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A comparative study on three quantitating methods of microalgal biomass

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Nannochloropsis oculata, Nannochloropsis salina, *Nannochloropsis oceanica*, *Phaeodactylum tricornutum* and *Cylindrotheca fusiformis* were cultured in liquid *f*/2 medium with aeration till the end of exponential growth phase and serially diluted into eight cell suspensions, and cell concentration, optical density and dry weight in each suspension were determined. The cell density was counted on a hemocytometer, while optical density was measured in the spectrophotometer at 750 nm and dry weight was assayed after a lyophilization procedure. We found significant linear correlations between cell density, optical density and dry weight. When the cell concentration was low, the correlation between optical density and cell density was stronger than that between dry weight and cell density. When the cell concentration was high, the dry weight method was more accurate than other two, thus being applicable to single and high quality measurement.

[**Keywords:** Microalgae, Optical density, Cell density, Dry weight]

Introduction

Quantifying microalgal biomass accurately and effectively is highly needed, as it is crucial for determining the growth performance of microalgae and understanding their physiological and biochemical processes and mechanisms[1-3]. To date, the widely adopted microalgal biomass assaying methods include direct cell counting using a Sedgewick-Rafter counting chamber or a haemocytometer[4] and measurement of optical density using a spectrophotometer[5], algal biomass by weighting[6], cell density using a flow cytometry[7] or particle counter[8] `and chlorophyll *a* content measurement[9-10]. Cell counting is believed to be the most accurate[11]; however, it is laborious, error-prone and inapplicable to filamentous, catenated and agglomerate microalgae[12]. Light absorbance is simple, fast and widely used; however, pigments and medium may cause variation, thus reducing its accuracy[10]. Flow cytometry and particle counter are widely used in recent years, as they can determine the biomass and size constituents

simultaneously[13]. Unfortunately, they are applicable only to single cell microalgae and may also be inaccurate for the proteiform microaglae, for example, *Dunaliella* sp.[14]. In addition, they are expensive and may not be available for many laboratories. Determination by dry weight and ash-free dry weight are the most reliable and generally applicable to large-scale cultivation of microalgae; however, they are tedious and time-consuming and hard to be automated[15]. It is necessary to establish a uniform method so that comparable results can be obtained by different studies, or the relationships between different biomass estimates can be established.

In this study, 5 microalgal species, *Nannochloropsis oculata*, *N. salina*, *N. oceanica*, *Phaeodactylum tricornutum* and *Cylindrotheca fusiformis*, were chosen for evaluating the biomass assaying methods available currently in order to establish a suitable method for determining microalgal biomass rapidly and accurately.

Materials and Methods

N. oculata, *N. salina*, *N. oceanica* and *P. tricornutum* was obtained from Key Laboratory of Mariculture of Chinese Ministry of Education; while *C. fusiformis* was provided by Researching Station of Ocean University of China. Five species were cultured in *f*/2 solution (pH 7.8) with aeration and at 25 ± 1°C and salinity 30 and under an irradiation of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a rhythm of 12 h light and 12 h dark. The algae were cultured to the end of exponential growth phase and diluted to eight continuous concentrations.

The microalgal dilution was counted for cell number with a haemocytometer, six times each dilution, and read at 750 nm for optical density on Hitachi U-3310 UV visible spectrophotometer, 3 times each dilution. When the value was > 1.0, the optical density was read again after further dilution[16]. The algae in 500 mL medium were centrifuged at 5 000 *g* for 20 min, washed with sterile double distilled water, lyophilized for 18 h and weighed. The software EXCEL was applied to process the data, draw the scatter plot and calculate the correlation coefficients.

Results

Correlation between OD_{750} and cell density

Cell density (y) and optical density (x) of all 5 species correlated positively and significantly (Fig. 1). For *N. oculata*, y ($\times10^6$ cells /mL) = $37.392x + 0.2013$ ($0.056 \leq x \leq 0.707$, $R^2 = 0.99$); for *N. salina*, y ($\times10^6$ cells /mL) = $30.426x - 2.9010$ ($0.062 \leq x \leq 0.673$, $R^2 = 0.99$); for *N. oceanica*, y ($\times10^6$ cells /mL) = $102.11x - 0.4065$ ($0.065 \leq x \leq 0.798$, $R^2 = 0.99$); for *C. fusiformis,* y ($\times10^5$ cells /mL) = $23.468x - 0.8270$ ($0.058 \leq x \leq 0.715$, $R^2 = 0.99$); and for *P. tricornutum,* y ($\times10^5$ cells /mL) = $79.311x - 3.1551$ ($0.058 \leq x \leq 0.623$, $R^2 = 0.99$).

There was an optimum concentration range for the linear relationship between optical density and cell density. When cell concentration was beyond the range, the linearity will not be credible. Our analysis showed that the highest optical density (OD_{750}) of *N. salina* and *P. tricornutum* was 0.794 and 0.724, respectively, and that of *N. oculata*, *N. oceanica* and *C. fusiformis* all was within the range.

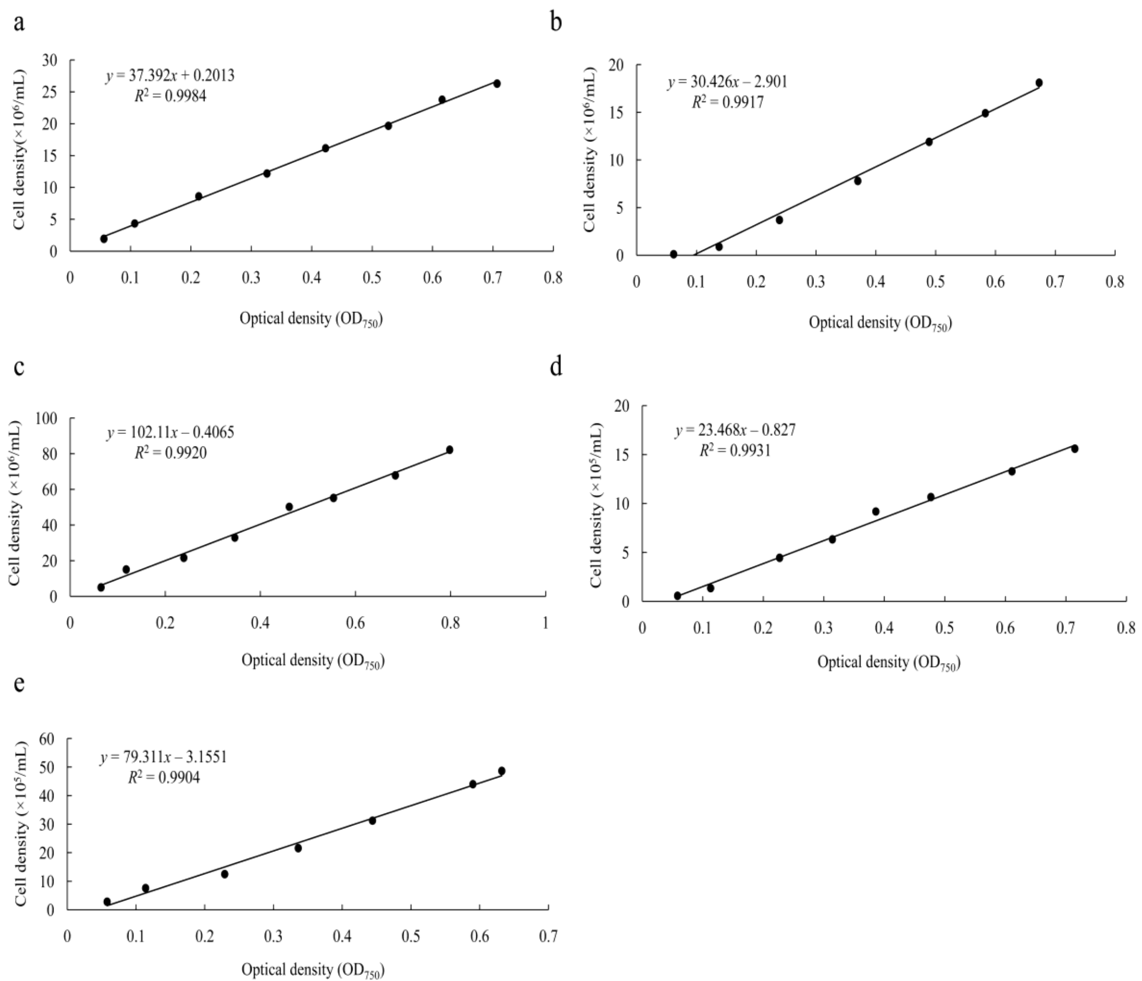

Fig. 1–Linear relationship between OD_{750} and cell density

a, *N. oculata*; b, *N. salina*; c, *N. oceanica*; d, *C. fusiformis*; e, *P. tricornutum*

Correlation between OD_{750} and dry weight

Dry weight (y) of all five species and their optical density (x) correlated positively and significantly when their concentration was appropriate (Fig. 2). For *N. oculata*, y (mg/mL) = 0.3788x + 0.0107 ($0.056 \leq x \leq 0.707$, $R^2 = 0.99$); for *N. salina*, y (mg/mL) = 0.2344x + 0.0436 ($0.062 \leq x \leq 0.673$, $R^2 = 0.98$); for *N. oceanica*, y (mg/mL) = 0.3038x - 0.0178 ($0.065 \leq x \leq 0.798$, $R^2 = 0.99$); for *C. fusiformis*, y (mg/mL)=0.4055x - 0.0150 ($0.058 \leq x \leq 0.715$, $R^2 = 0.99$); and for *P. tricornutum*, y (mg/mL) = 0.3020x + 0.0337 ($0.058 \leq x \leq 0.623$, $R^2 = 0.98$).

There was also an optimum concentration range for such these linearity relationships. It was found that the linear relationship existed within the concentration of 8 dilutions of *N. oculata*, *N. oceanica* and *C. fusiformis*, while the highest optical density of *N. salina* and *P. Tricornutum* (OD_{750}) was 0.794 and 0.724, respectively.

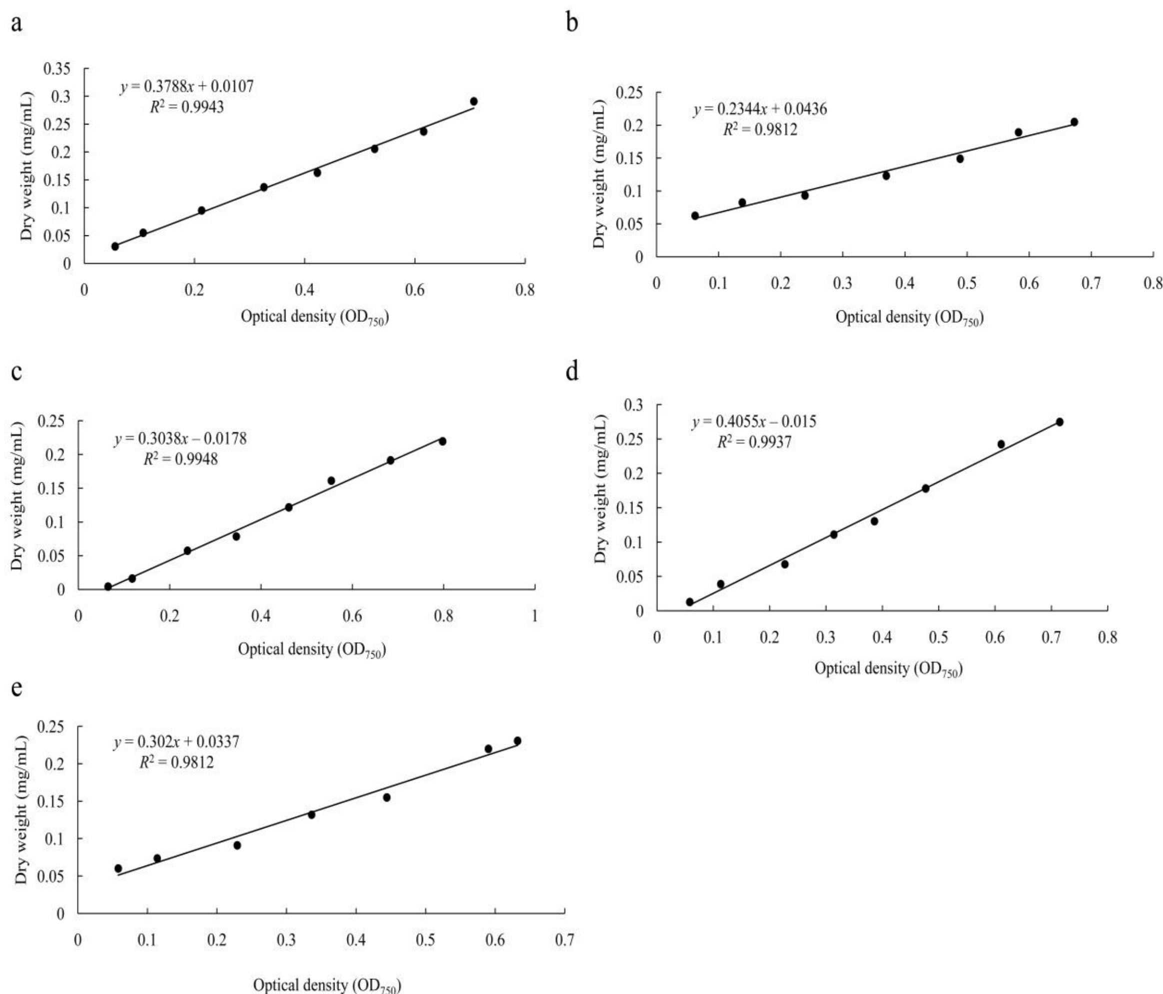

Fig. 2–Linear relationship between OD_{750} and dry weight

a, *N. oculata*; b, *N. salina*; c, *N. oceanica*; d, *C. fusiformis*; e, *P. tricornutum*

Correlation between dry weight and cell density

Dry weight (y) and cell density (x) were positively and significantly correlated as well for all five species when their concentration was appropriate (Fig. 3). For *N. oculata*, y (x10^6 cells/mL) = 98.031x - 0.7527 ($0.056 \leq x \leq 0.707$, R^2=0.99); for *N. salina*, y (x10^6 cells/mL) = 114.46x- 6.7698 ($0.062 \leq x \leq 0.794$, R^2 = 0.9838); for *N. oceanica,* y (x10^6 cells/mL) = 33.396x + 0.5810 ($0.065 \leq x \leq 0.798$, R^2 = 0.98); for *C. fusiformis,* y (x10^5 cells/mL) = 57.253x + 0.1241 ($0.058 \leq x \leq 0.715$, R^2 = 0.98); and for *P. Tricornutum,* y (x10^5 cells/mL) = 290.65x - 15.091 ($0.058 \leq x \leq 0.724$, R^2 = 0.98). All five strains showed good linearity when their concentration was within the diluted. Further work was needed to widen the concentration range.

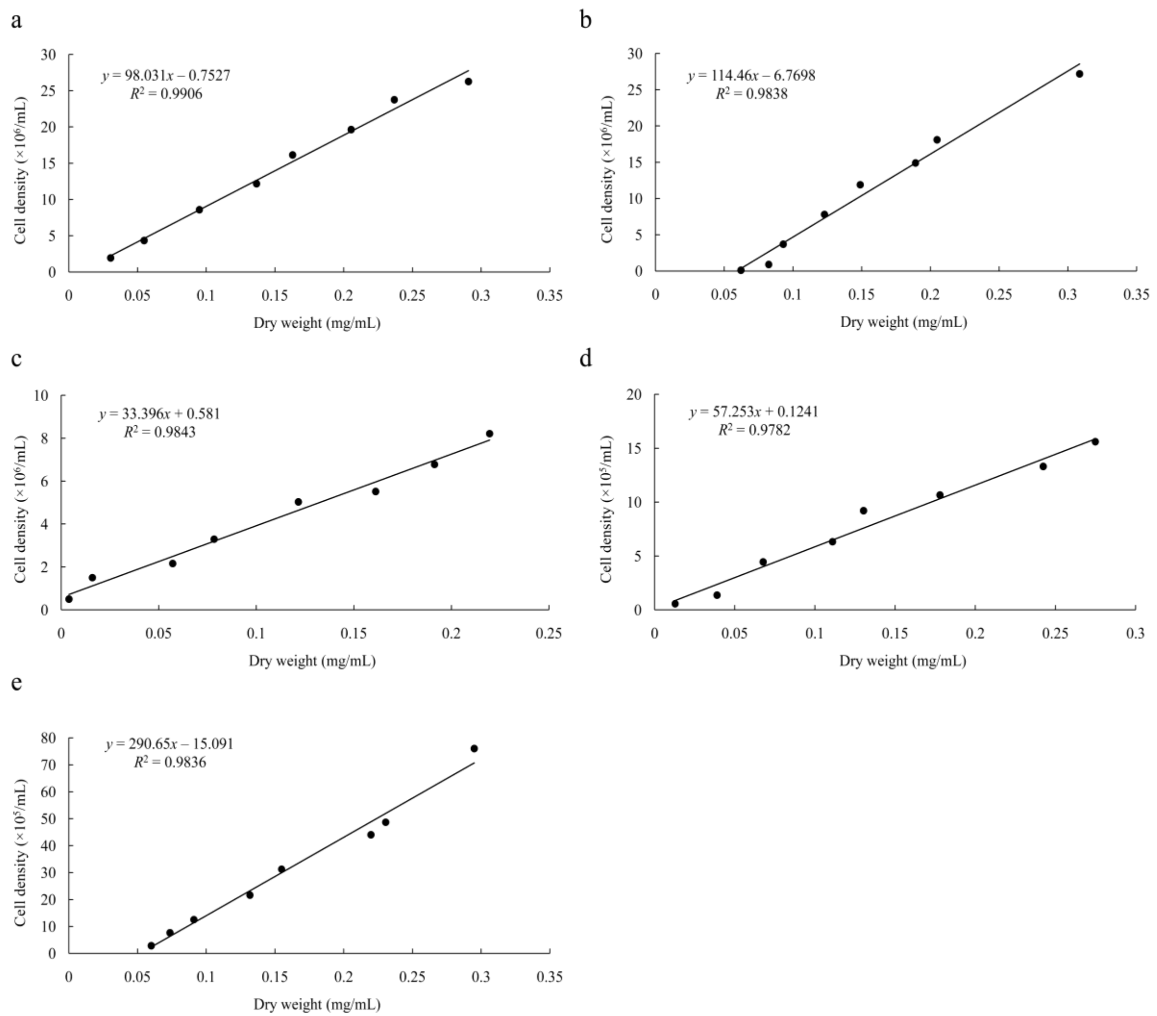

Fig. 3–Linear relationship between dry weight and cell density

a, *N. oculata*; b, *N. salina*; c, *N. oceanica*; d, *C. fusiformis*; e, *P. Tricornutum*

Discussion

Diverse methods have been developed for determining microalgal biomass; each describes the growth performance from a single aspect. In this study, good correlations between optical density, cell density and dry weight were found in five microalgae. Our findings made the conversion between these parameters possible; thus a fastest and easiest automatic method may be selected for use in microalgal culture practice.

We found that the linearity was significant only when algal concentration (optical density) was within an appropriate range, for example, from 0.062 to 0.673 for *N. salina* and from 0.058 to 0.623 for *P. tricornutum*. Further studies are needed for bounding the concentration range of *N. oculata, N. oceanica* and *C. fusiformis*. In general, at relatively low concentrations, correlation between optical density and cell density was better than that between dry weight and cell density, therefore measuring optical density may be applicable to continuous measurement. In contrast, measuring dry weight is more applicable to one-time measurement of farmed

microalgae, as it is more accurate when algal concentration is high.

Wavelength used in measuring optical density varied in early reports, and linearity appeared only within certain concentration ranges. Shen[17] measured the optical density of 15 microalgae at 680 nm and established the relationship between optical density and cell density. Zeng[18] selected 674 nm as the measuring wavelength and discovered a good linearity between optical density and cell density when the optical density was between 0.07 and 0.23. In *Dunaliella salina*, Hao[19] found that significant linearity exists between cell density, optical density and dry weight when the read at OD_{630} was between 0.07 and 0.35. These findings were obtained by selecting an optimal wavelength at which the optical density was measured. However, so called optimal wavelength may vary with the changes of growth stage and culturing condition. As documented earlier, the maximal absorbance of chlorophyll *a* existing in all microalgae ranges from 400 to 460 nm and from 650 to 680 nm[20-21]. Influence of chlorophyll should be taken into account in determining the biomass of pigment-rich species by measuring optical density. Liu[22] and Griffiths[10] suggested that the wavelength range with chlorophyll absorbance peak(s) should be avoided in drawing a standard curve. We found that 750 nm was appropriate for all microalgal species tested, as it revealed a good linearity between the optical density with cell density or with dry weight.

The optical absorption coefficient, namely, the slope of linear regression between optical density and cell density, is related to cell size. When the cell density was within the same order of magnitude, three Eustigmatophyceae species showed larger optical absorption coefficient than two diatoms which were larger in size. In addition, optical absorption coefficients among three Eustigmatophyceae species were different from each other, thus characteristics of microalgae may associate with optical absorption coefficient. Actually, type, content and distribution of microalgal inclusions, as well as microalgal movement may cause the change of slope[17], which was also documented in our study. Hence, even for congeneric species, equations need to be calculated separately.

At logarithmic growth phase, microalgal cells divide vigorously, thus senescent and dead cells and cell debris are scarce. The size and morphological characteristics of microalgal cells at this stage are extremely similar with each other. Our works provided a reference for the biomass assaying of microalgae at logarithmic growth phase. However, different standard curves may be needed for different growth stages in batch culture[10]. Liang[23] monitored the growth of four microalgae in batch culture and found that the ratio of dry weight to optical density increased with time. In monitoring the batch heterotrophic culture of *Chlorella vulgaris*, Liu[24] found that the ratio of dry weight to optical density increased first and then decreased. In large scale cultures of microalgae, the morphological characteristics and inclusion of cells may vary from time to time, thus their spectral characteristics and dry weight may change. These variations may be due to the change in medium and trophic mode. Accordingly, the relationship may need to be established, respectively, for culture media and trophic modes each.

Conclusion

Optical density was the best choice for measuring microalgal biomass at low cell concentrations, while dry weight was more suitable for determination of microalgal

biomass at high cell concentrations. Furthermore, it was strongly recommended that 750 nm as a promising wavelength for measuring optical density. These findings will help to study the growth performance of energy microalgae, which is the basis of exploiting biofuel from microalgae.

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