Bioresource Technology 243 (2017) 583–588

Contents lists available at ScienceDirect

Bioresource Technology

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

Efficient harvesting of marine *Chlorella vulgaris* microalgae utilizing cationic starch nanoparticles by response surface methodology

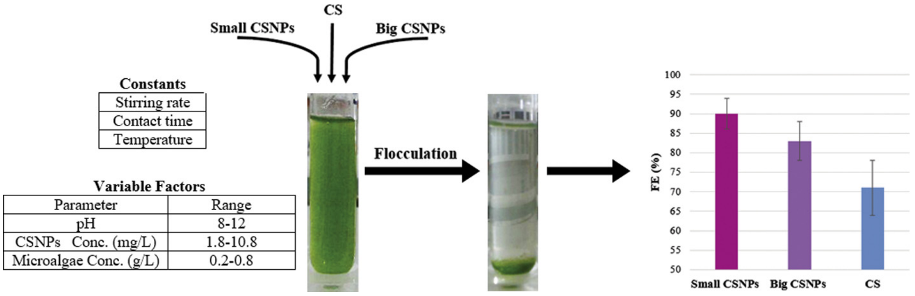
Mahya Bayat Tork [a], Rasoul Khalilzadeh [a], Hasan Kouchakzadeh [b,*]

[a] Department of Biotechnology, Malek Ashtar University of Technology (MUT), Tehran, Iran
[b] Protein Research Center, Shahid Beheshti University, G.C., Velenjak, Tehran, Iran

HIGHLIGHTS

- A suitable and cost-effective method for efficient microalgae harvesting developed.
- The flocculation potential of CSNPs assessed and optimized through the RSM.
- 62 nm CSNPs revealed 10% and 20% higher efficiency than the bigger ones and CS.
- CSNPs can be employed for large-scale harvesting of *Chlorella vulgaris* microalgae.

GRAPHICAL ABSTRACT



Constants

Stirring rate
Contact time
Temperature

Variable Factors

Parameter	Range
pH	8-12
CSNPs Conc. (mg/L)	1.8-10.8
Microalgae Conc. (g/L)	0.2-0.8

ARTICLE INFO

Article history:
Received 7 May 2017
Received in revised form 29 June 2017
Accepted 30 June 2017
Available online 3 July 2017

Keywords:
Chlorella vulgaris microalgae
Response surface methodology (RSM)
Flocculation efficiency
Cationic starch nanoparticles (CSNPs)

ABSTRACT

Harvesting involves nearly thirty percent of total production cost of microalgae that needs to be done efficiently. Utilizing inexpensive and highly available biopolymer-based flocculants can be a solution for reducing the harvest costs. Herein, flocculation process of *Chlorella vulgaris* microalgae using cationic starch nanoparticles (CSNPs) was evaluated and optimized through the response surface methodology (RSM). pH, microalgae and CSNPs concentrations were considered as the main independent variables. Under the optimum conditions of microalgae concentration 0.75 g dry weight/L, CSNPs concentration 7.1 mg dry weight/L and pH 11.8, the maximum flocculation efficiency (90%) achieved. Twenty percent increase in flocculation efficiency observed with the use of CSNPs instead of the non-particulate starch which can be due to the more electrostatic interactions between the cationic nanoparticles and the microalgae. Therefore, the synthesized CSNPs can be employed as a convenient and economical flocculants for efficient harvest of *Chlorella vulgaris* microalgae at large scale.

1. Introduction

Nowadays, the unicellular organisms, microalgae, are an attractive source of biomass due to their higher productivity than the agricultural crops and their use in applications such as food additives, animal feed and renewable fuels is being industrialized. They are particularly used in the production of fatty acids and biodiesel (Hultberg et al., 2014; Vandamme et al., 2012). Cultivation, biomass harvesting, and then extraction for the production of desired materials are the key steps in the process of microalgae production. Among them, microalgae harvesting is encountered with limitations including the low concentration in culture medium and their small size with mostly negative surface charge in a wide ranges of pH, consisting of nearly 30 percent of the total cost of biomass production (Wang et al., 2015; García-Pérez et al., 2014). Therefore, selection of an appropriate, efficient and economical harvesting method according to the scale of biomass that is going to be harvested can reduce the total production costs. The selected method

---

* Corresponding author.
E-mail address: h_kouchakzadeh@sbu.ac.ir (H. Kouchakzadeh).

http://dx.doi.org/10.1016/j.biortech.2017.06.181
0960-8524/

should be able to keep the microalgae medium free from toxic compounds (Barros et al., 2015).

In general, harvesting methods are classified into three main categories including biological (bio-flocculation), chemical (chemical flocculation) and physical (e.g., flocculation by filtration and centrifugation) (Barros et al., 2015). Although conventional chemical flocculants such as iron and aluminum salts have been extensively used as an inexpensive way to harvest large-scale microalgae, but using large amounts of synthetic chemical flocculants can have negative effects on the downstream processes (Salim et al., 2011; Granados et al., 2012; Rashid et al., 2013). Among the methods, bio-flocculation of microalgae by natural polymers can be considered as an effective method due to its simplicity, biocompatibility, non-toxicity and low cost. Flocculation also may occur as the result of a change in the environmental conditions such as pH, nutrients, temperature and dissolved oxygen that are not preferred to be employed for the large-scale harvesting of microalgae due to uncontrolled flocculation and unwanted changes in cell composition (Salim et al., 2011; Benemann and Oswald, 1996).

A number of high molecular weight compounds such as synthetic or natural polymers can be used to improve the efficiency of the flocculation process (Xu et al., 2013). Microalgae flocculation can be easily obtained through the electrostatic interactions and bridging among the cells utilizing these compounds. Although synthetic polymers such as polyacrylamide are more effective than inorganic salts, but their little biodegradability limits their extensive use (Billuri et al., 2015). In this case, indigenous materials such as chitosan, cassia gum or a cheaper alternative such as cationic starch (CS) can be employed (Rashid et al., 2013; Roselet et al., 2015). Flocculants based on anionic polymers are not proper for microalgae harvesting due to the negative charge of microalgae cell surface that prevents the formation of polymer-cell bridges. In contrast, experiments conducted with cationic flocculants showed promising results in microalgae harvesting 9 ’t Lam et al., 2014).

One of the natural flocculants that has increasingly been evaluated in recent years is the polysaccharide, starch. Its powerful potential for flocculation as well as its local abundance and low price make it possible to use this biopolymeric flocculants for microalgae harvesting even at large-scale (Le Corre et al., 2010; Gutiérrez et al., 2015). In comparison with the chitosan-based flocculants, starch as a cost-effective flocculants for microalgae harvesting will reduce the total production fee (Gutiérrez et al., 2015). In addition, starch can be chemically modified in order to be cationized that significantly increases its flocculation capacity (Hansel et al., 2014). Unlike flocculation by chitosan which happens at pH range of 4–8, it needs to be performed at higher pHs for achieving better results that keeps the positive charge of the CS (Billuri et al., 2015; Gerchman et al., 2017).

The use of nanoparticles in the processes of cultivation and harvesting of microalgae is an interested field of research. Besides providing a very large surface area, nanoparticles have unique physical, chemical and mechanical properties in the case of reactivity, adhesion, flexibility and resistance, which are different from the properties of their nano-particulate form. They can be used independently or in combination with cationic polymers for the flocculation of microalgae (Lee et al., 2015). Fe_3O_4 nanoparticles (Toh et al., 2014), Fe_3O_4-Polyethylenimine (PEI) nanocomposites (Hu et al., 2014), Al-aminoclay (Lee et al., 2013a), Aminoclay-wrapped nanoscale zero-valent iron (nZVI) (Lee et al., 2014a), composition of Aminoclay-TiO_2 composite (Lee et al., 2014b), Amino clay-humic acid composite (Lee et al., 2014c), nano-chitosan (Farid et al., 2013), chitosan-Fe_3O_4 composite (Lee et al., 2013b), and nanocrystalline cellulose (CNC) (Vandamme et al., 2015) are some example of nanoparticulate flocculants.

With the consideration of the potential of CS and nanoparticles for microalgae flocculation as mentioned before, cationic starch nanoparticles (CSNPs) can also be recommended as an effective flocculants. Accordingly, the potential of *Chlorella vulgaris* microalgae flocculation induced by biocompatible, biodegradable and economical CSNPs was evaluated and the process was optimized through the response surface methodology (RSM). In this regard, after the production and characterization of CSNPs, the main factors affect the flocculation efficiency (FE) and their levels were determined and the process was optimized using D-optimal for the design of the experiments. In addition, the effect of CSNPs size on FE was assessed under the obtained optimum conditions. Reaching an efficient harvesting of *Chlorella vulgaris* microalgae utilizing inexpensive CSNPs would be promising for the production of cost-effective products at large-scale.

2. Materials and methods

2.1. Materials

Soluble starch, Span80, liquid paraffin and $POC1_3$ were purchased from sigma (Steinheim, Germany). All other reagents were of analytical grade and used as received.

2.2. Culturing of microalgae

Chlorella vulgaris microalgae obtained from the Iranian Research Organization for Science. The defined Bold’s Basal Medium (BBM) was used as the culture medium of *Chlorella vulgaris* (Yah and Chang, 2012). The culture medium was adjusted to pH 6.8, autoclaved, inoculated and the flask incubated at 25 ± 2 °C for 7 days under constant stirring with continual illumination of 50 μmol photons m^{-2} s^{-1}. Growth rate of the microalgae monitored periodically by measuring the absorbance at 600 nm.

2.3. Synthesis of CSNPs

CSNPs were prepared by a water in oil emulsification technique as described elsewhere (Huang et al., 2013) after the modification of soluble starch and obtaining CS. Briefly, prepared CS dissolved in 10 mL of distilled water in a boiling water bath, sonicated for 30 min in order to obtain a uniform aqueous solution and then the pH adjusted to 10. The oily phase prepared by dissolving span80 in 100 mg paraffin under 250 rpm shaking at 30 °C for 30 min. Afterwards, the aqueous phase added dropwise into the oily phase under 1000 rpm shaking for 30 min. Subsequently, $POC1_3$ added and the solution stirred for another 4 h. By the end of the reaction, the resulting solution centrifuged (10 min, 10,000 rpm) three times and the nanoparticles were washed from the oily and water phases with re-dispersing in equal volumes of distilled water and ethanol using a probe sonication.

The amount of CS and $POC1_3$ affects significantly on the diameter of the nanoparticles. CSNPs with two different sizes were synthesized for the evaluation of their effect on microalgae FE by changing the amount of CS and $POC1_3$. In order to obtain CSNPs with different sizes, two dissimilar protocols in the case of quantities were used: (1) the amount of CS: 0.5 g, the ratio of oily phase/aqueous phase: 10, the amount of span80: 0.5 g, the amount of $POC1_3$: 10 μl, (2) the amount of CS: 0.2 g, the ratio of oily phase/aqueous phase: 10, the amount of span80: 0.5 g, the amount of $POC1_3$: 25 μl.

The potential of manufactured nanoparticles with different sizes for microalgae harvesting was initially evaluated in some pre-experiments and after the comparison of their efficiency, the best formulation was selected and used as the CSNPs in the