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Effect of harvesting methods on the reusability of water for cultivation of *Chlorella vulgaris*, its lipid productivity and biodiesel quality

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ABSTRACT

The large water footprint is one of the major bottlenecks for the sustainable microalgae-based biorefinery. In order to reduce the amount of water that is needed for mass cultivation of microalgae, the reusability of culture medium for further algal growth was tested. *Chlorella vulgaris* was cultivated in recycled medium that was obtained from harvesting microalgal cells by using either centrifugation or flocculation with $FeCl_3$ or alum. The present study shows that centrifugation and flocculation with $FeCl_3$ are equally effective (>90%) for harvesting *C. vulgaris* without any deleterious effects on algal growth when recycled media was used. However, even low concentration (<5 ppm) of residual alum was shown to inhibit microalgal growth. More interestingly, the recycled media obtained after centrifugation or flocculation with $FeCl_3$ had a positive effect on biomass and lipid productivity of *C. vulgaris*. Extracellular substances such as carbohydrate, proteins, or ferric ions in the recycled media appear to cause these positive effects. Furthermore, change of pH to 2–3 and washing with water were found to effectively remove the residual ferric ions that are present in either harvested biomass or biodiesel, respectively. These results suggest that the use of recycled medium for microalgal cultivation is possible, and the choice of harvest methods must be carefully made when the recycling of culture medium is considered for microalgal cultivation.

1. Introduction

The looming energy crisis due to the declining supply of fossil fuel and a series of environmental problems due to the emission of greenhouse gases resulted in increase of research and development investments in search for renewable and environmentally friendly alternatives [1]. Microalgae-derived biodiesel is an attractive alternative due to algae's ability to uptake CO_2 from the atmosphere and much greater biomass productivity compared to land plants [2,3]. However, the commercialization of microalgal biodiesel is hampered by the prohibitively high cost of biomass production that is incurred during the cultivation due to the costs of nutrients, water, and harvesting steps [4]. Moreover, the water footprint required for the production of 1 kg of biodiesel exceeds 3000 L [5]. It has been reported that microalgal biofuel can only be environmentally sustainable if water cost is reduced.

In addition, the report predicts that the production of 39 billion liters of microalgal biofuel will require approximately 6 to 15 million metric tons of nitrogen and nearly 1 to 2 million metric tons of phosphorus, if the remaining nutrients from algal cultivation are not recycled back into the system. Recycling water after harvesting can save up to 84% of water and 55% of essential nutrients such as nitrate and phosphate [5]. Therefore, recycling of the cultivation medium after harvesting of microalgal biomass is necessary for the economical and sustainable production of microalgal biofuels [6].

The choice of harvesting methods has a significant impact on the reusability of recycled water, as it will affect the quality of water after the harvest. Many different methods have been employed to harvest microalgal cells. Most common methods include the use of centrifugation, organic and inorganic flocculants, polymer, membrane filtration technology and dissolved air flotation [7,8]. Some methods such as centrifugation are very energy intensive, while organic polymers like chitosan are costly. Cationic polymers also have been used, but they are not as effective in saline culture mediums [9]. Other harvesting techniques such as electrolytic flocculation and electro-flotation also have been effectively applied with low energy cost [9,10]. An ideal harvest method must have high harvesting efficiency and low capital and

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operational costs, nor have negative impact on biodiesel quality. More importantly for the purpose of reusing the medium, the choice of harvest method must not introduce substances that are toxic to the cells nor have negative impacts on biodiesel quality.

Although there are many reports about the utilization of different harvesting methods, very few reports address the reusability of water after harvesting [11–15]. Therefore, the objectives of this study are 1) to evaluate the harvesting methods based on their harvesting efficiency, 2) to study the reusability of water after harvesting, 3) to investigate the effect of harvesting methods on quality of water, biomass and lipid productivity when recycled medium is used, and 4) to assess the impact of harvesting methods on the quality of biodiesel. For the harvesting methods in this study, chemical flocculation with $FeCl_3$ and alum was selected as a method for investigation due to low cost and energy requirement [16]. Centrifugation was used as a reference harvest method for comparison. Our results confirm that biomass and lipid productivity of *C. vulgaris* were enhanced only when centrifugation or FeCl3 harvesting methods were used to obtain the recycled medium.

2. Material and methods

2.1. Microalgae and culture conditions

Chlorella vulgaris (UTEX 265) was obtained from UTEX at the University of Texas, Austin (USA). Culture was maintained on solid agar plates or in liquid BG-11 medium [17]. *C. vulgaris* was grown axenically in 300 mL of BG-11 medium in 500 mL Erlenmeyer flask at 25 °C under a continuous illumination of 100 μmol m^{-2} s^{-1} using cool-white fluorescent lamps. The culture was continuously aerated by gentle bubbling of air containing 2% CO_2 with a flow rate of 150 mL/min in addition to constant shaking of 120 rpm in an orbital shaker. Microalgal growth was monitored for 10 days, and a small volume of the culture medium was sampled every day to monitor algal growth and nutrient uptake. The microalgal growth was estimated by measuring the optical density of the culture at 680 nm using a UV/Vis spectrophotometer (Beckman Coulter, model DU 730).

2.2. Harvesting methods and efficiency

Centrifugation and chemical flocculation with ferric chloride $FeCl_3 \cdot 6H_2O$ and alum ($KAl(SO_4)_2 \cdot 12H_2O$) were chosen to harvest microalgae. After flocculation, the floc and the growth medium were separated immediately. To evaluate the harvest performance, small amounts of culture were withdrawn before and after the harvesting and the optical density at 680 nm was measured. The harvest efficiency was calculated using the following equation:

$$\text{Harvesting efficiency}(\%)\text{R} = \left[1-\left(C_f/C_i\right)\right] \times 100,$$

where C_f and C_i is the final and initial optical density at 680 nm, respectively. To ensure the expediency of the harvesting methods, harvest efficiencies of different methods were evaluated based on the performance within 10 min.

2.3. Reuse of the culture medium after harvesting

The culture medium after harvest was reused for the next cultivation cycle. Prior to a subsequent cultivation, the spent medium was filtered using 0.2 μm with receiver flask filter system in order to avoid any contamination (Sartorius Stedim Biotech). The pH and nutrient concentration of the growth medium were readjusted to that of the original BG-11 by adding the necessary amount of inorganic supplement after the estimation of remaining nutrients in the spent medium. Inoculum density was adjusted to have equivalent starting cell density in the reused medium for the growth study.

2.4. Staining lipids using Nile Red

To visualize the lipid bodies, microalgal cells were stained with Nile Red as described by Chen et al. [18] with a minor modification. For staining, 50 μL of microalgal culture was briefly mixed with 30 μL of Nile Red dye diluted in 25% DMSO solution (5 μg mL^{-1}) [18]. The suspension was mixed via vortexing, then followed by 10 min incubation at 40 °C in dark before microscopy. Optical and fluorescent microscopic analyses were carried out using an inverted light microscope (Leica DM2500, Leica Microsystem, Switzerland) with 100× magnification. The images were taken with a camera (DFS 420C) and stored in a computer for further analysis.

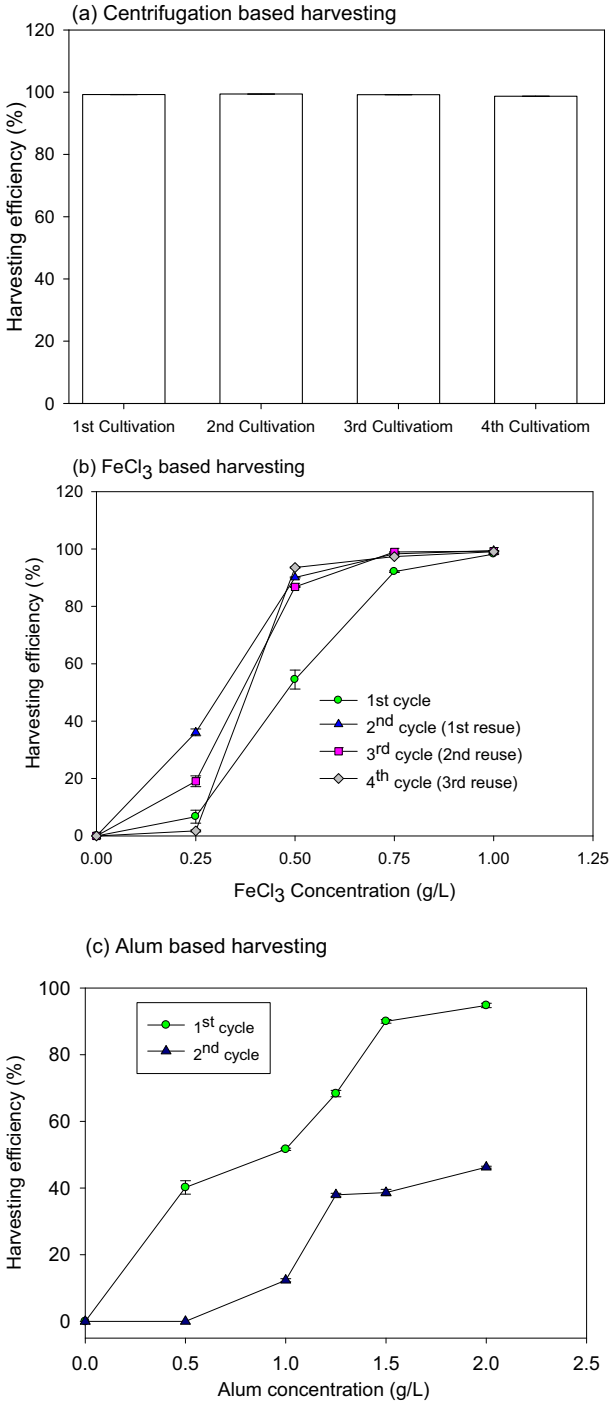

Fig. 1. Harvesting efficiency of (a) centrifugation, (b) $FeCl_3$, and (c) alum (n = 2).