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Marine Microalgae Biomass Harvesting Based on pH Alternation in *Nanochloropsis oculata*

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ABSTRACT: Marine microalgae *N. oculata* have been commercially used as live feed for aquaculture and nutritional supplements. An innovative process for flocculation of marine microalgae using NaOH was developed as a cost-effective alternative for preparing microalgae concentrate as well as non-toxic for the aquaculture industry and is suited for on-site production by hatcheries. *N. oculata* is an interesting microorganism in the field of marine biotechnology because of its high lipid content. However, harvesting of marine microalgae is a major obstacle for industrial scale and one of the promising harvesting techniques is flocculation. Using NaOH in the pH range 8.5 to 11 was studied with a turbidity of OD₆₆₀. The results of the algal flocculation by pH alteration were very effective at 10.5 to 11 pH on 8th day of the culture reached the flocculation efficiency up to 95.29 % with recovery of total lipid up to 27.27 %. In summary, this work is devoted to demonstrate that the optimization of the separation of microalgae from culture broth is mostly dependent on the pH. This method demonstrated their utility to harvest microalgae cells by economically, effectively and in an ecofriendly way.

KEYWORDS: Flocculation; pH, Efficiency, NaOH, Lipid.

I. INTRODUCTION

Currently, microalgae have been essentially commercialized and cultured for their copious applications including live feed for a wide variety of animals in aquaculture. Microalgae are also used in cosmetics, health and food products [1]. Microalgae are mainly used as a precious source of energy due to quick growth process, high oil production, low carbon dioxide (CO₂) emissions and high protein content as compared to the traditional crops [2,3]. Harvesting of microalgae is a major obstacle for industrial scale processing. Recently, research has focused on the harvesting and biomass recovery of microalgae [4,5,6,7], due to the characteristics of microalgae such as small cells size (2–20 μm in diameter), similar cells density to water, and large volumes of water need to be removed during microalgae biomass recovery. Hence, harvesting technique requires one or more solid-liquid separation steps, including the concentration and drying processes [8]. There are various concentration technologies applied which is mostly relied on complex and sophisticated approaches, for instance coagulation, flocculation, centrifugation, gravity sedimentation, filtration, Alfa Laval decanter technology and high pressure membrane molecular sieving [9,10,11]. However, these technologies have contributed to very high maintenance and operational cost. The total of 20- 30% biomass production cost applied due to the energy requirement for the machinery especially in large scale [12,13,14]. Additionally, harvesting of microalgae is difficult and energy intensive processes [15]. Therefore, an integrated approach is required in order to minimize the energy consumption in the harvesting process. Efficient system for harvesting is extremely essential for commercial production of microalgae.

Flocculation is one of the most economical and energy efficient methods for harvesting microalgae [16]. Harvesting microalgae by flocculation has been successfully applied in aquaculture [17], biofuel production [18], wastewater treatment [19] and silt treatment [20]. Flocculation methods result in higher particle sizes that enable gravity

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sedimentation, centrifugal recovery as well as filtration [21]. Flocculation is an effective process, that allows rapid treatment with great quantities of microalgae cultures [22]. Flocculation is the coalescence of separate suspended microalga cells into larger attached conglomerates. Initially, the cells are aggregated into greater particles, via the interaction of flocculants with the surface charge on the cells. Then, the aggregates coalesce into large flocs that settle out of suspension [17]. A large number of chemical products have been tested as, flocculants, including various inorganic multivalent metal salts [23] and organic polymer/polyelectrolytes [24]. In addition, recently some microbes have been applied to flocculating certain microalgae [25,26,27].

The flocculating reactions of an algal biomass are particularly effective to the pH. The removal of algae from the mass culture by flocculation has been studied. In this present study the flocculation of the *N. oculata* by altering the pH with NaOH was studied. However, use of this flocculated microalga has several applications including the utilization of cultivated cells for the production of biodiesel and other nutraceuticals products. Thus similar to other species of algal cultivation this method of cost effective harvest of microalgae by flocculation process will be effective and practical for removing the suspended cells from the culture broth. Microalgae cells carry negative charge that inhibits aggregation of cells in a culture broth. Preferably, the flocculants used should be inexpensive, nontoxic, and effective in low concentration. In addition, the flocculants should be selected so that further downstream processing is not adversely affected by its use. The objective of this work was to understand and optimize the flocculation of *N. oculata* using NaOH as the flocculants. To study the enhancement correlation between increased pH in the medium as well as the production of lipids in the *N. oculata*.

II. MATERIAL AND METHODS

Cultivation of marine microalgae

Nannochloropsis oculata was used as a model microalga for its ability to be concentrated by flocculation. The predominant species of marine microalgae, *N. oculata* was cultivated in 15 L glass tank filled with 29 ppt seawater containing f/2 medium for 9 days [28]. The cultured was stored at room temperature (20–24°C) with continuous illumination in laboratory. The aeration was supplied by bubbling air at constant pressure.

Sampling procedure

A microalgae culture 50 ml aliquots of culture of *N. oculata* was taken into 250 ml beaker and the pH was adjusted by the addition of 1M NaOH solution and the pH was measured with a bench top pH meter (Thermo Orion) with 0.01 accuracy. In This study the *N. oculata* culture has been adjusted the pH 8.50, 9.50, 10.50 & 11.00. The pH adjusted culture of *Nannochloropsis* was then transferred immediately into a 100 ml class-A measuring cylinder. The aliquots of samples were retrieved from the measuring cylinder and measured for turbidity by spectrophotometer at OD₆₆₀ (UV spectrophotometer, GENWAY 7315, UK).

Flocculation Efficiency

Flocculation efficiency as calculated by the following equation, flocculation efficiency (%) = $(1 - A/B) \times 100$, A: OD of sample, B: OD₆₆₀ of reference (without flocculants) [29]. The flocculation of batch settling is shown in the form of table:1 & fig:1, at different concentrations and at different pH conditions.

Determination of dry weight

Algae biomass concentrations reported in this study are expressed by algal dry weight. Dry weight was determined by filtering a fixed volume of the algae suspension through a pre-weighed filter with a 0.45µm porous membrane. Then the filter and algal cells were dried at 105°C for 48 h. Finally, the algal dry weight was calculated by subtracting the mass of the filter from the total mass.

Total lipid estimation

The flocculated microalgae cells were macerated in a tissue homogenizer with chloroform-methanol (2:1, v/v) and transferred to a separating funnel. Lipids were extracted, and separated into chloroform and aqueous methanol layers by

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the addition of methanol to give a final solvent ratio of chloroform: methanol of 2: 1 is a modification of the method by Bligh and Dyer. The chloroform layer was washed with 20 ml of 0.6% NaCl solution, and evaporated to dryness, total lipids were measured gravimetrically [30].

Data Analysis

For comparing and maintaining the uniformity and homogeneity, all the data were transformed into the same units and the results were expressed as standard deviation (SD). The significance of difference in the lipid recovery for microalgae subjected to varying pH and flocculation efficiency.

III.RESULTS AND DISCUSSION

Large scale cultivation of microalgae requires high energy inputs and is not economically feasible as harvesting accounts for more than 30% of the total cost. Flocculation is generally induced by addition of chemicals that interact with the negatively charged microalgae cell surface. These chemicals can induce flocculation through neutralizing the negative surface charge of the cells, connecting individual cells or forming a precipitate that binds the cells. In this study, effects of pH concentration on the efficiency of flocculation were evaluated. The influence of pH on flocculation efficiency was tested in a pH range from 8.5 to 11, Fig.1– pH treatment. A batch settling experiment using *N. oculata* with the same batch culture was investigated on day 2 to day 8. The settling was completed within an hour at pH 10.5 and 11, the settling velocity was slower in 8.5 and 9.5 pH. The flocculation of microalgae was not effective in pH 8.5 and 9.5. However, by the increasing the pH rapidly started settling.

Table 1: Flocculation efficiency (%) at various pH

pH	Flocculation Efficiency (%)				
	Day 2	Day 3	Day 5	Day 8	Average
8.5	54.66	62.5	75.42	84	69.15%
9.5	68.55	77.13	80.47	86	78.04%
10.5	70.07	77.44	80.47	87.2	78.80%
11.0	93.93	95.12	95.29	96.8	95.29%

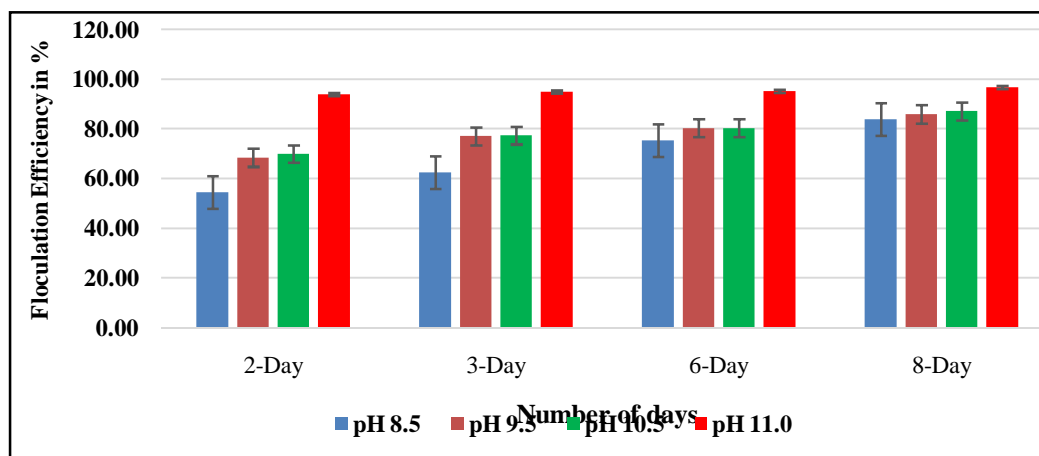


Fig 1: Comparison on performance of flocculation efficiency was tested in a pH range from 8.5 to 11, Flocculation efficiency of (%) = (1-AB) x 100, Data are mean values of replicates, error bars indicates standard deviation.

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The above table and figure shows no flocculation occurred in pH 8.5. At pH 8.5 the flocculation efficiency of 69.15 % was observed. At pH 11, the flocculation efficiency exceeded 95.29 %. This is similar with the study of [31], which agreed the settling time depends on the alkaline solutions. This indicates that *N. oculata* can be flocculated efficiently by increasing the pH using NaOH accelerated the mass settlement of algae which made the harvest easier. This observation is in agreement with previous studies [32]. Several studies have suggested that bivalent cations such as calcium and magnesium play a role in the flocculation process [33]. The use of flocculation induced by high pH for harvesting microalgae may have as advantage that the high pH effectively sterilizes the micro algal biomass as well as the process water. This may be advantageous when microalgae are used in wastewater treatment, as the high pH may kill pathogenic microorganisms [34]. These findings suggest that operation time will be an important process parameter to take into account in the development of a pH induced harvesting process for microalgae. Thus the NaOH can be used effectively to harvest *N. oculata* from large scale. with alkaline flocculation using NaOH was reported as most efficient for harvesting of *Chlamydomonas* and *Chlorella* [35]. The pH increase of culture medium could induce the flocculation efficiency of fresh water microalgae [36]. An optimum pH of 9.2 resulted in 95% of biomass harvest for *Botryococcus* [37].

In this study intended to identify the best conditions for lipid accumulation in *N. oculata*. This alga has been identified as a good candidate for the production of biodiesel and other fine chemicals on the basis of high lipid content and the fact that *Nannochloropsis* is tolerant to a range of environmental extremes including high salinity and pH.

Table 2. Percentage of total lipid content recovery at different pH, the control which was consider 100% reference value.

Samples	Total lipid (gm/ 50ml)	% of Lipid recovery
Control	0.0033	100.00 %
pH 8.5	0.0004	12.12%
pH 9.5	0.0004	12.12%
pH 10.5	0.0009	27.27%
pH 11.0	0.0007	21.21%

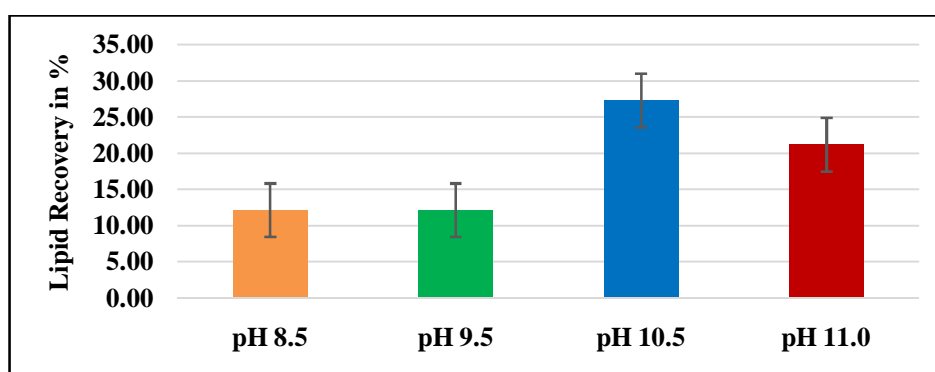


Fig 2: Lipid recovery in % of *N. oculata* cells, effect of NaOH with different concentration, data are mean values of replicates, error bars indicates standard deviation

The above table and fig shows the percentage of total lipid content recovery at different pH 8.5, 9.5, 10.5, 11 showed Table 2 & Fig 2. The high lipid was found at pH 10.5 and 11 produced most. The lipid content of *Nannochloropsis* was 27.27 %. which confirms the ability of *Nannochloropsis sp.* to accumulate large amounts of lipids when grown under alkaline conditions in efficient culture systems, these findings are in line with [38]. Higher lipid concentration signified

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that increase of pH increased the efficiency on marine microalgae *N. oculata*, which was compared with the control which was consider 100% reference value. The results from this study showed that flocculation induced by increase pH change is a useful method to harvest microalgae. At alkaline pH levels, the flocculation rates were higher in *Nanochloropsis*. This is similar with the study, Variations of the pH-shift process to recover protein from microalgae have been published on *Spirulina platensis* [39]. Lipid class analyses have demonstrated that the increase in lipid content is due to the increase in the production of neutral lipids [40]. Microalgae membrane and extracellular polymeric substances are mainly composed of polysaccharides, proteins, and lipids [41]. At low pH, the dissociation of carboxyl groups is inhibited, while the dissociation of amine groups is enhanced, so the negative surface charge of algae is weakened [42]. When pH was above 9, microalgae cells may release extracellular polymeric substances to protect themselves from the environment, which may cause the change of surface charges. However, our current findings indicated that the NaOH flocculation process under the optimized conditions gave the maximum efficiency as 95.29%. Once the pH 8.5 was decreased, it is noticed there was decrease in flocculation efficiency and as well as similar with lipid.

IV. CONCLUSION

An innovative process for flocculation of marine microalgae using NaOH was developed as a cost-effective alternative for preparing microalgae concentrate as well as non-toxic for the aquaculture industry and is suited for on-site production by hatcheries. Alkaline flocculation was highly effective for *N. oculata*, this could allow to adapt a semi-continuous inline harvesting system in a typical bioreactor with no need of a separation tank. Further it was found that cells when alkaline condition, that produced lipids at a higher rate which was clearly depicted by this study. This technique could be used for the large scale production and utilize lipids to generate biodiesel, live feed for aquaculture and nutritional supplements.

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