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Short communication

Rapid harvesting of freshwater microalgae using chitosan

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ABSTRACT

In this study, chitosan was used as a flocculant to harvest freshwater microalgae *Chlorella vulgaris*. The recovery efficiency of *C. vulgaris* was tested at various chitosan concentrations. 120 mg/L of chitosan showed the highest efficiency (92 ± 0.4%) within 3 min. The maximum concentration factor of 10 was also achieved at this dose of chitosan. The harvesting efficiency was pH dependent. pH 6.0 showed the highest harvesting efficiency (99 ± 0.5%). Measurement of zeta-potential confirmed that the flocculation was induced by charge neutralization. This study showed that a biopolymer, chitosan, can be a promising flocculant due to its high efficacy, low dose requirements, and short settling time.

1. Introduction

Microalgae biomass has been considered as an alternative source of biofuels since long [1–3]. Due to dilute nature of microalgae culture, harvesting, i.e. biomass recovery from growth medium, is one necessary step, which accounts 20–30% of total biomass production cost [4]. Cost-effective harvesting is challenging due to small size of microalgae cells (3–30 μm in diameter).

Several techniques are available for microalgae harvesting, such as centrifugation, sonication, filtration, air floatation, coagulation, and flocculation. Among these, flocculation is the most striking option, as it is simple and relatively cheap [5,6]. Flocculants such as aluminum and ferric salts can form flocs with microbial cells including microalgae. Despite high efficiency of such chemicals, their abundant use can contaminate microalgae biomass, which exhibit adverse effects on its subsequent uses such as feed for human and animals [5]. Natural polymers such as chitosan can be a promising alternative to address these challenges [7,8]. Chitosan is a linear poly-amino-saccharide, which is produced by alkaline deacetylation of chitin [9]. It is insoluble in water and soluble in acids. Generally, chitosan has a viscosity of 20–300 centipoises, molecular weight of (5–19) × 10^4, density of 0.15–0.3 g/cm^3 (in 1% acid solution), and deacetylation degree of 75–85% [10]. Chitosan has distinct advantages over commonly used flocculants for microalgae harvesting [7]. It is cheap, has high flocculation ability, and require low dose for harvesting [10,11]. It costs only 2 $US/kg. One kilogram of chitosan can effectively treat 500,0000 L of microalgae culture [12]. Moreover, it is biodegradable and has no toxic effects on some downstream applications such as feed for fish and animals.

Several factors such as flocculant type, flocculant dose, settling time, and culture pH affect the harvesting efficiency of microalgae [13]. pH also plays a vital role in flocculation process [3]. pH affects the flocculant interaction with microalgae, and thus, alters the harvesting efficiency [4].

In this experiment, we have explored the potential of chitosan as a flocculant for microalgae harvesting. The effects of flocculant dose, zeta-potential and pH, on harvesting efficiency are also demonstrated.

2. Materials and methods

2.1. Strain and growth conditions

Chlorella vulgaris AG30007 UTEX 0000265 was obtained from the University of Texas at Austin, USA. *C. vulgaris* was cultivated photo-autotrophically in 250 mL sterilized cell culture flasks in a shaking incubator, at 140 rpm, 25 ± 2 °C, and under the illumination of white (Light Emitting diodes (LEDs)). BG-11 medium was used as a growth medium [14].

The medium pH was adjusted by adding either 1 M H_2SO_4 or 1 M NaOH. Cell suspension was stirred for 10 min at 100 rpm on an orbital shaker. The mass cultivation of *C. vulgaris* was carried out in conical ended glass reactors (1 L). 5% (v/v) CO_2 enriched air was flushed into the reactors at an air flow rate of 100 mL/min. All experimental stuff was autoclaved for 20 min at 121 °C, before use.

2.2. Preparation of chitosan solution

Chitosan was obtained from Sigma–Aldrich (Republic of Korea). 100 mg of chitosan (dry weight) was mixed in 10 mL of 0.1% HCl solution with continuous stirring

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Table 1
Harvesting efficiency of microalgae using chitosan.

Chitosan concentration, mg/L	Harvesting efficiency, %	Concentration factor	References
80	75	N.A	[11]
40	95	N.A	[31]
80	70	N.A	[20]
250	9	N.A	[32]
20	80	19	[10]
100	20	4	[33]
80	86	N.A	[21]
120	99	10	This study

N.A: not available.

for 30 min [15]. The solution was diluted to 100 mL using deionized water to make final chitosan concentration of 1000 mg/L.

2.3. Harvesting

Harvesting experiments were performed at 1 g/L dry density of microalgae [16]. The experiments were carried out by jar test using 25 mL glass beakers. The beakers were filled with 20 mL of microalgae cells. A specific amount of chitosan was added in microalgae cells culture according to the experimental design. The mixture of cell suspension and chitosan was mixed at 50 rpm for 3 min only. After mixing, the algal cells were allowed to settle down. 0.5 mL sample was collected from the center of testing jar to measure optical density [17]. The optical density of the sample was measured at 600 nm by spectrophotometer (Beckman Coulter, model DU 730) equipped with a carousel cell holder. The optical density was calibrated against cell dry density [7]. Demineralized water was used as a reference to measure optical density. Biomass recovery efficiency was calculated as follows:

$$\text{Recovery\%} = \frac{OD_{t0} - OD_t}{OD_{t0}} \times 100 \quad (1)$$

where OD_{t0} is the optical density at time zero and OD_t is the optical density of the sample taken at time t.

The percentage ratio of chitosan to microalgae biomass was calculated as follows:

$$\text{\%age chitosan dose} = \frac{\text{Chitosan dose (mg/L)}}{\text{Microalgae biomass (mg/L)}} \quad (2)$$

All experiments were replicated to ensure the authenticity of data.

2.4. Concentration factor

Concentration factor of microalgal suspension was determined according to a method reported by Salim et al. [17].

$$\text{Concentration factor} = \frac{OD_{sed}}{OD_{t0}} \quad (3)$$

where OD_{sed} is the optical density of sediments after flocculation and OD_{t0} is the optical density at time zero.

2.5. Characterization

Zeta-potentials of chitosan treated samples were determined through electrophoresis method, reported by Banerjee et al. [1]. The cell surface of chitosan-treated *C. vulgaris* was examined via SEM, after drying. Microalgae flocs were observed under light microscope, before and after flocculation (Leica, model DM2500, Switzerland).

3. Results

3.1. Effect of chitosan dose

The effect of chitosan dose on harvesting efficiency of *C. vulgaris* was investigated. Various concentrations (30, 60, 90 and 120 mg/L) of chitosan were tested. The corresponding percentage ratios of chitosan dosages to microalgae biomass were 3%, 6%, 9% and 12%. A control experiment (without chitosan) was also performed as a reference. A dramatic decrease in optical density was found just after 3 min settling time. The optical density decreased with increase in chitosan dose. We have presented the decrease in optical density in terms of harvesting efficiency. The maximum decrease in optical density was found at 120 mg/L of chitosan and the lowest at 30 mg/L. The highest harvesting efficiency ($92 \pm 0.4\%$) was obtained at 120 mg/L (Fig. 1). The efficiency decreased with decrease in flocculant dose; the lowest efficiency ($32 \pm 0.5\%$) was found at 30 mg/L. The harvesting was almost complete within 3 min. This rapid harvest, even at low dose, is one distinct advantage of chitosan. Papazi et al. reported that only 60% flocculation efficiency was achieved with *C. vulgaris* using rather high dose of chemical coagulants, i.e., 1000 mg/L of $Al_2(SO_4)_3$ and incubation time of 6 h [18]. The harvesting efficiency achieved in this experiment using chitosan was higher than the reported data. Table 1 shows a comparison of harvesting efficiencies at various chitosan dosages, reported in several studies.

3.2. Concentration factor and zeta-potential

Concentration factor is a parameter to evaluate the degree of harvest. It is the ratio of cell culture density, before and after flocculation. This parameter obviously depends on flocculant dose. We measured the concentration factor at various chitosan dosages. An un-expected decreasing trend of concentration factor was found up to 30 mg/L of flocculant dose, we could not know the reason behind this phenomenon. However, it was in agreement with Salim et al.'s results [17]. At higher chitosan concentrations (>30 mg/L), the concentration factor increased (Fig. 1). The maximum concentration factor of 10 was achieved at 120 mg/L of chitosan.

Zeta-potential was negatively correlated with flocculant dose [4]. Fig. 1 shows that the zeta-potential decreased from -10.6 ± 0.3 mV (in control) to -18 ± 0.4 mV at 120 mg/L of chitosan. It verifies that the negative charge on microalgae cell was neutralized by the positive charge of chitosan. Generally, the zeta-potential of microalgae culture increases positively with flocculant dose. However, in our experiment, the decreasing trend of zeta-potential was likely due to dissociation of carboxylic acid groups of microalgae cells surface, which generated negative ions. Wu et al. also observed the decreasing trend of zeta-potential with increase in flocculant dose [4,19].

3.3. pH effect

Medium pH also affects the harvest efficacy of microalgae [20]. This study supported such observation. Using chitosan, the highest harvesting efficiency of $99 \pm 0.5\%$ (at 120 mg/L of chitosan) was obtained. It showed that pH 6.0 was the optimal pH [5]. The pH effect can be explained by physical property of chitosan and physicochemical interactions between chitosan and microalgae cells [21]. In fact, a change in pH is known to affect the flocculant structure. At neutral pH, the flocculant is present in coiled like structure. At acidic pH, it forms large flocs due to more positive charge, which work as ligands. As a result, flocculation efficiency increases [22]. We mixed chitosan solution in distilled water (data not shown) but the pH did not change much (as compared to chitosan solution adding in microalgae culture). It showed that the change in pH is induced by the reaction of chitosan with microalgae cells only.