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An efficient microalgal biomass harvesting method with a high concentration ratio using the polymer-surfactant aggregates process

Yin-Hu Wu[a,b,1], Li-Cheng Shen[c,1], Hong-Ying Hu[b], Nick P. Hankins[c,*], Wei E. Huang[a,*]

[a] Department of Engineering Science, University of Oxford, Parks Road, Oxford, OX1 3PJ, United Kingdom
[b] Environmental Simulation and Pollution Control State Key Joint Laboratory, State Environmental Protection Key Laboratory of Microorganism Application and Risk Control (SMARC), School of Environment, Tsinghua University, Beijing 100084, PR China
[c] Laboratory of Sustainable Water Engineering, Department of Engineering Science, The University of Oxford, Parks Road, OX1 3PJ, United Kingdom

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ABSTRACT

The high cost and energy consumption related to the downstream harvesting and dewatering process is one of the most important bottlenecks limiting the commercial production of microalgal bioenergy. In this study, a novel microalgal biomass harvesting technique has been developed using polymer surfactant aggregates (PSAs). This approach has been applied to three different microalgal strains and two cynobacterial strains with a recovery efficiency of over 80%. In particular, the recovery efficiency of *Chlorella* sp. ZTY4 with a biomass concentration of 1.43 $g{\cdot}L^{-1}$ can be as high as 99.9% using 360 $mg{\cdot}L^{-1}$ poly (acrylic acid) (PAA) and 4 mM (1432 $mg{\cdot}L^{-1}$) cetylpyridinium chloride (CPC). In addition, with this PAA and CPC dosage, the recovery efficiency of *Chlorella* sp. ZTY4 remains above 90% for biomass concentrations up to 2.5 $g{\cdot}L^{-1}$. Furthermore, the water content in the harvested biomass is below 70% with a corresponding concentration ratio of 231. The total flocculation time needed for this technique is 20 min. The optimum dosage ratio for PAA to CPC ranges from 90 to 100 mg/mmol. Based on these results, an efficient harvesting method with a high concentration ratio is proposed to simplify the whole downstream harvesting and dewatering processes of microalgal biomass.

1. Introduction

Microalgal biomass-based bioenergy is considered to be one of the most promising substitutes for fossil fuels due to a series of advantages, including an attractive energy density, compatibility with the existing sophisticated distribution and storage infrastructure, and higher energy production efficiency and lower land demand compared with terrestrial crops [1–3]. Several processes have been developed to convert microalgal biomass into bioenergy products, such as biodiesel, bio-crude oil, biogas and so on. However, regardless of the conversion process, the water content of microalgal biomass must be reduced below at least 90% before being converted into bioenergy [4,5], which means the biomass in an autotrophic cultivation system must be concentrated by a factor over one hundred. With the decrease of the water content in the harvested biomass, the conversion process would then be much easier and more efficient. Actually, one of the key factors limiting the commercial production of microalgal bioenergy is the high cost related to the downstream harvesting and dewatering processes [6], which typically contributes some 20%–30% to the total cost of the microalgal biomass production [7]. Microalgal biomass is difficult to recover from the culture, mainly because of: (1) low biomass concentration in the medium (typically around 1 $g{\cdot}L^{-1}$ in an autotrophic cultivation system), (2) the small size of microalgal cells (5–50 μm), (3) their negative surface charge and, (4) the similarity of the density of microalgal cells to the growth medium [8,9].

Several harvesting and dewatering approaches have been developed for microalgal biomass, including but not limited to micro-filtration, centrifugation, and flocculation followed by sedimentation or air flotation [8,9]. Micro-filtration and centrifugation are effective in recovering and concentrating microalgal biomass, but are energy-intensive and expensive to operate. These methods are acceptable only when harvested microalgal biomass is used to produce high-value products, such as long-chain polyunsaturated fatty acids (PUFAs), astaxanthin, carotenoid and so on [7,10]. For low-value bulk products, such as biodiesel, flocculation and flotation are the preferable options. However, there are lots of hydrophilic species on the microalgal cell

Abbreviations: PSA, polymer surfactant aggregates; PAA, poly (acrylic acid); CPC, cetylpyridinium chloride; poly DADMAC, poly (diallyl dimethylammonium) chloride; PEI, poly (ethyleneimine); OD, optical density; TOC, Total organic carbon

* Corresponding authors.
E-mail addresses: nick.hankins@eng.ox.ac.uk (N.P. Hankins), wei.huang@eng.ox.ac.uk (W.E. Huang).
[1] These authors contributed equally to this work.

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Table 1
Microalgal strains used in this study.

Strain	Description	Reference
Synechococcus sp. PCC 7002	Cyanobacteria	[a]
Microcystis aeruginosa	Cyanobacteria	[a]
Scenedesmus sp. LX1	Originally isolated by Li et al. from tap water	[23]
Chlorella sp. ZTY4	Isolated from the primary sedimentation tank of a wastewater treatment plant in Beijing, and now is identified by the 18S-rRNA method, named as *Chlorella pyrenoidosa* THUZTY1304, and preserved in China General Microbiological Culture Collection Center, CGMCC (No. 12521)	[16]
Selenastrum capricornutum	Microalgae	[a]
Chlamydomonas reinhardti	Microalgae	[a]

[a] Freshwater Algae Culture Collection of the Institute of Hydrobiology in Chinese Academy of Science (Wuhan, China).

membrane, such as membrane proteins and polysaccharides, which form a hydration layer. Furthermore, large amounts of water are trapped in the harvested microalgal biomass due to the intercellular capillary effect. During the conventional flocculation or flotation process, the removal of intercellular water in a hydrophilic system is difficult to achieve. As a result, flocculation alone followed by sedimentation or flotation could only achieve a concentration ratio ranging from 5 to 50. Further dewatering processes, such as filter pressing, drum drying and sun drying [9], are required before converting the harvested microalgal biomass to bioenergy. Considering the operational cost, energy consumption and complexity of the current harvesting approaches, it is necessary to develop novel cost-effective and consolidated techniques which can permit a high recovery efficiency of microalgal biomass with a sufficient concentration ratio for further conversion processes.

To achieve this purpose, a novel microalgal harvesting process has been developed using a colloidal structure called polymer-surfactant aggregate (PSA). The cationic surfactant, which contains a hydrophilic and cationic 'head' and a hydrophobic 'tail', can bind to the microalgal cell membrane via electrostatic and hydrophobic attractions. Due to the electrostatic attraction, the surfactant head is bound to the hydrophilic and negatively charged species of the cell membrane, such as polysaccharides and some proteins. Due to the hydrophobic attraction, the surfactant tail can bind to the hydrophobic parts of the cell membrane as well as to other surfactant tails, thus forming a surfactant bilayer. Both forces of attraction lead to the surfactant headgroups coating the microalgal cell membrane and displacing the associated water. The anionic polymer acts as a flocculant and a backbone for the formation of PSAs. The positively charged surfactant initially binds electrostatically to the negatively charged polymer, which leads to an increase in the local concentration of surfactant in the vicinity of the polymer chain. When the local concentration of surfactant is relatively high, small aggregates start to form onto the polymer chain as the backbone. This process has been successfully applied to recover charged species, such as heavy metal ions and metallic anions, from aqueous solutions [11–14].

There are three main characteristics of the PSA process that can be advantageous for harvesting microalgae: in-situ formation of nano-scale PSAs, self-flocculation and hydrophobicity. Firstly, PSAs contain both positive and negative charges in one structure when they form in-situ in an aqueous solution. Individual nano-scale PSAs contain a high surface to volume ratio, which can effectively bind to microalgal cells. In addition, when the microalgal cells are bound to PSAs, they also associate inter-cellularly with each other to form large flocs. This subsequently leads to self-flocculation of the microalgae loaded PSAs, forming visible-size flocs. The flocs can be easily recovered by coarse filtration, which obviates the need for an expensive membrane process or the relative long residence time for a sedimentation process. Lastly, when the microalgae-loaded PSAs flocculate, the polymer shrinks to form compacted flocs. The formation of a hydrophobic surfactant coating layer on the microalgal membrane cell also squeezes the intercellular water out of the flocs. Both effects reduce the amount of final intercellular water in the harvested algal biomass.

In this paper, 6 different microalgal strains, including *Synechococcus* sp. PCC 7002, *Microcystis aeruginosa*, *Scenedesmus* sp. LX1, *Chlorella* sp. ZTY4, *Selenastrum capricornutum* and *Chlamydomonas reinhardti*, were used to test the applicability of the PSA process. *Chlorella* sp. ZTY4 was selected for detailed investigation because this strain could grow mixotrophically using different kinds of wastewater as a resource and synchronously accumulate lipids within the cells [15,16]. Comparative studies in recovery efficiency and concentration ratio were conducted between the PSA process and several conventional flocculation methods using cationic polymers, the aluminium ion and ferric ion. The dosage between polymer and surfactant was optimised, based on previous application in the treatment of dilute solutions of metallic ions [13]. The effects of mixing time and cell density were examined to evaluate the potential industrial applicability. Based on the results, an efficient microalgal biomass harvesting method with a high concentration ratio was proposed.

2. Materials and methods

2.1. Microalgal strain

The strains used in this study are listed in Table 1.

All the microalgal strains except *Synechococcus* sp. PCC 7002 were maintained in a liquid BG11 medium as well as on agar plates containing BG11 medium in an artificial climate chamber (Incubators SI60, Stuart Equipment). *Synechococcus* sp. PCC 7002 was cultivated in A plus medium.

Colonies picked from the agar plate were first cultured in 200 mL of liquid BG11 medium (A plus medium for *Synechococcus* sp. PCC 7002) in an artificial climate chamber (Incubators SI60, Stuart Equipment) until the end of the initial growth phase (cultivated for 7 days). After this, a 10 mL sample of pre-cultivated algal culture was inoculated into 400 mL of BG11 medium (A plus medium for *Synechococcus* sp. PCC 7002) in a 500 mL Duran bottle with filtered air-bubbling. The cultivation conditions were as follows: light intensity 70 μmol photon $m^{-2} s^{-1}$ to 80 μmol photon $m^{-2} s^{-1}$; light/dark ratio 14 h:10 h, and temperature 25 °C (30 °C for *Synechococcus* sp. PCC 7002). After being cultivated for 15 days, the microalgal culture was used to test the harvesting methods.

2.2. Harvesting microalgal biomass by the PSA process

Poly (acrylic acid) (PAA) was prepared by diluting stock PAA solution (Sigma Aldrich, average MW ~250,000, 35 wt% in H_2O). A stock solution of 1 M cetylpyridinium chloride (CPC) was prepared using the powder (purity ≥99%, purchased from Sigma Aldrich UK). A calculated amount of PAA and CPC solutions were added to an algal culture with a known cell density (described in detail in the results). The solution was stirred by a magnetic stir bar at 200 rpm for a period of time (investigated in detail in this study) to achieve equilibrium, which was indicated by a transparent solution with precipitates at the bottom of