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Contributed Paper

Chlorophyll is not accurate measurement for algal biomass

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ABSTRACT

Microalgae are key primary producers and their biomass is widely applied for the production of pharmaceutics, bioactive compounds and energy. Conventionally, the content of algal chlorophyll is considered an index for algal biomass. However, this study, we estimated algal biomass by direct measurement of total suspended solids (TSS) and correlated it with chlorophyll content. The results showed mean chlorophyll-a equal to 1.05 mg/L; chlorophyll-b 0.51 mg/L and chlorophyll-a+b 1.56 mg/L. Algal biomass as 161 mg/L was measured by dry weight (TSS). In statistical t-tests, F-tests and all the tested growth models, such as linear, quadratic, cubic, power, compound, inverse, logarithmic, exponential, s-curve and logistic models, we did not find any discernible relationship between all chlorophyll indices and TSS biomass. Hence, the conventional method of chlorophyll measurement might not be a good index for biomass estimation.

Keywords: Microalgae; Biomass; Chlorophyll; Total suspended solids; Eco-tech

1. INTRODUCTION

Microalgae algae are highly diverse groups of organisms playing vital roles in ecosystem, not only as the primary producers, but also as symbionts with other organisms including bacteria [1-3]. They sequester CO_2 by photosynthesis and supply food and oxygen to the consumers of aquatic environments, and thus play central roles in biogeochemical cycles [4, 5]. Algal biomass serves as a sustainable raw material for producing pharmaceuticals, fertilizers, biofuels and food products [6]. Algae are ideal organisms for biological monitoring. Algal density, abundance, and diversity are ideal indicators of the health of aquatic ecosystems and water quality. Hence, algal biomass measurement is important in many biological and ecological studies and in microalgae industry.

Chlorophyll in plants, algae, and some bacteria is vital to the survival of the plant, animal and other kingdoms in nature. Chlorophyll is essential for photosynthesis, since it absorbs light energy, which is subsequently converted into chemical energy bound in biomass. Conventionally biologists, ecologists and industrialists consider chlorophyll as a reliable and standard algal biomass measurement [7-9]. Common chlorophyll (Chl) types in plants and algae are a, b, c and d. Chlorophyll-a (Chl-a) is found in all photosynthetic algae [10, 11], whereas chlorophyll-b (Chl-b) is confined to green and blue-green algae [12]. Chlorophyll-c is found in traces in green algae and abundant in diatoms and brown algae [13, 14]. Chlorophyll-d is found in marine red algae [15, 16]. In algal chlorophyll, Chl-a is the abundant species, while Chl-b is the minor species [17].

Amount of Chl has been used as a measure of algal biomass world-wide [18-21]. However, though there is a algal taxon-specific distribution of Chl species is evident, there exists a wide species-specific difference in the cellular concentrations of Chl [22, 23]. Such wide variation in Chl concentration questions the reliability of use of Chl amount as an index for algal biomass. Hence, in this study, we focused on Chl-a, -b and -a+b, to understand whether the chlorophyll amount is a reliable index for algal biomass.

2. Materials and Methods

The methodology adopted in this study is illustrated in (Figure 1). We performed the gravimetric method of dry weight to measure algal biomass directly, since we assumed that algal biomass was in the proportion of total suspended solid (TSS). We also performed the proxy measurement of chlorophyll index (Chl-a, Chl-b and Chl-a+b). Subsequently, the values were tested by t-tests and F-tests. We formed possible growth models such as linear, quadratic, cubic, power, compound, inverse, logarithmic, exponential, logistic and s-curve. We also did the ANOVA analyses.

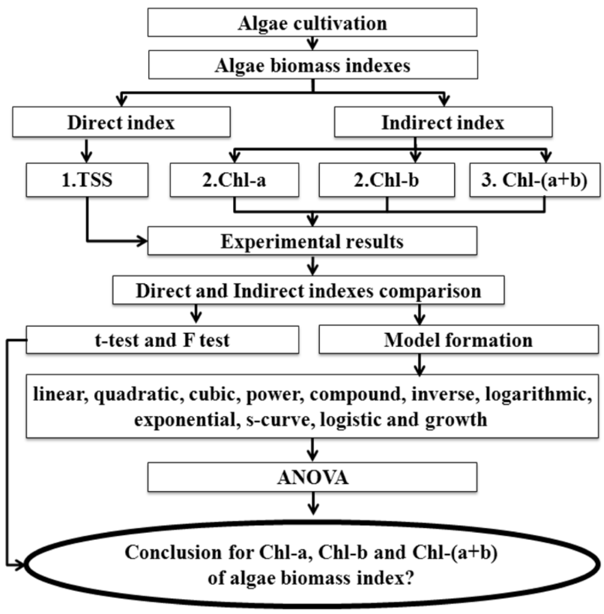

Figure. 1 A flowchart of methodology

2.1. Algal cultivation and biomass measurements

The samples were collected from triplicated reactors (P1, P2 and P3) in the Sustainable Resources and Sustainable Engineering research laboratory, Department of Soil and Water Conservation, National Chung-Hsing University, Taichung, Taiwan. The algae were grown as a mixed culture. The dominant algal species in the mixed culture were of the genera *Anabaena*, *Chlorella*, *Oedogonium* and *Oscillatoria*. The photo-bioreactors were illuminated through uorescent lamps and the cultures were grown in autotrophic conditions with 10 days detention time, for 20 months period, in batch-fed 4 liters continuously-stirred tank reactor (CSTR) at room temperature; the units were shown in (Figure 2). The daily feed of natural freshwater was collected from the Green River in the vicinity of the National Chung Hsing University, Taichung, Taiwan. The collected water was filtered through by 0.45 μm filter paper and used as the medium.

The algal biomass was measured by TSS with Whatman GF/C filter paper [24]. A total suspended cell (TSC) is the popular measurement in biology, but TSS is another way to measure algae biomass by weight directly and the measurement is convenient and efficient for bio-engineering determination. The amounts of Chl-a, -b with -(a+b) were determined following the method described by Becker [25].

Figure 2. Photo-bioreactor

3. Results and Discussion

3.1. Algal biomass indices

Since the feed (the natural river water) was filtrated through 0.45 µ filter paper, there were no algae seeding in the feed. In our study, done in triplicate, the mean biomass production was 0.16 g/L (Figure 3), while the mean amount of Chl-a average was 1.05 mg/L (Figure 4A). Since the determination of the Chl-a is relatively simple and straightforward, the amount of Chl-a is considered as an index of algal biomass and widely used as a proxy measurement. Results for Chl-b (a secondary pigment) and Chl-(a+b) were shown in Figure 4B and Figure 4C, respectively. The mean amount of Chl-b was 0.51 mg/L and the mean amount of Chl-(a+b) was 1.56 mg/L. Many earlier studies used the Chl-(a+b) to measure algal growth, as the biomass index [25–29].

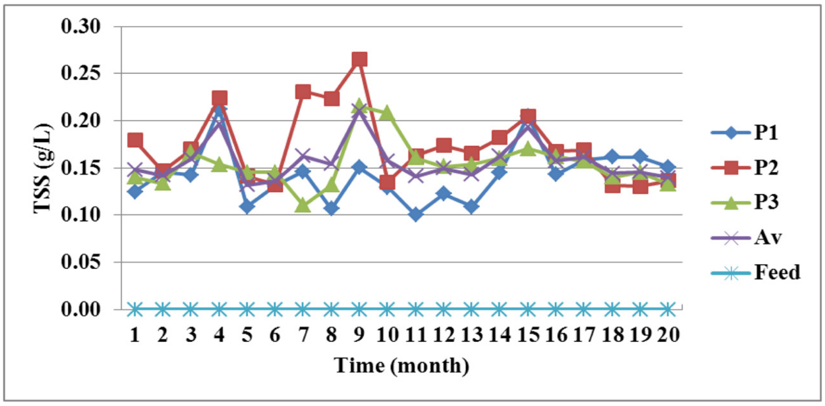

Figure 3. Total suspended solids of feed and reactors

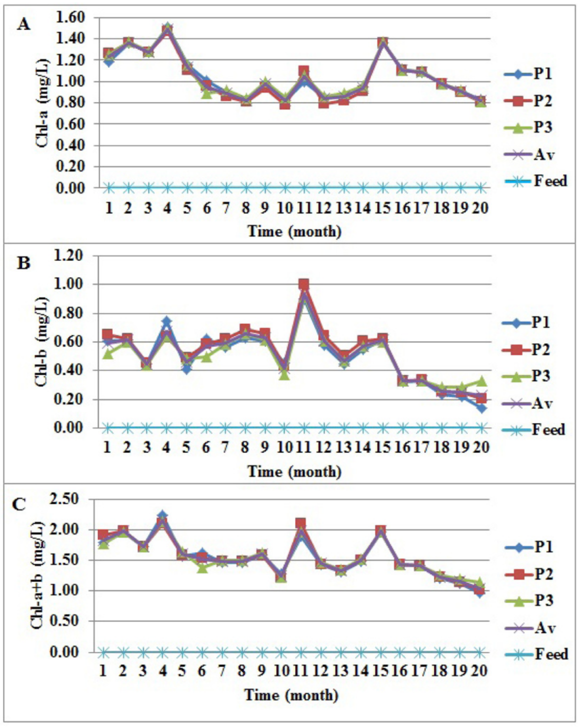

Figure 4. Chlorophylls of feed and reactors

3.2. Statistical analysis of biomass indices

For each paired index comparison of the TSS and Chl-indices, t-tests were performed as the initial trials in this study. The Chl-a/b/a+b are the popular indices of algal growth and biomass. However, they are relatively less direct, when compared to the indices of TSS. Hence, in this study, we compared the Chl-indices with the direct indices of TSS. The t-test results were shown in (Table 1); all tests were rejected to indicate that each index of Chl was different from biomass statistically. This situation can be attributed to the lack of discernible relationship between chlorophylls and biomass.

Amount of algal Chl and its efficiency in an aquatic ecosystem varies depending on the algal species, taxonomic composition and physicochemical and biological factors. Chl content and function could be strongly influenced by physiological shifts in intracellular pigmentation in response to changing growth conditions (light, temperature, pH and temperature). Hence, though Chl content can reflect the (relative) rate of photosynthesis, it may not account for the total accumulated biomass.

Moreover, Chl is not a reactant in the photosynthesis, but a biocatalyst [30-32], which may not be reliable index of algal biomass. Though Chl is a vital component of photosynthesis process, its amount is not crucial to the reaction rate [33]. A small quantity of Chl is sufficient to maintain photosynthesis. As a catalyst, Chl is required to carry on the photosynthesis just by its existence, not by its concentration.

In photosynthesis, Chl-a serves a dual role in oxygenic photosynthesis: light harvesting and converting energy of absorbed photons to chemical energy. While different chlorophylls are participants in photosynthesis in different photosynthetic organisms, Chl-a is present in all photosynthetic organisms [34]. Chl-b may be necessary for effective utilization of light energy, and it may stabilize the photosynthetic device in certain species [35]. However, more rapid oxygen evolution (on chlorophyll basis) in the cells with high Chl-a/Chl-b ratio is suggestive of relatively minor role for Chl-b in photosynthesis [26].

The t-test results revealed the lack of relationship between the amount of Chl and biomass. However, there was still a certain chance to make Chl as a good candidate of the index for biomass by distribution similarity mathematically. Therefore, we tried to investigate further with F-test, which was the best likelihood test of distributions to find any potential resemblance. However, the results demonstrated that the distributions were completely different, as shown in (Table 2).

Both t-tests of mean values and F-tests of distribution proved that each index of Chl and biomass was completely different and strongly suggest that Chl index may not be a reliable biomass index.

Table 1. t-test between TSS and chlorophyll.

Biomass / chlorophyll	t value	p-value	test result
TSS / Chl-a	33.05056	1.49E-18	reject
TSS / Chl-b	33.16413	1.39E-18	reject
TSS / Chl-a+b	32.94011	1.58E-18	reject

Table 2. F-test for difference and regression between TSS and chlorophyll.

F-test for difference					
Biomass/chlorophyll		F value		p-value	test result
TSS/Chl-*a*		10443.10433		3.13E-34	reject
TSS/Chl-*b*		14277.56104		1.61E-35	reject
TSS/Chl-*a+b*		4825.55339		4.79E-31	reject
F-test for regression					
dependentvariable	independent variable	R^2	F	p-value	test result
TSS	Chl-*a*	0.036	0.670	0.423	no regression
	Chl-*b*	0.002	0.038	0.848	no regression
	Chl-*a+b*	0.010	0.190	0.668	no regression

3.3. Linear regression test on chlorophyll and biomass

If Chl-a, -b and -(a+b) have any potential relationship with biomass, they can be used as biomass indices with the calibration curves. Accordingly, we checked the most popular linear relationship first. The results of linear regression tests were shown in (Table 2). There was no relationship between Chl-a, -b or -(a+b) and TSS. Consequently there was no linear regression between each Chl index and biomass.

3.4 Further relationships analysis

Further to establish any possible remote relationship between chlorophyll indices and biomass, we chose several alternative models such as quadratic, cubic, power, compound, inverse, logarithmic, exponential, s-curve, growth and logistic. The results were shown in (Table 3). All the model tests expressed no relationship whatsoever between Chl-a,-b and -(a+b) and the directly measured biomass TSS unfortunately.

Though Chl method is relatively convenient [36], the wide algal species-specific [22, 23,37], age-dependent [38] variations in Chl content undermine its reliability to consider as index for biomass. Depending upon the algal species, the cellular amount chlorophyll may range from 0.1% to 9.7% [36]. When compared to Chl-a, the cellular amount of Chl-b is too scarce [39] to be considered as a reliable index for biomass. In addition, several external factors, such as the presence of interfering compounds [37], nutrition and operation conditions [40], etc may influence the reliability of Chl amount as an index for biomass. Currently, there is no single analytical method available to resolve these problems [41]. Therefore the Chl-a method may not provide correct estimation of algal biomass all the time [42].

Table 3. Linear regression test.

Model	F	p-value	test result
linear	2.493	0.132	no regression
quadratic	1.991	0.167	no regression
cubic	2.188	0.143	no regression
power	2.049	0.169	no regression
compound	2.356	0.142	no regression
inverse	1.917	0.183	no regression
log	2.185	0.157	no regression
exp	2.356	0.142	no regression
s-curve	1.782	0.199	no regression
growth	2.356	0.142	no regression
logistic	2.356	0.142	no regression

4. CONCLUSIONS

Algal biomass in algal cultures was directly measured as TSS (dry weight) and indirectly measured as chlorophyll amount. The great discrepancy between these two sets of values necessitated statistical scrutiny and modellings. Statistical studies and modellings demonstrated that the proxy index of chlorophyll-a, -b and -(a+b) had no discernible relationship with biomass. Hence, though popular, the method of chlorophyll measurement as an index for algal biomass appears to be unreliable.

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