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Harvesting microalgae using ionic polyelectrolytes in an aqueous-organic two-phase system: Screening of separation parameters using model algal particles

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ABSTRACT

Separation and recovery of microalgae from the aqueous medium that they reside in is difficult as a result of the nature of the algal cells, i.e., small cell size, density close to water, low concentration, and ability to stay suspended in water due to surface potential. In this study, an easy technique that can separate microalgae from aqueous phase to a water-immiscible organic phase utilizing the natural zeta potential of microalgae is reported. The technique involves the addition of the positively charged electrolyte, Mono/Poly-(diallyl dimethyl ammonium chloride, DADMAC), which then interacts with the negatively charged biomass. The particle-bound electrolyte results in the formation of a hydrophobic ensemble which in turn migrates the particles from the aqueous phase to hydrophobic organic solvent phase. Approximately 80–85% of cellulose beads (model particles) and 80–82% of algae cells were displaced into hexane phase. Studies also indicated that the technique could be used to extract lipids without subjecting algal cells to disruption. The results pave the way for developing a low-cost method to dewater and separate whole algal cells by utilizing the inherent charges they carry. We anticipate the results being a starting point for the development of new separation technology targeting bioprocessing industry.

1. Introduction

The versatile nature of microalgae has made it an excellent choice as a source for numerous bio-products such as proteins, therapeutics, lipids, and other high-value polysaccharides [1]. But in the last few years, the focus has been primarily on lipids due to the high lipid yielding capacity of microalgae. Nonetheless, lifecycle assessment (LCA) and techno-economic analysis indicate that lipid recovery alone is economically unfeasible due to high energy demand for dewatering, drying and lipid extraction [2,3]. Although some biomass and oil productivity improvements have been achieved, algae-for-biofuels-platform remains unsustainable unless a low-cost downstream processing technique is developed and other high-value metabolites are coproduced.

Harvesting microalgae is technically challenging because of a number of reasons including 1) the nature of the algal suspension [4], 2) the dilute nature of the microalgae suspension (mass concentration less than 1 g L^{-1}) with densities close to that of water [5–8], and 3) their stability in dispersed states due to the negative charges they carry owing to presence of algogenic organic matter [9,10]. As the harvesting step could represent 20–30% of the biomass production costs, there is an absolute need to develop low-cost harvesting processes that could overcome all the physical, chemical and economic barriers [5,6,11–13].

Aqueous two-phase separation techniques are widely used to separate biological entities like microbial cells, proteins, genetic material and organelles, but the separation of these biomolecules in water-organic solvents is not practiced due to their low solubility in the organic phase [14]. Though the water-organic system has been studied for separation of biomolecules by Lovrien's group [15], the presence of the organic solvent resulted in the formation of a three-phase system. The biomolecules resided in an intermediate layer between the aqueous and organic solvent phases [16] which was proven to be quite challenging to separate. However, using the proposed technique, biomolecules can be wholly migrated into the organic solvent making the water-hexane phases easy to separate.

The solution proposed herein is to simplify the dewatering and separation of algal cells from aqueous media by adsorbing an oppositely charged amphiphilic polyelectrolyte on algal cells so that ensemble surfaces are hydrophobic and the algal-cells repel water molecules and migrate into hexane phase. The objective is to understand the migration

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behavior by capitalizing on particle's zeta potential and its ability to bind to the oppositely charged electrolyte and move the ensembles away from the water phase to a water-immiscible hexane phase. The charged particles and algal lipids can be easily recovered by evaporating hexane with relatively low energy input as compared to that required to evaporate water. In this work, the impact of various parameters like pH, type of electrolyte (monomer/polymer), the concentration of the electrolyte, system temperature, and equilibration time has on the migration percentage of functionalized beads from an aqueous medium to hydrophobic medium has been reported.

2. Material and methods

The chemicals used in this study were analytical grade. Sodium monochloroacetate was purchased from Sigma-Aldrich and sodium hydroxide and hexane from VWR chemicals. Asahi Kasei Chemicals Corporation provided cellulose beads (Celphere CP-102) to conduct this study. The average size of cellulose particles was ~106 μm with a bulk density of 0.83 g/cm^3. DADMAC and low molecular weight PolyDADMAC (< 100,000) were ordered from Sigma Aldrich. Concentrated microalgae (15% w/w) were obtained from Texas A&M AgriLife Extension (Pecos, TX, USA). Algae were stored under dark conditions in the refrigerator at 4 °C. During the experimentation, the microalgal suspension was diluted to 2% to mimic the natural conditions. Statistical analyses were done using JMP 13.

2.1. Carboxylation of cellulose particles

Using the process described by Magnus Bergh with a slight modification, carboxylation of cellulose particles was carried out [1,17]. The modification being, cellulose beads mixed with sodium hydroxide solution before introducing it in sodium monochloroacetate solution. This was followed by addition of isopropanol. Carboxylation of cellulose particles was confirmed using FTIR analysis (Fig. 1) and the net surface charge was quantified in a flow cell cuvette arrangement using a Beckman Coulter's Delsa nano C zeta-sizer.

The surface charge under certain condition determined the stability of the suspension. For this the zeta-potential had to be varied. The surface potential of the functionalized cellulose beads was varied by addition of acid or base.

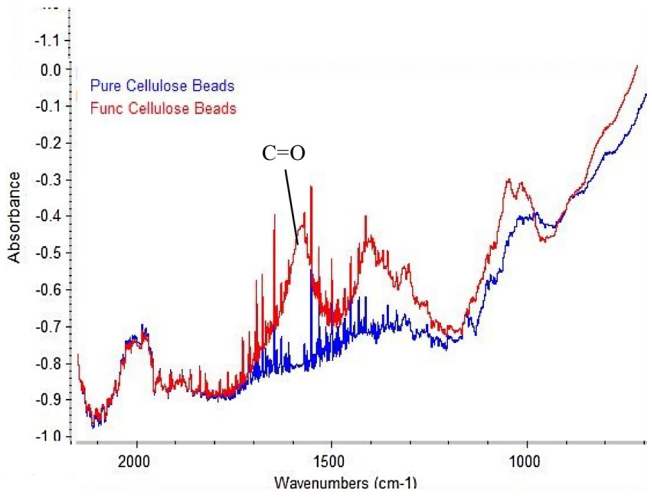

Fig. 1. FTIR output of functionalized cellulose particles. An asymmetric stretch vibration of COO^- near 1560–1610 cm^{-1} confirms the addition of ($-COO^-$) group on cellulose beads.

2.2. Quantification of particles migrated

An initial weight of 0.2 g of functionalized particles in 10 mL water (or 2% w/w) were used for studies discussed herein. Cationic polyelectrolyte/ surface modifier (hereafter referred as SM) was added to the aqueous suspension of particles followed by addition of hexane, and the suspension was mixed thoroughly. The system was kept undisturbed for a set amount of time. The resulting two phases were separated carefully and used for gravimetric analysis (TGA, via TA Instruments – Q50). The weight pan of the TGA was loaded with 50 μL of a sample from the aqueous phase. The furnace temperature was maintained at 70 °C for 15 min to evaporate hexane followed by 110 °C for another 15 min to evaporate water. The constant weight was attributed to the amount of cellulose particles that did not migrate into the hexane phase. The difference between the amount of cellulose particles in the original suspension and the amount of particles in water phase after migration was equated to be the amount of particles that migrated into the hexane phase.

2.3. FAME analysis

The analytical procedure was based on the transesterification of algal lipids to fatty acid methyl esters (FAME). Gas chromatography-mass spectrometry (GC/MS) was used to accurately quantify and identify the FAMEs obtained from algae samples per gram of ash-free biomass (AFBM). The amount of each FAME in the algae samples was calculated based on the use of internal standards.

2.4. Experimental design

Preliminary screening was done to identify significant factors impacting the migration behavior. Equilibration time, temperature, electrolyte type (polymer/monomer), pH, water to hexane ratio and electrolyte concentration were control variables and the amount of cellulose beads migrated was the primary response variable. A 2^{n-1} experimental design was used to identify significant parameters in the screening studies (Table 1). Three replicates for each experimental setup were done. The amount of particles migrated into hexane phase from the aqueous phase, and zeta potential (whenever applicable) were also used as response variables.

After identifying highly significant factors, more elaborate parametric studies were conducted to understand the migration behavior of the model particles. The impact of polyelectrolyte concentration (varied from 0.5 to 3% w/w of the particles) and system pH (at 6–8) on particle size, zeta-potential and amount of particle migration was studied based on the statistical significance resulting from the screening studies. Low polyelectrolyte concentrations and near neutral pH conditions were chosen looking at the high migration efficiency during the screening studies. The temperature was kept constant (at room temperature) considering the very low impact of it during the screening studies and the economics for large-scale algal separation. As the migration rate of the particles was observed to be rapid during the initial part of the migration process, to block the limiting factor, longer equilibration time was given for all the experiments.

Table 1
Factor and levels used for screening studies.

Factor	Level(s)
Type of Electrolyte Surface Modifier	PolyDADMAC and DADMAC
Electrolyte Surface Modifier (SM) concentration (w/w %)	2% and 5%
Temperature (°C)	4 °C and 20 °C
pH	4 and 11
Equilibration time (min)	60 and 180
Hexane: Water ratio (v/v)	1:1, 1:2 and 2:1