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Optimization of biomass production and nutrients removal by *Spirulina platensis* from municipal wastewater

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ABSTRACT

Using wastewater as a medium to cultivate microalgae is regarded to have economic and environmental potentials for producing biomass, recovering nutrients and treatment of wastewater. However, low strength of municipal wastewater may influence the optimum conditions for microalgae cultivation while comparing with standard media. In this study, to optimize the microalgal production and nutrients uptake from wastewater, the optimum conditions for microalgal autotrophic cultivation were predicted by using response surface methodology (RSM) and validated through laboratory experiments. The optimum conditions for the cultivation of *Spirulina platensis* in synthetic municipal wastewater (SWW) were the range of 8.8–8.9 for pH, the light intensity of 3300–3400 lx in the daily illumination time for 12 h when the temperature was set at $25 \pm 1\,^{\circ}C$ with the air-bubbling of 0.5 vvm. Under the optimum conditions, the yield of microalgal biomass and protein content was 262.50 mg/L, 46.02%, respectively. Nitrogen and phosphorus removal efficiencies from SWW were 81.51 and 80.52%, and the nutrients recovery efficiencies in the removed TN and TP were 92.58 and 94.13% in autotrophic culture. The mixotrophic cultivations enhanced the microalgal production, nutrients removal and recovery, and mitigated nutrients limitation. Moreover, the addition of 300 mg/L glucose in mixotrophic cultivation, as a low-cost alternative, was found to be a suitable medium for effective enhancement of microalgae growth, nutrients removal and recovery. These strategies can be a good reference for the enhancement of microalgal production and their application for the large-scale wastewater treatment under a controlled environment.

1. Introduction

Blue-green algae (cyanobacteria) are capable of producing valuable metabolites, such as proteins, fatty acids, and carbohydrates for feed additives and health products. Among filamentous cyanobacteria, *Spirulina platensis* is preferred for high biomass yield because of its relatively high cell growth rate and easy biomass recovery (Guerin et al., 2003; Hu, 2004). In addition, the ability to grow in alkaline media reduces the risks of contamination by other microorganisms (Ak, 2011).

However, the high cost of microalgae cultivation has limited the development at large-scale production. Approximately 80% of the total costs for microalgae cultivation are associated with excessive consumption of nutrients and water (Luo et al., 2016). Hence, using wastewater as a nutrient source to cultivate microalgae is regarded to have economic and environmental potentials for producing biomass, assimilating nutrients from wastewaters and abating water contamination. Moreover, this process would significantly reduce the cost of culture medium, and would not cause secondary pollution (Luo et al., 2016; Zamani et al., 2012).

In previous studies, a variety of wastewaters including municipal, industrial and agricultural wastewaters were used to investigate the nitrogen and phosphorus removal by microalgae. Different types of microalgae such as *Chlorella vulgaris*, *Scendesmus obliques*, and *Spirulina platensis* have been used for autotrophic, heterotrophic and mixotrophic cultivations in wastewaters (Ebrahimian et al., 2014). Among these species, *Spirulina platensis* has been known as one of the most widely used microalgae to treat various wastewaters, and it is able to readily uptake the nitrogen and phosphorus from wastewater through its cell membrane (Chaiklahan et al., 2010).

It has been demonstrated that the medium components largely affect the microalgal growth, biomass components and nutrient uptake, while low nutrients content in municipal wastewater generally results in low biomass productivity, limiting the integration of microalgae cultivation with wastewater treatment (Li

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et al., 2011; Sacristán de Alva et al., 2013; Zhang et al., 2014). Meanwhile, the optimum conditions for mass cultivation of *Spirulina platensis* were pH 9.0–10.0, temperature 25–30 °C, light intensity 72 μmol/m^2 s (4000 lx) and daily illumination time of 12 h with micro-aeration in mixotrophic cultivation (Markou and Georgakakis, 2011; Markou et al., 2016; Morocho-Jácome et al., 2015; Olguín et al., 2003; Pandey and Tiwari, 2010). However, these optimum conditions were based on sufficient and preferred nutrients in the medium for *Spirulina platensis* cultivation. We hypothesize that due to low nutrients and chemical compounds variation of municipal wastewater, the optimum conditions for microalgae cultivation maybe vary to some extent. To the best of our knowledge, with respect to the *Spirulina platensis* cultivated in municipal wastewater, there are no reported studies on strategic enhancement of the microalgal production, effective cell composition and simultaneous improvement of nutrients removal, and no knowledge on optimization of external conditions for the microalgal cultivation using response surface methodology (RSM). Furthermore, more evidence that the organic carbon affects the microalgal growth and wastewater treatment need to be explored.

Therefore, in this study, the cyanobacterium, *Spirulina platensis* is cultivated in a synthetic municipal wastewater for biomass yield and simultaneous wastewater treatment. The optimum conditions for the microalgal autotrophic cultivation were predicted by using RSM coupled with central composite design (CCD) and validated through laboratory experiments. The sole and interactive effects of pH, light intensity and daily illumination time were investigated based on the microalgal growth and nutrients removal efficiencies. Besides, the microalgal growth and nutrients removal in mixotrophic cultivation were explored and compared with autotrophic cultivation under the obtained optimum conditions. Our study aims to develop new strategies for microalgae cultivation for wastewater treatment.

2. Materials and methods

2.1. Microalgal strain, media, and cultivation

The microalgal strain used in this study, *Spirulina platensis* FACHB-431, was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). Microalgal cells were grown in Zarrouk medium which consisted of 13.61 g $NaHCO_3$, 4.03 g Na_2CO_3, 0.5 g K_2HPO_4, 2.5 g $NaNO_3$, 1.00 g K_2SO_4, 1.00 g NaCl, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.04 g $CaCl_2{\cdot}2H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.08 g Na_2-EDTA and 1 mL A5 trace metal solution per liter (Markou et al., 2014). The A5 trace metal solution contained 2.86 g H_3BO_3, 1.81 g $MnCl_2{\cdot}4H_2O$, 0.222 g $ZnSO_4{\cdot}7H_2O$, 0.39 g Na_2MoO_4, 0.079 g $CuSO_4{\cdot}5H_2O$, 0.049 g $Co(NO_3)_2{\cdot}6H_2O$ per liter of deionized water (Markou et al., 2014). The culture medium was autoclaved and the pH was adjusted to 9.5 using 0.1 M HCl and 0.1 M NaOH.

The microalgal seeds at the log-phase were gently centrifuged (3000 rpm for 5 min) and washed twice with deionized water. Then, the microalgal cells were re-suspended in flasks (2.5 L) containing 2 L of sterilized synthetic municipal wastewater (SWW) with initial microalgal inoculum concentration of about 0.05 g dry weight/L for initial microalgal cultivation in association with wastewater treatment. The composition of SWW is shown in Supplementary Table S1. To maintain stable microalga cultivation and to obtain more reliable results, synthetic wastewater was used as a medium in the present study instead of real wastewater. Furthermore, we investigated two different cultivation modes: autotrophic and mixotrophic. In order to elucidate the effect of organic carbon on the microalgal growth and nutrients removal from wastewater, synthetic wastewater was used to exclude the impact of organic carbon for an autotrophic culture which was latter compared with a mixotrophic culture. Generally, the composition of real wastewater widely varies and that can significantly influence the microalgal growth and subsequent nutrients removal by microalgae. At the preliminary experiment, we used real wastewater, but the nutrient concentrations of the real wastewater obtained from university campus varied greatly (data not shown), which resulted significant variations in cultivation results. In addition, real wastewater sometimes shows a lack of necessary and trace elements (Ebrahimian et al., 2014; Zhang et al., 2014).

Agitation of the culture broth was provided with filtered air by a membrane air pump at the rate of 0.5 vvm. The cultivation was carried out in a growth chamber and the culture temperature was kept constant at 25 °C (±1 °C). The cultivation flasks were exposed to 28 W fluorescent lamps as the light source.

The microalgal cultivation was conducted in two modes: autotrophic and mixotrophic cultivations. In autotrophic cultivation, carbon source was only inorganic carbon. The effects of pH, light intensity and daily illumination time on microalgal biomass yield, protein content, total nitrogen (TN) and total phosphorus (TP) removal were investigated by the cultivation of the microalga in SWW. Then the optimum conditions were predicted by RSM and validated by additional experiments. Meanwhile, autotrophic cultivation of the microalga in Zarrouk medium under the predicted optimum conditions was carried out as a control. In mixotrophic cultivation, both inorganic and organic carbon were added as the carbon source. The effects of organic carbon source on the biomass yield, protein content, TN and TP removal were investigated by adding specified amount of glucose (150, 300, 600 mg/L) to the SWW under the optimum conditions as autotrophic cultivation. Each of the microalgal cultivation was carried out in triplicate and the reported results were the subsequent average.

2.2. Evaluation of effective parameters on microalgae growth and nutrients uptake by RSM

The microalga was autotrophically cultivated in the SWW under different conditions of pH, light intensity and daily illumination time. The variation of light intensity was set by controlling the distance between flasks and fluorescent lamps and measured by light intensity the meter (Lutron-YK-10LX lux meter, Taiwan). The pH value was detected by a pH meter (Thermo-Scientific, USA). The variations of these independent factors were: pH (symbol A: 7.5, 8.5, 9.5); light intensity (symbol B: 2000 lx, 3000 lx, 4000 lx); daily illumination time (symbol C: 8 h, 12 h, 16 h).

The RSM coupled with CCD through Design Expert software (Version 8.0.6) was employed to investigate the effects of the parameters on the microalgal biomass yield, protein content, nitrogen removal efficiency (NRE) and phosphorus removal efficiency (PRE), and to predict the optimum conditions. The RSM is a combination of mathematical and statistical methods suitable for the analysis and modeling of problems in which an indicator of interest is influenced by several variables where optimization of this indicator is the objective. The experiments were designed and conducted under different conditions as shown in Table 1.

The original polynomial models based on the various parameters were fitted to experimental data using the least squares method and analysis of variance techniques in Design Expert software (Version 8.0.6). The models for microalgal biomass yield, protein content, NRE and PRE for the autotrophic cultivation of the microalga in SWW could be obtained by Eq. (1).

$$Y_0 = b_0 + \sum_i b_i X_i + \sum_{ii} b_{ii} X_i^2 + \sum_{ij} b_{ij} X_i X_j \qquad (1)$$

in which, Y_0 is the predicted biochemical indicator, X_iX_j are independent variables, b_0 is the offset term, b_i is the ith linear coefficient,