

To conclude, liquid tapioca waste can be used as a culture medium of microalgae *Navicula* sp. The best treatment for a maximum density of *Navicula* sp. for the medium was 20% dilution level of liquid tapioca waste without phosphate addition, with the average density of 31029.724 cell mL⁻¹.

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