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Rapid evaluation of algal and cyanobacterial activities through specific oxygen production rate measurement

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ABSTRACT

Extant respirometry enables rapid determination of sludge decay coefficients and chemical biodegradability in wastewater treatment systems. This study extends its use to phototrophic systems to determine phototrophic decay rate and photosynthetic activity through the measurements of specific oxygen uptake rate (SOUR) and specific oxygen production rate (SOPR), respectively. With a sufficient CO_2 supply (4.0 mM of $NaHCO_3$) and pH control (from 7 to 8) at the light intensity of 50 ± 5 μmol m^{-2} s^{-1} and the temperature of 23 ± 1 °C, the specific growth rates of cyanobacteria (*Microcystis aeruginosa*) and green algae (*Chlorella vulgaris*) were 0.92 ± 0.11 and 0.79 ± 0.14 d^{-1}, respectively. The decay coefficients of *M. aeruginosa* and *C. vulgaris* were 0.08 ± 0.04 and 0.08 ± 0.03 d^{-1}, respectively. Compared to batch phototrophic growth studies that often last longer than 10 days, the proposed SOPR measurement enables rapid determination of algal/cyanobacterial growth kinetics within minutes and is capable of determining phototrophic growth under different environmental and stress conditions (e.g., pH, nitrogen sources, chemical and metal exposure). As demonstrated here, ammonium was a preferred nitrogen source for the growth of *M. aeruginosa* and *C. vulgaris* because reducing power (energy) is needed to convert nitrate to ammonium before nitrogen uptake by the phototrophs. *M. aeruginosa* was more susceptible than *C. vulgaris* to inhibition by heavy metal copper. At the concentration of 10 mg Cu^{2+}/L, cupric ions had no effect on algal growth but inhibited cyanobacterial growth by 66.4%.

1. Introduction

Algae-based bioreactor techniques are being revived for wastewater treatment and nutrient removal while the harvested algal biomass may be used for broad applications such as biodiesel and fertilizer production (Mulbry et al., 2005; Su et al., 2011; Valigore et al., 2012; Zhu et al., 2013). Phototrophic growth studies provide critical information about the kinetics of phototrophic growth and their linkage to nutrient uptake, which are essential for the design and operation of algal ponds or photobioreactors.

Algal growth kinetics are often studied in batch experiments by determining the changes in biomass concentration (optical density or OD) (Li et al., 2011; Pivokonsky et al., 2014), cell numbers (Drábková et al., 2007; Lavoie et al., 2014), and chlorophyll *a* content (Linkous et al., 2000; Sterner and Grover, 1998). However, these experiments often require a long period (>10 days) of cultivation to differentiate the changes and the results can be easily affected by biomass debris formation (Robertson et al., 1998). Furthermore, the changes in water pH, nutrient availability, biomass concentration, and self-shading of light by algae affect algal growth during the cultivation period, which may lead to an underestimation or overestimation of growth kinetics.

Other techniques have been explored to determine algal growth kinetics by quantifying the photosynthetic products, such as oxygen or ^{14}C assimilation products from the Calvin cycle (Hancke et al., 2008; Lin et al., 2005). Oxygen evolution measurements with O_2 electrodes allow for oxygen production measurements in the light (Buffle and Horvai, 2000). An extension of this method is the microamperometric oxygen evolution measurements by determining photosynthetic oxygen evolution using microelectrodes (Matsue et al., 1993; Yasukawa et al., 1999). However, the insertion of microelectrodes could physically injure cells and trigger undesired intracellular reactions (Yasukawa et al., 1999). Direct chlorophyll fluorescence measurement provides a sensitive analysis of photosynthetic activity based on the short-term change in chlorophyll fluorescence after light exposure (Beutler et al., 2002; Dewez et al., 2005; Goldman et al., 2013). However, interference from light absorbing compounds, such as dissolved organic matter may cause a significant underestimation of photosynthetic activity (Goldman et al., 2013). On the other hand, ^{14}C-assimilation rate measurements

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reflect the activity of photosynthesis by quantifying the amount of dissolved inorganic carbon converted into cell biomass during photosynthesis. However, the ^{14}C techniques require the use of special equipment such as liquid scintillation counter and could result in significant variation in carbon fixed per unit chlorophyll due to nutrient limitation (Halsey et al., 2010). The variation of photosynthetic activities revealed by the above methods were not only caused by the use of different test endpoints, but were also affected by many important factors such as initial phototrophic cell density, light intensity and exposure time (Lin et al., 2005). Therefore, it is necessary to develop a rapid, simple and reliable method to determine the photosynthetic activity of phototrophs upon light irradiation.

Respirometry based on oxygen production has been proposed as a non-destructive and non-invasive approach to rapidly determine phototrophic activity (Buffle and Horvai, 2000; Park and Lee, 2001). Extant respirometry, which is reflective of conditions immediately before the assay, allows estimation of activated sludge growth kinetics and sludge decay rate coefficients by recording the dissolved oxygen (DO) profiles (Ellis et al., 1996, 1998). A high-throughput respirometric assay results in information-rich data, which can translate into high precision of estimated parameters (Chandran et al., 2008). The application of extant respirometry can be easily extended to phototrophic systems where the phototrophic activity and decay rate constant can be determined through the measurements of specific oxygen production rate (SOPR) in the light and specific oxygen uptake rate (SOUR) in the dark, respectively. Like SOUR measurement in extant respirometry, SOPR measurement is analytically facile because the continuous acquisition of oxygen production by the phototrophs can be fully automated to avoid sampling errors and bias. In fact, respirometric methods have been explored and evaluated in photosynthetic studies for biokinetic parameter estimation. For example, photosynthetic rates obtained from respirometry suggest that the growth of diatoms is inhibited at higher light intensities (Lee et al., 1982). The respirometric method has been proposed for algal growth inhibition screening (Chen et al., 2005; Fargašová and Drtil, 1996; Yeh and Chen, 2006). Unfortunately, previous methods to determine photosynthetic activity by measuring O_2 evolution are often ambiguous on what exact test devices are needed (e.g., the type and size of the bottles and whether or not the respirometric bottles should be filled completely without headspace) or test conditions such as carbon dioxide concentration in the mixed liquor, water pH and temperature, nitrogen source, light intensity, wavelength and light–dark period. The objective of this research was to develop a standard procedure to rapidly determine algal and cyanobacterial activities through SOPR measurement by taking into account these important factors affecting photosynthesis. The proposed SOPR measurement would, therefore, allow for determination of algal/cyanobacterial growth kinetics within minutes under different environmental and stress conditions (e.g., pH, nitrogen sources, chemical and metal exposure).

2. Materials and methods

2.1. Axenic culture of algae and cyanobacteria

Pure algal species *C. vulgaris* (Carolina Biological Supply Company, Burlington, NC) and pure cyanobacteria species *M. aeruginosa* (UTEX 2385, Austin, TX) were selected as model phototrophic organisms. *C. vulgaris* was cultivated aseptically in Bold's Basal Medium, which consisted of the following components per liter of water: 0.25 g $NaNO_3$, 0.025 g $CaCl_2{\cdot}2H_2O$, 0.05 g $MgSO_4{\cdot}7H_2O$, 0.10 g K_2HPO_4, 0.15 g KH_2PO_4, 0.025 g NaCl, and trace metals. *M. aeruginosa* was grown in modified Bold 3 N medium, which consisted of the following components per liter of water: 0.75 g $NaNO_3$, 0.019 g $CaCl_2{\cdot}2H_2O$, 0.10 g $MgSO_4{\cdot}7H_2O$, 0.07 g K_2HPO_4, 0.18 g KH_2PO_4, 0.03 mg NaCl, and micronutrients including vitamin B_{12} and trace metals. Both algae and cyanobacteria were cultured under continuous photon irradiance ($50 \pm 5\ \mu mol\ m^{-2}\ s^{-1}$) at $23 \pm 1\ ^\circ C$ on a solid agar plate and later in a liquid medium, respectively. All subsequent bioassays were carried out in Bold's Basal Medium with modification, such as pH adjustment, $NaHCO_3$ addition, and replacement of nitrate by ammonium as the nitrogen source. Stock cultures were maintained and transferred into fresh culture media once per month in Erlenmeyer flasks. Axenicity was tested periodically and visually with agar plating. Exponentially growing algae and cyanobacteria were first centrifuged ($9000 \times g$) for 10 min and washed twice with the Bold's Basal Medium before use. To ensure good sensitivity of oxygen production due to algal photosynthesis and to avoid cell self-shading, a cell density of 6×10^6–7×10^6 cells/mL with an algal or cyanobacterial biomass concentration ranging from 200 to 250 mg/L chemical oxygen demand (COD) was selected for all SOPR measurements. Such biomass densities were kept well below the carrying capacity of the growth media while achieving maximum test sensitivity and reproducibility (data not shown).

2.2. SOPR measurement to determine the specific phototrophic growth rate and decay coefficient

Phototrophic growth associated with oxygen production due to photosynthesis by algae or cyanobacteria was measured in duplicate at $23 \pm 1\ ^\circ C$ using a modified batch extant respirometric assay (Choi et al., 2008; Hu et al., 2002). Prior to the SOPR assay, the test culture or mixed liquor was purged briefly with nitrogen to reduce its initial DO concentration to approximately 1–3 mg/L. Sodium biocarbonate ($NaHCO_3$) was added to the test culture at a final concentration of 4 mM to ensure the CO_2-independent reactions of photosynthesis (details below). The mixed liquor pH was subsequently adjusted to 7.0 by the addition of 1 M HCl or 1 M NaOH. Aliquots (50 mL) of the test culture (with the biomass COD concentration ranging from 200 to 250 mg/L) were filled into the respirometric bottles with no headspace and the bottles were tightly capped. The mixed liquor in the bottles was stirred at 100 rpm to ensure complete mixing. With the bottles covered with aluminum foil, the test culture was kept in the dark for a short period before it was exposed to fluorescent light at an intensity of $50 \pm 5\ \mu mol\ m^{-2}\ s^{-1}$. An increase in the DO concentration in the respirometric bottle due to photosynthesis was measured by a DO probe (YSI model 5300A, Yellow Springs, OH) and continuously monitored at 4 Hz by an interfaced personal computer.

The dark–light cycles were repeated to determine the reproducibility of the assay. In each cycle, the test culture experienced about a 10-min dark period followed by a 20-min light exposure. Because of oxygen production due to the photosynthesis by phototrophs during the light period and oxygen consumption due to microbial endogenous respiration during the dark period, the mixed liquor DO concentration increased in the light period and decreased in the dark period. The endogenous respiration activity was therefore obtained through linear regression analysis of DO data in the dark. The oxygen produce rate (OPR) in the light was obtained in the same way after the correction of endogenous respiration. SOPR was determined by dividing OPR by the biomass concentration. Finally, the growth kinetics of phototrophs under the constant light condition were determined by the relationship between SOPR and specific phototrophic growth rate (Xu et al., 2014) as detailed in Supporting information.