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Short communication

Harvesting microalgae using the temperature-activated phase transition of thermoresponsive polymers

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A B S T R A C T

Microalga is a promising feedstock for biofuel, chemical, food, and animal feed; however, harvesting is a critical barrier for its commercial application. This communication demonstrates a new harvesting technology by utilizing the phase separation of thermoresponsive polymers and charged copolymers of N-isopropylacrylamide and allylamine. *Chlorella protothecoides* cells are separated from solution when the mixture of algae and polymers is heated above the lower critical solution temperature of polymers (~32 °C), where the polymer phase separates from the aqueous media and aggregates into a solid–gel phase. It was found that copolymer concentration, allylamine content (mol%) and charge (based on initial solution pH) affect the extent of polymer phase separation and alga separation efficiency. The copolymer containing allylamine with lower than 2.6 mol% displayed nearly complete algal cell separation at polymer concentrations of 25–50 mg/mL and pH 7. The results indicated that thermoresponsive polymers provide a promising technology for alga harvesting using recyclable and reusable materials.

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1. Introduction

Microalgae have been considered one of the most promising feedstocks to produce biofuels, chemicals, food, and animal feed due to their high productivity, high product yield (e.g., lipid), and high growth rate; however, a number of technical barriers render alga biofuel economically unfit [1]. The separation of algal biomass is considered the most problematic area and a key cost factor limiting the commercial use of algae. The cost contribution of harvesting could be in the range of 20–50% to the total cost of algal biomass [2–5]. Amer et al. [6] estimated that 90% of the equipment cost in the open alga production system comes from harvesting and dewatering.

A number of harvesting methods have been developed, including centrifugation, flocculation, filtration, and dissolved air floatation. At this time, there is no universal harvesting method because of high cost, high energy consumption, and/or chemical contamination (e.g., residual non-recyclable flocculants). To overcome these limitations, which is necessary for commercialization [7], we have developed a process to separate algal cells from their growth media by utilizing the thermally activated phase transition of responsive polymers. Poly-(*N*-isopropylacrylamide) (pNIPAM) exhibits a lower critical solution temperature (LCST) in aqueous systems, which results in polymer phase separation from aqueous media when the temperature is increased above 32 °C [8]. These polymers have attracted interest in drug delivery, polymeric biointerfaces and bioseparation because of the versatility in tailoring the interactions between the polymers and proteins/cells [9–12].

In this communication, we presented the efficiency of harvesting algae, specifically *Chlorella protothecoides*, using pNIPAM and its copolymer with allylamine (AA), pNIPAM-co-AA. *C. protothecoides* was chosen as a test species due to the potential for high oil production, biomass and oil productivity [13,14] and smaller cell diameter making the organism more of a challenge for harvesting. Fig. 1 shows an illustrative schematic of the phase transition in pNIPAM solutions, and how this phase separation can be utilized to harvest algal cells. The influence of heating temperature, polymer concentration, allylamine composition, charge (based on initial solution pH), on the alga harvesting efficiency is discussed.

2. Materials and methods

All chemicals were purchased from Sigma-Aldrich and Fisher Scientific and used as received unless otherwise noted. All solvents and acids for polymer synthesis were of analytical grade, and deionized (DI) water was purified with a Millipore Milli-Q water purification system (18 ΩM) equipped with a 0.22 μm Millipax filter. All water and water-based samples were stored under nitrogen to avoid ion contamination through the absorption of CO_2 from the atmosphere.

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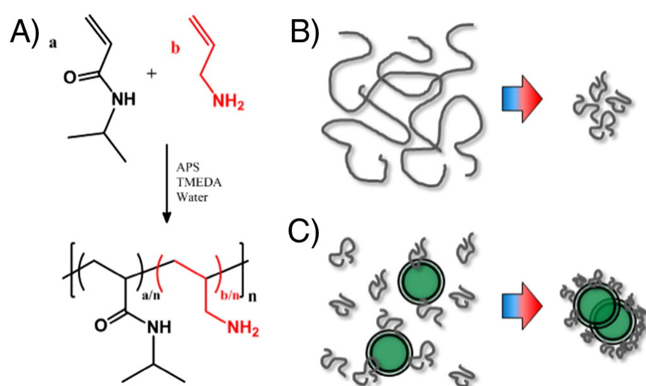

Fig. 1. (A) Thermoresponsive polymer synthesis; (B) thermally activated phase transition of thermoresponsive polymers in aqueous solution; and (C) the mechanism for alga harvesting in solution.

N-isopropylacrylimide (NIPAM) was recrystallized from hexanes and allylamine was distilled under a vacuum prior to use.

2.1. Preparations of microalgae

C. protothecoides (UTEX 256) was purchased from the Culture Collection of Algae at the University of Texas at Austin, TX (UTEX). The alga was cultured on a basal medium supplemented with 30 g/L glucose and 4 g/L yeast extract. The basal medium was modified from the BG-11 medium, with components per liter as follows: 0.7 g KH_2PO_4, 0.3 g K_2HPO_4, 0.3 g $MgSO_4 \cdot 7H_2O$, 25 mg $CaCl_2 \cdot 2H_2O$, 25 mg NaCl, 3 mg $FeSO_4 \cdot 7H_2O$, 0.01 mg Vitamin B1, and 1 mL of A5 solution. The recipe of A5 solution per liter is as follows: 2.86 g H_3BO_3, 2.5 g $MnSO_4 \cdot 7H_2O$, 222 mg $ZnSO_4 \cdot 7H_2O$, 79 mg $CuSO_4 \cdot 5H_2O$, and 21 mg $Na_2MoSO_4 \cdot 2H_2O$. The media was autoclaved at 121 °C for 20 min before inoculation. The initial pH of the media was adjusted to 6.8 with 5 M KOH before autoclaving. All cultures were incubated under heterotrophic conditions in 500-mL flasks containing 200 mL of culturing media at 28 °C, and flasks were kept in a shaking incubator (VWR 3500, Suwanee, GA) at 180 rpm in a dark room for 7 days until used. No extra air was supplied because shaking provided enough oxygen for alga growth in such small flasks.

2.2. Synthesis of pNIPAM-allylamine copolymer

Copolymers were synthesized using a free radical polymerization with prescribed ratios of NIPAM (8.0 g, 70 mmol) and allylamine (1–10 mol%, 0.7–7 mmol). Monomers were dissolved in 75 mL of degassed DI water in a 4 °C ice bath, to which the radical initiator ammonia persulfate (APS) was added in a 1:20 w/w ratio of initiator to monomer. Tetramethylethylenediamine (TEMED) was used as a catalyst in a 1:20 w/w ratio of catalyst to monomer. After addition of the monomers, initiator, and catalyst, the mixture was allowed to react for 24 h at room temperature (25 °C). The reaction was stopped and the APS and TEMED were removed by dialysis with the continuous exchange of fresh DI water over 3–4 days using 3500 MWCO cellulose membrane tubing. Water was removed by rotary evaporation and then the polymer was dried under a vacuum for 24 h. In some cases the polymer was further purified by dissolving the recovered solid in DI water, heating above the phase transition to reflux to perform a hot gravity filtration. The product (retentate) was dried under a vacuum for an additional 24 h. The resulting copolymers were stored under nitrogen at room temperature. The amine content in the copolymers in this study was varied between 1 and 6 mol%. The pNIPAM-co-AA copolymers were characterized and their physical properties are shown in Table 1.

Table 1
Physical properties of pNIPAM-co-AA copolymers.

Target NH_2 (mol%)	Actual NH_2 (mol%)	M_n	M_w	$D_{w/n}$
1.00	1.23	166,000	368,000	2.22
2.50	2.61	133,000	236,000	1.77
2.5 HF*	1.86	136,000	246,000	1.81
5.00	5.97	67,000	99,000	1.48

HF* designates that the material was purified via hot filtration.
M_n is number average MW.
M_w is weight average MW.
$D_{w/n}$ is dispersity.

2.3. Microalga harvesting with thermoresponsive polymers

Alga separation conducted by adding the polymer powder to the alga suspension was ineffective as the polymer required many hours to dissolve. Therefore, polymers were dissolved in DI water at room temperature overnight to make a series of polymer concentrations (20–200 mg/mL). To harvest algal cells, the polymer solutions were mixed with the alga broth (1:1, vol/vol) and allowed to stir for 10 min (or more), then subsequently heated to 40–70 °C for 10–30 min to induce polymer phase transition. When the temperature of mixture solutions increased above ~32 °C, the solutions turned a milky white color as the polymers underwent a phase transition. Depending on the solution and polymer characteristics, a fraction of the algal cells were separated from the solution. The supernatant was decanted from the vial and collected for optical density (OD) and algal biomass dry weight (BDW) measurements. Reported values of polymer concentration and alga OD/BDW were based on the values after mixing the equal volume of polymer solution and alga broth. Alga OD values represent averages of three measurements and were converted to BDW using a calibration curve. Alga harvesting efficiency is calculated as:

$$\text{Algae removed } (\%) = (BDW_i - BDW_f)/BDW_i \times 100 \quad (1)$$

where, BDW_i and BDW_f are initial and final algal biomass dry weights, respectively. All confidence intervals (CI) are calculated with 99% confidence (z-coeff = 2.57) using 3–4 unique measurements. Trials where no phase separation occurred were repeated at least 3 times and assigned a value of 0%. Algae removal values reported without a confidence range represent individual values.

The ionic content, expressed as $[NH_3^+]$ or % charge (ratio of NH_3^+ to NH_2 on allylamine) of copolymer solutions (either 1.2 mol% AA or 1.9 mol% AA) was varied by adjusting the starting pH of the 100 mg/mL polymer solution (pH 6–10) using 1 M HCl. At these pH values, the overall $[NH_3^+]$ was in the range of 12.9 mM (in pure DI water) to 16.9 mM (<pH 7, fully protonated amines) for copolymers with 1.9 mol% AA and between 6.6 and 10.7 for copolymers with 1.2 mol% AA. Similarly, the fraction of charged amine groups varied from 0.62 to 1.0 (fully protonated) over these conditions. The molarity of NH_3^+ is determined according to the following equation ($[NH_2]$ is based on amine content and polymer concentration):

$$pH = pK_a + \log_{10}([NH_2]/[NH_3^+]). \quad (2)$$

2.4. Analytical methods

OD measurement was carried out at 540 nm on a microplate reader (Bio-Tek synergy HT, Winooski, VT). The culture sample was diluted with a culturing medium before OD reading in order to obtain an OD value of less than 1.0. Alga concentration was measured by OD and converted to algal BDW using a calibration curve. To determine known BDW vs. OD, a certain amount of alga suspension was centrifuged to obtain an alga pellet, which was washed with DI water for three times to remove residual medium and dried in an oven at 105 °C for overnight

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