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Harvesting of *Spirulina platensis* by cellular flotation and growth stage determination

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ABSTRACT

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Aim: To investigate an effective harvesting method for *Spirulina platensis*.

Methods and Results: Eighty per cent of *S. platensis* cells in the logarithmic growth phase were harvested by flotation when the cells were set in a static condition for 2 h. The optimum harvesting time was about day 6 of cultivation. The flotation activity of *S. platensis* cells was enhanced by the addition of NaCl.

Conclusions: The harvesting of *S. platensis* by flotation is a cost-effective and straightforward method that can retain the algal quality. The optimum harvesting time of *S. platensis* can be predicted by the cellular protein to carbon ratio.

Significance and Impact of the Study: Flotation harvesting is also applicable to other cyanobacteria with gas vesicles.

Keywords: cyanobacteria, flotation, gas vesicle, harvesting, *Spirulina platensis*.

INTRODUCTION

Microalgae produce high-value compounds, such as pigments, proteins and vitamins, which can be used as feed additives, pharmaceuticals and nutraceuticals (Bubrick 1991; Gladue and Maxey 1994; Cartens *et al.* 1996), as such, a lot of research has already been conducted to find bioactive metabolites from cyanobacteria (Shimizu 2003). The recovery of the biomass is one of the critical steps involved in the commercial production of biomaterials from microalgae. Thus, the development of a harvesting method that is cost-effective, preserves quality and improves the culture process is important for commercial algal production. Gudin and Therpenier (1986) reported that 20–30% of the total cost is related to biomass recovery in algal production.

Various strategies have been suggested for harvesting large-scale algal cultures. For example, flocculation is triggered by adding flocculants to the algal culture to aggregate the microalgal cells and increase the particle size (Lee *et al.* 1998). However, the downstream processes include sedimentation, centrifugation and filtration, which involve the use of heavy equipment and result in algal damage as a result of the effect of rubbing that causes cell content leakage and a deterioration in quality. Furthermore, in the case of flocculation, the high concentration of metal salts, which is normally used as the flocculant, can have a negative effect on the quality of the final product if used for animal or human consumption. Thus, bioflocculants are being developed as an inexpensive and nontoxic alternative to chemical flocculants (Oh *et al.* 2001; Divakaran and Pillai 2002). Moreover, centrifugation is also a rapid method for harvesting microalgae.

Certain cyanobacteria with gas vesicles, such as *Microcystis*, *Anabaena* and *Spirulina*, are known to use their gas vesicles to move to a more favourable vertical position (Oliver and Ganf 2000), making them difficult to harvest using sedimentation and centrifugation. Although filtration is a simple and cheap alternative, it is only applicable to filamentous or colony-forming algae. Thus, a more effective

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harvesting method for cyanobacteria with gas vesicles is to take advantage of their natural flotation activity, which is essentially related to their physiology. For example, the flotation activity of *Spirulina platensis* is related to its gas vesicles (Rippka 1988). Accordingly, the present study investigates an effective harvesting method based on flotation activity and its enhancement by the addition of NaCl.

MATERIALS AND METHODS

Algal strain and culture conditions

Spirulina platensis NIES 46 was obtained from the National Institute for Environmental Studies (Japan) and grown in a SOT medium with the following composition: 16.8 g $NaHCO_3$, 0.5 g K_2HPO_4, 2.5 g $NaNO_3$, 1 g K_2SO_4, 1 g NaCl, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.04 g $CaCl_2{\cdot}2H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.08 g Na_2EDTA, 0.03 mg H_3BO_3, 0.025 mg $MnSO_4{\cdot}7H_2O$, 0.002 mg $ZnSO_4{\cdot}7H_2O$, 0.0079 mg $CuSO_4{\cdot}5H_2O$ and 0.0021 mg $Na_2MoO_4{\cdot}2H_2O$ in 1 l of distilled water. The *S. platensis* was cultivated in a 5-l photobioreactor containing 3 l of the medium, stirred at 50 rev min^{-1} at 25°C, and continuously illuminated at 200 μmol photons m^{-2} s^{-1} by cool-white fluorescent lamps. Air was supplied through the bottom of the reactor at a rate of 30 l h^{-1}. To determine the harvesting time with the maximum harvest yield and freshness, the productivity and chlorophyll *a* to dry cell weight ratio were measured during the cultivation of *S. platensis*.

Harvesting of *S. platensis* by flotation

To measure the flotation activity of the cells at different growth stages, the *S. platensis* culture was collected at different logarithmic growth stages. An aliquot (50 ml) of the algal culture was placed in a 50-ml cylinder with a 3-cm internal diameter. The test cylinder was stirred for 1 min at room temperature, then left to allow the *S. platensis* cells to float. The flotation activity was measured based on the change of absorbance of the algal culture. As the cells floated up to the water surface, the absorbance of the lower part decreased. The absorbance at 680 nm was measured at regular intervals using the algal cultures withdrawn at a height of one-third from the bottom. The flotation activity was then expressed in the form of the flocculation activity that showed the degree of algal removal from the middle of the water column (Kurane and Matsuyama 1994). That is, the flotation activity was calculated according to the following equation:

$$\text{Flotation activity}(\%) = (1 - \text{O.D.}_{\cdot t}/\text{O.D.}_{\cdot i}) \times 100$$

where $\text{O.D.}_{\cdot t}$ is the absorbance at time *t* and $\text{O.D.}_{\cdot i}$ is the initial absorbance.

Effect of cations on flotation

The effect of various metal cations on the flotation activity was tested. Algal cultures collected from the late logarithmic phase were mixed with 6.8 mmol l^{-1} of NaCl, $MgCl_2{\cdot}H_2O$, $CaCl_2{\cdot}H_2O$, $Al_2(SO_4)_3{\cdot}14–18H_2O$ and $FeCl_3$. The flotation activity of the cells was then measured after transferring the algal culture to a 50-ml cylinder.

Finally, some of the floating cells were placed in a 25-ml tight syringe, one end of which was blocked by a rubber stopper. Next, the syringe was pressurized several times against a solid wall to disrupt any gas vesicles, then the pressurized cells were compared with the floating cells using a light microscope and the flotation activity checked.

Analysis of biomass and cellular composition

Chlorophyll *a* was extracted using a chloroform–methanol mixture (2 : 1, v/v) and measured with a fluorometer (Turner 450; Barnstead/Thermolyne, Dubuque, IA, USA) (Wood 1985). Meanwhile, the dry cell weight was measured after the cells were filtered through a GF/C (Whatman, Kent, UK), washed with two volumes of distilled water, and dried for 4 h at 105°C. The cellular protein was measured using the method of Lowry *et al.* (1951) with bovine serum albumin as the standard, and the cellular carbon was measured using a total carbon analyser (TOC 5000-A, Shimadzu, Tokyo, Japan). To determine the total protein content, the cells in 1 ml of algal culture were disrupted by grinding for 150 s with a Mini-Beadbeater (Biospec Products, Bartlesville, OK, USA) using 1 g of glass beads (100 μm) at 4°C.

RESULTS

Growth of *S. platensis*

The *S. platensis* was cultivated in a 5-l photobioreactor. The dry cell weight and chlorophyll *a* both increased until about day 7, reaching a maximum of 1.9 g l^{-1} and 16.4 mg l^{-1} respectively (Fig. 1a). The ratio of chlorophyll *a* to the dry cell weight also increased until about day 5 and thereafter decreased. The productivity based on the dry cell weight was highest about day 6 (Fig. 1b).

Flotation activity of *S. platensis*

The culture of *S. platensis* was collected about days 4, 5 and 6, then the flotation activity was measured at various settling times. Overall, the flotation activity showed a tendency to increase with both the harvesting time and settling time (Fig. 2). The flotation activity increased until about day 6 and showed a maximum value of 80% after 2 h

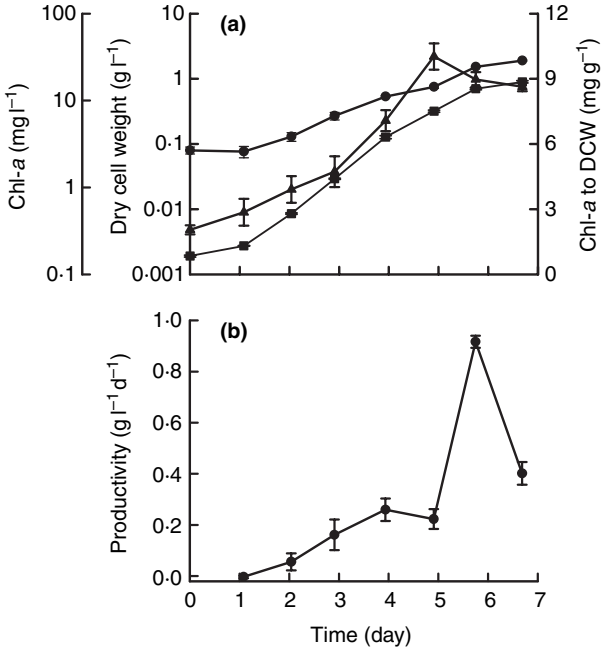

Fig. 1 Dry cell weight (●), chlorophyll *a* concentration (■), and ratio of chlorophyll *a* to dry cell weight (▲) of *Spirulina platensis* cultivated in SOT medium (a). Productivity on basis of dry cell weight (b)

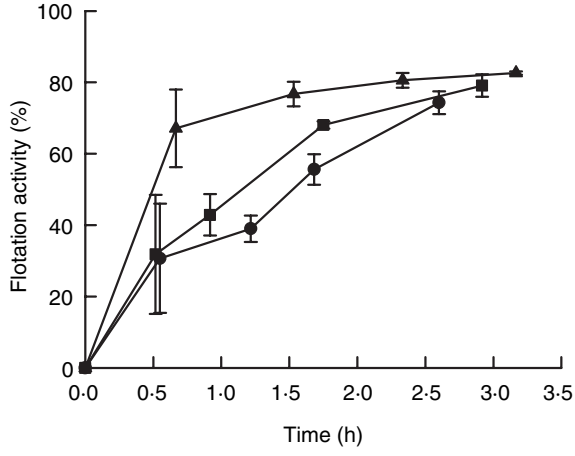

Fig. 2 Flotation activity of *Spirulina platensis* harvested at different growth stages. Algal cultures collected from exponential growth phase were left under static conditions in 50-ml cylinder. Harvesting time: day 4 (●), day 5 (■) and day 6 (▲)

of settling. The floating cells were easily harvested from the surface by skimming. After 10 h of settling, no observable cells were left in the middle or bottom of the cylinder. Conversely, the algal cells lost their flotation activity and exhibited a certain amount of precipitation and aggregation about day 7.

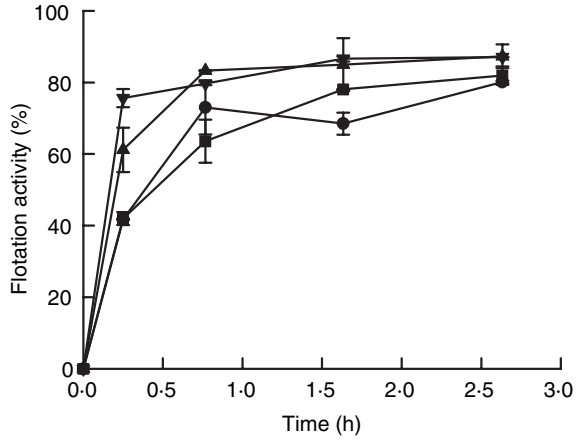

Fig. 3 Enhanced flotation activity of *Spirulina platensis* with addition of NaCl. Algal cultures collected from exponential growth phase (about day 6) were mixed with NaCl and then left under static conditions in 50-ml cylinder. NaCl concentrations: 0% (●), 1% (■), 2% (▲) and 5% (▼)

Increased flotation activity with cations

The effect of cationic salts on the flotation activity was tested. Algal cultures from the logarithmic growth phase (about day 6) were collected and Na^{+}, Ca^{2+}, Mg^{2+}, Fe^{3+} or Al^{3+} added to a final concentration of 6.8 mmol l^{-1}. The cells with Ca^{2+}, Al^{3+} and Fe^{3+} aggregated, yet there was no increase in flotation and some of the cells even precipitated. Thus, no flotation activity could be determined in the case of the cells with Ca^{2+}, Al^{3+} and Fe^{3+}. However, the flotation activities of the cells with Na^{+} and Mg^{2+} were enhanced.

In particular, the effect of the NaCl concentration on the flotation of *S. platensis* was further investigated with various settling times. On the whole, the flotation activity tended to increase with an increase of NaCl (Fig. 3). Plus, a two-way analysis of variance (ANOVA) confirmed the addition of 2% NaCl enhanced the flotation activity compared with 1% (equivalent to *c.* 170 mmol l^{-1}) NaCl ($P = 2.72 \times 10^{-6}$).

Monitoring flotation activity

The gas vesicles in cyanobacteria are mainly composed of a hydrophobic protein, then cyanobacteria control their buoyancy by regulating the carbohydrate content (Walsby 1994). As such, the cellular protein and carbon content were monitored at different growth stages to determine the optimum harvesting time. The cellular protein to carbon ratio increased until about day 5 along with cell growth (Fig. 4). The protein to carbon ratio was found to be a harvesting indicator that appeared prior to the maximum productivity about day 6, as shown in Fig. 1.

The structure of a gas vesicle is irreversibly degraded by high pressure. Thus, pressure was applied to the *S. platensis*

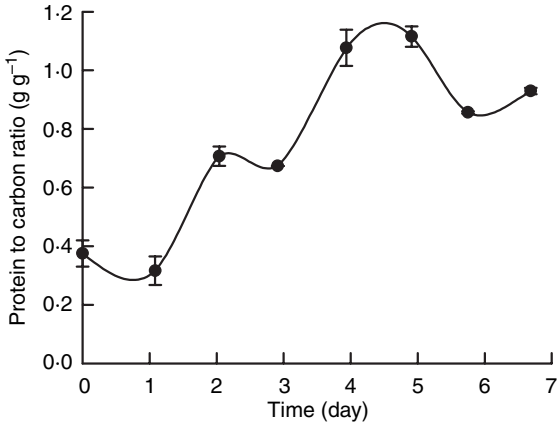

Fig. 4 Cellular protein to carbon ratio in *Spirulina platensis* at different growth stages

collected after flotation and the pressurized cells precipitated. No gas vesicles were seen in the pressurized cells when using a phase contrast light microscope.

DISCUSSION

The current research demonstrated the feasibility of harvesting *S. platensis* cells based on their natural flotation activity and growth stage determination. Mass cultured *Spirulina* is currently harvested by three stages of filtration, where the first screen filters out pond debris, the next screens harvest the microscopic algae, and the final filter thickens the *Spirulina* cells (Belay 1997). Meanwhile, Umesh (1984) previously described a column-gravity filter and rotary drum filter for harvesting *Spirulina*, and Divakaran and Pillai (2002) tested chitosan for the flocculation and filtration of *Spirulina* and other cyanobacteria. However, in all cases, it is unclear why the gas vesicles in the *Spirulina* cells do not provide buoyancy.

Therefore, the current study tested a flotation harvesting method for *Spirulina* and found that the flotation activity was positively related to the cultivation time at a certain point during the logarithmic growth stage (Fig. 2). The buoyancy of the gas vesicles in *S. platensis* cells has already been found to produce flotation activity (Oliver and Ganf 2000). However, pressurized *Spirulina* cells did not show any flotation or gas vesicles.

The change in flotation activity could be attributed to a change in the carbohydrate content while maintaining the same number of gas vesicles (Oliver and Ganf 2000), as Fig. 4 shows the tendency of a concomitant increase in the cellular protein to carbon ratio with the cultivation time. As such, it would appear that the synthesis of gas vesicle protein exceeded the carbohydrate accumulation during the late logarithmic growth stage. However, the cells in the stationary growth stage lost their flotation activity and could not be harvested by flotation. Moreover, the quality of the biomass in the stationary growth stage was lower in terms of its colour and smell. Thus, monitoring the cellular protein and carbon to determine the optimum harvesting time is necessary to increase the efficiency of flotation harvesting.

Generally, microalgal cells have negative charges on the surface that prevent the cells from aggregating. Thus, to reduce or neutralize the charge, the addition of a cation is preferred (Grima *et al.* 2003), and the addition of Na^{+} and Mg^{2+} was found to enhance the flotation activity of *Spirulina*. It has also been reported that the addition of Ca^{2+} increases the flocculation efficiency of *Chlorella vulgaris* (Oh *et al.* 2001). Yet, there has been no other report on the enhanced flotation or flocculation effect of NaCl. The flotation activity increased when 2% NaCl was added to the algal culture. One possible explanation for the current results is that the NaCl increased the density of the medium rather than neutralizing the negative charges.

As the cells float to the water surface without any mechanical force, harvesting by flotation does not cause any shear stress on the cells, which allows them to be used as an inoculum. Plus, the flotation harvesting method could also be used to harvest other cyanobacteria that have gas vesicles.

In conclusion, to harvest *Spirulina* based on its flotation activity, the productivity, chlorophyll *a* to dry cell weight ratio and cellular protein to carbon ratio can all be used as indicators to determine the optimum harvesting time. The maximum productivity of *S. platensis* was about day 6 of cultivation, which appeared to be the optimum time for harvesting. Meanwhile, the cellular protein to carbon ratio of *S. platensis* reached its maximum about day 5. Therefore, it would seem that the cellular protein to carbon ratio can be used as a parameter for predicting the optimum harvesting time. Furthermore, the addition of NaCl was found to enhance the flotation activity. Accordingly, it is suggested that flotation harvesting is a cost-effective and straightforward method for harvesting cyanobacteria without any deterioration of the harvested algal biomass.

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