Arab Universities Journal of Agricultural Sciences
(2023) 31 (2) 217-222
Website: http://ajs.journals.ekb.eg

Harvesting Microalgae by Chitosan as Sustainable Biopolymer

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https://doi.org/10.21608/AJS.2023.183957.1510

Received 4 January 2023; Accepted 23 May 2023

Short Communication

Keywords:

Biopolymer,
Chitin,
Sustainable harvesting,
Microalgae harvesting

Abstract: This study aimed to use the organic polymer, chitosan, as a flocculant for harvesting microalgae *Chlorella.* sp. Various polymer concentrations were tested. Within three min, 50 mg/L of chitosan showed the highest efficiency (98.9±0.5%), at pH 6.3, while the lowest efficiency (86.7±0.5%) was observed at pH 7.5. Harvesting efficiency in this study was chitosan concentration dependent. The present study showed that chitosan can be a promising and sustainable solution as an organic source and environmentally friendly flocculant. Additionally, it has excellent harvest productivity and requires minimum effort for quick outcomes.

1 Introduction

Throughout Earth's history and ecological evolution, microalgae have played a significant role in various aspects. They have the power to affect our future through a variety of potential applications that solve global problems (Rizwan et al 2018). Algae have unique biological and biochemical properties (Yan et al 2022), which made them able to compete and overcome previous obstacles in biotechnological applications and water treatment. (Rizwan et al 2018, Koyande et al 2019), as well as their adaptable biochemical features. Currently, harvesting of microalgae is the most technical and financial challenge in the production of microalgae biomass (Grima et al 2003, Wu et al 2012, Singh and Patida 2018). Low cell concentrations in cultures (0.5–5 g/L), a small cell volume (30 ml), the stability of the inoculum, and variations in cultivation conditions are the main causes (Vandamme et al 2010, Lam and Lee 2012, Singh and Patida 2018). Various techniques, such as sedimentation, sonication, filtering, air float, coagulation and gelation, may be employed to collect microalgae. The most notable choice among these is flocculation because it is clear and concise and cost-effective (Wu et al 2011, Pragya et al 2013). Microalgae and other microbial cells can form flocs with chemicals flocculants like aluminum and ferric salts; however, these chemicals have environmental adverse effects (Vandamme et al 2010). Chitosan, on the other hand, has advantageous features as a natural coagulant for harvesting microalgae since it is inexpensive, highly flocculating and only needs a small concentration to harvest (Lubián 1989, Şirin et al 2012). Additionally, it is a biodegradable compound and non-toxic to fish and animal feed. The efficiency of harvesting microalgae is affected by a variety of parameters, including flocculant type, concentration, settling time, and culture pH (Xu et al 2011). This research investigated the feasibility of collecting microalgae employing chitin as a bio-flocculant. In addition, the effects of acidity and coagulant amount on the efficiency of harvesting.

2 Materials and Methods

2.1 Species and growing factors

Chlorella sp. was obtained from the National Research Center, Algae Research Department. For culture preparations, in a flask of 5 L, *Chlorella* sp. was inoculated with 1L of sub-culture algal (0.660_{OD680}_cells/mL) in a 3L blue green eleven medium with a starting pH of 7.1. The flask was then incubated at 30-35°C for 20 days under 6500 lux/m^2 of continuous cool-white fluorescent light illumination with manual shaking for 5 minutes daily. It is possible to depend on the carbon dioxide and oxygen gases present in the surrounding air since the bottles were closed with a cotton plug (Al-Rikabey and Al-Mayah 2018).

2.2 Forming a solution of chitin

The chitin used in this experiment is from the source of the crab shells, The chitosan was purchased from the Roth Company. Ten mL of 0.1% HCl solution and 100 mg of chitosan dry weight were combined and continuously stirred for 30 min. Deionized water was used to dilute the solution to 100 mL, resulting in a final chitosan concentration of 1000 mg/L effective, they can produce toxic residues that affect algae cells In the case of re-use of cells for feeding, those residues also affect water quality (Vandamme et al 2012). A promising substitute for these synthetic materials is ecological polymers like chitosan (Yunos et al 2017, Heng et al 2009).

2.3 Harvesting

In triplicates, harvesting microalgae was conducted in bottles using 100 mL glass flasks containing 50 mL of microalgae cells. Chitosan concentrations were determined by the experimental design (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 mg/L). At just three minutes, the sample solution and chitin mixture were mixed at 50 rpm. The microalgae cells were stirred and then given time to settle. To test the initial concentration, a one-milliliter sample was collected from the testing flask's center (Salim et al 2012).

At a wavelength of 680 nm, the sample's light density was determined (Vandamme et al 2010) with a Spectrophotometer PG instrument, model T60 to determine the absorption coefficient, demineralized water was utilized as a standard (Rashid et al 2013, Yunos et al 2017). The following formula was used to get the chitin-to-algal biomass ratio:

$$Harvisting\ efficieny\ \% = \frac{ODt0 - ODt}{ODt0} \times 100$$

where the absorption spectrum at time zero is indicated by ODt0, and the amount of light absorbed by the sample at time t is denoted by ODt.

The following formula was used to get the chitin-to-algal biomass ratio:

$$chitosan\ Dose\ \% = \frac{chitosan\ dose\ mg/l}{microalgae\ biomass\ mg/l}$$

In this study, the mean of the replicates was obtained by Excel.

3 Results and Discussion

3.1 Effect of chitosan concentration on harvesting efficiency

Investigations were done to examine the effect of chitosan concentration on the efficacy of *Chlorella* sp. harvesting. Chitosan was evaluated at various concentrations (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 mg/L). Relevant chitosan concentrations to microalgae biomass ratios were (0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, and 5%). Results revealed how the absorption spectrum has declined in terms of the efficiency of harvesting; after only 3 minutes of settling time, a significant reduction in optical density was observed. As the chitosan concentration was increased, the optical density decreased. Chitin doses of 50 mg/L and 5 mg/L led to the greatest and lowest levels of UV absorption reduction, respectively. A yielding quality of 98.9 ±0.5% was attained at 50mg/L. Efficiency reduced as the flocculant concentration was decreased; the lowest efficiency (86.7 ± 0.5%) was discovered at 5mg/L. Within 3 minutes, the harvesting was practically finished.

3.2 Effect of pH on harvesting efficiency

The ability of microalgae to be harvested is similarly impacted by the media pH. This study indicated that at a concentration of 50 mg chitosan, the highest microalgae harvesting efficiency of (98.9 ±0.5%) was obtained. Results also showed that pH 6.3 was the optimum value and the least efficient harvest was (86.7±0.5%) at pH 7.5.

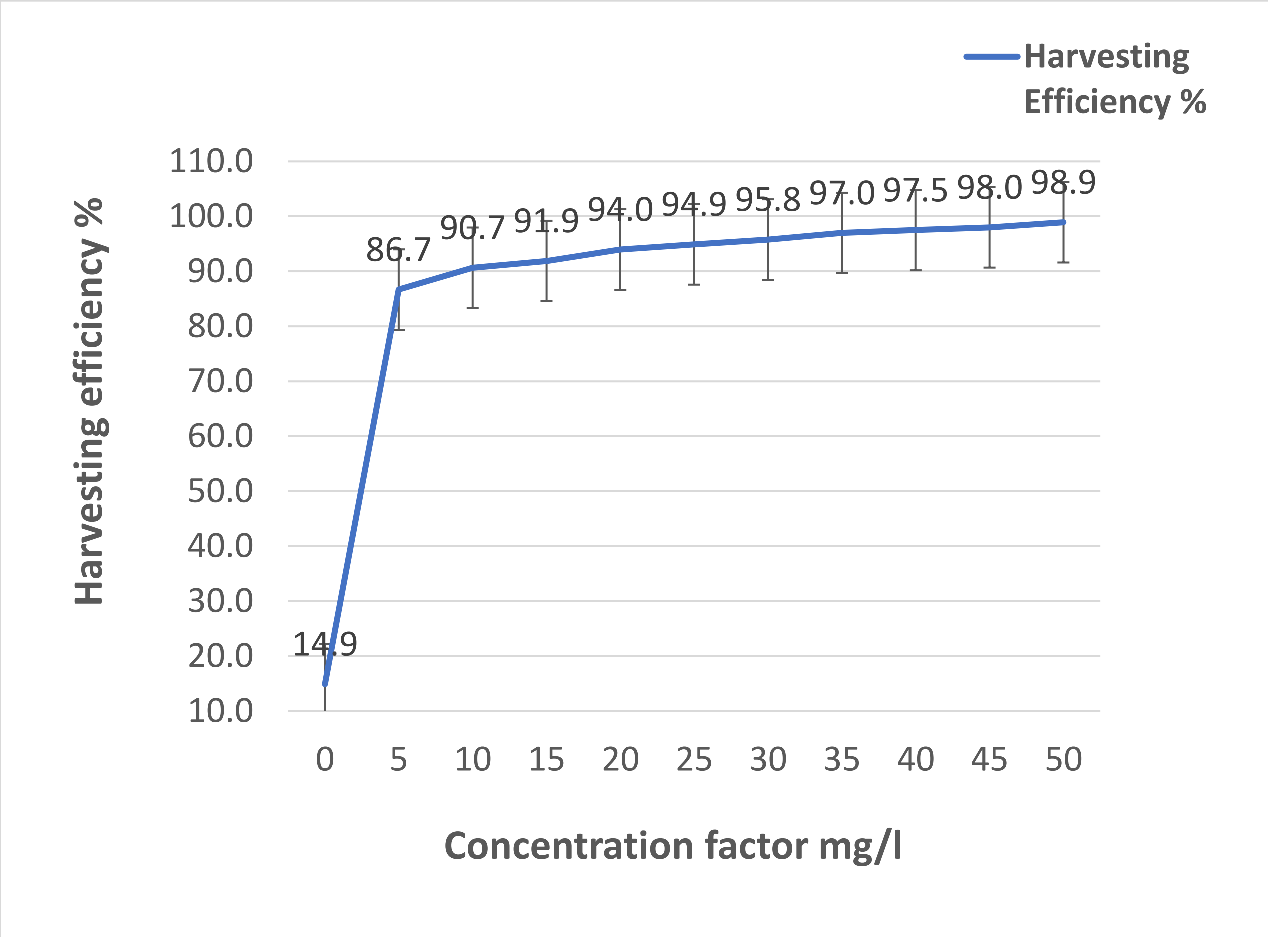

Fig 1. The effect of chitosan concentration on harvesting efficiency showed that the relationship between harvesting efficiency and chitosan concentration was a direct relationship.

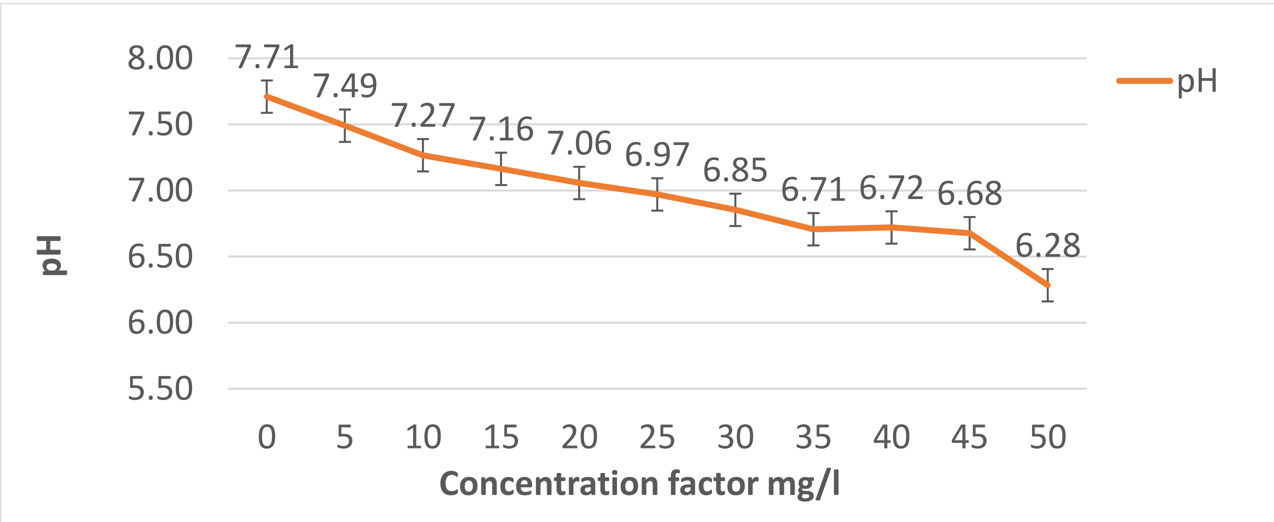

Fig 2. The effect of chitosan concentration on pH showed that the relationship between pH and chitosan concentration was inverse.

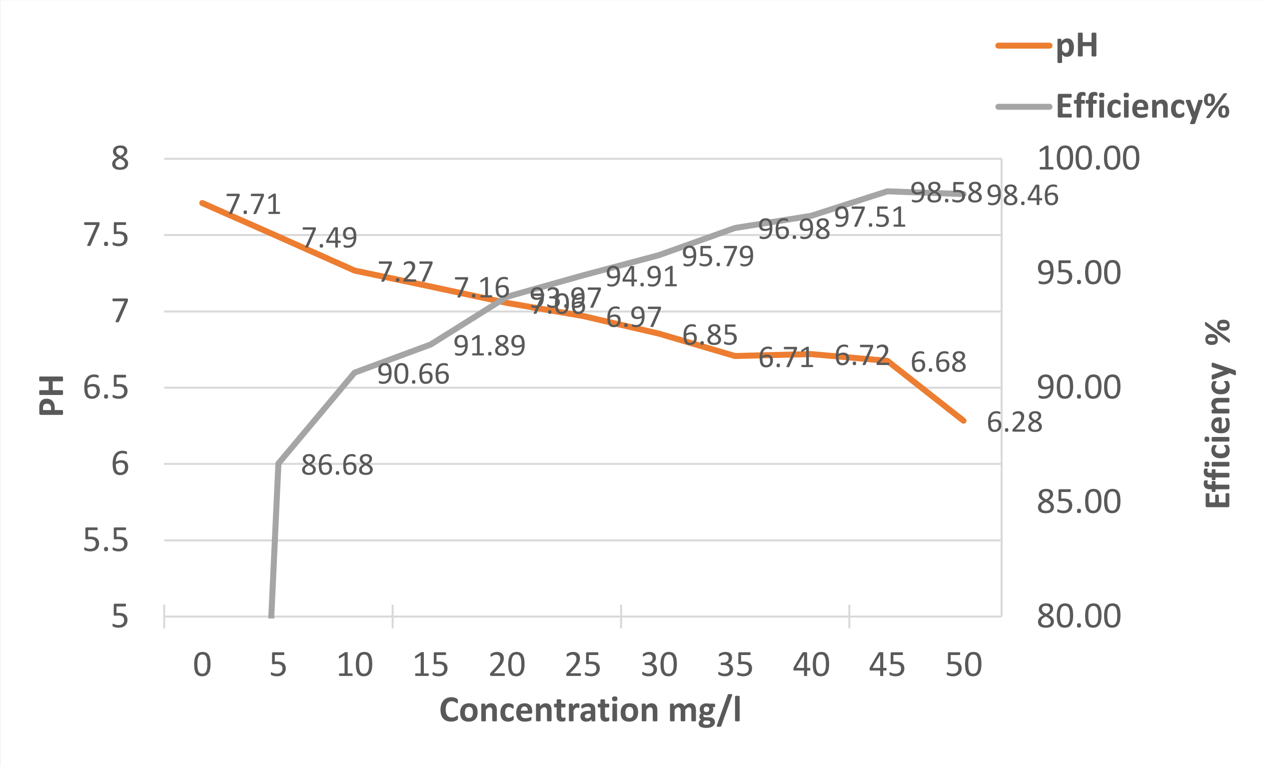

Fig 3. The interaction between the effect of pH and concentration on harvesting efficiency showed an increase in harvesting efficiency with decreasing acidity with increasing chitosan dose.

Harvesting efficiency in this study was affected by the interaction between chitosan concentration and pH change. Increasing the concentration of chitosan, the pH becomes more acidic whereas the highest harvesting efficiency was observed at pH 6.3, resulting from the use of 50 mg/L of chitosan. While pH 7.5 resulted from the use of 5 mg/L of chitosan, the harvesting efficiency decreased to the lowest rate.

The effect of pH results from the interaction between the charge of the algae's surface cells and the charge of the chitosan group, which leads to the precipitation of algae by chitosan (Kurita 1998, Pillai et al 2009, Salim et al 2012). High acidity increases the percentage of positive charges, while low acidity increases the percentage of negative charges, which affects the flocculation of algae cells by chitosan, which has a direct effect on the algae harvested (Morales et al 1985, Renault et al 2009, Xu et al 2011). These results are consistent with the observed less flocculation of algae cells by chitosan at a pH higher than 7, because of the increase in negative charges leading to increasing repulsion between algae cells and chitosan and thus decrease in the effectiveness of flocculation and harvesting of algae (Sionkowska et al 2004, Kim et al 2011).

4 Conclusions

Chitosan is one of the most promising sustainable, environmental, and economical solutions for harvesting microalgae, and has magnificent potential for microalgae culture biomass recovery. Chitosan has various advantages over commonly used flocculants, including a low concentration required and a quick settling time.

Acknowledgment

This work was financially supported by The Academy of Scientific Research & Technology (ASRT), the Egypt government's Ministry of Higher Education and Scientific Research, scientists of next-generation scholarship, cycle six (2019).

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