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Cells Harvesting of Tropic Ocean Oleaginous Microalga Strain *Desmodesmus* Sp. WC08

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Abstract

For biomass recovery of tropic ocean oleaginous microalgae strain *Desmodesmus* sp. WC08, eight flocculation methods (pH adjustment, $Al_2(SO4)_3$, polyacrylamide, $AlCl_3$, $Ca(OH)_2$, $FeCl_3$, alum and chitosan) were evaluated and optimized. The results indicated that ferric chloride, aluminum sulfate, aluminum chloride and chitosan exhibited high flocculation ability and their flocculation efficiency were all beyond 94% at the optimal dosage (0.15, 0.4 and 0.03 gL^{-1}, respectively). Chitosan displayed the most tremendous potential for biomass recovery from culture broth based on its feasibility and safety. Acetic acid and hydrous chloride, used to dissolving chitosan, had no significant difference in the flocculation efficiency. And when the pH of culture broth was set 5 or 6, the flocculation efficiency of chitosan was higher and the required flocculation time was less. More dose chitosan was needed to harvest the biomass following the cell growth stage. Overall, the optimal flocculation reagent for harvesting biomass of *Desmodesmus* sp. WC08 is chitosan, and its optimal flocculation conditions are: just when the pH of the culture broth was set to 6 and the dosage of chitosan was 0.03 g/L at the end of microalgae cultivation, more than 110g of algal biomass can be recovered just by per 1 g of chitosan. Meanwhile, there is little residual chitosan found in the final flocculation supernatant which can be reused to some extent.

Keywords: Microalgae *Desmodesmus sp*; Flocculation; Cells harvesting; Chitosan

Introduction

In recent decades, microalgae have become more and more attractive because of a wide range of applications. For example, algal biomass can be used as food source both for human and animal, fertilizer, biofuel, fine chemicals, pharmaceuticals and water treatment [1]. Especially for the purpose of biofuel, microalgae have been attracting much attention from the scientific community to government [2]. However, microalgal cells harvesting is a bottleneck technology all the time for all kinds of miroalgal applications based on algal biomass, due to their small size (5-20 μm), low culture concentration (0.5-5 gL^{-1}) in culture medium, and high cost (about 20-30% of total production cost) [3,4].

To date, there are several available techniques for microalgae harvesting, such as centrifugation, filtration, air floatation, gravity sedimentation, flocculation and so on. Centrifugation and filtration are two kinds of common solid-liquid separation technology, which are most usually used to collect microalgal slud. But centrifugation is an energy-intensive process and is unfit to be used in large-scale [5]. While filtration is ease to operate, the membranes can be rapidly fouled by extracellular organic matter and minority of microalgae which are vimineous or have big size may be more suitable [6]. Gravity sedimentation is convenient and cheap, but that is just for those microalgae which have good self-sedimentation nature. Air floatation is also confirmed to be efficacious for microalgae harvesting. However, in contrast, flocculation is most popular among these methods. Because it is effective, convenient and low-cost for harvesting microalgae from large quantities of culture broth [7]. Besides, it can largely improve harvesting efficiency of other separation technology by pretreatment.

Flocculation is a process in which freely-suspended cells are aggregated together to form large particles and cells can then be easily harvested by sedimentation [8]. Flocculation is usually induced by various factors, mainly including chemicals which are called flocculants, for example, aluminum and ferric salts, as well as cationic polymers. Different flocculants have different performance and effects on different microalgae strains. Many chemical flocculants are inexpensive and effective for variety of microalgae strains, but most of them are toxic and have some adverse effects on downstream process. Chitosan, a natural biopolymer, is a linear poly-amino-saccharide and has marked advantages over commonly used chemical flocculants [9]. It has high flocculation ability, and is non-toxic and biodegradable. However, the flocculation ability usually changes with the relative conditions to some extent, such as flocculant dosage, pH of culture medium, settling time and so on.

In our previous study, tropic ocean oleaginous microalgae strain *Desmodesmus* sp. WC08 was identified as one possible candidate for biodiesel production based on its biomass productivity, lipid productivity and fatty acid profile [10]. However, fewer studies were performed to investigate cells harvesting of tropic ocean microalgal species of the *Desmodesmus* genus. So in this work we have explored the harvesting potential of tropic ocean microalgae strain *Desmodesmus* sp. WC08 with different flocculants. In addition, the effects of dosage, sedimentation time, acid solution, pH of culture medium and growth stage on flocculation efficiency are also discussed. Finally, the effects of recycled water from harvesting process on the growth and lipid accumulation of microalgae *Desmodesmus* sp. WC08 are further investigated.

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Materials and Methods

Microalgae strain and growth conditions

Microalgae strain *Desmodesmus* sp. WC08 was obtained from Hainan University at Haikou, China. Microalgae were grown photo-autographically in BG-11medium added 20 gL^{-1} sea salt. The cells were grown in 250 cm^3 culture medium in a sterilized air-bubbled (from the bottom) transparent glass-tube (600 mm×30 mm i.d.). Mass cultivation was carried out in 1 L culture medium in a sterilized air-bubbled (from the bottom) transparent glass-tube (600 mm×60 mm i.d.). All cultures were incubated at room temperature and the light intensity was approximately 160 μmol m^{-2} s^{-1} with continuous illumination.

Effects of different flocculants with different dosages

Seven flocculants $Ca(OH)_2$, $Al_2(SO4)_3$, $FeCl_3$, $AlCl_3{\cdot}6H_2O$, $AlK(SO_4)_2{\cdot}12H_2O$, polyacrylamide, chitosan) and pH regulation, were used for collecting cells of microalgae *Desmodesmus* sp. WC08 from culture medium. The same dosages (0.10, 0.20, 0.30, 0.40, 0.50 and 0.60 gL^{-1}) were used for the flocculants $Ca(OH)_2$, $Al_2(SO4)_3$, $AlCl_3{\cdot}6H_2O$ and $AlK(SO_4)_2{\cdot}12H_2O$. The dosages of $FeCl_3$ was 0.03, 0.06, 0.09, 0.12, 0.15 and 0.18 gL^{-1}, respectively. The dosage of polyacrylamide was 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 g L^{-1}, respectively. And the dosage of chitosan was 0.01, 0.02, 0.03, 0.04, 0.06 and 0.08 gL^{-1}, respectively. The pH was set to 5, 6, 7, 8, 9, 10, 11 and 12, respectively. After adding the required amounts of flocculants on the 45 cm^3 microalgal culture medium in a 50 cm^3 test jar, the 0.5-1 cm^3 supernatant samples were taken and measured according to 2.5.1. The detection time of flocculation efficiency were set at 0, 15, 30, 60, 90 and 120 min, respectively, for all flocculation experiments. And every experiment was carried out triplicate.

Effects of flocculation conditions with using chitosan as the flocculant

Effects of pH of culture broth: When the pH of culture media was set 5, 6 and 7, respectively, with hydrochloric acid at different dosages (0.01, 0.02, 0.03, 0.04, 0.06 and 0.08 gL^{-1}, respectively), flocculation efficiency (E_f) of chitosan in different pH was measured and evaluated at 30 and 120 min, respectively.

Effects of acid solvents dissolving chitosan: Two kinds of acid solvents, acetic acid and hydrochloric acid, were used to dissolve chitosan for evaluating their each effects on pronation of chitosan. In this experiment, the dosages of chitosan were set to 0.02, 0.03 and 0.04 gL^{-1}, and the pH of culture broth was set to 6 for measuring E_f at 30 and 120 min, respectively.

Effects of culture time of cells: The effects of culture time on flocculation efficiency of chitosan were further investigated at 4th, 8th, 12th, 16th and 20th d from the culture beginning. In this test, the dosage of chitosan was 0.01, 0.02, 0.03, 0.04, 0.06 and 0.08 gL^{-1}, respectively. And chitosan was dissolved by hydrochloric acid and the final pH of culture broth was set to 6. All E_fs was measured at 30 min once the chitosan was added into the culture broth.

Supernatant reuse assay

Growth, biomass and lipid content were further investigated in the supernatant of culture broth flocculated by chitosan. Therefore, three culture medium (fresh brine BG11, culture broth flocculated by chitosan (FS), and FS + nutrient salt of BG11 medium) were compared under the same culture conditions of microalgae strain *Desmodesmus* sp. WC08. Cultivation was carried out in 250 cm^3 culture medium in a sterilized air-bubbled (from the bottom) transparent glass-tube (600 mm×30 mm i.d.). All cultures were incubated at room temperature and the light intensity was approximately 160 μmol $m^{-2}s^{-1}$ with continuous illumination. Cell growth was measured by the absorbance at 680 nm with a UV spectrophotometer. And the microalgae biomass were all collected by centrifugation (6000 g, 10 min) and dried by a freeze-dryer. Every experiment was carried out triplicate.

To quantify lipid contents, lipids were extracted using the solvent system of chloroform-methanol (2:1, v/v) following a slightly modified method of Floch [11], which was briefly described as follows. After freeze-drying algal cells, algal dry powder of 0.1 g was mixed with 2 cm^3 of chloroform-methanol (2:1, v/v) solvent and stirred for 30 min at room temperature. Supernatant was collected by centrifugation at 6000 g for 5 min, and the residual algal slud went through the same solvent extraction procedure twice to ensure that most lipid was extracted. All crude oil extract was combined together and 0.1% NaCl was added to give a final solvent ratio of chloroform: methanol: water of 8:4:3 (v/v/v) and mixed for 3 min, then centrifuged at 6000 g for another 10 min. The lower organic phase was collected and evaporated at room temperature on previously weighted glass capsules until constant weight. Then the weight of the crude lipid obtained from each sample was measured gravimetrically. Experiments were performed in triplicate.

Analytical methods

Determination of flocculation efficiency: 45 cm^3 of algal culture broth was placed in a 50 cm^3 test jar. Flocculant was added at the designated concentration and mixed rapidly for 30 s followed by slowly mixing for 3 min. After mixing, the algal cells were allowed to settle down. An aliquot of supernatant was taken from the test jar height of one-thirds to measure the optical density (OD_{680}) at different designed time. Fresh brine BG11 medium was used as a blank reference. Each assay was carried out in triplicate. E_f was calculated as follows:

$$E_f(\%) = (1 - OD_t / OD_o) \times 100$$

Where OD_o is the optical density of samples taken at original time and OD_t is the optical density at time t.

Determination of chitosan concentration: The quantitative determination of chitosan in the final supernatant of culture broth flocculated by chitosan was carried out in routine analysis method reported by Wischke and Muzzarelli [12,13].

Result and Discussion

Effects of different flocculants on flocculation efficiency

Flocculant dosage has been recognized as a crucial parameter in flocculation processes because it can influence both the rate and the extent of flocculation sedimentation [14]. And different flocculants have different flocculation ability on the cells harvesting for different microalgae species. Tropic ocean oleaginous microalgae strain *Desmodesmus* sp. WC08 is identified as a possible candidate for biodiesel production in previous study in our lab. Cells harvesting has been studied a lot for several microalgae species in large-scale cultivation. However, rare study was reported for this genus ocean microalgae strains. Cationic flocculants and pH regulation were investigated for the biomass harvesting of tropic ocean microalgae strain *Desmodesmus* sp. WC08.

The results were showed in Figure 1 for the flocculation efficiency of different flocculants. As Figure 1 showed, it displayed a worse flocculation ability found for using pH regulation, polyacrylamide, calcium hydroxide and alum to precipitate cells of microalgae

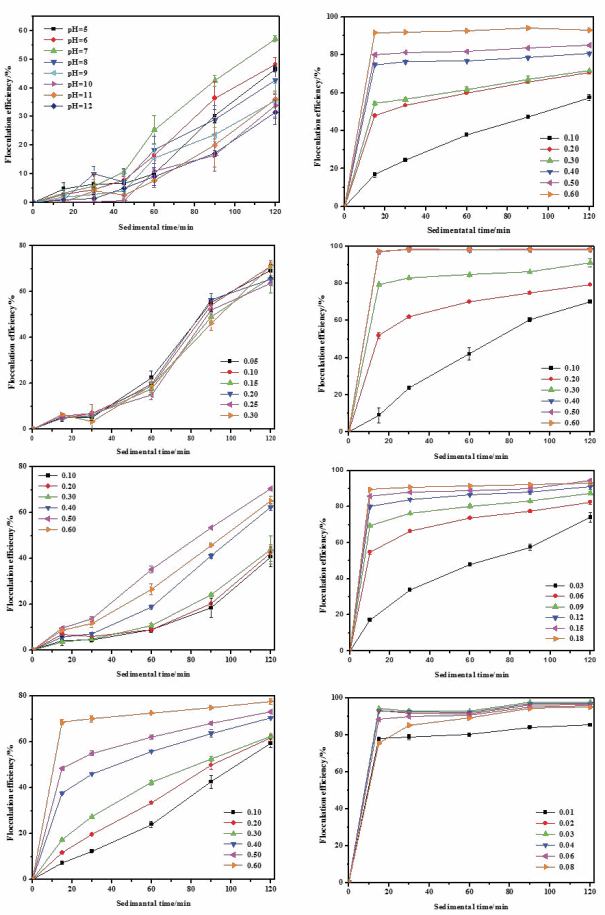

Figure 1: Effect of the different dosages (g/L) of eight flocculants, (A) pH, (B) $Al_2(SO4)_3$, (C) polyacrylamide, (D) $AlCl_3{\cdot}6H_2O$, (E) $Ca(OH)_2$, (F) $FeCl_3$, (G) $AlK(SO_4)_2{\cdot}12H_2O$ and (H) chitosan, at different sedimentation time on the flocculation efficiency of tropic ocean oleaginous microalgae *Desmodesmus* sp. WC08 at the end of cultivation.

Desmodesmus sp. WC08. For pH regulation (Figure 1A and 1B), the highest E_f [(57.20 ± 1.15)%] was got by pH 7 at 120 min. And when the pH was between 5 and 7, the flocculation efficiency at 120 min rose with the increase of pH and the situation was opposite when the pH was 8-12. So pH regulation was less effective for cells recovery of microalgae *Desmodesmus* sp. WC08. For polyacrylamide (Figure 1C), the flocculation efficiency increased with the sedimentation time extension. And there was no significant change (p>0.05) of flocculation efficiency among different concentrations of polyacrylamide. When the dosage reached 0.3 gL^{-1}, the highest E_f was just [(71.50 ± 0.90)%] at 120 min. So that is not a good choice for the cells harvesting of microalgae *Desmodesmus* sp. WC08. For calcium hydroxide (Figure 1D and 1E), when the dosage reached 0.5 gL^{-1}, the flocculation efficiency [(70.51 ± 0.70)%] was highest at 120 min, which had significant different differences (p<0.05) with 0.4 and 0.6 gL^{-1}. Alum, (Figure 1G), the flocculation efficiency rose with the increase of the dosage. The highest flocculation efficiency (77.75 ± 1.17)% was got by 0.6 gL^{-1} at 120 min, which had significant differences (p<0.05) with other concentrations. The flocculation efficiency of these four tested flocculation methods were all less than 80%, so they were unfit for cells harvesting of ocean microalgae *Desmodesmus* sp. WC08 because of their bad flocculation ability.

In contrast, the other four flocculants has exhibited the quite high E_f. Changes of these four flocculants were quite sharp within 15 min and then it either turned slowly or reached the balance. For aluminum sulfate (Figure 1B here), the highest flocculation efficiency was [94.08 ± 0.33%] obtained by 0.6 gL^{-1} of aluminum sulfate at 90 min. And it has significant difference (p<0.05) with other concentrations at different sedimentation time. For aluminum chloride (Figure 1D), after 15 min, the changes of E_f went up slowly for 0.1 -0.3 gL^{-1} of aluminum chloride, and it reached the balance and was overlapped for 0.4-0.6 g L^{-1} of aluminum chloride. So 0.4 gL^{-1} was a saturated concentration for aluminum chloride to harvest biomass of microalgae *Desmodesmus* sp. WC08. The highest flocculation efficiency [(98.13 ± 0.03)%] was obtained with such concentration at 120 min, which had no significant difference (p>0.05) with the other sedimentation time except for 15 min. Namely, when the dosage reached 0.4 gL^{-1}, over 98% of biomass can be recovered after 15 min. For ferric chloride (Figure 1F here), the optimal flocculation efficiency [(94.38 ± 0.24)%] was gained by 0.15 gL^{-1} of ferric chloride at 120 min. And there was no significant difference (p>0.05) among 0.15, 0.12 and 0.18 gL^{-1}. Meanwhile, the E_f of 120 min was significantly different (p<0.05) with others sedimentation time using 0.15 gL^{-1} of ferric chloride. For chitosan (Fig.1-H here), there were few differences among 0.02-0.08 gL^{-1}. After 120 min, the highest flocculation efficiency [(97.49 ± 0.22)%] was got when the concentration was 0.03 gL^{-1} and it was not significantly different (p>0.05) with 0.02 gL^{-1} of chitosan. Dosage which was beyond 0.03 gL^{-1} led to the lower flocculation efficiency. This phenomenon was also observed by other researchers and the reason might be that excess amino group of chitosan resulted in supersaturated positive charge in culture broth [15].

As Figure 1 and the analysis above showed, $AlCl_3$, $FeCl_3$, $Al_2(SO_4)_3$ and chitosan exhibited a high flocculation efficiency over 94% at a relatively short time, while the other four flocculation methods, especially for pH regulation, showed lower flocculation efficiency. The flocculation efficiency of $Al_2(SO_4)_3$, $FeCl_3$, $AlCl_3$ and chitosan sharply increased from beginning to 15 min of sedimentation with less dosage, especially for chitosan, which exhibited a similar flocculation ability with $AlCl_3$, while the dosage was just less than 1/10 of $AlCl_3$. It reported that flocculation by metal salts may be unacceptable if biomass was used in certain aquaculture (or other) applications [16]. Therefore, flocculants based on natural biopolymers were a safer alternative, example for chitosan, which is non-toxic, biodegradable, renewable and ecologically acceptable [17,18]. So it was more suitable for chitosan to flocculate the cells of tropic ocean microalgae *Desmodesmus* sp. WC08 based on its safety and environment friendly. However, if there are no strict demands or special needs for algal biomass in downstream processing, ferric chloride, aluminum sulfate and aluminum chloride would also be a good available choice.

Effects of flocculation conditions using chitosan

Effects of pH of culture broth: When the sedimentation time was at 30 min, the E_fs of different pH in culture broth using chitosan as the flocculant were showed in Figure 2A. The E_f was displayed as pH6 > pH5 > pH7, when the concentration was under 0.03 gL^{-1}. And both for pH 5 and pH 6, the highest flocculation efficiency was obtained when using 0.03 gL^{-1} of chitosan. Then the flocculation efficiency gradually decreased with the increase of chitosan concentration. For pH 7, the flocculation efficiency gradually increased with the increase of chitosan concentration. And when the dosage was beyond 0.04 gL^{-1}, the flocculation efficiency turned stable. Afterward, when the concentration was higher than 0.05 gL^{-1}, the flocculation efficiency was

exhibited as pH7 > pH6 > pH 5.

When the sedimentation time was at 120 min (Figure 2B), several changes were different with 30 min of sedimentation. When the concentration was less than 0.05 gL^{-1}, the E_f was exhibited as pH5 > pH6 > pH7. And then they tended to be no difference.

The flocculation mechanism of chitosan can be understood as neutralization and polymer bridging [19]. Because of faster reaction performance, neutralization may play a major role when the pH was lower (pH=5 or 6) in the culture broth. Hence, E_f of chitosan quickly reached the top. When the pH was higher (pH=7), the protonation of chitosan was not enough and the effect of neutralization was weak. So the other flocculation mechanism -bridging,may play a main role in the culture broth with higher pH. In this flocculation mechanism, for more flocculation, more dosage flocculants are needed for the action of bridging. So it needed more chitosan for obtaining the highest flocculation efficiency in higher pH of culture broth.

It is more suitable to harvest the microalgal cells when the pH of culture broth and the sedimentation time are 6 and 30 min, respectively. Although when the pH was 5, the E_f was also satisfying. However, it meant more acid needed to be added into culture media for lower pH regulation. And there were some serious challenges about equipment corrosion and environment pollution with using more acid. And less sedimentation time meant higher harvesting efficiency with the precondition of nearly same E_f. So the optimal pH and flocculation time for harvesting microalgae *Desmodesmus* sp. WC08 using chitosan are 6 and 30 min, respectively.

Effects of dissolving solvent of chitosan: It was reported that the structural behavior and solubility of chitosan in different acid solutions may be different and this differences may be caused by the nature of acid solutions and degree of deacetylation and amino group of chitosan [15,20]. The dilute organic acid was the common solution to dissolve chitosan and acetic acid was more often. Hydrochloric acid was one of most common acid used in most industrial production, which was more advantageous in price than acetic acid. And it was discovered that chitosan displayed high solubility in hydrochloric acid [21]. So it is imperative to investigate the flocculation performance of chitosan dissolved in hydrochloride acid solution for harvesting microalgae *Desmodesmus* sp. WC08.

Figure 3 showed the difference for flocculating microalgae *Desmodesmus* sp. WC08 at 30 min of sedimentation time with acetic acid and hydrochloride acid as the dissolving solvent of chitosan. There was no significant difference (p>0.05) for acetic acid and hydrochloride acid in different concentrations. The same results were also obtained for 120 min of sedimentation time (the data was not given). Although the flocculation efficiency was slightly higher with acetic acid as chitosan solvent than hydrochloride acid, it had no significant difference (p>0.05) for harvesting cells of microalgae *Desmodesmus* sp. WC08. It is a desired result, because it meant that hydrochloride acid can take place of acetic acid to be used as the dissolving solvent of chitosan to collect cells when microalgae *Desmodesmus* sp. WC08 is cultivated in large-scale.

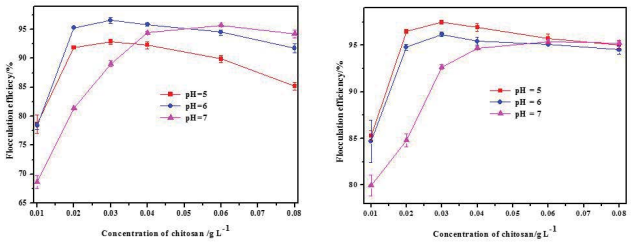

Figure 2: Effects of different pH on the flocculation efficiency of chitosan for biomass harvesting of tropic ocean oleaginous microalgae *Desmodesmus* sp. WC08 at the end of cultivation with different dosages at sedimentation time of 30 min (A) and 120 min (B), respectively.

Effects of cell growth time: Tropic ocean microalgae *Desmodesmus* sp. WC08 were cultured in artificial seawater BG11 medium for 4, 8, 12, 16 and 20 d in order to test the influence of the growth stage on flocculation efficiency using chitosan at pH 6. It is recognized medium composition, cell concentration and cell properties were changing with culture time [18]. So it is believed that cell growth stage may be an important factor impacting on flocculation of microalgal cells. So this test has been done to understand the flocculation performance of microalgae *Desmodesmus* sp. WC08 in different growth stage.

As shown in Figure 4, the dosage of chitosan increased with the culture time. The highest flocculation efficiency reached 91% by 0.01 gL^{-1} chitosan for the culture time of fourth and eighth day. And for the twelfth and sixteenth day, the highest flocculation efficiency was

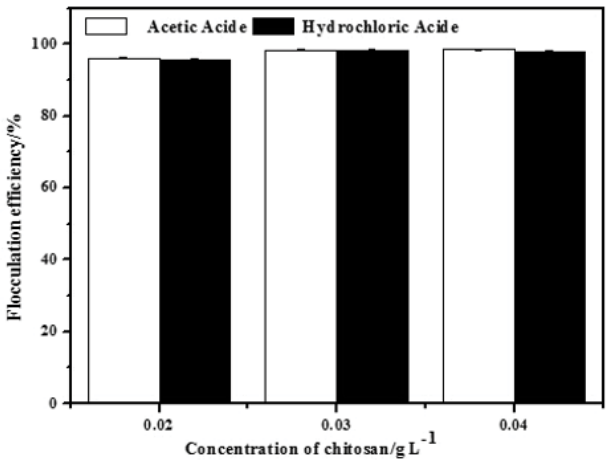

Figure 3: Effects of acid solution on the flocculation efficiency of chitosan for biomass harvesting of tropic ocean oleaginous microalgae *Desmodesmus* sp. WC08.

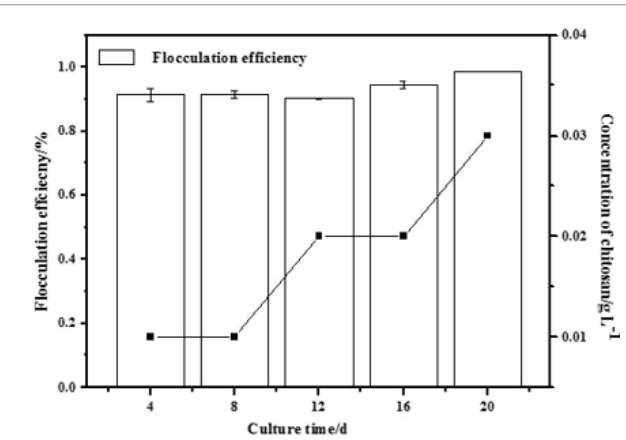

Figure 4: Effects of cells growth stage on the flocculation efficiency of chitosan for biomass harvesting of tropic ocean oleaginous microalgae *Desmodesmus* sp. WC08.

beyond 95% by 0.02 gL^{-1} chitosan. At the end of cultivation, the highest flocculation efficiency was beyond 98% by 0.03 gL^{-1} chitosan. Meanwhile, the biomass was 0.52, 0.74, 1.29, 2.42 and 3.18 gL^{-1} in 4th, 8th, 12th, 16th and 20th d of culture time, respectively. Based on biomass and flocculation efficiency, per 1g chitosan can recovery microalgal biomass of 47, 68, 58, 114 and 104 g in each culture time, respectively. So the optimal cell harvesting of tropic ocean microalgae *Desmodesmus* sp. WC08 is at end of cultivation (16-20d) and the optimal cells harvesting can be obtained beyond 110 g biomass just by per 1 g chitosan.

Supernatant reuse assay

Chitosan residual in the supernatant: After cells harvesting, residual chitosan in the supernatant of culture broth flocculated by chitosan was determined using dye-binding assay described Wischke and Muzzarelli [12,13]. And the relationship between chitosan concentration (mg/L) and (OD_{575}-OD_{750}) is obtained in this experiment as following equation:

$$C_{chitosan}\,(mg\,L^{-1}) = 81.64 \times (OD_{575} - OD_{750}) - 1.87\ (R^2=0.998)$$

According to this relation equation, the final residual chitosan was calculated just (4.12 ± 1.52) mgL^{-1} in the supernatant at the end of cell cultivation under the optimal flocculation conditions of chitosan (that is, the pH of culture broth was set to 6 and the dissolved solvent of chitosan is hydrochloride acid, and the original chitosan concentration was 0.03 gL^{-1}). It's showed that most chitosan play a role of sedimentation and settle down slowly by binding to microalgal cells. Namely, about 86.27% of chitosan added into the culture broth exists in the final recovered microalgal biomass and 13.73% of chitosan is free in the supernatant and nearly play little positive role in the process of flocculation.

Growth, biomass and lipid content in the recycled culture medium: If the supernatant could be recycled, it would be quite significant for saving water and reducing the cost of cultivation. It's reported that recycled water from its harvesting culture can be reused and has some positive effects on biomass production [22].

In Figure 5, it is showed that although the biomass and lipid yield of FS culture medium is lower than that in the fresh culture medium (BG11) and there is a significant difference (p<0.05) existing between them. And the recycled culture medium FS+BG11 can be reused well because of pretty good growth and lipid yield. The nutrition concentration is the main differences among the three culture mediums. Higher nutrition concentration in SF+BG11 leads to higher biomass and lipid yield than FS, but it is lower than fresh brine BG11 culture medium. Maybe it is because the BG11 salts in SF+BG11 is a little higher than fresh culture medium, so more effort must be taken to optimize the required additional nutrition salts in the supernatant. Besides, some metabolites in FS may be an important factor to play some role to lead to such a result, so more detailed research must be further studied for supernatant reusing.

Conclusions

Eight flocculation methods were investigated for harvesting tropic ocean oleaginous microalgae strain *Desmodesmus* sp. WC08. Ferric chloride, aluminum sulfate, aluminum chloride and chitosan exhibited quite high flocculation ability. Among these flocculants, chitosan held the most tremendous potential for efficient and safe biomass recovery from culture broth. However, if there are no strict demands or special needs for algal biomass in downstream processing, ferric chloride, aluminum sulfate and aluminum chloride will be also a good available choice for harvesting microalgae *Desmodesmus* sp. WC08.

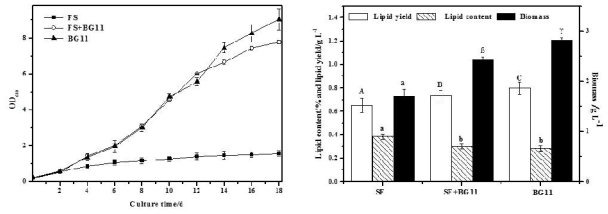

Figure 5: Growth curve (A), lipid content (B), biomass (B) and lipid yield (B) of tropic ocean oleaginous microalgae *Desmodesmus* sp. WC08 in SF, SF+BG11 and BG11 culture medium. Different letters on the bars indicate significant differences among different culture medium (p<0.05). And there is no significant differences (p>0.05).for the same letters on the bar.

Partial acid culture medium (pH5-6) was better for more efficient biomass harvesting of tropic ocean microalgae *Desmodesmus* sp. WC08 with less dosage of chitosan. Acetic acid and hydrous chloride have no distinct difference on the protonation of chitosan to harvest microalgae *Desmodesmus* sp. WC08. With increasing of microalgae growth time, more dosage of chitosan was gradually needed. And finally, more than 110g biomass of tropic ocean microalgae *Desmodesmus* sp. WC08 can be harvested just by per 1 g chitosan at the end of cultivation. In addition, there is little chitosan residues in the final supernatant of culture broth flocculated by chitosan and the final supernatant has quite great potential for recycling.

Acknowledgements

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