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EFFECT OF ANIONIC FLOCCULANT AND PH CHANGE ON THE FLOCCULATION EFFICIENCY OF PARACHLORELLA KESSLERI

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ABSTRACT
Microalgae have shown a great potential in the biotechnology field, however, high production costs have limited industrial applications. Biomass harvest is one of the major difficulties in microalgae cultivation due to high energy inputs which are needed to separate the cells from culture broth. Flocculation is considered to be a reliable method to improve cost-effectiveness in the down streaming processing. The effects of flocculation efficiency on harvesting Parachlorella Kessleri cultivated in 24 l photobioreactor were investigated by changing culture medium pH value and adding polyacrylamide flocculant (Magnafloc® 10). The flocculation experiments were conducted in 40 ml test tubes using culture broth obtained from the photobioreactor. The flocculation efficiency was evaluated by comparing the remaining cell density in the supernatant with the cell concentration before flocculation. The flocculation efficiency of 90 and 79% was obtained without flocculants and conducted under dark conditions for 24 hrs at 22ºC and 4ºC, respectively. For only 4 hrs settling time, 80% of flocculation efficiency was achieved by adjusting the culture pH to 10 using sodium hydroxide (NaOH) and adding Magnafloc® 10. By adjusting only pH value of the culture medium to 4 using Hydrogen Chloride (HCl), the measured flocculation efficiency was 97%. The addition of Magnafloc® 10 improved cell viability and slightly increase the flocculation efficiency. The anionic flocculant is only efficient when the pH of the cell culture was adjusted to a pre-defined value that induces cells immobilization and surface charge neutralization preceding flocculation process.

KEYWORDS: Flocculation, microalgae, Parachlorella Kessleri, pH, anionic flocculant.

INTRODUCTION
Microalgae are emerging to be one of the most promising, sustainable sources of biomass and oils for fuel, food and other co-products.[1] However, microalgal technology has yet to circumvent limitation related to the cost of production and processing to achieve high biomass productivity.[2]

One of the issues in large scale productions of microalgae is the development of effective technique to allow efficient separations of microalgae cells from culture medium as well as to keep their viability prior to be used subsequently.[3]

Several methods are available for dewatering and recovering microalgal biomass,[4], such as centrifugation, flocculation, gravity settling, microfiltration and dissolved air floatation.[5,6] The technology for microalgal biomass harvesting is still in its infancy and trials on suitable combinations of these methods are currently underway.[7] The use of the centrifugation technique on a large scale is not cost effective due the huge amount of power consumption.[8]

Flocculation is one of the most convenient methods for harvesting microalgae[9] due to its low costs compared to other methods such as centrifugation and filtration.[10]

Inorganic flocculants such as alum and iron chloride are efficient but are required in high doses and result in contamination of the biomass with aluminium or iron.[11] However, organic biodegradable flocculants such as polysaccharide[12] and polyacrylamide[13] do not contaminate the algal biomass and are often required in lower doses. They are usually high in molecular weight and water soluble organic compound, which can be anionic, cationic, or nonionic.[14]

Flocculation process has been applied in the harvesting of many microalgae type. Vandamme et al., have reported the efficiency of cationic starch to flocculate

fresh water microalgae such as Parachlorella and Scenedesmus using lower dose of flocculants.[15]

Recently, Liu et al., have developed a new flocculation method based on decreasing pH values (5- 1.5) of growth medium. The measured flocculation efficiencies were as high as 90% for some microalgae.[16] On the other hand, Knuckey et al. and Harith et al., have reported that adding flocculants prior to increase the pH of culture medium (8 - 11) promote precipitation and increased the harvesting efficiency substantially.[17, 3] The two methods induce no toxicity to the cells and eliminate the use of inorganic flocculants. However, both methods were only tested to small range of pH.

The objective of this study was to investigate the influence of adjusting pH combined with Magnafloc® 10 as a flocculant on the flocculation efficiencies of Parachlorella Kessleri. The effects of culture pH and flocculation temperature on the flocculation efficiency were also investigated.

The reason behind the choice of Magnafloc® 10 as a flocculant in this study is due to the work that has done on behalf of the Canadian Mine Environment Neutral Drainage (MEND). Regarding MEND report, Magnafloc® 10 is a non-toxic anionic polyacrylamide flocculant and is the most efficient common flocculant used for waste water treatment.[18]

MATERIALS AND METHODS

Microalgae and cultivation method

Microalgae, Parachlorella Kessleri, obtained from GEPEA UMR CNRS 6144, Université de Nantes, France was used throughout this study. The microalgae were cultivated in 24 l photobioreactor using modified BBM (Bold Basal Medium) medium.[19]

The photobioreactor produces high biomass concentration (>1 g/l/day).

The temperature within the photobioreactor was regulated at 22ºC by air-conditioning and aeration was provided by air bubbling. The pH of the medium was maintained around 8 by the initial quantity of bicarbonate added to the culture.

Cultures were grown under 16 hrs photoperiods by white fluorescent illumination (800-1000 lux). Cells were harvested at late growth phase (after 15 days of cultivation) for subsequent use in the flocculation experiments.

Flocculation and pH adjustment experiments

All flocculation experiments of Parachlorella Kessleri cells were carried out using 40 m1 test tubes (internal diameter = 16 mm and height = 180 mm). Initially, the microalgae cultures having a pH of 8 were allowed to settle in the dark at 2 different temperatures (4 and 22°C) and without flocculants.

Samples were taken at 2, 6, 18, 24 and 40 hours at 2 cm of above the base of the tube for evaluation of flocculation efficiency.

Subsequently, the effect of culture pH on the flocculation efficiency was carried out by adjusting the culture pH ranging from pH 4 to pH 10 using either Sodium Hydroxide (NaOH) or Hydrogen Chloride (HCl). The glass tube was vortexed thoroughly for 30 s as soon as the pH had been adjusted and allowed to stand for a specific time. At the end of flocculation time, surface water was collected for analysis.

The effect of an anionic flocculant was studied by adding 1 mg/l of Magnafloc® 10 (BASF, Canada Colors and Chemicals LTD, Ontario, Canada) to the culture where the pH was previously adjusted to the required value according to method as described above.

Stock solution of this flocculant was prepared by dissolving 2.4 g of Magnafloc® 10 into 1l of continuous swirling hot tap water. A wooden or plastic spoon or stirring stick was used to swirl the solution. Before transferring to a storage vessel, stock solution was allowed to stand covered overnight. Flocculants were added to the culture, followed with vigorous mixing for 2 min. Then, the stirring was stopped to allow the flocculation under gravity. At the end of flocculation time (4 hrs), surface water was collected for analysis.

Cells viability

For staining procedure, 1 ml of flocculated cells was treated with 50 µl of 1% (w/v) stock solution of Methylene blue. The samples were allowed to stand at room temperature for at least 30 min and cells were observed microscopically under light microscope. The dead cells were stained blue due to the penetration of the stain through the cell wall whereas the viable cells would retain their natural color due to intact cell wall. Cell numbers were counted using haemacytometer.

Flocculation efficiency

The flocculation efficiency was calculated by comparing the remaining cell density in the supernatant with the concentration before flocculation.[3] The flocculation efficiency (%) was calculated using the following equation:

$$\text{Flocculation efficiency (\%)} = \left[\frac{C_i - C_f}{C_i}\right] \times 100$$

Where C_i is the concentration of cell in suspension before treatment and C_f is the final concentration of cells in suspension.

Statistical analysis

All experiments were performed in triplicates and results are presented as mean ± SD. Differences were analyzed by the Student's t test and P values < 0.05 were considered significant.

RESULTS

Flocculation without flocculant

The flocculation of Parachlorella Kessleri culture without flocculant at two different temperatures is shown in Fig. 1. For both cases, flocculation efficiency was increased gradually with time and reached a maximum of about 93% after 40 hrs of incubation time. However, increased in flocculation efficiency with time was higher for flocculation carried out at 22ºC as compared to 4ºC. On the other hand, very stable flocculate at steady-state was observed for flocculation at 22ºC though a slight increase in flocculation efficiency from 90% at 24 hrs to 93% at 40 hrs were observed.

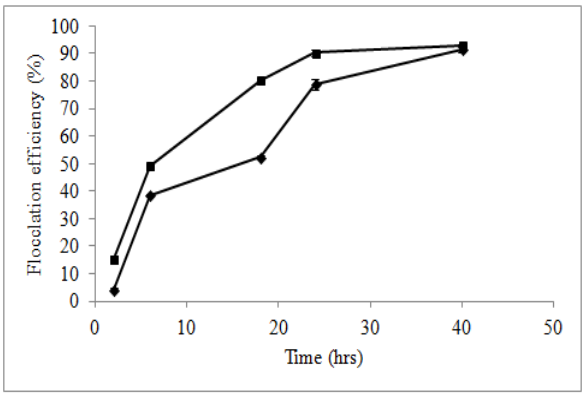

Fig. 1: Flocculation efficiency of Parachlorella Kessleri without flocculants at two different temperatures. Initial pH was 8 and not adjusted prior to the flocculation experiment. Symbols represent (■) 4ºC; (♦) 22ºC.
Error bars indicate standard deviation of three replicates.

Flocculation and cell viability with pH adjustment

The original microalgae media had a pH of approximately 8. The effect of pH on the flocculation of microalgae was determined by performing the flocculation process at pH 4, 6, 7, 8 and 10, respectively.

Figure 2 shows the flocculation efficiency of Parachlorella Kessleri cultures with pH adjustments prior to flocculation process. A flocculation efficiency of 97.12 ± 0.99% was obtained at pH 4 and decreased significantly to 60.51 ± 1.74% at pH 6 (Student's t test, P = 0.01). The change of pH culture from 6 to 10 has no significant effect on the flocculation efficiency. Overall, for these pH values the student t test used has shown no significant differences of flocculation efficiency (Student's t test, P > 0.3).

The results of cell viability of Parachlorella Kessleri after 4 hrs of flocculation without flocculant and at different pH are illustrated in Fig.2.

The highest viability (97.55 ± 0.82%) was obtained at pH 8 and gradually reduced to 84.45 ± 6.29%) at pH 4. At pH 10 the viability of Parachlorella Kessleri was reduced as well to (90.23 ± 6.39%).

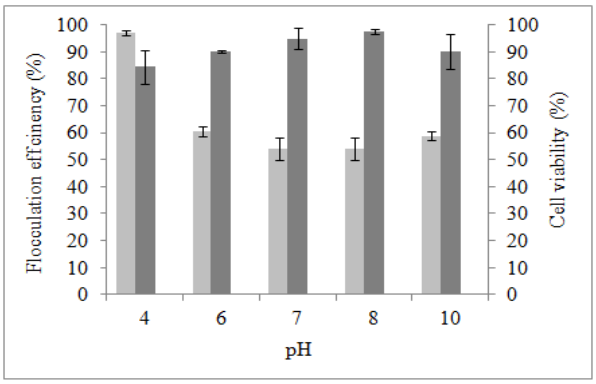

Fig. 2: Flocculation efficiency (light color) and cell viability (dark color) of Parachlorella Kessleri at different pH adjustment.
Error bars indicate standard deviation of three replicates.

Flocculation and cell viability with flocculant and pH adjustment

Fig. 3 shows the effect of pH on flocculation and viability of Parachlorella Kessleri while maintaining a constant concentration of the anionic flocculant.

The maximums of flocculation efficiency were obtained at pH 4 with (82.85 ± 2.41%) and at pH 10 (79.53 ± 3.44%). When adjusting the pH of cultures between pH 6 and 8, the flocculation efficiencies were almost the same and no significant differences were observed (Student's t test, P > 0.4). Interestingly, at pH 10, the flocculation efficiency increase from 58.81 ± 1.47% to 79.53 ± 3.44% after adding Magnafloc® 10.

On the other hand the cell viability was improved by the addition of Magnafloc® 10 as compared to culture without flocculant.

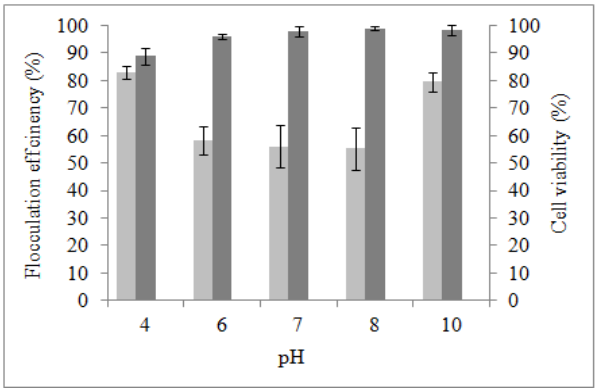

Fig. 3: Flocculation efficiency (light color) and cell viability (dark color) of Parachlorella Kessleri at different pH adjustment followed by addition of 1 mg/l of Magnafloc® 10. Error bars indicate standard deviation of three replicates.

DISCUSSION

Effect of temperature

In this study, it was found that temperature played a noticeable role in flocculation process.

The higher flocculation was occurred at temperature 22°C. This apparently can be explained by the theory of collision. With increasing temperature, there is a greater probability that the microalgae cells will collide due to the increasing mobility of the cellular particles. Increasing the number and frequency of collisions increases the number of possible interactions that can occur, which in turn improves the flocculation rates.[20-22]

Interestingly more than 90% of the microalgae cells were flocculated after 24 hrs of incubation time at 22°C and without any flocculants.

Effect of pH

Our results showed that microalgal flocculation increases when pH value reduced to acidic conditions (pH = 4). These findings corroborate previous reported study that hydrogen ion concentration influences both the surface charge density of the colloid and the action of the flocculant.[17] At low pH, the freshwater microalgae are closer to its isoelectric point and at this point the microalgal cells are more likely to flocculate.

Similar results were also reported by Liu et al.[16] The authors found that the optimum flocculation efficiency of Chlorococcum nivale, Chlorococcum ellipsoideum and Scenedesmus sp. was achieved at pH 4. They claimed that the mechanism could be that the carboxylate ions adhering on microalgal surface accepted protons when pH decreases and the negative charges were neutralized, resulting in hampering of the cells dispersing stability and subsequent flocculation of cells.

The pH adjustment between pH 6 and 10 did not enhance the flocculation of Parachlorella Kessleri. Similar behaviors were observed on other microalgae species such as Scenedesmus sp and Thalassiosira pseudonana.[23, 24]

Effect of pH adjustment and anionic flocculant

The addition of anionic flocculant seems to decrease the flocculation efficiency at pH 4. As reported by Uduman and coworkers, decreasing the pH brings an increase in the amount of Hydrogen ion in solution that binds to the negatively charged polymer, thus giving it a lower efficacy. The pH variation could also affect the optimum pH range required for successful polymer activity.[20]

At pH of 10, the addition of Magnafloc® 10 was effective and the flocculation efficiency reach 80%. It seems that at basic pH the electrostatic repulsions between the anionic flocculant and microalgae cells may reduce. Vandamme and coworkers reported similar results using Greenfloc 120 as flocculant. The authors found that the flocculation of Parachlorella was independent of pH in the pH range of 6 to 10 with an increase of flocculation efficiency at pH 10.[15]

However, it is worth mentioning that the flocculants were only effective when the pH of the microalgae culture was pre-adjusted to a certain value that promotes cells entrapment and surface charge neutralization prior to flocculation process.

Cells viability during pH decrease process

Viability of microalgal cells was determined by the Methylene blue assay and the cells seemed to be very resistant to relatively low pH values and few cells were found to be damaged. Cells viability was not significantly changed with pH but the highest viability (98%) was obtained at pH 8. Similar microalgae specie was isolated by Shimura and coworkers and they reported its resistance to acidic and alkaline conditions at a pH range of 3-11.[25]

After adding the anionic flocculant to culture medium, cell viability was slightly improved and maintains a similar viability over the adjusted pH range.

The dosage of Magnafloc® 10 used as well as pH changes seems to have no toxicity on Parachlorella Kessleri.

CONCLUSION

The flocculation efficiency of microalgae for separation of cells from the culture medium was greatly influenced by the pH. Adjusting the pH to 10 and adding Magnafloc® 10 improved the flocculation efficiency as well as the cell viability.

The results showed that chemical flocculation can maintain high cell viability and could be a method of choice due to rapid, inexpensive and simple method for harvesting large quantity of microalgae cells such as Parachlorella Kessleri from the culture broth prior to commercial formulation. Further research regarding the impact of pH induced flocculation on cell viability at large scale and recycling water in microalgae cultivation is needed.

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