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Relationship Between Pigment Concentration and Dry Weight in Determining Microalgae Abundance in Artificial Water Samples

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Abstract: Microalgae are aquatic organisms that can function as bioindicators of surface water quality. The concentration of chlorophyll a can be measured to determine the level of microalgae abundance in a body of water. Measurement of the dry weight of microalgae biomass is another method that can be used to determine the abundance of microalgae in a body of water. Both methods have their advantages and disadvantages. In measuring chlorophyll concentration, the presence of other compounds besides chlorophyll which can absorb light spectrum at certain wavelengths (wavelengths of 665 nm, 645 nm, and 630 nm) causes the measured absorbance value to be greater than it should be. The level of turbidity due to the content of suspended particles in water is a problem in the dry weight measurement method. Determination of the dry weight of biomass based on the chlorophyll concentration approach is the subject of this study. The results of simple linear regression analysis showed that the concentration of chlorophyll a and the dry weight of microalgae biomass in treatment A had a fairly strong positive correlation (Ra = 0.870), the tendency of which was to follow the linear regression equation Y = 302.35x + 17.121. Determination of dry weight based on the chlorophyll concentration measurement approach can be applied to water samples with suspended solids content that tends to be constant and inert (does not produce substances that can react with organic solvents during the chlorophyll extraction process). In addition, based on the results of data processing it can be concluded that the effect of the content of other suspended particles in the water samples did not show a statistically significant effect.

Keywords: Biomass Dry Weight, Bioindicators, Turbidity, Chlorophyll a, Microalgae

I. Introduction

Aquatic organisms are biotic components that require certain conditions to maintain their survival. Changes in the condition of a water body as a place to live can result in a change in the composition of aquatic organisms in the water body (Jha et al., 2008; Tampus, Annielyn D. Ermelinda G. Tobias, Ruben F. Amparado, 2012). Changes in composition that occur indirectly can describe the condition of a body of water, so it can be said that aquatic organisms are bioindicators of a body of water (Hosam et al., 2011; Pool et al., 2016). Microalgae are aquatic organisms that are sensitive to changes in nutrient content in waters, so they can be classified as bio-indicators of water quality (Gupta & Singh, 2011; Lan et al., 2012).

Kshirsagar (2013) states that the abundance of algal biomass in a body of water is affected by the concentration of phosphorus and inorganic nitrogen in that body of water. Lavoie, et al. (2004) conducted a study evaluating the effect of pollution on the Quebec (Canada) river stream caused by agricultural activities on benthic algae as a bioindicator of water quality. The results of this study indicate that periphyton can be used as a bioindicator in integrated water quality measurements (Torres et al., 2008), which can then be used as a monitoring strategy for the phytochemical quality of water. In addition, this organism is also a photosynthetic organism that contains chlorophyll so that it can absorb sunlight needed in the photosynthesis process (Carley, J., Pasternack, G., Wyrick, J., & Barker, J. (2012). Significant decadal channel change 58–67 years post-dam accounting for uncertainty in topographic change detection between contour maps and point cloud models Geomorphology, Caballero, Y., Cheva, et al., 2006; Zhang et al., 2012).

Quantification of chlorophyll content in microalgae cells can be used as an approach to determine the content of microalgae in a sample of surface water. In addition to the quantification of chlorophyll content, the determination of dry weight is also a method that can be used as an approach to determine microalgae content. This research was conducted to examine the relationship between the concentration of pigment (chlorophyll a) contained in photosynthetic organisms and the dry weight of their biomass (the dry weight determination approach is based on the measurement of chlorophyll concentration), as well as the effect of the content of other suspended particles on the results of measuring pigment concentrations in determining microalgae content. This is considered to need to be studied more deeply, considering that the diversity of matrix content in surface water can affect the results of measuring the content of aquatic organisms as a bioindicator of surface water quality.

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II. Material and Method

This research was conducted on a laboratory scale using artificial samples which were a mixture of isolated algae cultures and kaolin suspension in a certain ratio. Artificial samples are research objects whose composition is made in such a way as an approach to concluding at the end of the study.

Preparation phase

This stage includes the process of culturing microalgae which is a source of chlorophyll and making artificial water samples as research objects.

a. Mixed Microalgae Culture

The microalgae culture was obtained from the results of isolation in a previous study that was conducted from peat swamp waters in Kampar Regency, Riau Province, concerning research on potential microalgae screening for carbon dioxide capture and storage. The results of this study indicate that the genus Chlorella sp., Scenedesmus sp., and Ankistrodesmus sp. is the dominant isolate that can live together in an artificial medium under controlled conditions in the peat swamp waters. Based on the results of this study, it is assumed that the isolated microalgae consortium can represent the actual conditions of the peat swamp waters in Kampar Regency so that it can be used as a research object. Each genus is grown in a separate reactor until it reaches its optimum inoculum age.

According to Rinanti, et al (2013), the optimum inoculum age for the genus Chlorella sp., Scenedesmus sp., and Ankistrodesmus sp. respectively are 2 days, 5 days, and 3 days. The medium used was PHM liquid media which had a pH of 7, the culture process was carried out at room temperature. The growth reactor is equipped with an aeration unit with an air rate of 800 ml/minute and uses a lighting source in the form of a fluorescent lamp with a light intensity of 2500 lux, light/dark light period (24 hours / 0 hours). After the optimum inoculum age is reached, each genus is harvested and stored in a refrigerator to reduce further biological reactions. After all, genera were harvested, the cultures were mixed into mixed cultures with the ratio of Chlorella sp.: Scenedesmus sp.: Ankistrodesmus sp.= 2:1:1.

b. Artificial Water Samples

Referring to previous research conducted by Lindu, et al (2010), artificial water samples were prepared by dissolving kaolin in water with concentrations of 50 mg/L, 100 mg/L, and 200 mg/L. Stirring was carried out for 3 hours, then deposited for 17 hours, so that a relatively stable turbidity value was obtained according to surface raw water with low-medium turbidity levels (10 NTU to 50 NTU). The results of turbidity measurements showed values of 15 NTU, 40 NTU, and 55 NTU. In this study, kaolin suspension was prepared with a concentration of 500 mg/L, stirred for 3 hours, and deposited for 15 hours to obtain a turbidity value of 176 NTU, which was then made several dilution concentrations for experimental purposes. The kaolin suspension that has been prepared is then mixed with mixed microalgae cultures in certain ratios according to the needs of the experiment. Preparation of artificial water samples using turbidity measurement as an approach to determining the level of dilution to obtain artificial samples with the desired concentration.

The highest turbidity levels of microalgae suspension and kaolin suspension were determined to be ±170 NTU respectively (referring to the turbidity level of kaolin suspension). The same thing was done on artificial water samples. The suspension is the main suspension which is then diluted according to the purposes of the experiment, then an analysis is carried out on the chlorophyll content and dry weight of biomass for each dilution treatment. Trial Step In this study, several experiments were carried out as an approach to get the conclusion of the study. The experiments carried out included:

a. Determination of the chlorophyll calibration curve, carried out to determine the linearity of the spectrophotometer measurement results by making several dilution concentrations of 50%, 75%, 100%, 125%, and 150% through the turbidity measurement approach.

b. The dry weight determination approach is based on the measurement of chlorophyll concentration, carried out by measuring microalgae mix suspension samples at various dilutions. The measurements carried out included measuring the concentration of chlorophyll and the dry weight of the biomass.

c. Experiment on the effect of dissolved solids content (turbidity) on the determination of chlorophyll concentration due to the interference of absorption of the light spectrum. This experiment was carried out on artificial water samples which were a mixture of microalgae suspension and kaolin suspension with a certain ratio. The measurement results are compared with the data in experiment (b) to see their significance.

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All dilution treatments used the turbidity measurement approach in determining the concentration of the dilution. The highest dilution concentration (150% dilution) refers to the turbidity of the kaolin suspension, made close to ±170 NTU turbidity. The highest dilution concentration was then made several lower dilutions, namely 50%, 75%, 100%, and 125%.

Determination of Microalgae Content

Determination of the concentration of pigment (chlorophyll a) as an approach to determine the concentration of microalgae refers to the standard method of testing water and wastewater (section 10200 H) with some adjustments to the availability of equipment and materials in the testing laboratory (Aquatic Biology Laboratory). This method includes the process of extracting pigment from microalgae cells, followed by spectrophotometric quantification of chlorophyll. The extraction process begins with a concentration of microalgae suspension through a filtration or centrifugation process. In this study, suspension concentration was carried out by centrifugation method. The concentrated microalgae suspension was then crushed with the help of a crushing stone using 90% acetone as a solvent.

The maceration process was carried out in a centrifuge at 3000 rpm for 20 minutes. The chlorophyll extract in 90% acetone was then quantified using a Thermo scientific type spectrophotometer with the trichromatic method. The absorbance readings at three wavelengths of 665 nm, 645 nm, and 630 nm were then calculated using the Strickland and Parsons empirical equation. Referring to Henriques, et al (2007) that "Axxx" is the absorbance at a wavelength of xxx nm after subtracting the absorbance of the sample at a wavelength of 750 nm to the solvent as a measurement blank. “v” is the volume of solvent used (mL), “l” is the length of the spectrophotometer cell (cm) and “V” is the volume of sample extracted (mL).

Measurement of the absorbance of samples at a wavelength of 750 nm was not carried out in the experiment, because based on the standard method of testing water and wastewater (section 10200 H) measurement of samples at that wavelength is to measure the amount of absorbance by suspended particles other than microalgae. The absorbance data needed in this study is the total absorbance data extracted by ignoring the effect of absorbance by other suspended particles because this experiment also examined the effect of the content of other suspended particles on the results of measuring chlorophyll concentrations. In addition to measuring the chlorophyll concentration, another method used in this study was the gravimetric method for determining microalgae content to calculate microalgae biomass.

III. Results and Discussion

To make it easier to conclude, the measured data is presented in the form of tables, and graphs, and statistical data processing is also carried out to determine the effect of turbidity content on the chlorophyll measurement results.

Determination of the Calibration Curve

The data in table 1 is obtained from the results of measuring the turbidity level and chlorophyll concentration at various dilutions. The data is then plotted on a graph to observe the trend of turbidity level and chlorophyll concentration in the dilution treatment of Chlorella Sp suspension.

Table 1. Results of measurements of turbidity level and chlorophyll concentration in Chlorella Sp. At various dilutions

Dilution (%)	Turbidity (NTU)	Chlorophyll-a (mg/L)
50	46.9	1,196
75	80.1	0,975
100	11.0	2,040
125	137.0	2,334
150	163.8	3,192

Dilution of the microalgae suspension was carried out using a nephelometric turbidity measurement approach. Stirring the suspension before a measurement is carried out to ensure the homogeneity of the sample whose turbidity will be measured nephelometrically.

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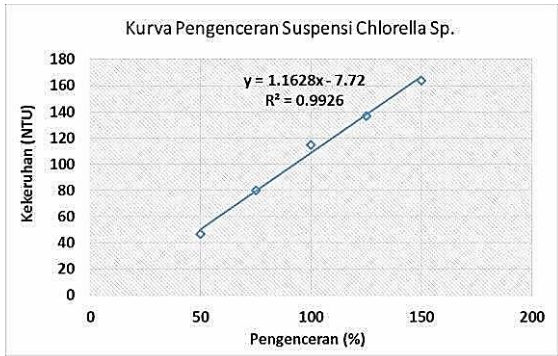

Figure 1. Linearity curve of turbidity level at various dilution concentrations

In addition, samples that have been homogenized must be measured as soon as possible to obtain a dilution curve that has a linear trend.

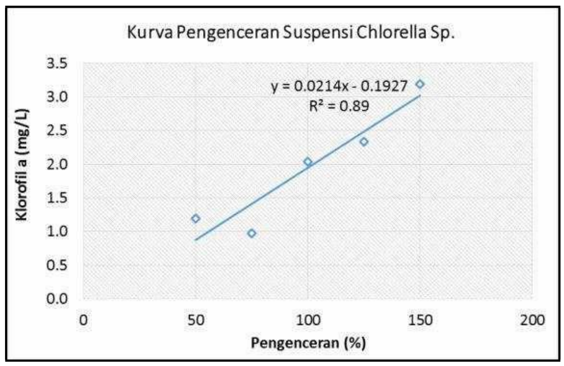

Figure 2. Chlorophyll concentration calibration curve at various dilution concentrations

The diluted sample was extracted with 90% acetone and then the absorbance was measured. The chlorophyll concentration was obtained by inserting the absorbance value from the measurement results into the Strickland and Parsons empirical equation. The calculation results are then plotted onto a graph to obtain a calibration curve to see the linearity of the results of spectrophotometric chlorophyll absorbance measurements. The sample used was a suspension of microalgae Chlorella Sp which was made up of several dilution concentrations. Based on the observations in Figure 1 and Figure 2, it can be observed that the turbidity level and chlorophyll concentration tend to follow the dilution level, so that artificial sample preparation can be carried out using a dilution approach based on turbidity measurements.

Dry weight determination is based on chlorophyll concentration

Referring to the standard methods section 10200 H which states that the concentration of photosynthetic pigments can be used as an approach to estimate the weight of phytoplankton biomass. The content of chlorophyll in microalgae is equivalent to 1% to 2% dry weight. The data in table 2 is the measurement result of turbidity level, chlorophyll concentration, and dry weight of microalgae biomass determined gravimetrically. Data on chlorophyll concentration and dry weight of biomass were then plotted on a graph to see the relationship between chlorophyll concentration and dry weight of biomass. The sample used in this treatment was a microalgae mix suspension which was a mixture of Clorella Sp., Scenedesmus Sp., and Ankistrodesmus Sp. with a ratio of 2:1:1 (treatment a).

Table 2. Microalgae Suspension Dilution Data (C:S: A = 2:1:1)

Dilution (%)	Turbidity (NTU)	Chlorophyll-a (mg/L)	Dry Weight (mg/L)
50	44,9	1,043	231,97
75	95,4	1,172	511,82
100	137,3	2,523	615,51
125150	157,3	2,680	783,11

In addition to the microalgae suspension mix, another sample measured was an artificial water sample which was a microalgae suspension mix with kaolin suspension added to it. In this treatment, 100 ml of the previously prepared microalgae suspension was added to each of the kaolin suspensions (treatment b). This is done as an approximation to surface water which in actual conditions contains a wider variety of suspended particles. Data on the measurement results in treatment a (table 2) and treatment b (table 3), were then plotted on a graph for further analysis.

Table 3. Artificial sample dilution data (microalgae mix suspension + 100 ml of kaolin suspension at each dilution

Dilution (%)	Turbidity (NTU)	Chlorophyll-a (mg/L)	Dry Weight (mg/L)
50	52,5	1,014	351,06
75	101,3	1,094	677,38
100	127,8	2,015	854,58
125	144,0	2,329	962,91
150	160,2	2,743	1071,24

Based on the measurement results and data processing in table 2 and table 3, it is known that the measured chlorophyll content is around 0.2% to 0.5% of the dry weight of the biomass. This value is lower when compared to the percentage of chlorophyll a in the standard methods section 10200 H which states that the chlorophyll content in microalgae is equivalent to 1% to 2% dry weight. This can be caused by several factors such as not optimal chlorophyll extraction process which may be caused by lack of extraction time, incomplete maceration process, or other non-technical factors that may occur in the research process. All of the above has been tried to be anticipated in the research process, namely by carrying out the chlorophyll extraction process referring to standard methods which of course have been validated, so that the validity factor of the analytical method can be ascertained not to be a problem.

In addition, each sample analyzed is treated with the same and consistent method and treatment to obtain consistent analysis data (so that consistent conclusions are obtained). In their research (Beatrice-Lindner et al., 2018; Castillo López et al., 2015; Pool et al., 2016; Zhao et al., 2013) the wet biomass concentration value of Nannochloropsis sp. of 10.98 g/L for Walne's medium, and 5.10 g/L for Guillard's medium. While the chlorophyll content extracted from dry biomass on microalgae Nannochloropsis sp. the Walne culture medium was higher than the Guillard medium with a total chlorophyll value of 353.045 µg/mL. So the best chlorophyll production is cultivated using Walne culture media (Reis Batista et al., 2015; Suriya Narayanan et al., 2018).

In Figure 3, both treatments, treatment a and treatment b, show that the chlorophyll concentration has a positive correlation with the dry weight of microalgae biomass. In this case, the two variables are not factors that influence each other, the correlation in question is that based on the graph in Figure 3 there is a relationship between chlorophyll concentration and biomass weight so chlorophyll concentration can be used as an approach in determining the dry weight of biomass based on the linear regression equation $y = 302.35x + 17.121$ for treatment a and the linear regression equation $y = 342.68x + 153.25$ for treatment b. This is indicated by the value of $R_a = 0.870$ ($R^2 = 0.757$) for treatment a and $R_b = 0.924$ ($R^2 = 0.857$) for treatment b (correlation coefficient of treatment a and treatment b), which statistically means that these two variables have a positive correlation quite tight.

According to them (Barros et al., 2015), sediment particles that float in the water and move without touching the bottom of the water are called MPT (Suspended Solid Material). The value of the MPT concentration is affected by input from river flow (Ahmed et al., 2013; Ebeling et al., 2006; Tampus, Annielyn D. Ermelinda G. Tobias, Ruben F. Amparado, 2012), and sediment resuspension, so the value MPT can affect brightness. The sediment resuspension is influenced by hydrodynamic processes in the sea such as currents and tides (Jackson et al., 2003; Suratman et al., 2014). The high MPT will affect the lack of sunlight entering the waters which causes less optimum photosynthetic processes in these waters (Bonnelye et al., 2004; Habib et al., 1997; Sui et al., 2010). The disrupted process of photosynthesis causes the primary productivity of these waters to be disrupted (Arifin et al., 2018; Mohamed et al., 2015; Othman et al., 2012). The sources of MPT in estuary waters generally come from several activities along the river flow, such as industry, recreation, and settlements (Eddiwan et al., 2020; Lacoul & Freedman, 2006; Ogle & Lotz, 2001; Usharani et al., 2010; Yogendra & Puttaiah, 2008).

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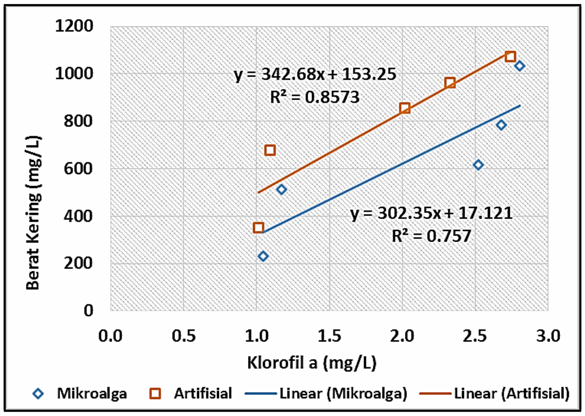

Figure 3. The relationship curve of chlorophyll a concentration with a dry weight of biomass.

Furthermore, in addition to observing the correlation between the two variables, in Figure 3 it can also be observed that the dry weight measurement results in treatment b (artificial water sample) are higher than the dry weight measurement results in treatment a (microalgae mix sample) in each dilution treatment. This clearly shows that the measurement of dry weight is strongly influenced by the content of other suspended particles in the water sample being analyzed. So it can be said that under certain conditions when there are differences in the concentration of other suspended particles in several water samples, the determination of dry weight based on the chlorophyll concentration approach can no longer be carried out. In other words, this approach can only be applied to water samples whose suspended solids content tends to be constant.

From the research results. It was reported that the many activities along the Semarang West Flood Canal River triggered the condition of the sea waters to change. The most affected condition is turbidity which causes high concentrations of Suspended Solid Material (MPT) in estuary waters and interferes with the entry of sunlight into the waters. From the results of the study, it was found that the highest concentrations of MPT, chlorophyll-a, and DIP were in the estuary area and decreased the further away the river mouth was with concentrations of 70.17-151.50 mg/L, 0.03-2.67 respectively. µg/L and 0.71-0.98 µM. Based on the results of Principal Component Analysis (AKU) showed that MPT affected chlorophyll-a by 8.7% ($r = 0.392$; $P < 0.05$) and dissolved inorganic phosphate by 54.46% ($r = 0.931$; $P < 0.01$). The low correlation between MPT and chlorophyll-a is due to the input of large amounts of inorganic particles into the body of the West Flood Canal River which makes the river waters turbid. So the turbidity of the waters causes the release of phosphate in the sediment which is utilized by phytoplankton for the photosynthesis process so that what is contained in MPT besides sediment particles is also phytoplankton.

The Effect of Other Suspended Solids Content on the Measurement of Pigment Concentration Two different types of treatment have been carried out on the experimental object to determine the extent to which the suspended solids content influences the measurement of pigment concentration. At this stage, statistical analysis was carried out on the measurement data in treatment a (dilution of the microalgae mix sample) and treatment b (dilution of artificial water samples). Statistical analysis was carried out using an independent sample T-test using SPSS 17.0 software. The principle of this statistical analysis is to compare the averages of two independent data groups (not related to each other) to see the difference based on the average difference between the two data groups. The data being compared is the measurement data for treatment a (table 2) and the measurement data for treatment b (table 3). In table 4 it can be observed the results of the statistical analysis that has been carried out in both data groups at the 95% confidence level.

According to researchers (Astill et al., 2015; Chirocentridae et al., 2006; Lan et al., 2012; Rubinoff et al., 2010), chlorophyll sources in Indonesia are very abundant, but their utilization is very minimal. One solution that can be used is the extraction of chlorophyll from microalgae, namely the extraction of chlorophyll from Nannochloropsis sp. So far, Nannochloropsis sp. is only used as biodiesel which requires high energy. Therefore the utilization of Nannochloropsis sp. in the field of extraction can add value to the use of microalgae Nannochloropsis sp. The highest average chlorophyll content was 0.000886 mg/g while the highest average chlorophyll b was 0.00067015 mg/g. The best treatment test was carried out using the Zeleny method, namely at 10 ml of acetone with a stirring time of 30 minutes.

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Table 4. Statistical test results data (independent sample T-test).

Levene's Test for Equality of Variances			Chlorophyll a	
			Equal variances assumed	Equal variances not assumed
		F	.374	
		Sig.	.471	
T-test for Equality of Means	95% Confidence Interval of the Difference	T	.400	.400
		df	8	7.880
		Sig. (2-tailed)	.700	.700
		Mean Difference	.20580	.20580
		Std. Error Difference	.51483	.51483
		Lower	-.98139	-.98453
		Upper	1.39299	1.39613

In table 4 it can be seen that the significant value in the variance test is 0.471, this value is greater than α (0.05), which means that the variances of the two data groups are identical. The significance value in the t-test is 0.700, this value is greater than α (0.05) which means there is no significant difference between the two treatments. This shows that based on the data in this experiment, statistically, the content of other suspended particles as a cause of turbidity has no significant effect on the determination of chlorophyll concentration by spectrophotometry. It is necessary to carry out further research on this issue, given the varying content of suspended particles in water. In this study, kaolin, which is assumed to be suspended particles other than microalgae, can be said to not release pigments which could interfere with the results of determining chlorophyll concentrations. On the other hand, if other suspended particles are found that can release pigment when the extraction process is carried out with organic solvents, this will certainly greatly affect the results of chlorophyll measurements.

IV. Conclusion

Based on the results of the experiments conducted in this study, it can be concluded that there is a fairly close positive correlation between chlorophyll concentration and the dry weight of microalgae biomass, the tendency of which is to follow the linear regression equation $y = 302.35x + 17.121$. Determination of dry weight based on the chlorophyll concentration measurement approach can be applied to water samples with suspended solids content that tends to be constant and inert (does not produce substances that can react with organic solvents during the chlorophyll extraction process). In addition, based on the results of data processing, it can also be concluded that the effect of the content of other suspended particles in the water sample does not show a significant effect (statistically). Further research is needed regarding the effect of the content of other suspended particles in water samples in determining the concentration of chlorophyll contained in microalgae.

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