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A new processing scheme from algae suspension to collected lipid using sand filtration and ozonation

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ABSTRACT

Algae-derived biofuels are increasingly seen as an alternative source of energy with potential to supplement the world's ever increasing demand. A great challenge exists today in energy input and costs to procure algal lipid from a cultivated suspension, which generally requires steps such as concentration, filtration, dewatering, grinding, and solvent extraction. Using well practiced sand filtration and ozonation processes, we sought to reduce processing steps and streamline the operations in one vessel. The specific *Chlorococcum aquaticum* suspension was acidified to pH 3.3 to promote agglomeration prior to biomass collection by sand filtration. The algae-loaded filter bed was drained of free water and added with methanol and ozonated for 2 min to rupture the cell membrane to accelerate release of cellular contents. The methanol solution containing the dissolved lipid product was collected by draining, while the filter bed was regenerated by further ozonation when needed. The results showed 95% collection of the algal biomass from the suspension and a 16% yield of lipid from the algae, as well as restoration of filtration velocity of the sand bed via ozonation. GCMS identification of the extract showed primary products in the forms of long-chain largely saturated hydrocarbons of 16 to 20 carbons. The new technique streamlines individual steps in the procurement of algal lipid from the suspension that is potentially an improvement over existing energy-intensive methods.

1. Introduction

World energy consumption has been steadily growing by 2.5% in the past decades, of which fossil fuels accounted for 87% and renewable energy for merely 2.1% most recently [1]. Microalgae are seen as a potential source of renewable energy [2,3] as they contain lipids that can be converted to various forms of biofuels via thermochemical and biochemical processes [2,4]. Algae for biofuels are particularly appealing because of their high photon conversion efficiency, lipid content [5,6], growth rate [7], convertibility into biodegradable biofuels, and carbon sequestration potential [8]. They are increasingly seen with potential to supplement the world's energy demand [8–12]. Lipid extraction from microalgae for energy purpose involves several critical steps that include collection, dewatering, grinding, extraction, and conversion. Presently, the cost of harvesting algae from growth media is critical [13]. Techniques to concentrate algae incur high capital and energy costs [14], commonly including coagulation/flocculation [15], microscreening [15,16], and centrifugation [8]. As a wet biomass decreases conversion efficiency [17], dewatering presents an additional challenge [14,18].

In this work, we investigated the use of sand filtration and ozonation through a series of streamlined steps to obtain the lipid product from a cultivated algal suspension of *Chlorococcum aquaticum* (1–10 μm). Both the sand filtration and ozonation processes have been well practiced in the water treatment industry for decades [19,20]. Conventional filtration under pressure or vacuum was useful for removing large algae (>70 μm) such as *Coelastrum proboscideum* and *Spirulina platensis* [21], but less so for microalgae approaching bacterial dimensions (<30 μm) such as *Scenedesmus*, *Dunaliella*, and *Chlorella* [3,14,22]. Using no chemical coagulants, Naghavi et al. [23] removed 97% of algae *Scenedesmus quadricauda* (30–40 μm) from water with a fine sand/silt filter (0.064–0.335 mm with a clean bed head loss of 7.3 cm). A recent review on algal biomass dehydration showed only a minimal evidence of the use of sand filtration for algae harvesting [24]; the challenge identified in this review paper was that only large algal particles could be harvested. Ozone is a powerful oxidant and a disinfectant [25] that removes contaminants including algal toxins [26–28]. It has been shown to rupture cell wall causing release of intracellular contents into the liquid medium [29–31]. Miao et al. [30] ozonated algae *Microcystis aeruginosa* that caused rupture and release of cytoplasm, resulting in volatile organics and assimilable, dissolved organics in the water. Our prior research showed ozonation via compression and decompression cycles in succession as a means to deliver ozone increased cell rupture efficiency even at a smaller ozone dose and shorter contact time [31]. We report here results of a new processing scheme that combines sand filtration and ozonation in one vessel for the collection and extraction of algal lipid. The process streamlines necessary steps of chemical coagulation, filtration,

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dewatering, mechanical grinding, and solvent extraction — a series of energy and cost intensive steps that presents a great challenge in procuring algal biofuel today.

2. Materials and methods

2.1. Algae

C. aquaticum (UTEX: 2222) from The Culture Collection of Algae (University of Texas, Austin) was used for its rapid growth [18] and wide temperature tolerance [32]; it is a top lipid producer at 54 mg lipid/L/day [7]. It was cultivated in the laboratory in a 60-gal aquarium at room temperature of 25 ± 2 °C. A modified Bristol medium was used to provide essential nutrients for the algae, which contained $NaNO_3$ (2.94 mM), $CaCl_2$–$2H_2O$ (0.17 mM), $MgSO_4$–$7H_2O$ (0.3 mM), K_2HPO_4 (0.43 mM), KH_2PO_4 (1.29 mM), and NaCl (0.43 mM). Illumination was by placing above the aquarium a T5 high-output light fixture housing four 48-in fluorescent tubes totaling 216 W (*Sun blaze*).

2.2. Pretreatment

The algal suspensions were pretreated by ozonation (contact at 150 mg O_3/g TSS), ozonation followed by pH adjustment (contact at 150 mg O_3/g TSS, then pH adjusted to 11.7 or 3.6), or only pH adjustment (to pH 3.3) to examine any changes in particle size. The adjustment of pH was via manual addition of 2 M of H_2SO_4 or NaOH solution.

2.3. Sand filtration

Sand filtration was used for collection of pretreated algal suspensions. The sand filter was constructed of a polycarbonate column of 19 cm in diameter and 38 cm in height packed with 6 layers of sieved sands increasing in size with depth: ≦53 μm (thickness of 2 cm at the top), 53–250 μm (4 cm), 250–430 μm (4.5 cm), 430 μm–1.2 mm (3 cm), 1.2–2.0 mm (3 cm), and 2.0–20 mm (4.5 cm). The bed depth and area were of 21 cm and 270 cm^2, respectively. Various volumes of algal suspensions (e.g., 4 L of pretreated sample and 50 L of ozonated sample) were added at the column top, and the effluents collected at the column bottom. Backwashing was through a reversed flow of distilled water. During filtration, a constant hydraulic head (via constant height of water standing above the sand surface) was maintained by a siphon. The filtration velocity was tracked throughout.

2.4. Ozonation treatment

Algal suspensions were obtained from consecutive steps of cultivation in tank, pretreatment and collection by sand filtration, and backwashing from the sand bed. Ozonation treatment was performed in two different modes. In one mode, the suspensions were ozonated through conventional bubbling of ozone gas into the 1-L suspension in an open Erlenmeyer flask; in another mode, ozone was delivered via successive cycles of compression and decompression of the ozone gas into the 1-L algal suspension in a closed, pressure-resistant, stainless-steel reactor of 1.5 L. [31,33]. The reactor featured a gas vent and a pressure gauge at the top, inlet and outlet at the bottom, and a magnetically coupled stirrer. The reactor was loaded with an algae suspension. A pressure cycle began with the compression stage when the inlet valve was opened to admit an O_3/air mixture driven by a compressor (GAST) at a desired flow rate. The gas passed through a diffuser plate at the reactor bottom and through the liquid to pressurize the closed headspace to reach 150 psi; once reaching it, the pressure was rapidly released by opening the outlet solenoid valve at the reactor top. The time it took for the reactor to reach the designated pressure depended on the headspace volume and gas flow rate (e.g., reaching 150 psi in 10 s at 2 L min^{-1}); decompression time varied with venting speed but was typically controlled at 2–3 s. The compression–decompression cycle was repeated multiple times. At the end of pressure cycles, the reactor content was stirred for 3 min. Alternatively, rupturing of algae collected in the open sand bed was by addition of methanol followed by bubbling of ozone at the column bottom. Ozone gas was generated at 1.5% (v/v) at 2 L/min by an ozone generator (Model T-816, Polymetrics) being fed with dry, filtered oxygen operated at 105 V; the O_3 concentration was measured by an Indigo colorimetric method [34].

2.5. Sand filtration, rupture, and extraction in one vessel

After individual steps of pretreatment, sand filtration, and ozonation (Sections 2.2 to 2.4) were tested, their combined operation in one vessel was performed. Fig. 1 shows a new processing scheme from the cultivated algal suspension to procured lipid in one vessel. A smaller sand filtration column of 7 cm in diameter and 15 cm in height was used to test the integral process: filtration of algae, ozonation rupture of cells, and solvent extraction of lipid in the same vessel. The sand bed was of 6.5 cm in depth and 40 cm^2 in filtration area, packed with 4 layers of sieved sands (from top): ≤53 μm (1.5 cm), 53–150 μm (2 cm), 150–250 μm (1.5 cm), and 250–425 μm (1.5 cm). Algae suspensions and distilled water (500 mL in each run) were passed through the column; distilled water was passed through the column before and after each filtration run to determine filtration velocity resulting from increasing head loss. A constant hydraulic head (7.5 cm above the sand surface) was maintained as long as possible during the runs, and the filtration velocity tracked. After filtration that entrapped algae in the sand bed, the drained bed was added with 90 mL of methanol (solvent height reaching 2 cm above the sand surface) and ozone gas (1.5% O_3) was introduced from the bed bottom at 2 L/min for 2 min. After ozonation, cell contents including the lipid were released from ruptured algae into the methanol, and the solution was collected by draining. It should be noted that for simplicity reason at this development stage of the single filtration-rupture vessel, ozonation was carried out by purging in a continuous mode, and operation via pressure cycles were not used for this single vessel.

2.6. Regeneration of sand filter

The sand filter for pretreated algae was readily regenerated at the end of each filtration cycle (designated at 4 or 50 L throughput) by reversed flow of water at 9.6 cm/min. The single vessel for filtration and rupturing of algae was regenerated by passing ozone through the bed for 2 min at 2 L/min when filtration velocity dropped by 78%. However, when ozonation for 2 min did not fully restore the filtration velocity, prolonged ozonation of 5 min was used which confirmed full restoration of filtration velocity.

2.7. Analysis

Gravimetric solid analyses including total suspended solids (TSS), volatile suspended solids (VSS), total solids (TS), volatile total solids (VTS), volatile dissolved solids (VDS) were measured to determine algal contents [35]. The differences in solids after various operations such as filtration, ozonation, and solvent extraction were used to determine filtration, rupture, and yield efficiencies for the individual steps as well as for the combined operation of the single vessel. Chemical oxygen demand (COD; HACH) and soluble chemical oxygen demand (sCOD; HACH) before and after algae rupture were used to determine rupture efficiencies under different ozonation conditions. Particle size and zeta potential of various algal suspensions at different pHs were measured by means of dynamic light scattering and laser Doppler microelectrophoresis, respectively, with the instrument Zetasizer Nano ZS (Malvern Instruments).