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Energy and operating cost assessment of competing harvesting methods for *D. salina* in a β-carotene production process

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ABSTRACT

A new evaluation concept to assess microalgal harvesting methods was introduced using β-carotene production with *Dunaliella salina* as a case study. Detailed experiments were conducted, analyzing different effects caused by the first harvesting step. As harvesting methods, centrifugation and flocculation by electrolysis, addition of aluminium sulfate (alum), ferric chloride or pH increase via NaOH were considered. A special focus was set on the influence of the harvesting method on interlinked units of the whole process system. Thereby, the samples harvested by NaOH flocculation negatively affected the reusability of the separated medium for cultivation and the efficiency of β-carotene extraction. Furthermore, a process model was developed to estimate the overall process energy demand and the operating costs based on the experimental data. In the case of *D. salina* centrifugation without flocculation was identified as the most cost effective harvesting method. In consequence, the here developed concept provides a general platform to evaluate the quality of microalgal harvesting procedures in a reliable manner.

1. Introduction

Dunaliella salina is one of the richest sources of natural β-carotene. In fact, this pigment can contribute up to 12% to the algal dry weight [1]. The halotolerant microalga is already industrially farmed in large shallow lakes or open raceway ponds in Australia and Israel [2,3]. The ability to produce valuable products like β-carotene under the harsh environmental conditions of high salinity and high solar irradiation makes it more resistant against contamination and therefore an ideal production organism. One main obstacle of large scale cultivation of *D. salina* is the low growth rate of this species. The low cell concentration in the cultivation medium determines the applied harvesting procedure in an industrial process. This step is essential to concentrate the biomass for subsequent processes, in particular the extraction of pigments. Thus, it is one of the critical process units regarding costs and energy demand of an industrial algae production process [4].

In the past, several harvesting methods were investigated for *D. salina*. For example, a mechanical harvesting method was patented by [5]. Here, a suspension of *D. salina* was filtered through diatomaceous earth. However, filtration is not the most favorable harvesting method for *D. salina*, since this algal species lacks of a rigid cell wall. Consequently, mechanical forces affecting the alga during filtration can lead to cell disruption and loss of valuable β-carotene. Another harvesting method is based on the hydrophobic effect which was observed if *D. salina* was incubated in medium containing at least 3 M NaCl [6]. In a high saline environment the alga is able to adsorb on hydrophobic surfaces like glass wool. This method is commercially realized on large scale in the Australian *D. salina* production site [4].

Flocculation is a further method to dewater algal biomass which can be induced by different mechanisms. For instance, multivalent metal cations provided by the addition of aluminium or ferric salts or the sacrificial electrode during electrolysis [7,8] interfere with the negatively charged surface of the alga. As a consequence, a neutralization of the negative surface charge occurs, leading to decreased electrostatic repulsion between the unicellular algae and finally to the formation of multicellular flocs. Based on the works of [9,10] it seems promising to flocculate *Dunaliella* cells via autoflocculation due to pH increase of the algae culture. This type of flocculation is accompanied by the precipitation of magnesium and calcium hydroxides which induce flocculation through charge neutralization, bridging or sweeping [11]. So far, the main industrially used harvesting method for algae biomass is the conventional centrifugal sedimentation since it is highly efficient, independent from the type of microalgae [12]. However, centrifugation has a high energy demand [13] which leads to high harvesting cost due to the processing of large volumes of the low concentrated microalgal cultures.

Besides the efficiency and cost consideration of the harvesting method, it is also important to analyze how other interlinked process steps are affected by the harvesting procedure. First experimental research work in this direction was done by investigating the reusability of culture medium after the harvest [14]. Recycling of separated nutrients

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and water can drastically reduce costs for cultivation and make-up water. Another important investigation was done by [15] who analyzed the contamination of the biomass and product extract with the applied flocculant. Remaining flocculant concentrations in the product are undesired; especially in pharmaceutical or food applications.

From the theoretical point of view, a detailed process analysis for industrial application was done by [16]. In their work an economic, sustainability and energetic model was developed to compare various methodologies of each process unit for microalgae bio-diesel production. In a more recent work by [17] the economic viability of algal biodiesel was evaluated by taking into account technical uncertainties using techniques of global sensitivity analysis. A major disadvantage of usual process models is, that they mostly use organism-unspecific assumptions. However, every microalgae strain shows differences in growth rate, product content and downstream behavior. Consequently, simply applying standardized assumptions will not be an adequate approach to conduct a realistic analysis for a specific microalgae bioproduction process.

Although there are numerous experimental approaches to identify an optimal harvesting concept for microalgae, so far none integrates the experimental data in a theoretical process analysis to get a reliable comparison of the different harvesting methods. The present study aims for a detailed process analysis to investigate the influence of the harvesting method on the overall process energy demand and operating costs. For this purpose, a process model for β-carotene production with *D. salina* was developed. For the validation of the model, specific laboratory scale experiments were carried out, allowing the determination of some crucial, strain specific process parameters, in particular dewatering efficiency, concentration factor and recovery of extracted pigments. The present contribution focuses on the first dewatering step, especially on the flocculation processes realized via electrolysis, increase of pH via NaOH or addition of metal salts. The impact of the harvesting method on interlinked process units was analyzed. For this purpose, the recyclability of the cultivation medium and the influence of the harvesting method on the extraction efficiency of β-carotene were investigated in detail.

2. Materials and methods

2.1. Strain and cultivation conditions

D. salina (CCAP 19/18) was grown photoautotrophically in 1 L shaking flasks filled with 0.5 L of the growth medium (pH 7.5) previously described by [18]. The cultivation occurred in a rotary shaking incubator (Multitron, Infors AG, Switzerland) at 3.5% CO_2 in air, a temperature of 26 °C and a shaking frequency of 100 rpm. A day/night cycle was used for cultivation by applying 16 h light (75 μE m^{-2} s^{-1}) and 8 h darkness per day. To introduce carotenogenesis, *D. salina* was cultivated in a flat panel photobioreactor (FMT 150, PSI, Czech Republic) under continuous high light conditions (1500 μE m^{-2} s^{-1}) at 26 °C and nitrogen depletion. For this purpose, 1000 mL culture medium was inoculated with approximately 1×10^6 cells mL^{-1} from a culture cultivated in shaking flasks as described above. During the cultivation the pH level was hold constant at 7.5 by adding 1 M NaOH and 1 M HCl. The culture was aerated with 400 mL min^{-1} of a synthetic air mixture containing 3.5% CO_2.

2.2. Analytical methods

For determination of algal growth and harvesting efficiency an UV/Vis spectrophotometer was used (Specord S600, Analytik Jena AG, Germany). Therefore, the optical density of the samples was measured at 735 nm against the pure culture medium. The cell concentration of *D. salina* cultures was determined with a cell counter (Cellometer Auto T4, Nexcelom Bioscience LLC., USA). The pH values of the suspensions were measured with a pH electrode (Seven Easy, Mettler Toledo, USA).

2.3. Harvesting procedure

All harvesting experiments were carried out under lab scale conditions at a pH of 7.5 and a culture density of approximately $OD_{735\ nm} = 1$. The corresponding biomass concentration (approximately 0.3 g_{dw} L^{-1}) is comparable to the concentration of *D. salina* in the first harvesting step used in industry [6]. Cultures with higher densities were adjusted to $OD_{735\ nm} = 1$ by dilution with the culture medium. The pH was adjusted by adding 1 M NaOH and 1 M HCl. The used flocculant concentration or electrolysis parameters were previously optimized with regard to the best harvesting efficiency under minimal flocculant dosage (data not shown).

2.3.1. Electrolysis

For the electrolysis two glass chambers ($5.5 \times 10.8 \times 12$ cm^3) equipped with two Al-electrodes (44 cm^2 active area in the culture broth) and a magnetic stirrer were filled with 300 mL culture; respectively. During electrolysis a current of 150 mA was applied with a power supplier (peqPOWER 250, Peqlab, Germany) for 5 min under mixing with a frequency of 250 rpm. The values of voltage were recorded with a multimeter (UT71B, UNI-T, Germany). Afterwards, mixing continued for additional 10 min. After a settling time of 2 h, samples were taken from the middle of the suspension height to measure the absorbance and estimate the harvesting efficiency η_h (see Eq. (3)). The concentration factor CF was optically determined according to Eq. (4). The energy consumption E_h and the mass of aluminium m_{Al} dissolved during the electrolysis were calculated according to Faraday's law using the following equations:

$$E_h = \frac{U \cdot I \cdot t}{V_E} \tag{1}$$

$$m_{Al} = \frac{M_{Al} \cdot I \cdot t}{z \cdot F} \tag{2}$$

with U and I as voltage and current density, respectively, applied during electrolysis with an electrolysis time t to a volume V_E. M_{Al}, z and F stand for the molar mass of Al, the charge number of aluminium ions (+3), and the Faraday constant, respectively.

2.3.2. Flocculation via aluminium sulfate

To investigate flocculation caused by the addition of $Al_2(SO_4)_3 \times 16\ H_2O$ (alum, 0.1 M stock solution), experiments were carried out in duplicates. For this, 150 mL culture suspension was filled in beaker glasses. After the addition of alum with a final concentration of 0.6 mM, the cultures were mixed for 10 min at 250 rpm, followed by 2 h sedimentation. Estimation of η_h and CF was done as described in Section 2.3.1.

2.3.3. Flocculation via ferric chloride

To harvest *D. salina* with $FeCl_3 \times 6\ H_2O$ (0.1 M stock solution) duplicates of beaker glass experiments were performed similar to Section 2.3.2. Cell suspensions were adjusted to a final concentration of 1 mM $FeCl_3 \times 6\ H_2O$.

2.3.4. Flocculation via pH change

Duplicates of beaker glasses filled with 150 mL culture suspension were prepared. To initiate flocculation via pH change, NaOH (5 M stock solution) was added to the cultures to reach a final concentration of 20 mM. The addition of NaOH led to an increase of the pH value of approximately 12. After addition of NaOH, the samples were treated and analyzed as previously described in Section 2.3.2.

2.3.5. Centrifugation

Centrifugation was used as comparative harvesting method as well as to further concentrate the flocculated *D. salina* cells prior to pigment