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Short communication

Combined effects of nitrogen levels and *Daphnia* culture filtrate on colony size of *Scenedesmus obliquus*

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ABSTRACT

Increased colony size facilitates the harvest of *Scenedesmus* cells from culture media; as such, factors that stimulate colony enlargement for production should be identified. This study aimed to investigate the combined effects of nitrogen levels and *Daphnia* culture filtrate on the colony size of *Scenedesmus obliquus*. Results showed that the colony formation of *S. obliquus* induced by *Daphnia* culture filtrate was promoted under low nitrogen levels. Colony size increased by approximately 50%–100%, which suggested that low nitrogen and zooplankton infochemicals synergistically affected the colony formation of *S. obliquus*. Furthermore, increased colony size and high settling velocities enhanced the suitability of populations for centrifugation and filtration; thus, these populations could be helpful for harvest. According to the results, addition of *Daphnia* culture filtrate at 3 days before *S. obliquus* was harvested could be useful. Therefore, the proposed method could be applied to facilitate efficient harvest of *Scenedesmus* biomass.

1. Introduction

Microalgal cultivation has been considered as a feasible method to produce valuable chemical compounds and biodiesel [1]. Efficient biomass harvesting is also a key step for the mass production of valuable substances from microalgae [2,3]. Microalgae may be harvested commercially using centrifugation, filtration, and flocculation, either used individually or in combination [3]. Filtration is suitable for colony-forming microalgae, such as *Scenedesmus*, which has been developed and cultivated as a very important alga for biodiesel feedstock because this organism can grow in various types of wastewater and also exhibits high biomass, lipid, and carbohydrate contents [4]. However, separation and recovery of *Scenedesmus* from culture media have been considered as a critical step in algal biomass production because of small size and retaining stability in a dispersed state [5]. As such, efficient and low-cost downstream processes should involve methods in which colony size of *Scenedesmus* cells is increased to harvest these cells from culture media. Therefore, factors that stimulate this increase in aggregate size during production should be identified. From this view, it is essential to develop ecological technologies to change the morphological trait of *Scenedesmus*.

Potential grazing pressure from herbivorous zooplankton may be a feasible way to increase the colony size of *Scenedesmus*. *Scenedesmus*, usually dominated by unicells and paired cells, can be induced to form large colonies by *Daphnia* culture filtrates [6–8]. Furthermore, extracellular polysaccharides increase the stickiness of the cell surface and contribute to cell aggregation in algal species [9–11]. Extracellular polysaccharides can be stimulated under lower nitrogen conditions [12,13]. Considering these observations, we hypothesized that colony formation of *Scenedesmus* may be enhanced by synergistic effects between low nitrogen and zooplankton infochemicals. To test this hypothesis, we used *Scenedesmus obliquus* and examined its morphological response to the combination of nitrogen levels and *Daphnia* culture filtrate. If our results support our hypothesis, this colony-enlarging approach likely facilitates the harvest of *Scenedesmus* from culture media for downstream processing.

2. Materials and methods

2.1. Test organisms and Daphnia culture filtrate preparation

S. obliquus strain FACHB-416 was axenically maintained in liquid BG-11 medium at 25 °C in a 12 h:12 h light:dark cycle at 40 μmol photons m^{-2} s^{-1}. The zooplankton grazer *Daphnia magna* was cultured in beakers and fed with *S. obliquus* (5×10^5 cells mL^{-1}) under the same conditions as described above. *Daphnia* culture filtrate was then obtained as previously described [14,15]. In brief, *D. magna* cultures were collected, washed to remove surface residual medium, and allowed to fast in water for at least 12 h; afterward, *D. magna* cultures were incubated at a density of 200 ind L^{-1} and fed with sufficient *S. obliquus*. After 24 h, *D. magna* cultures were removed; water from these cultures was filtered through a 0.10 μm membrane filter (Millipore Corporation, USA) and test water containing *Daphnia* infochemicals was produced.

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For the control filtrate, a culture of *S. obliquus* without *Daphnia* was subjected to the same procedures as producing *Daphnia* culture filtrate. Nitrate and phosphate concentrations in *Daphnia* culture filtrate and *Scenedesmus* culture filtrate were adjusted to the same levels before these filtrates were used in the experiments.

2.2. Experimental design

To minimize intracellular nutrient storage and nitrogen in the medium, *S. obliquus* cultures (50 mL) in exponential growth were harvested by centrifugation (6000 $\times g$ for 15 min) and then re-suspended in nitrogen-free BG-11 medium for two days, which was repeated twice in four days. After four days, the cells were collected by centrifugation, and the pellets were inoculated into modified BG-11 medium with different nitrogen contents (32, 16, 8, 4, and 2 mg L^{-1}) in 250 mL flasks. The corresponding average measured nitrogen contents were 33.05, 14.69, 7.75, 3.51, and 2.09 mg L^{-1}. The initial algal concentration in the treatment groups and the control group was approximately 5.5×10^4 cells mL^{-1}. Each flask contained 135 mL of *S. obliquus* suspension and either 15 mL of additional *Daphnia* culture filtrate or 15 mL of *Scenedesmus* culture filtrate. The experiments were run in three replicates for seven days under the conditions as described in Section 2.1. The cultures were shaken manually twice a day to maintain culture homogeneity.

2.3. Measurement of growth rate and colony size of S. obliquus

The number of cells per particle of *S. obliquus* was measured in all of the flasks during the experiment to determine the combined effects of nitrogen levels and *Daphnia* culture filtrate on morphological changes in *S. obliquus*. Samples (2 mL) were collected daily and fixed in Lugol's solution (2%). Abundance was determined using a hemocytometer (Tianlong XB-K-25; Jiangsu, China) under a microscope (Olympus 6V20WHAL; Tokyo, Japan). The numbers of cells per particle were calculated by counting at least 600 particles (i.e., unicells, two-celled, four-celled, eight-celled, and the rest) under a light microscope. Growth rate was determined as the slope of logarithm abundance versus time from the samples collected daily [11].

2.4. Statistical analyses

Data were presented as means $\pm$ 1 SE. Growth and cells per particle were analyzed by two-way ANOVA. Statistical analyses were performed in SigmaPlot 11.0.

3. Results and discussion

S. obliquus grew well under all nitrogen levels in this study. Two-way ANOVA results indicated that adding *Daphnia* culture filtrate did not affect the growth of *S. obliquus*; this result is consistent with that described in other studies [8,14,15]. Although nitrogen level significantly affected the growth rate of *S. obliquus* (Table 1), the differential degree was very low; in particular, growth rate only decreased by approximately 6% from 0.54 d^{-1} at 32 mg L^{-1} N to 0.48 d^{-1} at 2 mg L^{-1} N (Fig. 1).

In the treatments with added *Daphnia* culture filtrate, the proportion of eight-celled colonies increased rapidly and reached the peak on day 3

Table 1
Summary of two-way ANOVA of the effects of nitrogen level and *Daphnia* culture filtrate on the growth rate of *S. obliquus*.

Source of variation	*DF*	*SS*	*MS*	*F*	*P*
Nitrogen level	4	0.0188	0.00471	3.303	0.031
Daphnia culture filtrate	1	0.000881	0.000881	0.618	0.441
Nitrogen level × *Daphnia* culture filtrate	4	0.000337	0.0000842	0.0591	0.993

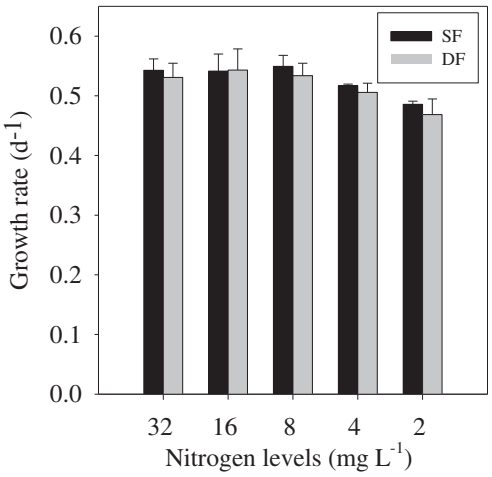

Fig. 1. Growth rates of *S. obliquus* populations incubated under different conditions during the 7-day experiment. Vertical lines represent one SE. SF: *Scenedesmus* culture filtrate; DF: *Daphnia* culture filtrate.

(Fig. 2); this peak was evidently higher than that of the cultures with added *Scenedesmus* culture filtrate. This observation was in line with that described in other studies [6–8,14,15]. The effect of nitrogen level was also determined in this study. The proportions of cells in colonies at low nitrogen levels (2 mg L^{-1} N) were higher than those at high nitrogen levels (4–32 mg L^{-1} N) after day 3. Rapid morphological responses of *S. obliquus* in the experiments were also indicated by the mean number of cells per particle (Fig. 3). In general, colony size increased by approximately 50%–100% in the cultures with added *Daphnia* culture filtrate. Two-way ANOVA results showed that low nitrogen levels and *Daphnia* culture filtrate significantly affected the mean numbers of cells per particle of *S. obliquus*; significant synergistic effects were detected between nitrogen levels and *Daphnia* culture filtrate on colony formation at the end of the experiments (Table 2).

The colony formation of *S. obliquus* in this study was likely a consequence of increased polysaccharide content in cells, as demonstrated in other studies [16,17]. Moreover, nitrate stress can possibly induce an increase in carbohydrate content [18]; excess carbohydrate is preferentially channeled by algal cells to synthesize high amounts of products, such as polysaccharides [19], thereby contributing to cell aggregation. Therefore, colony formation of *S. obliquus* induced by *Daphnia* culture filtrate was promoted under low nitrogen levels probably because high amounts of polysaccharides were produced by *S. obliquus* under low nitrogen conditions. This result suggested that nitrogen level and *Daphnia* culture filtrate elicited a combined effect on the colony size of *S. obliquus*; this finding is similar to the phenomenon observed in *Microcystis* [20]. Furthermore, large colonial populations exhibit higher settling velocities than unicellular populations [15]. Both increased colony size and higher settling velocities enhance the suitability of populations for centrifugation and filtration; thus, these factors could be considered to harvest *S. obliquus*. Our results also showed that *Daphnia* culture filtrate should be added 3 days before *S. obliquus* was harvested from culture media. Furthermore, from the view of increasing carbohydrate content and facilitating harvest of *Scenedesmus*, such colony-enlarging approach is really an effective method to satisfy both sides.

4. Conclusions

In summary, *S. obliquus* can grow well in different nitrogen levels. Colony formation of *S. obliquus* induced by *Daphnia* culture filtrate can be promoted under low nitrogen levels (2 mg L^{-1} N); this result suggested that nitrogen level and *Daphnia* culture filtrate elicited a