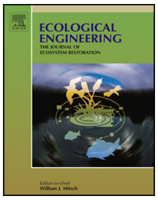
Ecological Engineering 92 (2016) 236–242

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng



Optimization of microalgal bead preparation with *Scenedesmus obliquus* for both nutrient removal and lipid production

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ARTICLE INFO

Article history:
Received 12 June 2015
Received in revised form 6 March 2016
Accepted 20 April 2016
Available online 29 April 2016

Keywords:
Microalgal immobilization
Scenedesmus obliquus
Water treatment
Nutrient
Biomass energy

ABSTRACT

The integration of wastewater treatment and biofuel production with immobilized microalgal beads has attracted much concern for both environmental and economic benefits. However, high cost of bead preparation hinders the large-scale application of this technology. To optimize microalgal bead preparation, an orthogonal array design with microalgal species *Scenedesmus obliquus* covering four factors including sodium alginate concentration, $CaCl_2$ concentration, bead size and initial algal density were employed in this study considering both nutrient removal and lipid productivity. The results show that the optimized conditions of microalgal bead preparation were as following: sodium alginate concentration of 4% (w/v), $CaCl_2$ concentration of 2% (w/v), bead size of 4.7 mm and initial algal density of 0.2×10^6 cells mL^{-1}. The highest algal biomass productivity and lipid yield were recorded as up to 18.9 mg L^{-1} day^{-1} and 0.091 g L^{-1}, respectively. Furthermore, the removal efficiency of TN, TP and NH_4^+-N was 63.12%, 99.06% and 92.02%, respectively, with the final effluent quality meeting the fifth-level of Chinese Surface Water Quality Standards. This technique therefore provides a potential methodology for integrative wastewater treatment and biofuel production.

1. Introduction

During the previous two decades, the surface water in China was rapidly degraded with the booming industrialization and increasing sewage discharge. Eutrophication was one of the most critical problems in surface waters of China (Qiu et al., 2010). The secondary effluent from various municipal wastewater treatment plants (MWWTPs) was identified as the main cause of eutrophication in natural waters (Ruiz-Marin et al., 2010). Consequently, the Ministry of Environmental Protection of China (MEP) raised the MWWTP effluent quality to Class 1A of *Discharge Standard of Pollutants for Municipal Wastewater Treatment Plants* (MEP, 2002a), with the maximum discharging concentrations for total nitrogen (TN), total phosphorus (TP) and ammonia nitrogen (NH_4^+-N) being 15 mg L^{-1}, 0.5 mg L^{-1} and 5 mg L^{-1}, respectively. The existing MWWTPs in China mainly adopt the conventional activated sludge process with the secondary effluent quality of Class 1B. It is therefore difficult for them to meet the new discharge standard (He and Xue, 2010). Nevertheless, even the Class 1A is not strict enough to protect the aquatic environment in some area. Around the Taihu Lake (30°55′40″–31°32′58″N, 119°52′32″–120°36′10″E), for example, more stringent criteria were adopted to mitigate the serious eutrophication problem (Qiu et al., 2010). Furthermore, the criteria in the future are expected to be close to the *Surface Water Quality Standards* (MEP, 2002b). It is therefore urgent to develop a secondary treated wastewater purification technology to meet more stringent effluent discharge criteria.

Wastewater treatment using microalgae has been in practice for decades, especially in areas with appropriate climate conditions throughout the year, such as relatively high temperature and adequate sunshine (Aravantinou et al., 2013). Immobilized microalgae are more efficient than free-living algal cells in removing nitrogen and phosphorus from a variety of wastewater (Lau et al., 1997; Liu et al., 2012). Up to date, various immobilization techniques have been developed, for example, flocculant agents, chemical attachment and gel entrapment, among which the alginate gel entrapment method is most feasible due to high diffusivity, low polymer cost and fast immobilization process (De-Bashan, 2009; Pittman et al., 2011; Liu et al., 2012). In this study, therefore, the alginate gel entrapment method was adopted for microalgal immobilization.

The coupling of wastewater treatment and microalgal biofuel production shows a great potential for commercial application in

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http://dx.doi.org/10.1016/j.ecoleng.2016.04.009
0925-8574/

the near future (Brennan and Owende, 2010; Xin et al., 2010; Ji et al., 2013). To simplify the separation of microalgae from wastewater for biofuel production, the microalgal immobilization technology can be employed (Lam and Lee, 2012a). Up to date, the cost of the immobilization matrix is, however, still high (Christenson and Sims, 2011; Lam and Lee, 2012a). For example, the cost of sodium alginate (Sinopharm Chemical Reagent Co., Ltd, China) is approximately CNY7.2 (US$1.16) for 1000 mL algal beads. In addition, most studies on microalgal immobilization focused on pollutant removal efficiency and its affecting factors (Ertuğrul et al., 2008; Jin et al., 2011; Liu et al., 2012). The optimization of microalgal bead preparation is however seldom reported.

This study, therefore, aims to obtain the most cost-efficient method for microalgal bead preparation covering four factors, i.e., sodium alginate concentration, $CaCl_2$ concentration, bead size and initial algal density, via an orthogonal array experiment design considering both nutrient removal and lipid production.

2. Materials and methods

2.1. Microalgal species and culture conditions

The microalgal species *Scenedesmus obliquus* was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences. The algae was cultured in conical flasks (1L) containing 500 mL BG11 medium as described in Chu et al. (2013). All the culture medium used in this study was autoclaved at 121 °C for 30 min. Then the culture was maintained at 23 ± 1 °C under a light intensity of 8000 lux with a 12 h:12 h light:dark (L:D) cycle, and pH of 7.2–7.6.

The artificial wastewater was made with a modified dauta medium (Kaya et al., 1995), with the following compositions ($mg\,L^{-1}$): NH_4Cl, 38.20; $NaNO_3$, 60.70; K_2HPO_4, 7.35; $MgSO_4{-}7H_20$, 2.50; $FeSO_4{-}7H_2O$, 1.00; $CaCl_2{-}2H_2O$, 2.50; $NaHCO_3$, 250.00; Na_2CO_3, 5.00; $Na_2EDTA{-}2H_20$, 1.10; $Na_2MoO_4{-}2H_2O$, 0.041 and $ZnSO_4{-}7H_2O$, 0.02. The initial pH of artificial wastewater was maintained at 7.5 ± 0.1. To supply sufficient carbon source for microalgal growth without CO_2 pumping, the sodium bicarbonate concentration in artificial wastewater was set as 250 $mg\,L^{-1}$ (El-Sheekh et al., 2013). The concentrations of TN (20 $mg\,L^{-1}$), TP (1 $mg\,L^{-1}$) and $NH_4{}^+$-N (10 $mg\,L^{-1}$) in the artificial wastewater were prepared in accordance with the Class 1B of Chinese National Discharge Standard.

2.2. Preparation of microalgal beads

After a ten-day culture, the microalgal cells at exponential growth phase were harvested by centrifugation at 1970*g* for 10 min at 20 °C. The microalgal residuals were washed twice with distilled water and re-suspended in distilled water to get a concentrated algal suspension. The concentrated algal suspension was then immobilized in alginate matrix with the following method. In brief, the cell suspension was mixed with sodium alginate solution at a volumetric ratio of 1:1 to yield an algal–alginate suspension. Then the mixture suspension was dropped into $CaCl_2$ solution with a peristaltic pump (model BT100-2J, Hebei province, China) at a flow rate of 3.2 $mL\,min^{-1}$ to produce uniform immobilized microalgal beads. Finally, the beads were kept in $CaCl_2$ solution for 12 h for hardening.

2.3. Orthogonal array design and analytic strategy

The orthogonal array design is based on Taguchi method (Yang and Tarng, 1998). It aims to reveal the optimized conditions (Wei et al., 2013). However, the optimized conditions obtained through traditional analytic methods depend only on the final experimental outcome without considering the dynamic change of target variable parameters during the whole experiment. In fact, a closer analysis of the target variable parameters during the whole experiment provides us more information and helps to make more rational judgment for optimized conditions.

Therefore, firstly we analyzed the dynamic changes of target variable parameters in each experimental group and obtained the preliminary optimization conditions. Secondly, we evaluated the significance level of each factor and got the optimization conditions for each target variable parameter based on the final outcomes. Lastly, we obtained the final optimization conditions considering all target parameters.

2.4. Experiment set-up

Four factors and three levels for each factor with nine combinations for microalgal bead preparation were studied by orthogonal array design of $L_9\ (3^4)$ (Table 1). The setup of each level of different factors was based on previous studies on single factor effect (Tam and Wong, 2000; Jin et al., 2011; Liu et al., 2012).

The volume of the microalgal beads was determined by the volume of water they discharge in a measuring cylinder. The algal beads with a volume of 50 mL were put into culture conical flasks (1L) containing 500 mL artificial wastewater. The flasks were then incubated in an illuminated incubator at 23 ± 1 °C under a light intensity of 12,000 lux with a photo period of 12 h:12 h and an initial pH of 7.5 ± 0.1. Blank flasks without any bead but with an equal volume of artificial wastewater were used as the control. All groups were carried out in duplicates and cultured for 10 days.

The whole experiment lasted for 10 days, which was divided into two stages. The first five days was a semi-continuous culture stage, during which 40% of culture solution were replaced each day with fresh artificial wastewater. The nutrient concentration in the culture solution was maintained at a relatively high level to promote the growth of the immobilized algae. The following five days was a batch culture stage, during which the culture solution was not replenished to stimulate lipid production through nutrient limitation (Chu et al., 2013).

2.5. Analytical procedures

2.5.1. Sample collection and nutrient analysis

In the first five-day stage, culture samples of 200 mL were taken daily from each conical flask for nutrient analysis and an equal volume of fresh artificial wastewater was added. In the next five-day stage, culture samples of 30 mL were collected each day. The samples were filtered through cellulose acetate membrane filters (0.45 μm pore size, 47 mm in diameter). Then, the filtrates were diluted and analyzed for TN, TP and $NH_4{}^+$-N according to the *National Water and Wastewater Quality Monitoring Method of China* (SEPA, 2002).

2.5.2. Determination of immobilized microalgal growth rate

The immobilized microalgal beads were dissolved in sodium citrate solution (0.2 M) and centrifuged at 1970*g* for 10 min. The cell suspension was washed and re-suspended twice with distilled water. The absorbance was measured by a spectrophotometer (UV-5100, Shanghai, China) at 560 nm (Martínez et al., 2000). In addition, the amount of bead leakage was measured directly via the absorbance of culture solution.

The correlation between light absorbance at 560 nm (OD_{560}) and the dry weight biomass (DW) of *S. obliquus* was pre-determined, which is shown in Eq. (1):

$$DW(g\,L^{-1}) = 2.5786 \times OD_{560} + 0.0166,\ R^2 = 0.995 \tag{1}$$