Moisture Displacement and Simultaneous Migration of Surface-functionalized Algae from Water to an Extraction Solvent Using Ionic Polyelectrolytes

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Abstract

Effective moisture removal and the ability of algal biomass to be compatible with lipophilic solvents are key to making the algal lipid extraction processes economically viable. Here we report a simple technique to chemically remove moisture from algal water while forcing above 95% of the algal cell mass to migrate to a (hydrophobic) lipid extraction solvent. The technique is based on functionalization of algal cell surface with a water-soluble cationic polyelectrolyte, Poly(diallyldimethylammonium chloride)(PolyDADMAC) that has hydrophobic ligands in its structure. Studies indicate that algal cells continued to displace moisture even after migrating into the solvent phase - continuing to perform the chemical drying step. The general results indicate that the technique could be extended to systems that require separation of particles with charged surfaces - by using a surface active ionic polyelectrolyte with appropriate charge and ligand combination(s).

Keywords:

Algae, Dewatering, Polyelectrolytes, separation, harvesting

Introduction

Microalgae is a promising source of lipids for biofuel production and fatty acid, pigment, separation for nutritional and pharmaceutical production[1, 2]. However, one major problem of lipid extraction from microalgae is caused by the huge amount of associated water [3-5]. Solvent extraction is an important step in lipid separation [6]. However due to large amount of moisture contact between algal cells and organic extraction solvent gets hindered. About 99.9% weight of the algae broth in an algal culture is water [3]. For conventional solvent extraction, a significant portion of this moisture is needed to be reduced during pretreatment [7, 8]. For example, during initial coagulation and sedimentation steps, the moisture content is reduced to approximately 90% [3]. However further reduction of moisture requires costly centrifugation and drying operations [3, 9, 10] and is impractical due to high energy requirements [9, 11, 12].

During our preliminary investigations, it was observed that algal broth treated with ca. 1% (w/w) polyDADMAC cationic polyelectrolyte repelled water and migrated to the organic solvent (Figure 1). Based on these encouraging results, this study was conducted to elucidate the effect of the molecular weight of the surfactant, solvent effects and time on moisture removal and solid separation characteristics of surface modified microalgae.

Figure 1: Effect of surfactant on miscibility of algae with chloroform. (a) 0.1 % TSS algae with solvent (b) 0.1 % TSS algae mixed with polyDADMAC surfactant and then mixed with solvent (c) 10 % TSS algae with solvent and (d) 10% TSS algae mixed with surfactant and then mixed with solvent

Methodology

Nannochloropsis oculata algal samples that has been subjected to nitrogen deprivation for increasing lipid content was obtained from algal culturing facility in Pecos, Texas. Lipid content of the samples is around 12 % (w/w dry basis). These samples were concentrated to 22% TSS (Total Suspended Solids) using centrifugation, prior to shipping using centrifugation. Upon arrival, samples were refrigerated at 18 °C until used.

PolyDADMAC (Sigma Aldrich) with three different molecular weights (High: 400,000-500,000, Medium: 100,000-200,000 and Low: <100,000) were used as the surfactant. All surfactant samples were diluted to 20 g/l solid content using deionized water for ease of transferring and to increase the measurement accuracy.

Algal samples were diluted to 10% TSS using deionized water before the experiment. 10 ml of the 10% TSS algal sample was transferred to a 30 ml screw cap vial and 500 µl surfactant was added to the algal sample. After vortexing it for 1 minute at 10 000 rpm, 10 ml of the solvent; Hexane, chloroform or dichloromethane; was added. Mixture was vortexed for 1 minute at 10 000 rpm and left to settle for 1 hour or 24 hour time period. During this time period mixture separates in to two layers: solvent phase and aqueous phase. For experiments with hexane, aqueous phase separates to the bottom while for experiments with chloroform or dichloromethane, aqueous phase separates to the top.

Aqueous layer was removed using a pipette and the volume recorded (V_{aq}). After vortexing the aqueous layer for 10 seconds, 1 ml of the layer ($V_{aq,0}$) was transferred to a pre-weighed, pre-ashed aluminum dish. If the aqueous layer volume was very small, removing aqueous layer from solvent phase without disturbing the interface becomes difficult; therefore, only a portion of the aqueous layer ($V_{aq,0}$) was used for dry weigh measurement (to ensure that the aqueous portion does not include any solvent). The remainder of the aqueous layer was separately siphoned in to a 1 ml graduated pipette and allowed to separate (in the pipette). Then the amount of aqueous portion was noted and summed with $V_{aq,0}$ to obtain

63 V_{aq} value. Then, aluminum dish was dried in an oven until constant weight to obtain the residue weight.
64 The same procedure was followed for the solvent phase.

65 Graphic summary of the experimental procedure is illustrated in Figure 2.

66 TSS of the aqueous layer, Solid content of aqueous layer, TSS of solvent layer, and the solids content of
67 solvent layer was calculated using equations 1 to 5.

68 Transfer efficiency of solids from aqueous medium to solvent phase were calculated using equation 5.

69 TSS of the aqueous layer TSS_{aq} can be calculated using equation (1)

$$TSS_{aq} = \frac{W_{aq,d}}{W_{aq,0}}$$

70 Where $W_{aq,0}$ is the weight of $V_{aq,0}$ and $W_{aq,d}$ is the dry weight of that sample

71 Solid content in aqueous layer (S_{aq}) can be calculated using equation (2)

$$S_{aq} = W_{aq,d} \times \frac{V_{aq}}{V_{aq,0}}$$

72 TSS of the solvent layer TSS_{sp} can be calculated using equation (3)

$$TSS_{sp} = \frac{W_{sp,d}}{W_{sp,0}}$$

73 Where $W_{sp,0}$ is weight of the solvent volume $V_{sp,0}$ removed for TSS measurement and $W_{sp,d}$ is the dry
74 weight of that volume.

75 Solid content in solvent layer can be calculated using equation (4)

$$S_{sp} = W_{sp,d} \times \frac{V_{sp}}{V_{sp,0}}$$

76 V_{sp} was derived from equation (5)

$$V_{sp} = V_T - V_{aq}$$

77 Where V_T is the total volume of the mixture, calculated by adding the volumes of algal broth and solvent
78 volumes.

Figure 2: Summary of the methodology

Results and discussion

We conjecture that the cationic nature of poly-DADMAC and the presence of hydrophobic ligands are responsible for the cell migration behavior that was observed. Poly-DADMAC is polar-enough to be soluble in (algal) water [13]. However, once algae are encountered, positively-charged ends of Poly-DADMAC get attached onto the negatively-charged algal cell-wall (with a measured zeta potential of -27mV) exposing the hydrophobic ligand ends to the exterior (Figure 3). This makes the algal cell surface hydrophobic - now repelling water. The organic solvents that were used were hydrophobic and thus, the now "hydrophobitized" algae will "attract" and migrate toward the solvent molecules. Since the solvent and water are immiscible, aqueous-phase gradually displaces to the top (due to the density difference between lighter water and heavier chloroform and dichloromethane or vice versa in the case of lighter hexane) leaving algal cells behind in the solvent-phase.

92

93 **Figure 3: Pictorial depiction of possible mechanism - hydrophilic algal surface (or chemical moiety) can be functionalized to**

94 **be hydrophobic via cationic polymer attachment (or vice versa) by modulating the ligands of the surface active polymer.**

95 Figure 4 shows the TSS in aqueous and solvent phases after 2 or 24 hour settling times. It is apparent

96 that the solvent has a significant effect on the TSS in solvent layer. Hexane shows higher Total

97 suspended solids content compared to other two solvents. Molecular weight of the polymer or the settling

98 time does not play a significant role in deciding the TSS in solvent phase. None of the tested parameters;

99 i.e. solvent, polymer chain length or settling time had an effect on the TSS in aqueous phase

Figure 4: TSS in aqueous and solvent phases. (a) Solvent phase TSS after 2 hour of settling, (b) Solvent phase TSS after 24 hour of settling, (c) aqueous phase TSS after 2 hour of settling and (d) aqueous phase TSS after 24 hour of settling

From above results it is clear that there is a decrease of TSS in aqueous phase when solvent is introduced (from 10 % TSS). However, it is necessary to consider the volume fractions of the two phases. Figure 5 illustrates the variation of volume of the aqueous phase after separation (note that the total system volume is 20 ml). After 2 hours of settling, the volume of aqueous phase was about 5% of the total volume of the mixture. However, after 24 hours of settling, it was possible to achieve up to 35% removal of aqueous phase. This implies that moisture i.e., water that was embedded in cytosol, continues to get removed from the system even after the cells have migrated into the solvent phase. It should be noted that the broader error bars are attributed to variability associated with separating the two layers with adequate precision. However, it is noted that the error values are relatively small when compared with the total volume of the mixture.

Figure 5: volume of aqueous phase (a) after 2 hour settling time and (b) after 24 hour settling time.

The amount of total solids in each phase (calculated using equations (2) and (4)) are depicted in Figure 6. It is clear that the amount of solids in the aqueous layer is negligible after settling. This implies that this polyDAD based algal cell migration system is quite effective.

Figure 6: Solid content in each phase as a percentage of total solids in the mixture. Independent axis represents combinations of surfactant molecular weight (High, Medium and Low) and solvent (c: chloroform, d: dichloromethane, h: hexane)

Conclusions

Migration of algae treated with a cationic polyelectrolyte, PolyDADMAC surfactant from aqueous phase to organic phase was investigated as a means of removing moisture. All three organic solvents responded positively to the cell migration scheme with over 95% (w/w) of cell migration from aqueous phase to solvent phase. Of the three solvents, hexane performed best by allowing the highest amount of cell migration. It was observed that the molecular weight of surfactant does not have an impact on the degree of cell migration or the quantity of water displaced. It was also noted that the system continued to displace moisture even after the cells had migrated into the solvent phase (continuing to perform the chemical drying). The ionic polyelectrolyte based particle migration technique could be adopted to separate particulate matter with charged surfaces using a polyelectrolyte or a surface active agent with appropriate ligand-charge combination(s). The feasibility of using this technique in bioseparations, especially, in high value product separation from complex biological fluids should be further investigated.

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