International Journal of Electrical and Computer Engineering (IJECE)
Vol. 14, No. 6, December 2024, pp. 6248~6257
ISSN: 2088-8708, DOI: 10.11591/ijece.v14i6.pp6248-6257

Algae content estimation utilizing optical density and image processing method

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Article Info

Article history:

Received Jan 5, 2024
Revised Jul 11, 2024
Accepted Jul 17, 2024

Keywords:

Algae
Image processing
Non-invasive
Optical density
RGB analysis

ABSTRACT

One of the factors that influence shrimp cultivation is the presence of algae. Precise knowing algae content in the pond is essential for effective management. Most research in the field of algae species carried out by researchers were observing *Chlorella Sp.* more than the other algae species, with a particular emphasis on substance concentrations. This study proposed non-invasive techniques for quantifying algae abundance, utilizing optical density (OD) and image processing (IP) methods. Three different algae species are frequently found in Indonesia i.e., *Chlorella Sp.*, *Thalassiosira Sp.*, and *Skeletonema Sp.* are used as sample. Those samples are cultured and prepared in a certain volume with a certain quantity. For experimental and observation purposes, those samples are then diluted into water based on percentage value. The experimental results provided RGB values, which were then used to establish polynomial equations. To verify these equations, two approaches were employed: synthetic image analysis and evaluation using additional data. The mean average error (MAE) was found to be 3.467 for IP method and 3.513 for OD method. It shows that IP method give better result compared to OD method in this study. However, it is very possible that the two methods will complement each other.

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1. INTRODUCTION

Shrimp is one of the aquaculture commodities which is currently experiencing an increase in production due to the high market demand. One of the important factors in the shrimp farming process is the water quality of the cultivation [1], [2]. Water quality problems often have a negative impact on shrimp, especially the cause of various diseases [3] and the possibility of harvest failure [4]. Water quality usually associated to chemical and physical parameters which is affecting shrimp growth, such as temperature, salinity, pH, and dissolved oxygen [5]. In another case, water quality is also associated with the presence of algae (good algae and bad algae) in the environment [6]. In the growth ecosystem, algae role as natural food for shrimp in addition to artificial feeding [7]. Algal metabolism will affect the levels of nitrite, nitrate phosphate and ammonia in the pond which maintains the pH value of the water and increases dissolved oxygen in the water. This condition typically enhances the growth performance of shrimp [8]. However, the presence of harmful algae [9] or an overpopulation of beneficial algae [10], [11] in the pond can negatively

impact the pond environment, leading to shrimp diseases and potential crop failure. Therefore, monitoring algae levels is crucial.

Basically, microalgae will affect the color of the water in the pond, making it possible to measure algae content based on the color of the water [12]. In cultures of bacteria and other unicellular microorganisms, optical density (OD), also known as absorbance or turbidity, is frequently used as a quick and non-destructive measurement of biomass [13], [14]. The mass or number of cells in a suspension can directly affect how much light is absorbed by the suspension. Image processing (IP) is used by adopting the OD method. The image analysis technique used color, specifically the red-green-blue (RGB) and grey models, allows for the discovery of a direct relationship between algal concentration and its RGB value [15]–[17]. Measurements and machine learning are frequently combined in their implementation.

Some related research on the same topic has been done. For instance, Jia *et al.* [18] utilized a multiwavelength laser diode monitor to measure cell density in microalgae. They employed wavelengths of 650 and 685 nm to estimate chlorophyll content and cell concentration, while a wavelength of 780 nm was used for turbidity measurements. The tested species were *Chlorella Sorokiniana* and *Scenedesmus Obliquus*. The findings indicated that measurements using optical density were good indicators for monitoring transitions of microalgae growth and detecting disturbances within the culture system. Metsoviti *et al.* [19], employed OD method using 2 wavelengths 420 to 520 nm and 580 to 680 nm to measure the amount of *Chlorella vulgaris* which is cultivated by irradiating white and red light emitting diode (LED). The result of OD measurement can be used to determine the rate of growth and the composition of biomass, protein, and lipids. Salgueiro *et al.* [20] applied digital IP method to establish a relationship between dry mass weight and light intensity of *Chlorella vulgaris*, to quantify its levels during growth process in a photobioreactor using RGB and greyscale analysis.

OD and IP have been used in previous studies by different researchers to estimate algae densities under varied circumstances. Moreover, there exists a significant deficiency in studies that precisely quantify the types of algae present in shrimp pond environments, particularly in Indonesia. The objective of this study is to compare non-invasive techniques i.e., optical density and image processing to estimate the biomass of algae. Due to limited information on *Skeletonema Sp.*, and *Thalassiosira Sp.*, this study will focus on these species, along with *Chlorella Sp.*, which is commonly found in Indonesia. This study is carried out under the same environmental conditions. During this phase of the study, algae specimens are sourced from laboratory cultures rather than directly from a pond. Each species will be diluted in pure water to create samples with varying concentrations, ranging from 0% to 100%, increasing by 20% increments for individual-species samples. In the case of mixed samples, the pure water will be replaced by other blended samples. The subsequent sections are structured as follows: section 2 provides insight into the prototyping, methods used, and experimental procedures. In section 3, we analyze the measurement results and the shape of the trend in the equations. Section 4 contains a discussion on the review of the measurement results. Finally, section 5 concludes the result of this study.

2. METHOD

This research builds upon the previous study [21]. In the research, a new portable measurement system with a new setup was developed, and a specific quartz cuvette cell unit Class A container was used instead of an acrylic container. The flow system diagram can be seen in Figure 1(a) and prototype is shown in Figure 1(b).

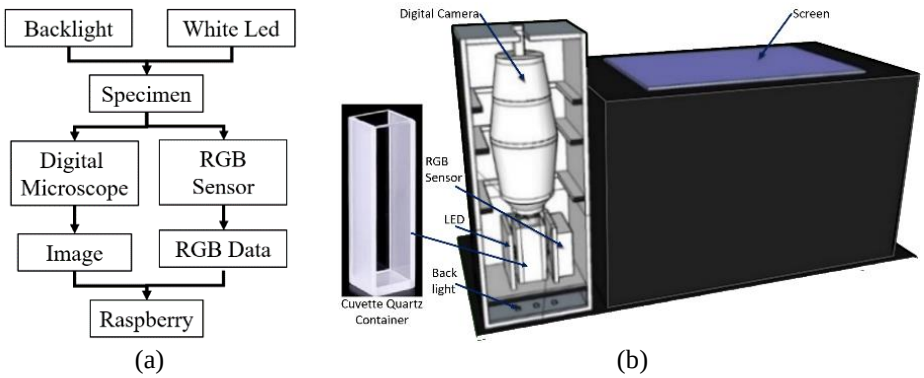


Figure 1. System design (a) flow diagram and (b) prototype

2.1. Optical density

In the OD measurement, the sample will be placed between the white LED and the RGB sensor. Light sources use a certain wavelength because algae have different color and amount of chlorophyll [22]. Since 3 different types of algae are used as samples, utilizing white LED will give an appropriate way to measure. Even though white LED will affect algae growth, however, the algae growth will not significantly change the amounts of algae in a relatively short measurement time [23]. After passing the sample, intensity values of white LED will be measured in the RGB color space using the TCS74325 sensor. The RGB sensor response will be recorded by a microcontroller and transmitted to a Raspberry Pi. The sensor response in the form of intensity is taken based on the Lambert-Beer law [24] according to the (1):

$$netOD = -log_{10} \frac{I}{I_0} \quad (1)$$

where $netOD$ is the intensity of the optical density, I is the intensity that captured on the sensor when the sample is not water (in this case algae) and I_0 is when the sample is water. For this experiment, I and I_0 have ranged between 0-255 according to the standard color scale in the RGB color space [25]. To simplify the comparison, only the I value will be compared for each channel so that the optical density equation becomes (2):

$$netOD_{(R,G,B)} = I_{(R,G,B)} \quad (2)$$

where $netOD_{(R,G,B)}$ is OD from 3 different channel and $I_{(R,G,B)}$ is captured RGB intensity on the sensor.

2.2. Image processing

The second method, i.e. IP, samples are placed between a backlight and digital camera. Since the measurement space is a dark space, a white backlight is needed to emphasize intensity captured by camera from the top of the space. Then, IP is used to extract color features [26] using a color histogram [27]. However, if all the image resolution data is considered, the value of the histogram will be too large. To simplify it, a simplification process is carried out by utilizing the mean statistical methods [28], based on (3), (4) and (5):

$$mean\_red = \frac{\Sigma_{i=1}^{n} R_i}{n} \quad (3)$$

$$mean\_green = \frac{\Sigma_{i=1}^{n} G_i}{n} \quad (4)$$

$$mean\_blue = \frac{\Sigma_{i=1}^{n} B_i}{n} \quad (5)$$

where R_i, G_i, B_i are the R, G, B value of the i pixel. While $\Sigma_{i=1}^{n} R_i$, $\Sigma_{i=1}^{n} G_i$, $\Sigma_{i=1}^{n} B_i$ the sums of the R, G, B values of all pixels in the image and n is the total number of pixels in the image. The image is reduced to a single mean intensity value through alternate measurement processes that minimize noise.

2.3. Algae culture and measurement procedure

The samples, *Chlorella Sp.*, *Thalassiosira Sp.*, and *Skeletonema Sp.* are obtained from Balai Besar Perikanan Budidaya Air Payau (BBPBAP) Jepara, and thus prepared and cultured by Fisheries Pathology Department of Politeknik Kelautan dan Perikanan Sidoarjo with cell contents of *Chlorella Sp.* is 710×10^4 cells/ml, *Skeletonema Sp.* is 43×10^4 cells/ml and 216×10^4 cells/ml for *Thalassiosira Sp.* as shown in Figure 2. Each sample will be measured in a 4 ml cuvette utilizing various concentrations (0% to 100%). This cuvette has 2 clear glasses and 2 opaque glasses on its side, with dimension $w \times l \times h$ is 12.5×12.5×45 mm. The measuring equipment and materials were sterilized using an autoclave to eliminate any microorganisms that could disrupt the culture activities.

The sample inside cuvette container is placed into the measuring chamber. For OD method, the white LED and RGB sensor are placed on both right and left side facing clear glass. The sample is then illuminated by white light, which on the other side RGB sensor is placed to record the optical density response, reflecting the sample's characteristics. Similarly, in the IP method, backlight illuminated the sample from bottom side of the sample, and the response is captured by a digital microscope that is placed on the top side of the sample. Composition of sample is shown on Table 1.

Figure 2. Algae samples

Table 1. Sample composition between algae and water (H_2O)

Concentration (%)	Composition
0	4.0 ml water
20	3.2 ml water + 0.8 ml algae
40	2.4 ml water + 1.6 ml algae
60	1.6 ml water + 2.4 ml algae
80	0.8 ml water + 3.2 ml algae
100	4.0 ml algae

2.4. Algae sample phycology

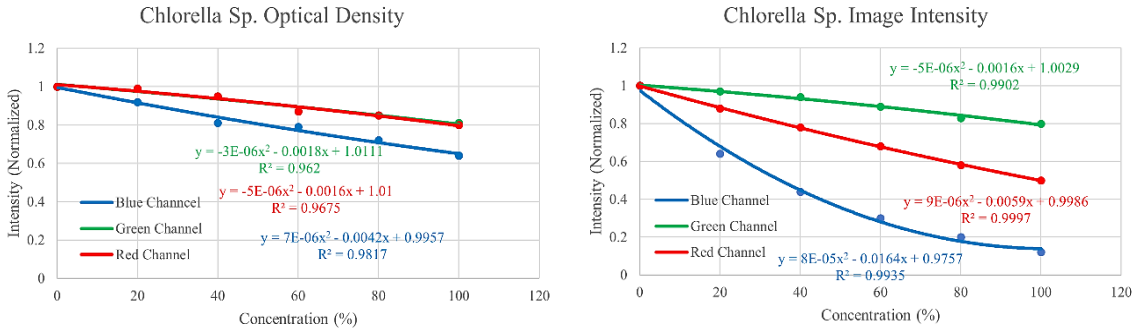
Before conducting experiments on algae samples, it is essential to understand what causes differences in their colors. Pigments are one of the fundamental properties responsible for variations in color in living organisms, especially in photosynthetic algae [29]. With this foundation, we aim to examine the characteristics of the test species, particularly in terms of pigments. *Chlorella Sp.* belongs to the green algae family. Naturally appears green, with a predominant content of chlorophyll a and b (in a 3:1 ratio) [30] and lower levels of carotenoids compared to other types of green algae. Chlorophyll a tends to give a blue-green color, while chlorophyll b imparts a blue-yellow hue [31]. Lutein is the most abundant carotenoid in *Chlorella Sp.* [32], giving it a natural yellow color [33], although not as pronounced as chlorophyll. In contrast to *Chlorella Sp.*, *Skeletonema Sp.* belongs to the diatom family and is primarily characterized by its chlorophyll and the carotenoid fucoxanthin [34]. Chlorophyll remains the predominant component [35], but due to the morphological structure of diatoms, the fucoxanthin pigment covers the chlorophyll, resulting in a dominant brownish-yellow coloration [36]. Like *Skeletonema Sp.*, *Thalassiosira Sp.* is a group of diatoms with the same primary components. The basis for categorizing *Skeletonema Sp.* and *Thalassiosira Sp.* lies in their morphological shape and size, where the cell size of *Skeletonema Sp.* is larger than that of *Thalassiosira Sp.* [37], [38]. Additionally, there is a difference in the content of the fucoxanthin pigment, with more of this pigment present in *Thalassiosira Sp.* compared to *Skeletonema Sp.* [39], [40], resulting in its physical appearance being more brownish in color compared to *Skeletonema Sp.*

3. RESULTS AND DISCUSSION

This section provides discussion based on the measurement results. The measurement results for *Chlorella Sp.* are shown in Figure 3, with OD displayed in Figure 3(a) and IP in Figure 3(b). Figure 4 presents the measurement results for *Skeletonema Sp.*, with OD results in Figure 4(a) and IP results in Figure 4(b). Figures 5(a) and 5(b) display the OD and IP measurements for *Thalassiosira Sp.*, respectively. Figure 6(a) shows the OD measurement results, while Figure 6(b) shows the IP measurement results for a mixed specimen of *Chlorella Sp.* and *Thalassiosira Sp.* Some studies [18]–[20] were focused on measuring the growth rate of algae species to produce microalgae products for the industry. Typically, linear estimation models were used [18], [20] whereas in this study, a polynomial model was employed, offering a more flexible curve shape for estimation purposes. Additionally, this study compares the performance of optical density methods [18], [19] and image processing [20] for estimating algae content using mean absolute error (MAE) values.

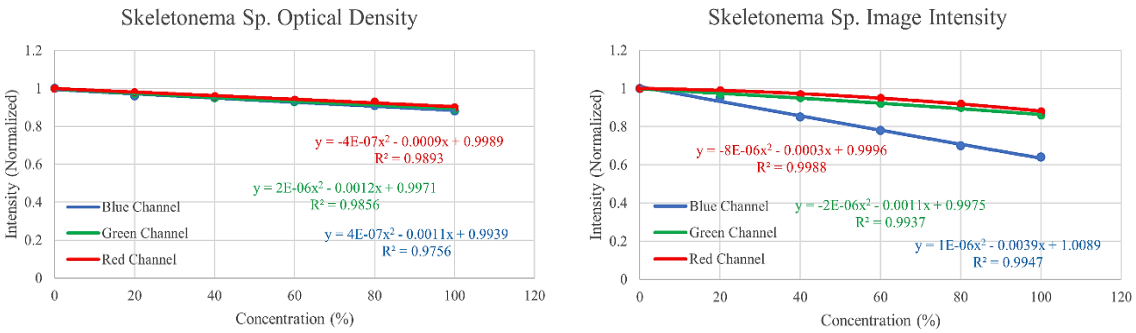
The measurement results of *Chlorella Sp.* as shown in Figure 3 exhibit a similar trend, with the dominant channel consistently appearing in the green channel, followed by red and then blue one. However, the gaps between each channel from OD method show narrower compared to the result from IP method. In terms of physical appearance, *Chlorella Sp.* exhibits a prominent dark green color, as discussed in the previous section and shown in Figure 2. The trend of its intensity tends to decrease as the concentration increases across all measurement methods. This is due to increment of its opacity, leading to the sensor absorption diminishing with rising concentration. A similar outcome for *Chlorella Sp.* measurement was also observed in the studies by

Sarrafzadeh *et al.* [15] and Salgueiro *et al.* [20]. Nonetheless, there exist distinctions in gradient variations between our findings and the studies conducted by Sarrafzadeh *et al.* [15] and Salgueiro *et al.* [20], which can be attributed to variations in the geographic characteristics of the algae samples [41] as well as differences in the sample densities used. Results for *Skeletonema Sp.* are presented in Figure 4. It shows the red channel being the most dominant, followed by green, and then blue for both OD and IP methods. The gaps between each channel are wider as the concentration of algae increases. The gap between the red and green channel is much narrower compared to the gap between green and blue channel. The dominance of the red and green channels indicates a brown color, which is in accordance with the phycology of *Skeletonema Sp.* compared to *Chlorella Sp.*, the red channel is more dominant than green channel, while blue channel is less dominant. This is because *Skeletonema Sp.* is known for its physically transparent yellow appearance, primarily due to the presence of carotene pigment (Fucoxanthin) in the chloroplast [42]–[45] as clearly shown in Figure 2.



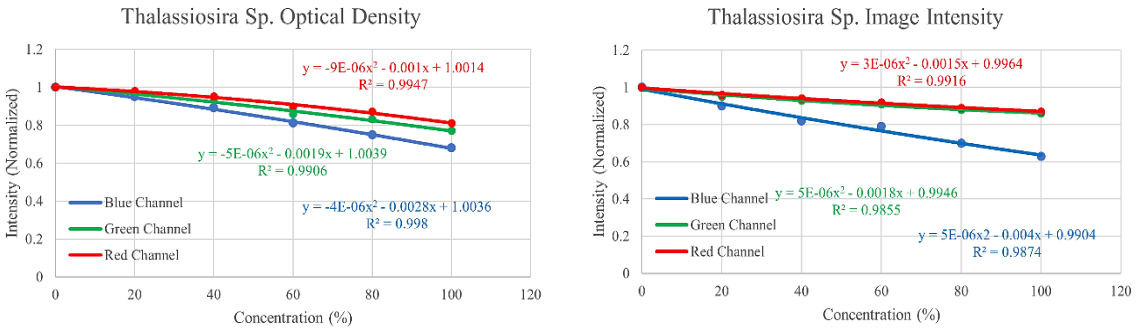
(a) (b)

Figure 3. *Chlorella Sp.* measurement result (a) OD and (b) IP



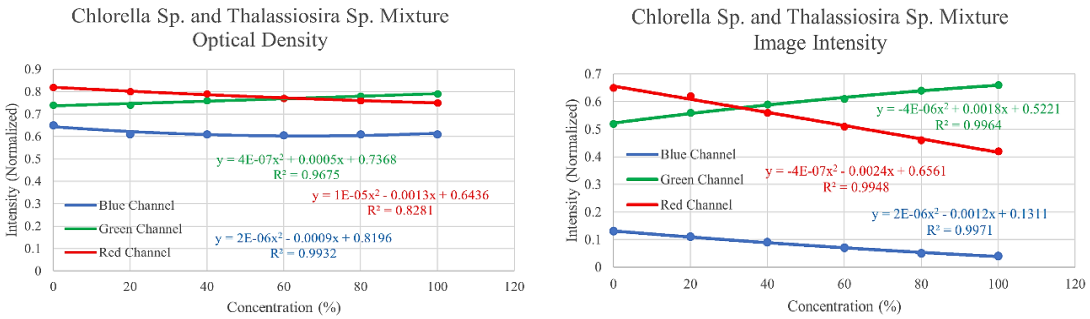
(a) (b)

Figure 4. *Skeletonema Sp.* measurement result (a) OD and (b) IP



(a) (b)

Figure 5. *Thalassiosira Sp.* measurement result (a) OD and (b) IP



(a) (b)

Figure 6. *Chlorella Sp.* and *Thalassiosira Sp.* mixture measurement result (a) OD and (b) IP

Figure 5 shows *Thalassiosira Sp.* measurement results, which is like *Skeletonema Sp.* measurement results, where the red channel being the most dominant, followed by green, and then blue for both OD and IP methods. However, the slope of the graph from *Thalassiosira Sp.* measurement shows steeper than *Skeletonema Sp.* measurement results. The dominance of the red and green channels indicates that *Thalassiosira Sp.* is shown by brown color, as illustrated in Figure 2. This closely resembles *Skeletonema Sp.*; however, *Thalassiosira Sp.* tends to have a more pronounced brown hue due to its higher carotene levels when compared to *Skeletonema Sp.* [39], [40], [46].

The measurements of the mixed sample are depicted in Figure 6. The graphical profiles indicate a decrease in the concentration of *Thalassiosira Sp.*, starting from 100% and decreasing to 0%, while the concentration of *Chlorella Sp.* shows the opposite trend, increasing from 0% to 100%. As shown in the graph, the green and red curves intersect at 60% for OD method and 35% for IP method, which ideally should occur at 50%. This difference may be due to the varying cell content between *Chlorella Sp.* and *Thalassiosira Sp.*, as already mentioned in section 2.2.

Based on those measurements, several data based on percentage of sample and equations are obtained as shown in Figures 3, 4, and 5. Utilizing data based on percentage of sample, we may construct the synthetic colors obtained from the measurement result to evaluate both OD and IP methods. Since we normalized maximum data, i.e., 255 into 1, then it is needed to bring back the number from 1 into 255 for all red, green, and blue channel. If we take the case of *Chlorella Sp.*, based on Figure 3, for OD method, 0 % is obtained by 1×255 for red, green, and blue channel. 20 % is obtained by 0.98×255 for red channel, 0.972×255 for green channel and 0.92×255 for blue channel, and so on. By doing a similar way, synthetic colors from IP method are obtained. Table 2 shows the synthetic image based on OD and IP methods. From this table, synthetic image from IP method is close to the real image of *Chlorella Sp.* sample shown in Figure 2 compared to that from OD method. By doing same manner for *Skeletonema Sp.* and Thalassiosira Sp, IP method is close to the real image of *Skeletonema Sp.* and *Thalassiosira Sp.* compared to that from OD method.

Second evaluation, we utilize the equations shown in Figures 3, 4, and 5. For this purpose, we took the case of *Chlorella Sp.* The equations shown in Figure 3 are inverted to produce new equations, (3) to (8).

$$YCH_{ROD} = -260.24\,CH_{ROD}{}^{2} + 22.143\,CH_{ROD} + 246.8 \quad (6)$$

$$YCH_{GOD} = -25.95\,CH_{GOD}{}^{2} - 416.3\,CH_{GOD} + 451.23 \quad (7)$$

$$YCH_{BOD} = 151.33\,CH_{BOD}{}^{2} - 532.07\,CH_{BOD} + 380.49 \quad (8)$$

$$YCH_{RIP} = 70.509\,CH_{RIP}{}^{2} - 304.88\,CH_{RIP} + 234.27 \quad (9)$$

$$YCH_{GIP} = -698.35\,CH_{GIP}{}^{2} + 786.03\,CH_{GIP} - 85.738 \quad (10)$$

$$YCH_{BIP} = 122.48\,CH_{BIP}{}^{2} - 247.46\,CH_{BIP} + 125.68 \quad (11)$$

where (6), (7), and (8) are used for calculating *Chlorella Sp.* concentration based on OD methods for red, green, and blue channel respectively. While (9), (10), and (11) are used for calculating *Chlorella Sp.* concentration based on IP method for red, green, and blue channel respectively. YCH is the output based on concentration (%), while CH is the intensity, based on red, green, and blue channel. For instance, if we aim to determine the *Chlorella Sp.* concentration through the optical density method, we can utilize (6) for red

channel, (7) for green channel and (8) for blue channel. The output of those equations will show concentration base on red channel noted by YCH_{ROD}, green channel noted by $YCH_{GOD,}$ and blue channel noted by YCH_{BOD}. The concentration of *Chlorella Sp.*, then is obtained by summing $YCH_{ROD}+YCH_{GOD}+YCH_{BOD}$ divided by (3). The mean values across these three channels are then calculated to obtain the MAE. MAE values for *Chlorella Sp.* are detailed in Table 3. To test the accuracy of the (3) to (8), another concentration, i.e. 10% and 50% are used for observation under the same system. The smallest error value is observed in (7), with a testing data of 10 and an error value of 1.02. On the other hand, the largest error value is found in (8), with testing data 10 and an error value of 5.601. MAE from OD model is 3.513 while the IP model is 3.467. MAE across all models is 3.489. It shows that the image processing method produces a smaller average error compared to the optical density method.

Despite the findings, this study solely discusses algae levels based on their concentration, without exploring calculations per cell or dry mass, which require specialized equipment. Moreover, this study also faces challenges with mixed-species specimens, although it has been successfully carried out. Further investigation and analysis are necessary to accurately determine the quantity of algae content, not solely relying on its color levels. Our research highlights that both methods are applicable solely for estimating algae levels. However, this is restricted to just three algae species. Future research could broaden the range of algae specimens for analysis. Furthermore, the estimation model is limited to polynomial models. In the future, incorporating other regression models or comparing them with machine learning estimation models may be possible. Moreover, alternative measurement techniques such as spectral or spectrophotometry measurements could be considered. In conclusion, it can be inferred that the application of IM and IP can determine algae levels influenced by their color intensity, consistent with a revaluation using synthesized images.

Table 2. Synthetic image comparison from chlorella measurement

Concentration (%)	OD synthetic image	IP synthetic image
0		
20		
40		
60		
80		
100		

Table 3. *Chlorella Sp.* MAE polynomial equation

Equation	AC (%)	PC (%)	AE	MAE	
(1)	10	8.703	1.297		
	50	55.934	5.934		
(2)	10	8.98	1.02	MAE_{OD}=3.513	
	50	55.540	5.540		
(3)	10	4.398	5.601		
	50	51.685	1.685		MAE_{OV}=3.489
(4)	10	8.268	1.731		
	50	55.403	5.403		
(5)	10	7.978	2.021	MAE_{IP}=3.467	
	50	46.326	3.673		
(6)	10	6.0992	3.900		
	50	54.0728	4.072		

Note: AC - actual concentration; PC - predicted concentration; AE - absolute error; MAE - mean absolute error MAE_{OD} - mean absolute error OD; MAE_{IP} - mean absolute error IP; MAE_{OV} - mean absolute error whole model.

4. CONCLUSION

This manuscript proposed portable measurement instrument to predict algae content. Based on the result of this study, it is found that OD measurements and IP can be used to measure algae, without the need

for traditional laboratory tests such as dry mass measurements. From the measurements, a polynomial model curve was successfully obtained that reflects the relationship between the concentration of algae and the channel values of R, G and B based on OD and IP methods. The result of synthetic image shows that IP method provide better result compared to OD method. Furthermore, the result of the polynomial equation also shows that IP method provide better result compared to OD method. It shows that MAE are 3.467 for IP method and 3.513 for OD method. It's conceivable that the two methods may offer complementary insights, but in this instance, the IP method proves superior in accurately depicting the measured algae compared to the OD method. This prototype is portable, allows to measure samples outside of laboratory, to save costs and time. However, the results may not be as accurate as measurements on dry mass. For better improvement, some study may be done in the future research such kinds of color spaces, utilizing machine learning, expand dataset, or combine with alternative measurement methods such as spectral analysis to enrich the data.

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