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A comparison of feasible methods for microalgal biomass determinations during tertiary wastewater treatment

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ABSTRACT

One of the most important parameters for monitoring, designing and optimizing the algae-based wastewater treatment system is the biomass concentration. The determination method of microalgal biomass concentration in the culture medium by Volatile Suspended Solids (VSS), as a conventional method, was found inconvenient because it takes long time to obtain the results. In this study, the performance of five feasible methods for microalgal biomass determinations was evaluated and compared. The coefficient of determination (R^2) of Optical Density (OD), Total Suspended Solids (TSS), turbidity and wet weight was 0.9903, 0.9698, 0.975, 0.9794 and 0.9922, 0.9686, 0.9649, 0.9694 for *C. vulgaris* and *Phormidium* sp., respectively. After comparing with the VSS method during wastewater treatment process, OD is recommended as a reliable method for biomass determinations for green (*C. vulgaris*) and blue-green (*Phormidium* sp.) algae.

1. Introduction

Microalgae have received more and more attention in recent years as an alternative bioresource and substrate for biofuel (e.g., biodiesel and bioethanol) or bioenergy (e.g., biogas) production (Krirolia et al., 2013; Oswald, 2003). Furthermore, their high biomass productivity comparing with land plant and the capacity to fix CO_2 through photosynthesis and all year round production together with no need for herbicides and pesticides, make microalgae-based technology more attractive (Nurdogan and Oswald, 1995; Schenk et al., 2008).

Carbon, nitrogen and phosphorus are the essential macronutrient elements for microalgal growth. Besides, several inorganic ions are universally required for algae, such as SO_4^{2-}, Mg^{2+}, Ca^{2+}, K^+, Na^+ and Cl^- (macronutrients) and iron, manganese, zinc, cobalt, copper, and molybdenum (micronutrients) (Andersen, 2005). Based on these growth requirements, different artificial mediums (such as TAP, BG11, LC Oligo, KC or Tamiya media) are used for microalgae cultivation (Chia et al., 2013; El-Sheekh et al., 2013; Su et al., 2012). However, municipal or industrial wastewater offers a cost-effective growth medium for microalgae, which could fulfill the requirements of algal growth (Su et al., 2011). Furthermore, in the conventional wastewater treatment systems, nitrogen and phosphorus were removed and returned to environment without recovery or reuse (Oehmen et al., 2007; Tchobanoglous et al., 2003). Using municipal wastewater for microalgal growth could realize high value-added biomass accumulation, wastewater treatment and resource recovery and reuse.

Microalgal biomass concentration is needed to monitor for the estimation of the algal growth rate and production, as one of the most important parameters for designing, monitoring, modelling and optimizing the microalgae cultivation systems. Volatile Suspended Solids (VSS) is generally considered to be the most precise expression of microalgal biomass, which is widely used as a direct parameter for indicating microalgal biomass concentration when municipal wastewater was fed (Li et al., 2011a,b). VSS are determined by measuring the mass of oven-dry solid retained by the filters that has been dried at 105 °C and volatilized at 505 °C (DEV, 2002). Prior to the assay, the filter should be prepared at 505 °C for a few hours to combust any adsorbed organic material (Gates et al., 1982). Furthermore, drying the sample at 105 °C and combusting it at 505 °C add more time to the analysis procedure. The Optical Density (OD) procedure has been established as the primary determination to express microalgal biomass weight in the artificial cultivation medium by transferring OD into biomass dry weight through a calibration curve developed via OD and dry weight (Arbib et al., 2013; Chia et al., 2013; El-Sheekh et al., 2013; San Pedro et al., 2013). Besides, other fast determinations such as Total Suspended

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Solids (TSS), turbidity, wet weight and Chemical Oxygen Demand (COD) have also been used as indirect methods to express biomass (Bullock et al., 1996; Contreras et al., 2002; Gates et al., 1982; Sladecek and Sladeckova, 1963; Su et al., 2011). However, when using these methods for microalgal biomass determination during wastewater treatment, whether the wastewater would influence the results of the measurements is largely unknown.

In this paper, for the first time, five biomass determination methods, including TSS, OD, wet weight, turbidity and COD were evaluated and compared in the municipal wastewater (from secondary clarifier) feeding system for both blue-green and green algae.

2. Material and methods

2.1. Microalgae cultivation

Blue-green algae (*Phormidium* sp.) and green microalga (*Chlorella vulgaris*) were chosen to investigate due to their high potentials in nutrient removal (Su et al., 2012). *Phormidium* sp. was obtained from Institute for Cereal Processing Ltd. (Germany) and grown on BG11 at room temperature (around 20 °C). *Chlorella vulgaris* was obtained from Scandinavian Culture Collection of Algae & Protozoa (Denmark) and grown on a modified MWC media at room temperature (around 20 °C). 5 L glass beakers were used as photo-bioreactor with a consistent mixing (300 rpm, VWR 984VW0CSTEUS, USA). A fluorescent lamp (Philips TL-D36w/840, Poland) was used to irradiate from the side of the reactors in a light: dark cycle of 12:12 h, to mimic natural solar day-night cycle, with 7000 Lux (measured at the side of bioreactor with TES-1335 Digital light meter). 5 L of wastewater, collected from the effluent of the second clarifier in the wastewater treatment plant of Holthusen (Germany) was used as growth medium. The characterization of the wastewater used here was: total COD 30.20 ± 2.50 (mg O_2/L), total kjeldahl nitrogen (TKN): 26.40 ± 0.70 (mg N/L), NH_4^+–N: 25.20 ± 0.30 (mg/L), PO_4^{3-}–P: 1.74 ± 0.12 (mg/L), NO_3^-–N: 0.75 ± 0.06 (mg/L) and NO_2^-–N: 0.10 ± 0.06 (mg/L). The initial algae concentration of the two microalgae was 0.20 g/L (VSS).

2.2. Five microalgal biomass determinations

At the end of the operation, *Phormidium* sp. and *C. vulgaris* were harvested and centrifuged (13000*g*) in the late exponential phase, washed 3 times with tap water to remove the residual nutrients and concentrated as stock solution. The stock solution of the two model microalgae strains were diluted in different times with tap water to test the VSS, OD, turbidity, wet weight, TSS and COD, respectively. TSS and VSS were analyzed according to DIN ISO 11465 (DEV, 2002). Turbidity was measured (Turbidity photometer, Dr. Lange, Type-Nr. LPG239, Germany) according to DIN EN27027 (DEV, 2002). COD was analyzed according to DIN 38409-H 41(44). The OD of *Phormidium* sp. and *Chlorella vulgaris* was determined with photometer (MERCK SQ118, Germany) at 680 nm. Wet microalgal biomass was obtained by centrifuging 25 mL sample at 13000*g* for 20 min (Thermo Scientific Heraeus Multifude X1, Germany). The empty centrifugation tubes and tubes with pellets after centrifugation were weighted. The microalgal wet weight was calculated as the weight difference between the tube with pellet and empty tube.

2.3. Nutrient analytical methods

TKN was determined according to DIN EN 25663-H11. NH_4^+, total phosphorus and dissolved phosphorus (PO_4^{3-}) were determined according to DIN 38406-E5-1 and DIN EN ISO 6878-D11 (DEV, 2002) using an UV/Vis Spectrometer (Perkin Elmer, Lambda 40, USA). NO_3^- and NO_2^- were determined using an Ion Chromatograph (Dionex DX-100, USA) according to DIN EN ISO 10304-1 (DEV, 2002).

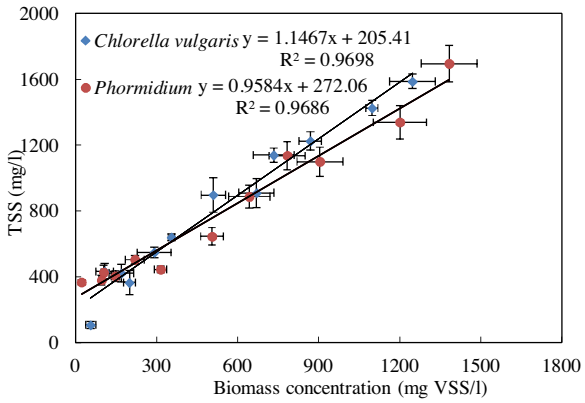

Fig. 1. Correlation between biomass concentration (VSS) and total suspended solid (TSS).

3. Results and discussion

3.1. The correlation between VSS and TSS

Algal biomass could be estimated gravimetrically by both TSS and VSS (Ramaraj et al., 2015). TSS is to test the total concentration of suspended (non-soluble) solids, which is used as a routine parameter to assess the performance of wastewater treatment process (Tchobanoglous et al., 2003). It is obtained by measuring the increase in the weight of the glass-fiber filter after drying the residues filtered through the glass-fiber filter to a constant weight at a temperature between 103 °C and 105 °C (DEV, 2002). VSS is determined by measuring the decrease in the weight of the filter for TSS after igniting at 550 °C (DEV, 2002). The value of TSS in this study includes the dry weight of microalgal biomass, unfilterable organic matter and unfilterable inorganic matter in the sample. The value of VSS in this study includes the dry weight of microalgal biomass, undissolved organic matter in the sample. Therefore, the value of TSS is higher than that of VSS.

Fig. 1 shows the correlation between VSS and TSS for *Phormidium* sp. and *Chlorella vulgaris*. There is a strong relationship between TSS and VSS ($R^2 > 0.96$), which might be due to the low concentration of undissolved inorganic matters in the effluent of the secondary clarifier. Although both TSS and VSS could be used to estimate the concentration of microalgae biomass, TSS is time-saving and convenient while VSS is more precise than TSS. The less inorganic matter in the sample, the more precise information could be obtained from TSS. Using the correlation between TSS and VSS as a direct index to test microalgal biomass could skip the process of ignition at 550 °C, and thus reduce the operation time. It suits for the cultivation medium with the low mineral solids content.

3.2. The correlation between VSS and OD

Fig. 2 shows the correlation between VSS and OD. Among the tested algal biomass measurement methods, OD is the most accurate measurement method for biomass concentration as the strongest correlation was obtained ($R^2 > 0.99$) for both of the tested microalgae.

OD is a convenient indirect parameter to express biomass concentration in microbial cell suspension (Griffiths et al., 2011), which is widely used in different algae-based systems (Borde et al., 2003;