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Measurement of algae concentration by using Color identification

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Abstract

The measurement of algae concentration in water plays a crucial role in environmental monitoring and assessment. Traditionally, this quantification has been accomplished using methods such as gravimetric analysis or spectrophotometry. However, in recent years, innovative approaches have emerged to enhance the accuracy, efficiency, and cost-effectiveness of this process.One such advancement is the utilization of RGB (Red-Green-Blue) light absorption as a means to estimate the concentration of algae. The RGB color model, a fundamental representation of colors, involves the blending of three primary light beams to produce a wide spectrum of colors. Building upon this principle, Benavides et al. (2015) conducted pioneering research that demonstrated the potential of RGB light absorption as a viable method for approximating algae concentration. In their study, Benavides et al. established a correlation between the component weights of the RGB color space and the presence of algae in water. This correlation was particularly evident when employing the YCbCr color space representation, which facilitated a quantitative link between the RGB values and algae concentration. This innovative approach hinged on the selection of a suitable RGB light sensor. The researchers opted for the Arduino KY-016 3-color LED module, a cost-effective solution that seamlessly integrated an RGB LED and a light-to-voltage converter, based on the Arduino UNO R3 platform.The implementation of this low-cost device opened up new avenues for estimating algae concentration with remarkable accuracy. By leveraging the RGB light sensor's capabilities, the researchers were able to establish a practical framework for estimating algae concentration that holds potential for widespread application. This innovation carries significant implications for environmental monitoring, as it offers a streamlined and accessible method for assessing water quality and potential ecological imbalances caused by excessive algal growth. In conclusion, the research conducted by Benavides et al. showcases a paradigm shift in algae concentration measurement. Through the inventive integration of RGB light absorption and the YCbCr color space representation, coupled with the utilization of the Arduino KY-016 3-color LED module, the study introduces a novel, cost-effective approach to estimate algae concentration in water bodies. From the experiment data there is a tight exponential correlation between correlation between the RGB (-Ln(I/Io)) and the biomass concentration (mg/L) of y = 15.153e0.4813x with a Pearson's correlation coefficient R2=0.973. This breakthrough holds promise for revolutionizing environmental monitoring practices, enabling more efficient and widespread assessment of aquatic ecosystems and contributing to the preservation of water resources. Keywords: Algae concentration, RGB light absorption, Environmental monitoring

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INTRODUCTION

Conventional methods for measuring algae encompass, direct counting utilizing microscopy (Hobbie, Daley, and Jasper 1977), spectrophotometry (Helena et al. 2011), protein concentration assessment (D'Souza and Kelly 2000)) (Rausch 1981), or fluorescence-based techniques (Hobbie, Daley, and Jasper 1977). However, these techniques often suffer from drawbacks such as time-intensive procedures, destructiveness when reliant on sampling, and notable expenses in terms of procurement and maintenance costs. More recent advancements have introduced alternatives like photo-electrochemical sensors(Frisulli et al. 2023, Meireles et al. 2008, Sahle et al. 2019,Jeon et al. 2013) and the quantification of O2 generation (Konstantin Bloch 2011), Sample dry weight measurement (Li and Mira De Orduña 2010) and O2 generation assessment (G. Cogne1,∗, Ch. Lasseur2, J.-F. Cornet1 2014) have also gained attention.

Notably, Benavides et al.'s (2015) study uncovered the potential of RGB light absorption as a means to estimate algae concentration, offering a promising avenue for improved measurement techniques. Given this context, there is a clear impetus to develop cost-effective sensors suitable for online biomass monitoring. This study seeks to validate the feasibility of a sensor founded on an RGB color generator and a light sensor to discern surface color. Similar applications have already employed RGB sensors, such as gauging wine or oil color through optical fibers, exhibiting satisfactory outcomes through economical implementations.

The paper is structured as follows: the subsequent section introduces the materials and methods deployed in this study. The data captured by the RGB sensor is correlated with biomass concentration (Miguel A. Pérez 2010). The proposed sensor's performance is juxtaposed with a commercial optical online sensor, both concurrently operating in the same batch culture within a photo-bioreactor for spirulina platensis algae. The potential utility of the RGB sensor in monitoring and control is further demonstrated by crafting a software sensor for biomass concentrations.

The Mechanism of Cell Destruction by Ultrasonic Cavitation

Ultrasonification of high intensity creates cavitation that could generate more damage to the cells, extracellular surface becomes paler and rough. Gelatinous sheath became more uneven, and there were various fragmented debris. Cell interior becomes black. All of thylakoids and bubbles will be broken, dissolved and disappeared. Cells which is present as partial block or homogenized structure. Some algal cells become pyknotic , but most cell died. The main mechanism of high intensity ultrasound is the cavitation effect, undergone by mechanical effect simultaneously (Fan et al. 2014).

The difference in the nature of the products formed at differrent frequencies is associated with the relative life time of the cavitation bubbles and the radicals. Cavitation micro-bubbles which are created in high-frequency fields have longer life time. The ultrasonic oxidative chemical events are located in an area where the maximum exchange between the air and gaseous atmosphere and the solvent occurs, and that is not always where maximum cavitation is located.

The combination of two simultaneous ultrasonic frequencies (20 kHz and 1 MHz) in a ultrasonic design, showed a synergy interaction of the cavitation bubbles. This increased the cavitation bubbles. The synergizing design, was named ''Orthoreactor", two ultrasonic fields (20 kHz and 1.7 MHz) were placed orthogonally (Gogate, Tayal, and Pandit 2006).

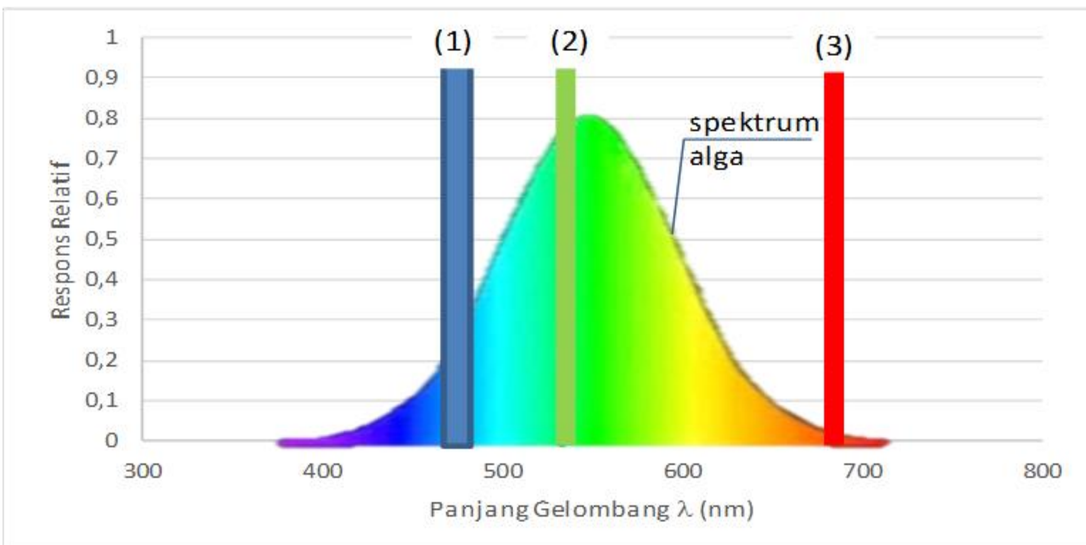
In Spirulina after ultrasonic irradiation, part filaments fractured (Hao et al. 2004), it means that damaged cells prevent further proliferation.

Maesuring The Algae Reduction

RGB sensors is another alternative for determining the algae concentration based on spectrophotometry measurement. For the past decade RGB sensor have already been used in several related applications, such as the measurement of wine, oil and coffee, and it is showing satisfactory results with a low cost implementation (Benavides et al. 2015).

This sensor range of detection is in the visible light spectrum, with a peak is at 780 nm, with the detail of the RGB colors. The wave length of red is 580 nm, green is 540 nm and for blue is 450 nm. Comparison to the typical wave length of light distribution and RGB can be seen in figure 1.

Figure 1. typical wave length of light distribution and RGB



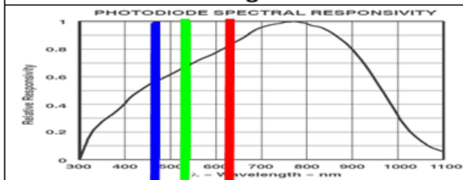
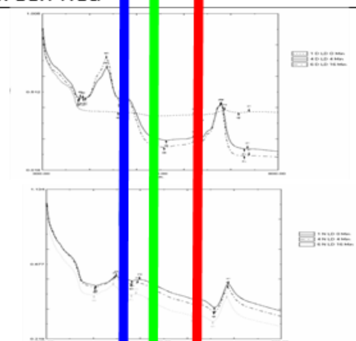
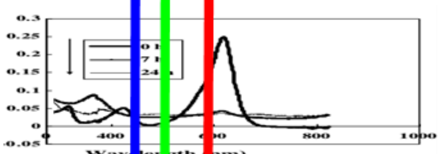
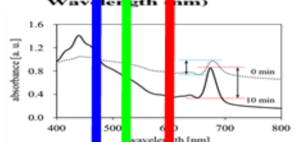
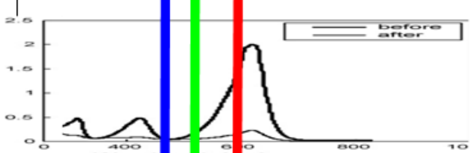
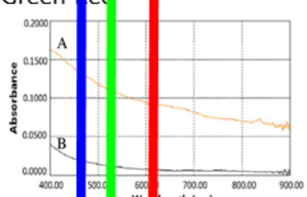
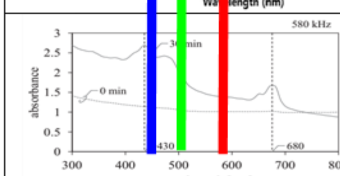
Based on several studies for algae reduction, the absorption of colors by different types of algae are different. Table 1 shows that most algae absorbs green and red color at various concentration.The algae that absorbs green and red color are:

- Dunnaliella salina sp/Chloropyta and Nannochloropsis sp oculata ➔ treated by Ultrasonic 20 KHz (Nowotarski et al. 2012).
- Chaetoceros gracilis sp ➔treated by Ultrasonic 4300 KHz (Kurokawa et al. 2016)
- Cosmarium sp ➔ Biological decolorization (Daneshvar et al. 2007)
- Chlorella sp ➔Biological decolorization (Daneshvar et al. 2007)

The algae that absorbs blue, green and red color are:

- chlorophyll-a ➔Magnetic Coagulant (Liu et al. 2013)
- Chlamydomons ➔Ultrasonic 20 KHz (Yamamoto et al. 2015)

Table 1. Characteristic Of RGB Absorb at Various Concentration

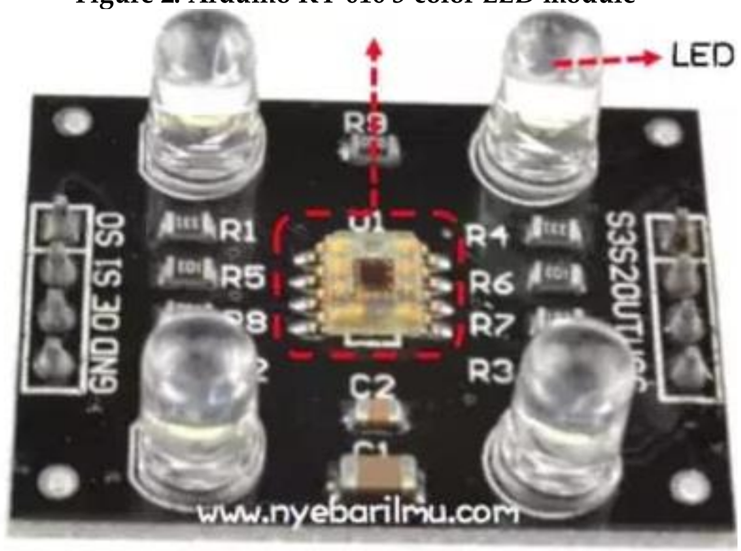
Wave Length distribution	Reference	Species	Treatment
	(Benavides et al. 2015)		
Green-Red			
	(Nowotarski et al. 2012)	Dunnaliella salina sp/ Chloropyta absorbs Green-Red Nannochloropsis sp oculata absorbs Green-Red	Ultrasonic 20 KHz
	(Daneshvar, Ayazloo, et al. 2007)	Cosmarium sp absorbs Red-Green	Biological decolorization
	(Kurokawa et al. 2016)	Chaetoceros gracilis sp Sensitive to Red-Green	Ultrasonic 4300 KHz
	(Daneshvar, Khataee, et al. 2007)	Chlorella sp Sensitive to Red-Green	Biological decolorization
Blue-Green-Red			
	(Liu et al. 2013)	chlorophyll-a absorbs Blue-Green-Red	Magnetic Coagulant
	(Yamamoto et al. 2015)	Chlamydomons Concordia sp absorbs Blue-Green-Red	Ultrasonic 20 KHz

In liquid media, light reflection produce low quality color detection. To improve this condition, a mirror is installed on the opposite side of the sample, this could enhance the reflected light of the low and medium turbidity.

RGB, consist of three light beams (Red, Green and Blue) for a certain color it must be superimposed. Each of the three beams represent a component of that color, and each of them can have an arbitrary intensity, from fully off to fully on, in the mixture.

The RGB color model is additive in term that the three light beams are added together, and their light spectra add, superimposed wavelength for wavelength, to make the final color's spectrum. Components are weights in R, G and B as a color space representation in YCbCr (International Telecommunication Union 2002).

Figure 2. Arduino KY-016 3-color LED module



The Arduino KY-016 3-color LED module (see figure 2) as a color detection device usually are used for solid surfaces color detection, the light will reflect back in accordance to the surface color.

$$I_n=\alpha_R.R+ \alpha_G.G+ \alpha_B.B \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

Where I is the light intensity

$$0<.R<255, 0<G<255, 0<B<255 \ldots\ldots\ldots\ldots\ldots 2$$

$$\alpha_B+ \alpha_G+ \alpha_R=1$$

The absorbance (ABS_{RGB}) of this medium can be estimated using the following Beer–Lambert law (Hardesty and Attili 2010):

$$ABS_{RGB} = -ln\left(\frac{I}{I_0}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

Theoretically the biomass concentration can be linearly correlated to the absorbance of the RGB intensity:

$$Biomass[mg/L] = ABS_G \cdot m + b \ldots\ldots\ldots 4$$

Or

$$Biomass[mg/L] = m.e^{ABSG} \ldots\ldots\ldots 5$$

Where *m* and *b* are the parameters obtained from the highest correlation coeficient. Therefor by using equation 4 the initial light absorbance is:

Considering that the dominant color is green, the correlation focuses only on the Green channel when assessing light intensity. Therefore, following equation 4, the color composition becomes αR = 0, αG = 1, αB = 0, resulting in:

In = 0.R + 1.G + 0.B = αG.G ………………………………...…….(Equation 5)

Where I represents the intensity of transmitted light color. As a result, light absorption according to equation III-28 becomes:

RGB = -ln(I/I_o) = -ln(G/Go) …………………………........…….(Equation 6)

Top of Form

The RGB light sensor selected in this implementation is the color sensor from ***RGB module Sensor***, and RGB number computer program. For RGB light sensor the microcontroler, Arduino KY-016 3-color LED module, and Arduino UNO R3 light-to-voltage color sensor converter will be used. Through this low-cost device, RGB light sensor hopefully could be used to estimate the concentration algae in water.

Measuring Correlation between Algae Biomass and the Light Intensity Absorbance

The Algae species and culture

Spirulina Plantesnsis colonies was provided by Environmental Laboratorium University Pelita Bangsa, the algae are to be placed in a constant-light incubator to inactivate the culture. The temperature was set to 15°C, light intensity to 500 lux, constant 24 hours. The algae will be initially cultivated in the prepared monoculture.

Experiment Setup

The disrupted cells then will be combined with untreated cells, and three separate calibration samples with 0%, 10%, 25%, 75% and 100% of disrupted cells will be analyzed. Relationship between fractions disrupted and maximum fluorescence absorbance relative to an untreated control are measured (equation 5) after electrocoagulation and settled for 1 hour the biomass is then analyzed using gravimetric method is expected to fulfill equation 6.

The Data and the result

By comparing the RGB measurement data with algal biomass concentrations acquired through gravimetric methods, we can examine the presence of significant trends or correlations between color information and the quantities of algal biomass. Utilizing statistical analyses such as regression or correlation allows us to quantify the underlying nature of this relationship. These analytical techniques offer a framework for statistically assessing both the strength and direction of any potential association between the RGB measurements and the corresponding algal biomass concentrations. The data obtained from gravimetry is as shown in table 2.

Table 1. Biomass concentration for each diluted concentration

No	Water Volume	Dilution Percentage	Weight Before Oven (g)	Weight after Oven (g)	Concentration of algae (mg/L)
			w1	w2	(w1-w2)/L
1	ALGA 1000 ML	100%	24.5192	24.5623	43.1
2	ALGA 750 ML+ AQUADES 250 ML	75%	20.2513	20.2878	36.5
3	ALGA 500 ML+ AQUADES 500 ML	50%	20.1034	20.136	32.6
4	ALGA 250 ML+ AQUADES 750 ML	25%	20.7312	20.7567	25.5
5	ALGA 100 ML+ AQUADES 900 ML	10%	20.1415	20.1622	20.7

Source: Lab data result

Table 2. The correlation between Biomassand Color Transmission Intensity

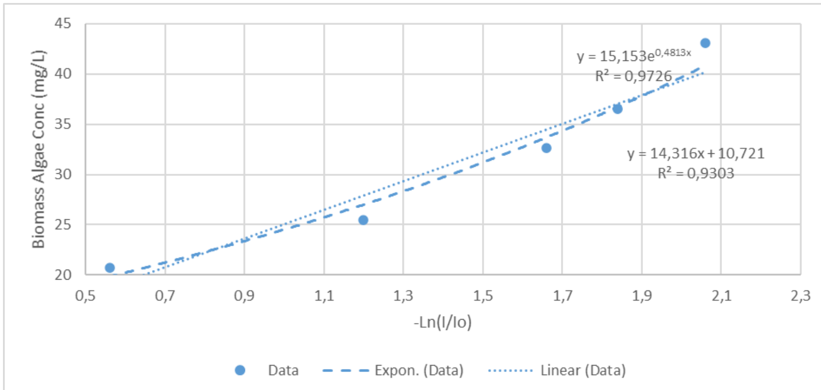
Biomass		RGB transmission intensity			
	mg/L	R	G	B	$-\ln(I_G/I_{Go})$
I	43.10	7	8	10	2.06
	36.50	8	10	12	1.84
	32.60	10	12	15	1.66
	25.50	15	19	21	1.20
	20.70	31	36	42	0.56
Io	0.00	63	63	85	-

The correlation formula quantifies the strength and direction of the relationship between algal biomass and RGB concentration. It is typically represented using correlation coefficients, such as Pearson's correlation coefficient (R^2) or Spearman's rank correlation coefficient (ρ). These coefficients range from 0 to 1, with positive values indicating a positive correlation (as one variable increases, the other tends to increase) and zero values indicating a negative correlation (as one variable increases, the other tends to decrease). The formula for Pearson's correlation coefficient (R) can be seen in the

$$R = \frac{[\mathrm{n}\sum \mathrm{x}2 - (\sum \mathrm{x})2]/[}{\mathrm{n}\sum \mathrm{y}2 - (\sum \mathrm{y})2]\mathrm{n}\sum(\mathrm{xy}) - \sum \mathrm{x}\sum y}$$

Here, n represents the number of data points, y is the algal biomass concentration data (mg/L), and x is the RGB concentration data in terms of $-\ln(I_G/I_{Go})$. The sums are calculated over all data points. Therefor by measuring the RBG the concentration of the algae can be estimated.

The correlation between the RGB (only the Green color intensity) and the biomass concentration can be express in the graph shown in figure 2.



The graph in figure 3 shows that there is a two trend of correlation between the RGB_G (-Ln(I/Io)) and the biomass concentration (mg/L)

- The Linear of $y = 15.153e^{0.4813x}$with a Pearson's correlation coefficient R^2=0.9732.
- The exponential of y = 14,316x + 10,721with a Pearson's correlation coefficient R^2=0.9303.

Therefore the exponential equation is more suitable for estimating the biomass by detecting the RGB_G color intensity.

Practical Use in Spirulina Plantensis Cultivation

The practical application of algae measurement in Spirulina cultivation business holds significant value for optimizing growth, quality control, and overall operational efficiency. Here are a few key areas where algae measurement plays a crucial role:

Biomass Monitoring

Algae measurement allows Spirulina cultivators to monitor the biomass density in the culture. By quantifying the algae concentration, they can ensure that the culture is thriving and growing at the desired rate. This information helps in adjusting nutrient levels, light exposure, and other cultivation parameters for optimal growth.

Harvest Timing

Accurate measurement of algae concentration assists in determining the right time for harvesting. This ensures that the highest biomass yield is obtained, while also preventing overgrowth that could lead to competition for resources or changes in water quality.

Nutrient Management

Algae measurement provides insights into nutrient uptake and utilization by Spirulina. By analyzing how quickly the algae consume specific nutrients, cultivators can adjust the nutrient supply to maintain a balanced and productive culture.

Quality Control

Monitoring algae concentration helps maintain consistent quality in the Spirulina product. Controlling the algae concentration ensures that the desired nutritional content, color, and other characteristics are achieved for both human consumption and other applications.

Efficient Resource Utilization

Precise measurement helps in using resources like water, nutrients, and energy more efficiently. Avoiding overfeeding or wasteful resource use not only reduces costs but also minimizes environmental impact.

Research and Development

Algae measurement provides valuable data for research aimed at improving cultivation techniques, increasing yield, and enhancing the nutritional profile of Spirulina. It aids in understanding how various factors impact growth and quality.

Automation and Data-Driven Decisions

Integrating measurement technologies with automation systems allows for real-time monitoring and control. Data collected from measurements can be used to make informed decisions to optimize cultivation conditions.

Regulatory Compliance

In industries where Spirulina is used for food, pharmaceuticals, or other products, accurate algae measurement is crucial to meeting regulatory standards and ensuring consumer safety.

Scaling Up

Algae measurement becomes even more critical when scaling up production. Maintaining consistent algae concentration across larger cultivation systems is essential for uniform growth and maintaining quality.

Cost Efficiency

Efficient measurement and management of algae concentration contribute to reducing operational costs by minimizing resource wastage and maximizing yield.

Incorporating precise algae measurement techniques into Spirulina cultivation business practices enhances the quality, yield, and sustainability of production, ultimately benefiting both the business and consumers.

Environmental Application Of Algae Measurement

Algae measurement has several important environmental applications due to the vital role that algae play in various ecosystems. Here are some key areas where algae measurement is applied for environmental purposes:

Water Quality Assessment

Monitoring algae concentration in aquatic ecosystems helps assess water quality. Certain types of algae, such as harmful algal blooms (HABs), can indicate pollution or nutrient imbalances in water bodies. Tracking algae levels assists in identifying potential contamination and implementing necessary remediation measures.

Eutrophication Monitoring

Algae measurement is crucial for detecting eutrophication, which is the excessive enrichment of water bodies with nutrients like nitrogen and phosphorus. Elevated nutrient levels can lead to rapid algae growth, disrupting aquatic ecosystems and potentially causing oxygen depletion and fish kills.

Early Detection of Harmful Algal Blooms (HABs)

Some algae species produce toxins during blooms, posing risks to aquatic life, human health, and wildlife. Monitoring algae concentration enables early detection of HABs, allowing authorities to issue warnings, close affected areas for recreation, and take measures to mitigate their impact.

Climate Change Indicators

Algae measurement contributes to understanding the effects of climate change on aquatic ecosystems. Changes in algae composition and distribution can provide insights into shifts in water temperature, nutrient availability, and overall ecosystem health.

Biodiversity Studies

Different types of algae thrive under specific environmental conditions. Monitoring algae diversity and abundance aids in assessing the health and diversity of aquatic ecosystems, which in turn reflects the overall ecological balance.

Aquaculture Management

Algae are a primary food source for many aquatic organisms in aquaculture. Monitoring algae concentration helps manage the health and growth of these organisms, ensuring they receive the appropriate nutrients.

Bioremediation

Certain algae species are used in bioremediation to absorb pollutants from contaminated water bodies. Accurate algae measurement is essential for evaluating the effectiveness of bioremediation strategies.

Research on Carbon Sequestration

Algae play a role in carbon sequestration, absorbing carbon dioxide from the atmosphere during photosynthesis. Measuring algae biomass and growth rates aids in understanding their contribution to mitigating climate change.

Environmental Impact Assessment

Algae measurement is part of environmental impact assessments for activities like construction, dredging, or industrial discharge that might affect aquatic ecosystems. It helps predict and monitor potential impacts on algae populations and the broader ecosystem.

Resource Management

In bodies of water used for recreation, fisheries, or irrigation, algae measurement informs sustainable resource management practices by preventing overgrowth and maintaining balanced ecosystems.

Overall, algae measurement is an essential tool for understanding and managing the health of aquatic ecosystems, ensuring water quality, and mitigating the environmental impacts of human activities.

CONCLUSION

Transformation in Algae Concentration Analysis: The extended research conducted by Benavides et al. signifies a revolutionary advancement in the realm of algae concentration measurement. By amalgamating RGB light absorption and the YCbCr color space representation, in conjunction with the Arduino KY-016 3-color LED module, a groundbreaking and effective technique is introduced for accurately estimating the concentration of Spirulina Platensis algae. Enhanced Accuracy and Efficiency: This innovative approach offers enhanced accuracy and efficiency in estimating algae concentration. By capitalizing on RGB light absorption and advanced color space representation, the method streamlines the assessment process. Cost-Effective Monitoring Solution: The use of the Arduino KY-016 3-color LED module demonstrates a cost-effective solution for algae concentration estimation. This affordability paves the way for wider adoption and utilization, making accurate assessment accessible to a broader range of stakeholders. Spirulina Platensis algae cultivation: An exponential equation could be a more appropriate approach for estimating biomass by utilizing RGBG color intensity to determine Spirulina Platensis algae concentration. Consequently, as a result, the application of this method has the potential to significantly improve the accuracy and precision of Spirulina Platensis algae cultivation, particularly for commercial purposes. Versatile Applicability: The success of this innovative approach suggests its versatility and potential applicability across diverse water bodies and environmental settings. The framework can be adapted for real-time monitoring of aquatic ecosystems. Timely Ecological Intervention: This novel method empowers timely intervention by providing accurate and accessible means to assess water quality. It aids in the detection of excessive algal growth and supports the implementation of measures to restore ecological balance. Democratizing Water Quality Assessment: The accessibility of the Arduino KY-016 3-color LED module democratizes water quality assessment. By making precise estimation more affordable, the approach enhances the collective effort to monitor and protect water resources.

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