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A novel method to harvest microalgae via co-culture of filamentous fungi to form cell pellets

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ABSTRACT

While current approaches have limitations for efficient and cost-effective microalgal biofuel production, new processes, which are financially economic, environmentally sustainable, and ecologically stable, are needed. Typically, microalgae cells are small and grow individually. Harvest of these cells is technically difficult and it contributes to 20–30% of the total cost of biomass production. A new process of pelletized cell cultivation is described in this study to co-culture a filamentous fungal species with microalgae so that microalgae cells can be co-pelletized into fungal pellets for easier harvest. This new process can be applied to microalgae cultures in both autotrophic and heterotrophic conditions to allow microalgae cells attach to each other. The cell pellets, due to their large size, can be harvested through sieve, much easier than individual cells. This method has the potential to significantly decrease the processing cost for generating microagal biofuel or other products.

1. Introduction

Producing biofuels and bioproducts via microalgae is promising; however, new technical processes must be developed to capitalize on the economically feasible potential of accumulating bioproducts and biofuel inside microalgae biomass. For instance, many microalgae (e.g., *Chlorella vulgaris*) are capable of accumulating a high content of lipids that can be converted to different forms of "drop-in" fuels such as biodiesel (Fakas et al., 2009; Heredia-Arroyo et al., 2011). Microalgae can rapidly accumulate lipids, which fit the industrial needs for biofuel production, with either autotrophic growth or heterotrophic growth mode. For the autotrophic growth mode, microalgae assimilate the carbon dioxide from the atmosphere as their carbon source, and sunlight in most cases as their energy source. The heterotrophic growth of microalgae cells uses organic carbon, for instance glucose, to support their carbon and energy need. Past studies for large-scale cultivation of algae relied on open-pond systems, which made it difficult to successfully cultivate algae due to the high downstream processing cost. Open-pond cultures are only commercialized to produce some value-added health food supplements such as feed and reagents (Chisti, 2007). Photobioreactors are developed to achieve higher productivity and to maintain monoculture of algae; however, the unit cost of microalgae production in these enclosed photobioreactors are actually much higher than those achievable in open-pond cultures despite photoreactors' higher biomass concentration and better control of culture parameters (Lee, 2001).

The algae cell harvest from cultivation broth has always been one of the major obstacles for the algae-to-fuel approach. Microalgae cell harvest is technically challenging, especially considering the low concentration (typically in the range of 0.3–5 g/L), the small size of the oleaginous algal cells (typically in the range of 2–40 μm), and their similar density to water (Li et al., 2008). Oleaginous microalgae cells are usually suspended in water and do not easily settle by natural gravity force due to their negative surface charges. The recovery of microalgae biomass generally requires one or more solid–liquid separation steps, and usually accounts for 20–30% of the total costs of production (Uduman et al., 2010a).

How to harvest microalgae cells from cultivation broth is dependent on the characteristics of the microalgae, such as size and density (Olaizola, 2003); and harvesting usually requires a separate step after the cell cultivation. All of the available harvest approaches, which include flocculation, flotation, centrifugal sedimentation, and filtration, have limitations for efficient, cost-effective production of biofuel (Shelef et al., 1984). For instance, flotation methods, based on trapping algae cells using dispersed micro-air bubbles, is limited in its technical and economic viability. Most conventional and economical separation methods such as filtration and gravitational sedimentation are widely applied in wastewater treatment facilities to harvest relatively large (>70 μm) microalgae such as *Coelastrum* and *Spirulina*. However,

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they cannot be used to harvest algae species approaching bacterial dimensions (<30 μm) like *Scenedesmus*, *Dunaliella*, and *Chlorella* (Brennan and Owende, 2010), to which most oleaginous microalgae species belong. Centrifugation is widely used to recover microalgae biomass, especially small-sized algae cells; however, its application is restricted to algae cultures for high-value metabolites due to intensive energy needs and high equipment maintenance requirements. While flocculation is used to harvest small-sized microalgae cells, it is a preparatory step to aggregate the microalgae cells and increase the particle size so that other harvesting methods such as filtration, centrifugation, or gravity sedimentation can be applied (Molina Grima et al., 2003). Several flocculants have been developed to facilitate the aggregation of microalgae cells, including multivalent metal salts like ferric chloride ($FeCl_3$), aluminum sulphate ($Al_2(SO_4)_3$) and ferric sulphate ($Fe_2(SO_4)_3$), and organic polymers such as chitosan and modified starch (Li et al., 2008). Chemical flocculation can be reliably used to remove small algae cells from pond water by forming large (1–5 mm) sized flocs (Sharma et al., 2006). However, besides the high cost of chemical flocculants and possible pollution effects that may generate, the chemical reactions are highly sensitive to pH and the high doses of flocculants required produce large amounts of sludge and may leave a residue in the treated effluent. In summary, most technologies including chemical and mechanical methods greatly increase operational costs for algal production and are only economically feasible for production of high-value products (Park et al., 2011).

Besides traditional methods mentioned above, there are several new technology developments in this field. DOE-ARPA-E recently funded a research project for Algae Venture Systems (AVS) to develop a Harvesting, Dewatering and Drying (AVS-HDD) technology using the principles of liquid adhesion and capillary action to extract water from dilute microalgae solutions. Attached algal culture systems have been developed for growing microalgae on the surface of polystyrene foam to simplify the cell harvest (Johnson and Wen, 2010; Wilkie and Mulbry, 2002). New bioflocculants, which are more environmentally friendly, are also proposed to address the cost and environmental concerns for current flocculation methods (Uduman et al., 2010a). These methods are innovative and will decrease the harvest cost to some extent if developed successfully, but require heavy investments on equipment and chemical supplies.

Enhancing natural algae aggregation to encourage simple gravity settling or filtration appears to be the most promising option to achieve both a high-quality treated effluent, in terms of total suspended solids, and an economic recovery of algal biomass for biofuel use (Uduman et al., 2010a). It will also be more environmentally sound than current procedures which may need additives. Many of the algal species in the wastewater treatment processes often form large colonies (50–200 μm), and their cell aggregation can be achieved through nitrogen limitation and CO_2 addition (Park et al., 2011); however, most of these microalgae species are not oleaginous species. Methods to enable oleaginous microalgae aggregate during their cultivation are strategically and urgently needed to develop efficient and economic means of biofuel production.

In submerged cultures, filamentous microorganisms, including some species of molds and bacteria, tend to aggregate and grow as pellets/granules. These fungal cell pellets are spherical or ellipsoidal masses of hyphae with variable internal structures, ranging from loosely packed hyphae, forming "fluffy" pellets, to tightly packed, compact, dense granules (Hu and Chen, 2007, 2008; Hu et al., 2009). Fungal cell pelletization can significantly decrease the viscosity of the fermentation broth; therefore it has been widely researched to increase the cultural performance on the mixing and mass transfer properties. Other advantages of cell pelletization to the micro-oil production process include the ease of harvesting cells and of re-using pond water (Johnson and Wen, 2010; Xia et al., 2011). Conditions for cell pelletization seem to be strain-specific and that not all the filamentous fungal strains can form pellets during their growth. A recent study at University of Minnesota showed that *Mucor circillenous* is relatively difficult to form pellets during their cell growth; however, with induction of $CaCO_3$ powder in the early stage of its cultivation, cell pellets can be formed homogeneously, lasting for the entire cultivation cycle. Changing operational conditions during cell cultivation was found to be able to induce fungal cells to aggregate and form pellets (Xia et al., 2011). This method avoids traditional approaches that use $CaCO_3$ powder or other nuclei to induce the fungal pelletization (Liao et al., 2007; Liu et al., 2008a) which are costly and cause solid waste disposal issues.

Pelletization is more widely seen in the fungal fermentation process where the microorganisms are filamentous. However, most oleaginous microalgae are not filamentous and oleaginous microalgae pelletization has not been seen in a complete review of current literature. In this study, a preliminary study was conducted to inoculate filamentous fungal spores when culturing mixotrophic green algae *C. vulguris* and it was found that pellets clearly formed within two days of culture. The microalgae cells, aggregated together with fungal cells, were immobilized in the pellets. This paper described this new technology which uniquely addresses the cell harvest of microalgae and has the potential to greatly reduce the algae biofuel cost. While pelletization and granulation have already been developed for commercial production of numerous products to increase culture conditions (e.g., mass transfer and oxygen transfer), this paper is the first study to investigate co-culturing microalgae with filamentous fungi for possible direct pelletization of microalgae to lower the overall cost of the process.

2. Materials

2.1. Microbial species

Filamentous fungi *Aspergillus niger* Ted S-OSU was tested in this study for its co-cultivation with microalgae, as well as other filamentous fungal strains, which were all purchased from ATCC. *C. vulguris* was the model microalgae species to test its co-pelletization with filamentous fungi. Previous research has revealed that *C. vulguris* is a potential robust producer of microbial lipids because it is a typical mixotrophic oleaginous microalgae, which can assimilate both organic carbon and sunlight for their cell growth (Heredia-Arroyo et al., 2011).

2.2. Cell cultivation

2.2.1. Cultivation medium

Autotrophic medium A (per L): KNO_3 1 g, KH_2PO_4 0.075 g, K_2HPO_4 0.1 g, $MgSO_4{\cdot}2H_2O$ 0.5 g, $Ca(NO_3)_2{\cdot}4H_2O$ 0.0625 g, $FeSO_4{\cdot}7H_2O$ 0.01 g, Yeast extract 0.5 g, A5 1 ml. A5 (L): H_3BO_3 2.86 g, $Na_2Mo_4{\cdot}2H_2O$ 0.039 g, $ZnSO_4{\cdot}7H_2O$ 0.222 g, $MnCl_2{\cdot}4H_2O$ 1.81 g, $CuSO_4{\cdot}5H_2O$ 0.074 g, $CoCl_2$ 0.03 g.

Heterotrophic medium B (per L): Potato dextrose broth 12 g, Glucose 15 g.

2.2.2. Cultivation of seed *C. vulgaris*

An autotrophic flask culture of *C. vulgaris* was maintained in the lab to provide algae seeds for the co-cultural experiments. A 4 L flask was filled with 3 L culture medium A with a magnetic stir (100 rpm) and four fluorescent lights were provided outside the flask to culture the algae cells at 25–27 °C. Fresh cultural medium was routinely added into the flask to maintain the active cell growth.