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Research paper

Biomass harvesting and concentration of microalgae scenedesmus sp. cultivated in a pilot phobioreactor

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ABSTRACT

Samples of a culture of *Scenedesmus* sp. grown in a pilot raceway photo bioreactor were processed in the laboratory by flocculation and centrifugation, to assess the efficiency of solid concentration of both methods. Three flocculation agents were tested, ferrous sulfate ($FeSO_4$), ferric chloride ($FeCl_3$) and aluminum sulfate $Al_2(SO4)_3$ using concentrations of 0.05, 0.2, 0.8, 1.5, 3.0 y 7.0 g L^{-1} and pH range between 4.0 and 11.0 for the better concentration response of each flocculant. Additional flocculation tests were carried out to measure the sedimentation kinetics of the concentrated biomass. All these tests were performed with culture samples of 800 cm^3 for 12 min of stirring and 10 min of sedimentation with three repetitions. In the case of centrifugation the testing was carried out using a laboratory centrifuge run at speeds of 1500, 1800 and 2200 rpm with culture samples of 300 cm^3 for 15 min and three repetitions. In order to quantify the efficiency of the concentration, initial and final turbidity of the cleared water and concentrated portion were measured. The flocculation experiments showed that a limit of maximum concentration efficiency of 97.9% that was reached with 1.5 g L^{-1} of $Al_2(SO_4)_3$ at pH of 8.5 and an average sedimentation velocity of about 2.7 cm min^{-1}. Testing also showed that $FeSO_4$ was the worst flocculant agent in the range tested. With respect to the centrifugation test, the solid concentration efficiency varied from 95.2% at speed of 1500 rpm up to 96.0% at speed of 2200 rpm.

1. Introduction

In a scenario of a steady increase in demand for fuel worldwide, evaluation studies report that energy consumption from fossil fuels account for 88% of total energy consumption, while nuclear energy and hydropower corresponding to 5 and 6% of total primary energy consumption respectively [1]. Fossil fuels are the largest contributor of greenhouse gases into the biosphere, not only contribute to global warming but also other impacts on the environment and human life [2]. This situation has led to the introduction of new alternative sources of energy, with a strong emphasis on the search for raw materials for the production of biomass that can be converted into biofuels, which are estimated to emit 40% less CO2 than fuel fossils [3]. One alternative source of biomass is microalgae for the production of different types of biofuels, including biodiesel, bio ethanol and bio methane [4]. Microalgae are photosynthetic microorganisms that can grow rapidly up to a biomass concentration ranging about 2 g L-1 and can live in extreme conditions due to the characteristics of their cellular structure, allowing use of non-arable land and use no potable water consumption, decreasing and preventing displacement of food crops. Microalgae can be grown using sunlight, CO_2, nutrients and photosynthetic agents. The CO_2 of the atmosphere may be fixed or may be obtained from a concentrate such as industrial flue gases source. Normally microalgae are grown in shallow ponds or closed ponds, exceeding seven times the productivity of open ponds. It has been used successfully different types of closed raceway and tubular photobioreactors for the production of large volumes of microalgae biomass. Microalgae are also used for food and cosmetic related items [5] and in the pharmaceutical industry, because some species of microalgae produce bioactive compounds as antioxidants, antibiotics, and toxins [6]. Likewise can be used as nutritional supplements for human consumption due to its high content of protein, vitamins and polysaccharides [7]. Some microalgae even have a higher content of amino acids introduced by the conventional foods such as *Scenedesmus spinosus*, which has lysine levels higher than the FAO pattern [8]. On the other hand, they can be used as potential source of oil for the production of renewable biodiesel that might be able to meet a fraction of the global demand for transport fuels.

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Currently biodiesel is usually produced from oilseeds such as soybeans, rapeseed, etc. wonder, however microalgae can be a suitable alternative feedstock for the biofuel, because some species produce high amounts of oil, which can be extracted and used for this purpose where the oil levels in the biomass can be overcome in some cases 80% of the weight of the dry mass, common values range between 20 and 50% by weight of the dry matter [9]. Among oil-containing species *Scenedesmus* sp presents an interesting amount of polyunsaturated fatty acids (PUFAs) and other interest for the production of biodiesel, with total oil content in the approximate range of 17–21% [10]. This microalgae has a capacity of carbon fixation particularly high [11]. The main companies involved in the development of microalgae biofuels in the world are in the United States which has 78%, and then there is Europe with 13% and other 9% [12]. The recovery of biomass requires one or more stages of solid–liquid separation to allow concentration of this biomass initially very dilute near 1% in weight. For example, in a culture of the microalgae *Scenedesmus* sp. with Bristol nutritional medium, Lara [13] achieved a concentration of 0.11% dry mass. The most commonly used harvesting methods for the algal biomass are flocculation and centrifugation [14]. Flocculation is the binding in large clusters of originally divided solids in suspension, and is a process widely used in industry for the removal of suspended solids. Microalgae carry a negative charge that prevents them from self-aggregation, which can be counteracted by the addition of chemicals known as flocculants. Cationic flocculants are chemicals that coagulate algae without affecting its composition. The flocculants used commonly are $Al_2(SO4)_3$ (aluminum sulfate), $FeCl_3$ (ferric chloride) and $Fe_2(SO4)_3$ (ferric sulfate), which vary in efficiency, being directly related to the ionic charge of the flocculant [15]. Several studies have achieved efficiencies high solids concentration as Knuckey et al. [16], who used ferric chloride, with an optimum flocculation pH of 6.0with different types of algae, achieving an efficiency of concentration of 85–95%. Tuan et al. [17], adjusted the culture pH achieving efficiencies greater than 90%. Moreover, the method of solid–liquid separation by centrifugation is certainly the preferred method for harvesting algae [18]. For instance, Morris et al. [19] used successfully centrifugation to concentrate biomass of different species of algae such as *Chlorella vulgaris*. The overall objective of this study was to evaluate the method of separation of biomass in the cultivation of microalgae *Scenedesmus spinosus* and in order to propose separation methodologies for industrial microalgae biomass production.

2. Materials and methods

The biomass used was obtained from a culture of microalgae *Scenedesmus spinosus* from a Chilean endemic strain collected by the Biology Department of the University of Concepción, Chile, produced in a Raceway bioreactor of 1.4 m^3 with a transparent polycarbonate cover of 3 mm thick. The biomass was grown in a Z8 culture medium, under controlled conditions of pH, CO_2, and nutrients. The culture at harvesting had a pH of 6.5, a turbidity of 259 NTU and a biomass concentration of 0.4 g L^{-1}. Once cultivated, experiences of microalgae biomass separation from the aqueous medium was performed by the flocculation and centrifugation. These tests were conducted at the Laboratory for Quality Control, Faculty of Agricultural Engineering, Universidad de Concepción, Chile.

2.1. Flocculation

Flocculation tests were run using a flocculation laboratory equipment, brand Stuart, and model SW6, which can simulate the flocculation stages and results of this process as on large scale. The equipment ensures repeatable conditions between six samples in beakers with the same stirring conditions, such as the agitator rotational speed and time for each repetition. The flocculants used were aluminum sulfate, ferric chloride and ferrous sulfate. The steps for selected operating conditions were as follows:

1. The same following conditions were applied to the six samples in parallel: (a) stirring at 120 rpm a period of 1 min for aggregation, (b) stirring at 25 rpm a period of 12 min for flocculation,(c) settling time of 10 min.
2. Determination of the optimal rate of each flocculant by varying the rate of 0.05 g L^{-1} to 7 g L^{-1} as shown in Table 1.
3. Determination of the optimum pH for each flocculant, maintaining fixed the previously determined rate of flocculant. The pH was varied in the range of 4–11 and adjusted with NaOH and HCl 1N, before the addition of the flocculant.
4. Determination of the efficiency of solids concentration in flocculation, taking as reference the turbidity of 0.44 NTU of distilled water, assuming that this value corresponds to the efficiency of 100% and that the initial turbidity of the solution corresponds to 0% efficiency. Thus the relationship to calculate the concentration efficiency is given by Equation (1) as:

$$E = \frac{T_i - T_f}{T_i} \times 100 \quad (1)$$

where E is the concentration efficiency in % and T_i and T_f are the initial and final turbidity of the culture sample expressed in NTU units.

5. Obtaining the sedimentation rate curve of each flocculant once completed the procedures 3 and 4.

In Table 1, the different testing parameters are shown. All values utilized are within range recommended by Fuentes et al. [15].

2.2. Centrifugation

Centrifugation tests were performed on two types of centrifuge: a laboratory centrifuge of four tubes 75 ml each, brand Daemon, IEC model HN–SII and an industrial nozzle disc centrifuge, brand Alfa-Laval, model WSPY 207TGP-74-50.

2.2.1. Laboratory centrifuge

The rotation speed for maximum concentration efficiency in the recovering of microalgae biomass from the aqueous medium was determined, by testing speeds of 1500, 1800 and 2200 rpm while maintaining a constant operation for 15 min, followed by the removing of microalgae biomass concentrate from the clarified aqueous medium at the end of each test.

2.2.2. Nozzle disc centrifuge

The nozzle disc centrifuge utilized provides standard conditions of 5500 rpm speed and a discharge of microalgae concentrate every 10 min with a feeding rate of 14 L min^{-1}. Three trials at the beginning, middle and the end of centrifugation were performed, taking samples of 200 ml of the clarified remaining solution for subsequent turbidity measurement.

2.3. Concentration efficiency in flocculation and centrifugation

In the assessment of concentration efficiency by the two methods, the turbidity was measured in NTU (Nephelometric Turbidity Unit) with a turbidimeter brand Hach Lange, model 2100p. After completing the concentration process, 200 ml samples