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A comparative study on flocculating ability and growth potential of two microalgae in simulated secondary effluent

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HIGHLIGHTS

- The self-flocculating property of *Chlorococcum* sp. GD was firstly reported.
- *Chlorococcum* sp. GD had excellent flocculating ability.
- The excellent flocculating ability of *Chlorococcum* sp. GD was related to EPS.
- Pollutants in secondary effluent were effectively removed by *Chlorococcum* sp. GD.
- The lipid content of *Chlorococcum* sp. GD was high.

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ABSTRACT

The flocculating ability was an important property to microalgal harvesting, especially in secondary effluent. In this study, the flocculating ability of two microalgae, *Chlorococcum* sp. GD and *Parachlorella kessleri* TY, was evaluated after 10 d of cultivation in secondary effluent. After 180 min of settling, the flocculating ability of *Chlorococcum* sp. GD and *P. kessleri* TY was 84.43% and 16.23%, respectively. It was suggested that *Chlorococcum* sp. GD was an excellent self-flocculating microalgae. The mechanism on self-flocculating of *Chlorococcum* sp. GD was probably related to hydrophobic extracellular polymeric substances (EPS). Besides, compared to *P. kessleri* TY, the nitrogen and phosphorus removal efficiency of *Chlorococcum* sp. GD was high, which was up to 66.51% and 74.19%, respectively. *Chlorococcum* sp. GD also had high lipid content and biomass concentration. Therefore, *Chlorococcum* sp. GD could be regarded as a promising candidate for microalgal cultivation and harvesting in secondary effluent.

1. Introduction

The secondary effluent from wastewater treatment plants (WWTPs) is commonly rich in nitrogen and phosphorus owing to the defect of process itself or inadequate operation. As described in literatures, the total nitrogen and total phosphorus concentration of secondary effluent range from 5 to 30 mg/L, and from 0.2 to 3 mg/L respectively (Wu et al., 2014), which are much higher than the threshold of nitrogen and phosphorus causing the eutrophication in streams (Chambers et al., 2012). Therefore, it is essential to develop some technologies for advanced treatment of secondary effluent.

Microalgae as the largest photoautotrophic group of plant taxa have high photosynthetic efficiency, rapid growth rate, and strong adaptability. Many microalgae are rich in oil, which can be further processed into biodiesel (Maity et al., 2014). Also, microalgae can metabolize nitrogen and phosphorus via different metabolic pathways (Cai et al., 2013). Therefore, microalgal-based biotechnology has been gradually developed for biodiesel production and wastewater treatment over the past few years (Rawat et al., 2013; Zeng et al., 2015).

As an interesting topic, the integration of microalgae-based advanced treatment of secondary effluent and lipid production has been widely concerned in recent years (Arbib et al., 2014; Ji et al., 2013; Li et al., 2010; Sydney et al., 2011; Yang et al., 2011). However, there is a difficulty on microalgal harvesting from secondary effluents due to the relatively low cell density (Ji et al., 2013; Li et al., 2010). Currently, microalgal harvesting methods include centrifugation, chemical coagulation, gravity sedimentation, filtration, flotation, electrophoresis technique, and immobilized technique (Barros et al., 2015). Although these techniques can effectively harvest microalgal biomass from wastewaters, the huge consumption of chemicals and energies is a major challenge.

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Also, some chemicals show a certain degree of biomass toxicity (Rawat et al., 2013). Consequently, all drawbacks above limit the use of these technologies for large-scale harvesting of microalgae, especially in secondary effluent.

Self-flocculating microalgae is a kind of microalgae, which can aggregate together to form large particles by themselves. Then, the aggregate can be easily harvested by sedimentation. Owing to environmental friendliness and no additional economic costs, screening and cultivating self-flocculating microalgae has been regarded as an alternative way to harvest microalgae. Till now, a few investigations have reported that some microalgae, such as *Ankistrodesmus falcatus*, *Chlorella vulgaris*, *Ettlia texensis*, *Scenedesmus obliquus* and *Tetraselmis suecica*, belong to self-flocculating microalgae (Alam et al., 2014; Guo et al., 2013; Salim et al., 2012, 2013, 2014). Especially, the flocculation efficiency of *E. texensis* after 3 h of sedimentation was around 90% (Salim et al., 2013). Nevertheless, the diversity of self-flocculating microalgae is low. Also, the flocculation performance of self-flocculating microalgae in secondary effluent has not been reported. Thus, it is important to screen more self-flocculating microalgae and evaluate their flocculating ability after cultivation of them in secondary effluent.

Compared to a large number of microalgae successfully cultivated in municipal wastewater, industry wastewater, agricultural wastewater and anaerobic digestion wastewater (Chen et al., 2015; Morales-Amaral et al., 2015), the number of microalgae adapting to secondary effluent is limited. The main groups focus on *Botryococcus*, *Chlorella* and *Scenedesmus*, and have low biomass production without any supplement of exogenous carbon sources (Li et al., 2010; Sawayama et al., 1992; Yang et al., 2011). Thus, it is essential to screen more microalgae suitable for growth in secondary effluent, and evaluate the potential of lipid accumulation and biomass production.

In the present study, two microalgae isolated from Shanxi Province are evaluated on flocculating ability, growth potential and pollutants removal efficiency in simulated secondary effluent. It is expected that the finding of this investigation can provide some bases for microalgal cultivation and efficient biomass recovery in secondary effluent.

2. Methods

2.1. Microalgal strains and cultivation

Chlorococcum sp. GD and *Parachlorella kessleri* TY were isolated from the moss *Entodon obtusatus* and soil of Shanxi Province, respectively. The details were in accordance with methods described by Rasoul-Amini et al. (2009) and Zhang et al. (2014). The two microalgae were cultivated in BG11 medium with the following compositions: 1500 mg/L $NaNO_3$, 40 mg/L $K_2HPO_4{\cdot}3H_2O$, 75 mg/L $MgSO_4{\cdot}7H_2O$, 20 mg/L Na_2CO_3, 27 mg/L $CaCl_2$, 6 mg/L citric acid monohydrate, 6 mg/L ammonium ferric citrate, 1 mg/L Na_2EDTA, and 1 mL trace metal solution (2.86 mg/L H_3BO_3, 1.81 mg/L $MnCl_2{\cdot}4H_2O$, 0.222 mg/L $ZnSO_4{\cdot}7H_2O$, 0.079 mg/L $CuSO_4{\cdot}5H_2O$, 0.050 mg/L $CoCl_2{\cdot}6H_2O$, 0.39 mg/L $Na_2MoO_4{\cdot}2H_2O$). At the end of cultivation period, the culture was centrifuged at 5000 rpm for 5 min. Then, the pellet was washed with deionized water and centrifuged at 5000 rpm for 5 min. After that, the pellet was suspended in the simulated secondary effluent for inoculation, and the initial microalgal biomass concentration was about 80 mg/L. The two microalgae were cultivated in 2 L conical flasks with batch mode, and conical flasks were stirred at 160 rpm. The temperature was set at 25 °C. The fluorescent lamps were used to provide incident light intensity with 3000 lux and light/dark period was 14 h/10 h. All these experiments were performed in triplicate.

The simulated secondary effluent in this study was mainly composed by $C_6H_{12}O_6$, $NaNO_3$, KH_2PO_4, $NaHCO_3$, NaCl, $MgSO_4$, $FeSO_4$, $CaCl_2$, H_3BO_3, $MnCl_2$, $ZnSO_4$, Na_2MoO_4, $CuSO_4$ and $Co(NO_3)_2$, and the chemical oxygen demand (COD), nitrate and total phosphorus concentrations were around 40 mg/L, 6.5 mg/L and 0.7 mg/L, respectively.

2.2. Flocculating ability test

The flocculating ability test was carried out according to Alam et al. (2014) with some modification. After 10 d of cultivation, the culture of the two microalgae was harvested. 25 mL culture was distributed in 25 mL cylindrical glass tubes, and followed by gently mixing for 1 min at room temperature. An aliquot of the culture was withdrawn at a height of two-thirds from the bottle when the culture was settled for 30, 60, 120 and 180 min, respectively. After that, the optical density of above aliquots was measured at 680 nm. The flocculating ability was calculated according to the equation as following:

$$\text{Flocculating ability} = (A - B)/A * 100\%$$

where A and B were the optical density (OD_{680}) of the aliquot before and after flocculation.

2.3. Extraction and analysis of EPS

The EPS were extracted according to the procedure from Yang and Li (2009) with some modifications. After 10 d of cultivation, microalgae suspension was dewatered by centrifugation at 5000 rpm for 5 min. The pellet was then washed with deionized water and centrifuged at 5000 rpm for 5 min. The washed pellet was diluted with deionized water and was heated to 80 °C for 30 min. Subsequently, the mixture was centrifuged at 10,000 rpm for 10 min. After that, the supernatant was filtered with 0.45 μm acetate cellulose membranes and the filtrate was regarded as the EPS fraction. Proteins were measured by the coomassic brilliant blue method (Bradford, 1976) using BSA as the standard. Carbohydrates were measured by the Anthrone method (Gaudy, 1962) with glucose as the standard.

2.4. Excitation-emission matrix (EEM) fluorescence spectroscopy

EEM spectra of microalgal EPS were measured with F-280 fluorescence spectrophotometer (Gangdong, China). Spectra were collected with subsequent scanning of emission spectra from 250 to 550 nm at 1 nm increments by varying the excitation wavelength from 200 to 450 nm at 10 nm increments. Excitation and emission slits were maintained at 5 nm and the scanning speed was set at 1200 nm/min. The voltage of the photomultiplier tube (PMT) was set to 700 V. The software Origin 8.0 was employed for handling EEM data.

2.5. Relative hydrophobicity (RH) of microalgae

The RH of microalgae was measured by following the protocol from Zhang et al. (2007) with some modifications. After 10 d of cultivation, 10 mL of hexadecane was mixed with 20 mL of microalgal suspension and the mixture was inverted for 10 min at room temperature. Then, the mixture was settled for 30 min, and the two phases separated completely. After that, the aqueous phase was transferred. The relative hydrophobicity was expressed as the ratio of the optical density (OD_{680}) in the aqueous phase after emulsification ($OD_{680\text{-E}}$) to the optical density (OD_{680}) in the aqueous phase before emulsification ($OD_{680\text{-O}}$). The RH was evaluated as

$$\text{RH} = (1 - \text{OD}_{680-\text{E}}/\text{OD}_{680-\text{O}}) * 100\%$$