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Enrichment of highly settleable microalgal consortia in mixed cultures for effluent polishing and low-cost biomass production

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ABSTRACT

Microalgae cultivation is a promising technology for integrated effluent polishing and biofuel production, but poor separability of microalgal cells hinders its industrial application. This study intended to selectively enrich settleable microalgal consortia in mixed culture by applying "wash-out" pressure, which was realized by controlling settling time (S_T) and volume exchange ratio (VER) in photo-SBRs. The results demonstrated that highly settleable microalgal consortia (settling efficiency>97%; SVI = 17–50 mL/g) could be enriched from indigenous algal cultures developed in WWTP's effluent. High VER was the key factor for the fast development of settleable microalgae. VER was also a controlling factor of the algal community structure. High VERs (0.5 and 0.7) resulted in the dominance of diatom, while low VER (0.2) facilitated the dominance of cyanobacteria. The settleable microalgal consortia were very efficient in phosphorus removal (effluent PO_4^{3-}-P<0.1 mg/L; removal efficiency>99%), which was largely attributed to intensive chemical precipitation of phosphate induced by high pH (8.5–10). However, the high pH decreased the bioavailable inorganic carbon, resulting in incomplete nitrate removal (effluent NO_3^--N = 2.2–4 mg/L; removal efficiency = 61–79%) under high VERs and low lipid content (up to 10%) in the settleable microalgae. This problem could be resolved by sparging CO_2 or controlling pH. Overall, this study demonstrated a simple and effective method to overcome the separation challenge in scale-up of microalgae biotechnology for advanced wastewater purification and biofuel production.

1. Introduction

Discharge of wastewater loaded with nutrient (nitrogen, N; phosphorus, P) into water bodies leads to eutrophication and imposes a serious threat to aqueous environment. Therefore, tertiary treatment for nutrient removal is now generally mandatory worldwide. Typical nutrient discharge limits are 10–15 mg/L for total nitrogen (TN) and 0.5–1 mg/L for total phosphorus (TP), which are still much higher than the thresholds (TN < 1.2 mg/L; TP < 0.1 mg/L) causing eutrophication in streams (Chambers et al., 2012). Therefore, it is required to further reduce the nutrient content in treated effluents. For example, the Dutch guidelines for discharging effluents to sensitive water bodies are 2.2 mg N/L and 0.15 mg P/L (Boelee et al., 2014). Similarly, the latest 'Integrated discharge standard of water pollutants' (DB11/307-2013) of Beijing sets a discharge limit of 0.2 mg/L for TP. These ever more stringent discharge limits challenge our current wastewater treatment plants (WWTPs) seriously, leading to the urgent need for cost-effective post treatment systems.

Established technologies (denitrifying biofilters, chemical precipitation, membrane separation, etc.) to meet this requirement are not sustainable since they are energy- and/or resource-intensive (Boelee et al., 2014). Alternatively, microalgae cultures provide an elegant solution. Microalgae are eukaryotic or prokaryotic photosynthetic microorganisms. They assimilate a significant amount of nutrient for growth, thus reducing the nutrient to a very low level without any chemical addition (Abdel-Raouf et al., 2012; Boelee et al., 2014). More attractively, many microalgae store a large amount (20–50% cell dry weight) of fixed carbon (CO_2) in the form of neutral lipids, which can be readily converted to biodiesel (Hu et al., 2008). Therefore, microalgae culturing has been widely recognized as a promising solution towards sustainable wastewater purification and biofuel production (Christenson and Sims, 2011).

Microalgae cultivation methods include suspended (open

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ponds, closed photobioreactors) and immobilized cultures (matrix-immobilized, photo-biofilm) (Christenson and Sims, 2011). For industrial application, open suspended cultures are the most economically viable way (Gonzalez-Fernandez and Ballesteros, 2013). However, suspended cultures face a major challenge – separation of microalgal biomass from its growth medium (i.e. treated wastewater) (Christenson and Sims, 2011). Most microalgae are small-sized microorganisms (generally, 2–30 μm) and negatively charged in suspension, which make them very difficult to separate from their growth medium (Gonzalez-Fernandez and Ballesteros, 2013). As a result, the biomass will be entrained in the flow and washed-out from the system, which bring on several consequences: (1) the high level of algal biomass in the effluent directly deteriorates the effluent quality; (2) it leads to very low culture density (0.2–0.6 g/L) and subsequently very low treatment capacity (Christenson and Sims, 2011); (3) the high cost (20–50% of biomass production) of harvesting (by flocculation, centrifugation, etc.) is a major limitation to the economical use of microalgae for biofuel production (Milledge and Heaven, 2013). Therefore, improving microalgae's separation efficiency is essential to achieve both efficient wastewater treatment and cost-effective algae biofuel production.

Some microalgae (*Desmodesmus* sp., *Micractinium* sp., *Actinastrum* sp., *Chlorococcum* sp., etc.) can form large settleable colonies (50–200 μm), which enable simple and effective algal biomass separation by gravity sedimentation (Lv et al., 2016; Park et al., 2011b). Several previous studies demonstrated that applying hydrodynamic selection pressure could increase the dominance of the readily-settleable microalgal species in the form of large algal bio-flocs (Park et al., 2011a, 2015, 2013; Valigore et al., 2012). However, the formation of these algal bio-flocs was achieved either with the aid of floc-forming bacteria (Park et al., 2011a, 2013; Valigore et al., 2012; Van den Hende et al., 2011a) or by seeding rapidly-settleable alga (Park et al., 2015). In post-treatment, the number of floc-forming bacteria is limited due to lack of organic carbon source. In addition, inoculum of rapidly-settleable microalgae is not easily available, since indigenous algal cultures are generally poorly settleable. Compared to a large number of settleable microalgal-bacterial flocs successfully cultivated in primary wastewater (Park et al., 2011a, 2013; Valigore et al., 2012; Van den Hende et al., 2011a), there is still limited information on the development of self-settling microalgal consortia in treated effluents (Aneesh et al., 2015).

Thus, the primary goal of this study was to investigate whether settleable microalgal consortia could be selectively enriched from indigenous mixed algal cultures developed in treated effluent. Then, the nutrient removal performance of the enriched microalgal consortia was assessed. Finally, the bioenergy potential of the settleable algal biomass was evaluated based on the cellular composition.

2. Method and materials

2.1. Wastewater and inoculum

Synthetic wastewater contained N and P in typical species and concentrations of tertiary effluent was used as the culture medium. The synthetic wastewater was prepared by dissolving known amounts of inorganic compounds and trace elements and vitamins in non-sterile tap water. The composition followed the recipe described in Boelee et al. (2011). The tap water also contained certain amount of nutrient and minerals, which resulted in the final concentrations of the synthetic wastewater (Table 1).

The inoculum was wastewater born mixed algal culture (Fig. 1a). Basically, glass beakers were fed with non-sterile effluent (Table S1) from a lab scale SBR treating domestic wastewater, and left on windowsill. With sunlight, microalgae naturally developed, reaching a biomass concentration (TSS) of approx. 0.3 g/L after two months' cultivation. Microscopic observation (Fig. 1b and c) revealed that the mixed culture was dominated by unicellular green algae species (*Chlorella* sp. and *Scenedesmus* sp.), followed by cyanobacteria *Chroococcus* sp. Few filamentous cyanobacteria *Oscillatoria* sp. and diatom *Melosira* sp. were also observed.

2.2. Experimental setup

Six laboratory scale photo-bioreactors were constructed with 2-L glass beakers. The photo-bioreactors were operated in batch mode (Fig. S1) with a total cycle time of 1 day. The cycle started with a long mixing & reacting phase after feeding wastewater. Following that, the mixing was stopped for a certain time and the suspension could settle. Finally, a certain amount of the supernatant (V_D) was discharged as the effluent and next cycle repeated. The hypothesis was that the settling time (S_T) and the volume exchange ratio (VER, discharged volume/total volume) together would create a hydrodynamic selection pressure. By such, non-settleable species would be washed out, and settleable species would become dominant in the mixed culture. According to the VER, the six photo-bioreactors were divided into three groups: Group one, No.1 and No.2, with a VER of 0.2; Group two, No.3 and No.4, with a VER of 0.5; and Group three, No.5 and No.6, with a VER of 0.7. Within each group, two different settling time (10, 60 min) were applied respectively. This was based on the consideration that different settling time and VERs would result in different hydrodynamic pressure and nutrient loading rates, thus affecting the settling property and algal community structure. Mixing was provided by overhead stirrers at 200–250 rpm. Illumination was provided continuously at 3000 Lux (ca. 40 μmol/m^2/s) with cool white fluorescent lamps. At the start-up, 2 L mixed microalgal culture (in section 2.1) was inoculated to each reactor. The photo-bioreactors were then operated for 134 days under laboratory conditions (Table 2). To achieve high biomass accumulation, no algal biomass wastage was performed throughout the whole experiment period, except for biomass loss via periodic sampling.

2.3. Analysis and calculations

2.3.1. Microalgal growth, community and settleability

Microalgal growth was monitored in terms of total suspended solids (TSS, g/L) and chlorophyll-a levels in the photo-bioreactors. TSS was determined according to the Standard Methods (APHA, 1999) after filtering 10 mL sample through a pre-weighed Whatman membrane filter (0.45 μm). Chlorophyll *a* (Chla, mg/L) were extracted with 10 mL of 90% (v/v) methanol at 75 °C for 5 min and determined spectrophotometrically using the equation described by Zhang et al. (2008). Settleable biomass productivity was calculated by Eq. (1) after the settling efficiency reached >97%:

$$\text{Productivity}\ (\text{mg/L/d}) = \left(\text{TSS}_{r,\,t2} - \text{TSS}_{r,\,t1}\right)/\Delta t \qquad (1)$$

where $TSS_{r,\,t1}$ and $TSS_{r,\,t2}$ are the biomass concentration (TSS) in the reactor at day t_1 and t_2 respectively; $\Delta t = (t_2 - t_1)$, d.

Microscopic images were taken on daily basis using an optical microscope (Zeiss Axioskop 40) to observe the community changes. Microalgal species were identified into genera based on the morphological characteristics illustrated in Bellinger and Sigee (2015). Settleability of the microalgal biomass were characterized by settling efficiency and sludge volume index (SVI). Settling efficiency was calculated by Eq. (2) and monitored on daily basis. SVI was measured at the end of the study and determined according to