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ORIGINAL RESEARCH

Flocculation of microalgae using calcium oxide nanoparticles; process optimization and characterization

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Abstract The massive energy required for biomass gathering makes current commercial microalgal biofuel manufacturing economically unsustainable. A present harvesting method is provided as an inexpensive and energy-efficient way; nano-flocculation by employing nanoparticles to concentrate and flocculate microalgae of interest. In this study, we used calcium oxide nanoparticles (CaONPs) derived from waste products of the Fayoum Sugar Factory (FSF) as a low-cost flocculent for a microalgae flocculation process that was tuned using three control parameters: temperature, flocculent dose, and medium pH values. Furthermore, FSF wastewater was employed as a nourishment source for microalgae production. FT-IR, X-RD, Zeta potential, and TEM were used to further identify and characterize CaONPs. According to the current data, using CaONPs under the best conditions for algae harvesting (pH 9.8 and temperature 45 °C with 250 mg/L) resulted in 99.3% algal coagulation. The advantages of the current method are that it is safe, rapid, does not require the addition of chemicals, is simple and effective, sustainable, and is cost-efficient because there are no costs associated with pre-treatment of the biomass.

Keywords Microalgae . Nano-flocculation . Biomass harvesting . Calcium oxide nanoparticles . Microalgae cultivation

Introduction

Microalgae are a huge distinct group of photosynthetic microorganisms with simple cellular features, varying from unicellular to multicellular structures; they are rapid-grower organisms with minimal nutrient requirements only water and sunshine (Khan et al. 2018). They could be extensively cultivated in various areas and conditions, both in freshwater, saltwater, and wastewater, as a result of their extreme tolerance to adverse environmental stress (Zhu et al. 2014). Recently, the great significant financial importance of microalgae was associated with their use in utmost daily life activities, industrial and marketing sectors including aquaculture, food processing, fertilizers, cosmetics, pigments, wastewater management, pharmaceuticals furthermore, anti-microbial and anti-tumor potentials (Hosikian et al. 2010; Chew et al. 2017; Li et al. 2020). Microalgae biomass has a great concern in the biological compounds used for the synthesis of many products, that are considered a fundamental source for biofuels (Costa et al. 2020; Fathy et al. 2021). As well as they used as nutritious supplements owing to their superior content of proteins,

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lipids, and carbohydrates (Borowitzka 2013). However, there are great challenges that complicate the biodiesel production from microalgae associated with harvesting of small-sized cells additionally, huge attractive force among the cells of the negative charges on the algae cell wall, which plays a great role in the harvesting process by flocculation (Roy and Mohanty 2019).

Harvesting is the collection of microalgae biomass from the culturing media and this process accounts for approximately 3% to 30% of the total cost of biodiesel production from microalgae (Brennan and Owende 2010; Fasaei et al. 2018). Harvesting technology selected along with cell age, shape type, density, and size of the microalgae cell indicated the costly and complexity of microalgae production (Danquah et al. 2009). Microalgae harvesting had been performed either with chemical, physical, biological, or electrical techniques; generally, there was no single authorized and well-documented applicable methods (Suparmaniam et al. 2019; Beckett and Le 1990; Paralkar and Edzwald 1996). Furthermore, other procedures including, filtration, centrifugation, alkaline flocculation, electro-coagulation, chemical, bio-flocculation, flotation, and sedimentation were used (Fathy et al. 2020; Gerde et al. 2014; Branyikova et al. 2018).

Previous studies used gravitational forces for sedimentation; this process is dependent on cell size and biomass density; challenges of sedimentation technique as physical harvesting method depend on cell size, so small cells with sizes ranging from 4-5 µm in the large scale harvesting efficiency became very low (Roy and Mohanty 2019). Other harvesting techniques include centrifugation, in which substances are gathered based on density fluctuation as centrifugal force forces big particles away from the axis and small particles toward the axis (Christenson and Sims 2011). The challenges of centrifugation technique as a physical harvesting method that requires a lot of energy, such as the harvesting process of *Scenedesmus* sp., 1 KWh m^{-3} is required (Mathimani and Mallick 2018). Using nanoparticles to collect microalgae is thought to be an environmentally friendly, cost-effective technology that could replace traditional, high-cost harvesting methods (Fathy et al. 2020; Wang et al. 2016). Calcium oxide is a low-cost catalyst with high basicity and non-corrosive qualities that is incomparable to homogenous base catalysts. Similarly, they require just modest reaction conditions to yield a high number of products in a short period of time (Zabeti et al. 2009).

The current study focused on the use of industrial waste products of sugar beet to improve culture growth in addition to calcium oxide nanoparticles for harvesting microalgae, this process considers an unexpensive, feasible, and time-saving procedure.

Materials and methods

Microalgae cultivation

Synechocystis sp. PCC 6803 was obtained from Botany and Microbiology Department, Faculty of Science Beni-Suef University, Egypt as it identified by Elsayed (2017), and then it was cultivated on Wuxal media, molasses of sugar beet, and wastewater of FSF to obtain high yield biomass. Wastewater was analyzed chemically and physically in Tabbin Institute Metallurgical Studies Central Lab for the Studies Industrial Pollution (TIMS/ CLISP), Cairo, Egypt, and (Tables 1 and 2), molasses was analyzed chemically and physically in the quality control laboratory of FSF (Table. 3 and 4). and Wuxal media it is a commercially available fertilizer Wuxal- Universal dünger liquid plant fertilizer (8 % N, 8 % P2O5, 6 % K2O, 0.01 % B,0.004 % Cu, 0.02 % Fe, 0.012 % Mn, 0.004 % Zn; Wilhelm Haug GmbH & Co. KG, Germany) as reported by (Elsayed et al. 2017). Briefly, *Synechocystis* sp. PCC 6803 was cultured in 2000 mL Erlenmeyer flasks and was left to grow at 30 °C for an alternative 12 h light and 12 h dark. Growth curves of microalgae on three different media (Fig.1) were established by optical density using a spectrophotometer (Shimadzu® 1800, Corp 01417, Japan) at 700 nm.

CaONPs synthesis and characterization

Limestone ($CaCO_3$) was obtained from FSF, Egypt. CaO nanoparticles were produced from the decomposition of $CaCO_3$ in a lime kiln at 1100 °C (Asadi 2006). CaONPs were characterized by Transmission Electron Microscope (TEM, JEOL Ltd., Japan). XRD configuration was verified using P-analytical empyrean X-ray diffraction using Cu Kα radiation (wavelength 0.154 cm^{-1}) at an accelerating voltage of 40 kV, current of 35 mA, scan angle of 5–75° range, and scan step of 0:02°. The crystal size average was calculated applying

Table 1 Chemical analysis of wastewater used in microalgae cultivation

Parameter	Result	Unit
BOD (5d, 20 °C)	10.1	ppm
COD Cr	20.4	ppm
Dissolved oxygen	6.2	mg\L
Total dissolved solids	857	ppm
Total suspended salts	20	ppm
Ammonia as NH_3	0.532	ppm
Total nitrogen	0.542	ppm
Total phosphorus	0.4	ppm

Table 2 Physical analysis of wastewater used in microalgae cultivation

Parameter	Result	Unit
Temperature	26.76	°C
pH	7.56	

Table 3 Chemical analysis of molasses used in microalgae cultivation

Parameter	Result	Unit
Sucrose	48	%
Protein	9	%
Betaine	5	%

Table 4 Physical analysis of molasses used in microalgae cultivation

Parameter	Result	Unit
Ash	10	%
Water	20	%
pH	7.5	
Density	1400	kg\m^3

Scherer's equation $D = 0.94\,\lambda/\beta\cos\theta$ (Patterson 1939). where D, was the crystallite size, λ, was the X-ray wavelength, β, was the broadening of the diffraction peak, and θ was the diffraction angle. FT-IR was used to assess the chemical bond vibrations of samples. FT-IR spectra were recorded on a Bruker (Vertex 70 FT-IR) spectrometer in the range from 400 to 4000 cm^{-1}.

Optimization of the harvesting process

Synechocystis sp. PCC 6803 culture was left to grow and form biomass. In the first parameter optimization, the pH value was 7.8 and the temperature was 15 °C, both were constant factors, while CaONPs were used in different doses (0.0025 g, 0.005 g, 0.0075 g, 0.01 g, and 0.0125 g per 50 mL algae culture). While, in the second parameter optimization, CaONPs amount was 0.01 g and temperature 15 °C, both were constant factors, while varying pH factors at 6.8, 7.8, 9.8, 11.8, and 12.6. Finally, in the third parameter optimization, CaONPs value was 0.01 g and pH were 9.8, both were constant factors, but the temperature was the changing factor at 15 °C, 30 °C, 45 °C, and 60 °C.

Estimating harvesting percentage

The spectrophotometer was used to measure the optical density at wavelength 700 nm both before and after adding calcium oxide nanoparticles to microalgae culture. The harvesting percentage was calculated using the following equation:

$$\text{Harvesting percentage} = \frac{\text{ODb} - \text{ODa}}{\text{ODb}} \times 100 \quad \text{(Equation 1)}$$

Whereas ODb was the optical density of the culture before the addition of calcium oxide nanoparticles,

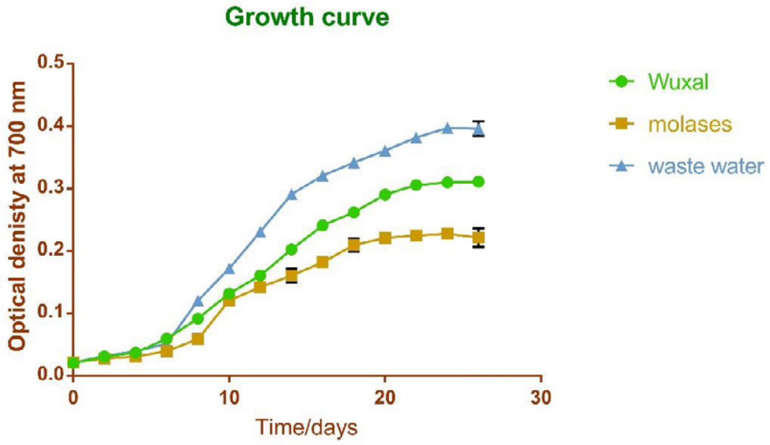

Fig. 1 Shows an investigation of the *Synechocystis* sp. PCC 6803 growth curve, which was established by spectrophotometer for Wuxal, molasses, and wastewater cultures.

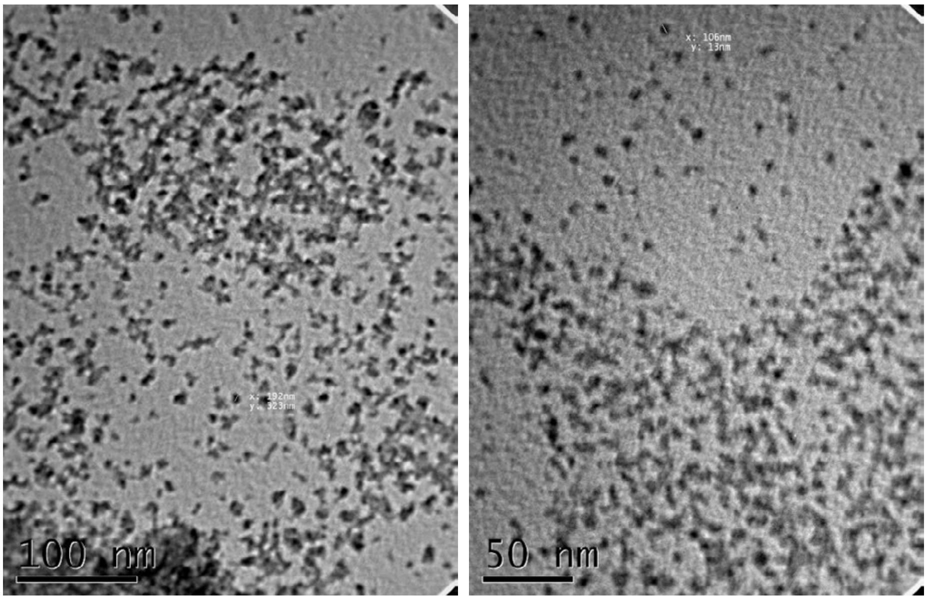

Fig. 2 Shows a TEM image of CaONPs with a regular and spherical shape

ODa was the optical density after the addition of calcium oxide nanoparticles.

Statistical analysis

The harvesting percentage was set up on a completely randomized design with 3 replications. The collected data were analyzed by nonparametric tests (chi-square test) using a statistical package for social sciences (SPSS, Inc., version 22.0, Chicago, IL, USA).

Results and discussion

Biomass of *Synechocystis sp*. PCC 6803 at three different media, Wuxal, molasses, and wastewater the growth curve was established by the spectrophotometer, and it was investigated that the high yield biomass was produced during the application of wastewater (Fig. 1).

TEM image of CaONPs (Fig. 2) was demonstrated that the shape of CaONPs, appeared as regular and spherical with a size less than 50 nm. In addition to that, XRD has investigated the structure of CaONPs peaks (Fig. 3), they were consistent with the peaks of standard CaO.

XRD patterns showed broadening of the peak's indicative of the ultrafine nature of the crystallite. The

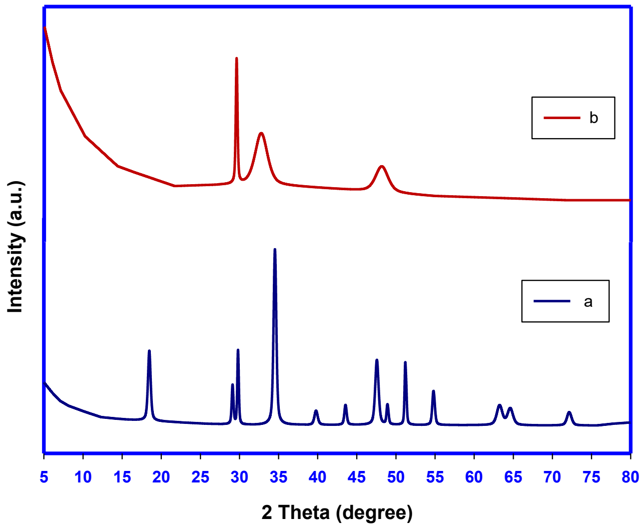

Fig. 3 XRD images of (a) CaONPs from the Fayoum sugar factory and (b) flocculated microalgae

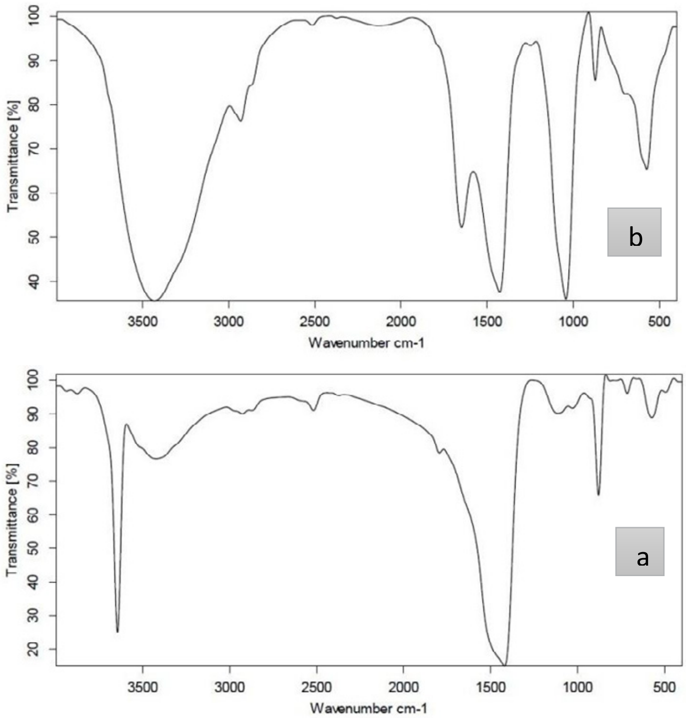

Fig. 4 Shows the FTIR spectra of (a) CaO nanoparticles and (b) flocculated microalgae

crystallite size calculated using Scherrer's formula was about 24 nm. The size distribution was presented between 13 nm and 49 nm. Zeta potential analysis is a measure of the charge attraction/repulsion between particles in solution, suspension stability, and gives us information about the reason for aggregation and dispersion.

The zeta potential of CaONPs was 31.4 mV. The higher value of ZP indicated that high attraction among nanoparticles and algal cell negative charge, that lead to forms coagulate algae cells. Infrared spectroscopy was verified the chemical purity of CaONPs powders, the bands were contributed to carbonate and hydroxyl that were markedly displayed in the spectrum (Fig. 4a). The sharp band at 3645 cm^{-1} resembled the OH bonds from the remaining hydroxide (Roy and Bhattacharya 2011). Bands at 1420 cm^{-1} and 879 cm^{-1} have corresponded to the CO bond (Nyquist and Kagel 2012). The wide and strong bands at 573 cm^{-1} were related to the CaO bonds (Park et al. 2002).

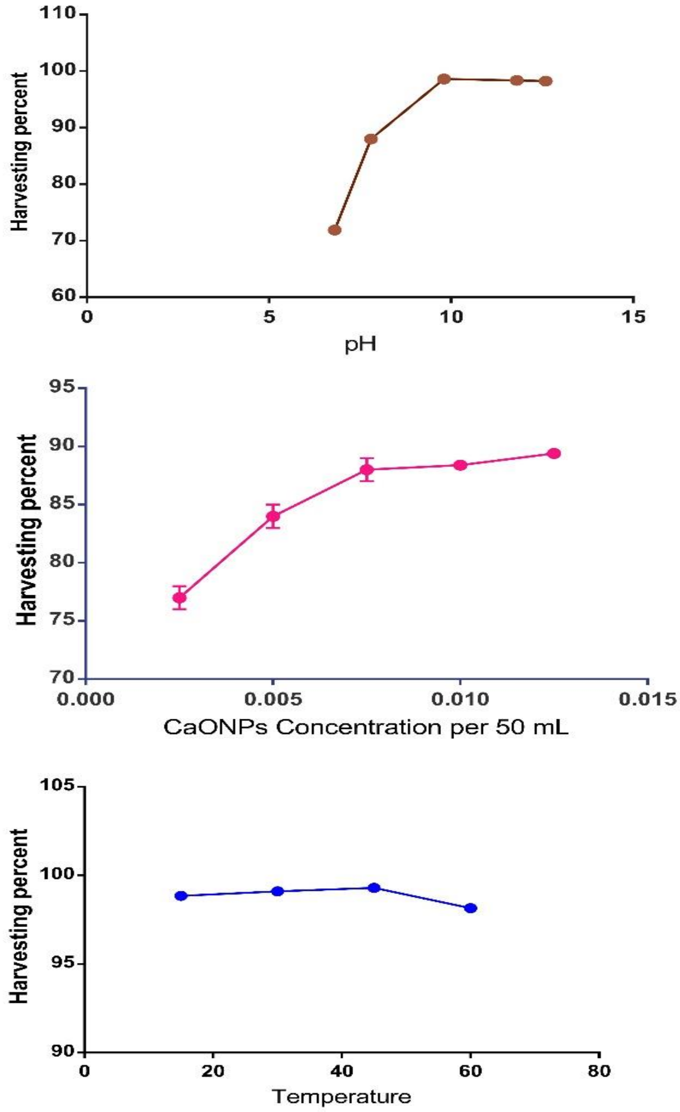

Fig. 5 Improving *Synechocystis sp*. PCC 6803 harvesting by varying CaONPs concentration, pH shift, and temperature variety consequently

The relation between the harvesting of *Synechocystis sp*. efficiency and CaONPs amount (Fig. 5), was raised with increasing flocculent concentration from 0.0025 to 0.0125 mg per 50 mL algae culture that was harvested by 89.4%. While depending on pH flocculent (Fig. 5), the best harvesting pH was between 9.8 and 12.6 yielding 98.6% of algae harvesting. In addition to that ranging temperature, the best harvesting percent was 99.3% at 45 °C. The XRD of flocculent algae (Fig. 3b) was indicated the presence of three peaks, while FT-IR of the precipitated algae (Fig. 4b). So, the most suitable condition for algae harvesting at pH 9.8 and temperature 45 °C with 250 mg/L produced 99.3% of algae coagulation.

Microalgae cell's membrane contains a negative charge owing to its high concentration of the important neutral sugars, 74.0%, uranic acid, 24.0%, proteins, 16.0%, and glucosamine 15.0% (Blumreisinger et al. 1983). Subsequently, the stability of microalgal cultures was dependent on the forces relating between the particles themselves as well as on the forces interacting between the particles and the water, microalgae could be considered as hydrophilic bio-colloids (Tenney et al. 1969). The flocculation percent in this study was very good compared to Elgiddawy et al. (2017), who used zinc aluminum-layered double hydroxide nanosheets to fluctuate *Chlorella vulgaris* by 96.0% efficiency. While Fathy et al. (2020) harvested microalgae by silver nanoparticles with an effectiveness of 97.2% for *Chlorella lobophora*. Other studies as Suparmaniam et al. (2019) used chicken eggshells to harvest *C. vulgaris* by the proficiency of 60.0%, while You et al. (2019) used cationic polyacrylamide to coagulate *C. vulgaris*

by 94.0% productivity rate.

Conclusion

The current work proposes a very viable approach for harvesting microalgae from FSF waste from CaONPs. According to the results of the zeta potential test, calcium ions in CaO nanoparticles flocculent were the key component for flocculation and operated as charge neutralization due to their positive charge. Furthermore, the recent findings demonstrated that calcium oxide nanoparticles could be efficiently used as a flocculant for harvesting *Synechocystis* sp. PCC 6803 at a pH change of 9.8 to 12.6 and temperature of 45°C, which increased harvesting *Synechocystis* sp. PCC 6803 by 99.3%. Furthermore, the precipitation techniques of calcium oxide nanoparticles for flocculation of microalgae would manage the traditional high-cost harvesting methods that lead to exposing the full potential of microalgae as promised and enthralling feedstocks in multipurpose exploitation such as pharmaceutical industries, commercial applications, and biofuels production.

Conflicts of interest The authors declare that they have no conflict of interest

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