Biocatalysis and Agricultural Biotechnology 9 (2017) 145–155

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Ionic effects on microalgae harvest via microalgae-fungi co-pelletization

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ARTICLE INFO

Keywords:
Co-pelletization
Biomass recovery
Algae harvest
Chlorella vulgaris
Aspergillus niger

ABSTRACT

Microalgae harvesting is a difficult and costly unit operation, hampering commercial microalgae based processes. Various innovative approaches have been under research investigations. Algae harvesting using microalgae/fungi co-pelletization where microalgae cells are attracted to filamentous fungus and form co-pellets during their co-culture has been proved to be an innovative approach that might be both cost effective and sustainable. Yet, the mechanism behind the co-pelletization based algae harvesting is still not known. Understanding its mechanism will significantly facilitate future developments to decrease the processing cost for generating biofuel and other microalgae based bio-products. Considering that the ionic conditions greatly affect the flocculation performance of microalgae cells, studies were conducted to compare the co-pelletization performance at different pH values and ionic strengths in order to evaluate the surface charge changes at the different conditions tested. Zeta-potential measurements indicated that (1) both microalgae and fungi have low negative zeta-potentials regardless of the pH of the bulk solution (< −10 mV) (2) fungi can have a positive electric charge at low pH (pH=3). The results suggest that it might be possible that the degree of repulsion and dispersion between these organisms is low at certain conditions, for instance, at higher concentrations of Ca^{2+} (> 0.1 g/L) the surface charge of fungal and microalgae cells were less negative (> −5 mV for fungal hyphae and > −12 mV for microalgae) at higher concentrations of magnesium (0.5 g/L), which might have facilitated the attraction between them.

1. Introduction

Microalgae are not only an ideal feedstock for biofuel production (Chisti, 2008, 2013; Li et al., 2008) but also for the production of a wide variety of high value metabolites due to their high growth rate, high photosynthetic efficiency, high biomass production, and the possibility of year-round cultivation (Goncalves et al., 2013). Unlike traditional oilseed crops, algae can provide more cell biomass with higher lipid content while using less water and land (Stephens et al., 2010), and more importantly, cultivation of algal biomass can be performed on non-agricultural land, thus avoiding competition with agricultural production. Ideally, CO_2 from a power plant (Wang et al., 2008) or any manufacturing industry (chemical, petrochemicals, iron and steel, cement, paper and pulp) can serve as the carbon source for microalgae growth, mitigating greenhouse gas production. Also, industrial wastewaters can serve as the nutrient source (Chisti, 2013; Christenson and Sims, 2011), all of which could potentially decrease the production cost. But one of the bottleneck in commercialization of algae based technologies, especially for the biofuel industry, is the high cost of current harvest technologies which make the process uneconomical (Brennan and Owende, 2010; Dassey and Theegala, 2013; Milledge and Heaven, 2013; Olaizola, 2003). Microalgae harvesting is a labor and energy intensive process (20–30% of the total cost of production) and technically challenging, especially because microalgae cells have low densities (typically 0.3–0.5 g/L), small size (typically in the range of 2–40 μm) and a similar density to water (Barros et al., 2015; Danquah et al., 2009; Grima et al., 2003).

Conventional methods of microalgae harvesting include flocculation, flotation, centrifugal sedimentation, and filtration (Barros et al., 2015), and at times a combination of the above methods may be required, depending on the characteristics of the microalgae, such as size and density (Milledge and Heaven, 2013). Recently, alternative harvest technologies like attached growth of algae (Christenson and Sims, 2012; Johnson and Wen, 2010; Liu et al., 2013) and bio-flocculation methods involving bacterial flocculants (Oh et al., 2001) or using flocculating algae (Salim et al., 2011), were studied to reduce the algae processing cost and reduce chemical addition (Christenson and Sims, 2011).

Algae harvesting using pelletization via filamentous fungus (Fig. 1) (Gultom and Hu, 2013; Gultom et al., 2014; Zhang and Hu, 2012) represents an innovative approach to address both the cost and sustainability issues in algal biofuel production and also has potential

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http://dx.doi.org/10.1016/j.bcab.2016.12.007
Received 26 September 2016; Received in revised form 30 November 2016; Accepted 4 December 2016
Available online 13 December 2016
1878-8181/

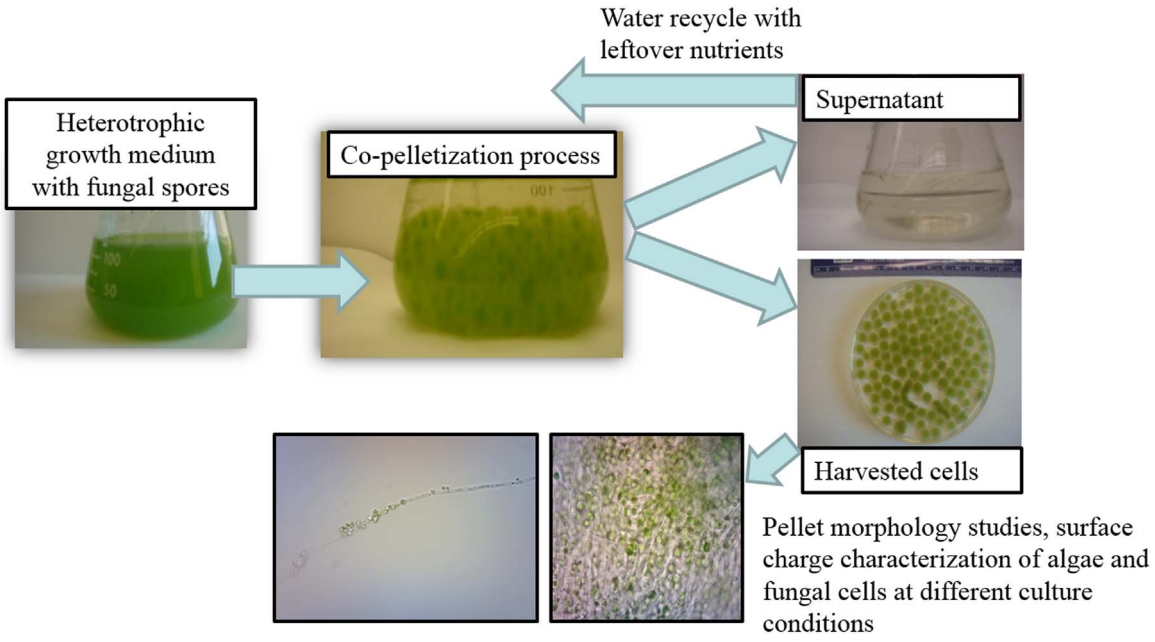

Fig. 1. Co-pelletization process to harvest microalgae via co-culture of filamentous fungi to form cell pellets under heterotrophic conditions.

direct commercial applications. This new process can be applied to microalgae cultures in both autotrophic and heterotrophic conditions (Zhang and Hu, 2012; Zhou et al., 2013), to allow microalgae cells and filamentous fungi to attach to each other.

There may be many factors affecting the interaction between the microalgae and fungi which causes the algae-fungi coagulation/flocculation. Among the different factors, the different surface charges of fungi and microalgae could significantly affect attraction between the organisms. It is widely known that microalgae carry a negative charge in their cells (Henderson et al., 2010) and because of this characteristic, flocculation and coagulation is broadly employed to harvest microalgae by applying a positively charged chemical compound. These processes usually are greatly affected by the ionic strength of the culture medium as these ions may pose competition for attraction sites. The objective of this work is to study the surface charges of fungi and microalgae at different pH and ionic strength in the co-pelletization process under heterotrophic conditions. The zeta potential of the fungal pellets and the microalgae cells was measured to quantify the cell surface charge. Medium pH, different ionic strength of calcium (Ca^{+2}), magnesium (Mg^{+2}) and sodium (Na^{+}) were used to influence the ionic strength of the medium due to their positive charge.

2. Materials and methods

2.1. Microalgae and fungal strains

The microalgae strain *Chlorella vulgaris* (UTEX 2714) and the fungal strain *Aspergillus niger* (Ted S-OSU) were used in this study. The microalgae strain was cultivated at room temperature (25 ± 2 °C) with a light intensity of approximately 100 μmol/m^2·s in a sterile BG11 medium (Andersen, 2005). *A. niger* was grown in a potato dextrose medium in a Petri dish at 37 °C and the spores were collected from the dish using sterile water and stored at 4 °C until use.

2.2. Cell cultivation

Experiments were conducted in a 250 mL Erlenmeyer flask with 100 mL working volume in an incubator shaker at 27 ± 2 °C for a period of 72 h at 150 rpm (Innova 2000, New Brunswick, USA). Control flasks are the monocultures consisting of only microalgae and fungal strains, included in each set of experiments in duplicate. The composition of the culture medium used for the co-culture contained: 2g glucose/L, 1g KNO_3/L, 0.075g KH_2PO_4/L, 0.1g K_2HPO_4/L, 0.5g $MgSO_4{\cdot}2H_2O$/L, 0.0625g $Ca(NO_3)_2{\cdot}4H_2O$/L, 0.01g $FeSO_4{\cdot}7H_2O$/L, 0.5g Yeast extract/L, 2.86 mg H_3BO_3/L, 0.039 mg $Na_2MoO_4{\cdot}2H_2O$/L, 0.222 mg $ZnSO_4{\cdot}7H_2O$/L, 1.81 mg $MnCl_2{\cdot}4H_2O$/L, 0.074 mg $CuSO_4{\cdot}5H_2O$/L, and 0.03g $CoCl_2$/L (Gladue and Maxey, 1994). For the inoculation, algal cells and fungal spores were counted using a hemocytometer (Hausser Scientific, Horsham, USA) under light microscope (DC5-163, National Optical, USA). The initial microalgae inoculation concentration was 2.55×10^9 cells/L and the initial fungal spore inoculation was 8.50×10^6 spores/L.

2.3. Experimental design

The surface charges of the cells were tested via zeta potential measurement at different compositions of culture media, pH, and ionic strength to study the co-pelletization process and the surface charge of the cells through zeta potential measurement. The algal harvest efficiency, percentage of algal and fungal biomass in the pellets, concentration of total fungal biomass and microalgae biomass in the co-culture and the pure culture was measured at different culture conditions (i) pH (5–9, uncontrolled pH); (ii) different concentrations of Ca^{+2} (0–0.1 g/L); Mg^{2+} (0–0.5 g/L) and NaCl (1–30 g/L). The quantity and diameter of pellets in the co-culture and the pure culture (only fungi) at different conditions were also studied. The effects of Ca^{2+} and Mg^{2+} on the surface charge of microalgae and fungi under acidic conditions (pH 3) in the culture medium were also studied.

2.4. Sample analysis

After 72 h of cultivation, biomass composition, distribution of the pellets (average pellet diameter and number of pellets) and glucose concentration in the culture liquid were analyzed. The total cell biomass was determined gravimetrically by drying the samples at 105 °C for 6 h. In order to differentiate the algal and fungal biomass in co-culture pellet samples, algal biomass was determined indirectly by measuring chlorophyll-a (Chl-a) concentration. Chlorophyll concentration was determined spectrophotometrically (Shimadzu UV spectrophotometer, UV-1800, USA) by extractions with methanol solution