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Effects of the absence of nitrogen and phosphorus on the growth rate and total chlorophyll content of a freshwater microalgae *Scenedesmus* sp.

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ABSTRACT

The objective of this study was to determine the effect of nitrogen and phosphorus on the growth and total chlorophyll content of a freshwater microalgae *Scenedesmus* sp. Growth behavior under modified Chu-10 medium without nitrogen source showed strong inhibition with growth rate reduced to 72.84% after 96 hour of treatment. There was about 10.24% inhibition of growth when the algae were cultured in a modified Chu-10 medium without phosphorus source. The photosynthetic pigment such as total chlorophyll content decreased at the same extent after 96 hour of culture in modified Chu-10 medium without the nitrogen source but not decreases in the medium without phosphorus source and the inhibition was highest in the medium without the nitrogen source.

Key words: Nitrogen; phosphorus; total chlorophyll; *Scenedesmus* sp.

INTRODUCTION

Microalgae are diverse group of prokaryotic and eukaryotic photosynthetic organisms. They are widely found in both marine and freshwater environments and can grow quickly due to their simple structure.[1] They are able to grow when all the necessary growth factors are sufficient in the environments. The factors include optimal light intensity, pH stability, consistent temperature, high availability of nutrients.[2] Even in the adequate growth factors each species of microalgae display different growth performance. Among nutrient factors, nitrogen is known to have a strong influence in the growth of the microalgae *Scenedesmus* sp. This green alga is common in freshwater lakes and it can occur both as single cell and colonies when maintained in laboratory cultures.[3]

In general, *Scenedesmus* sp. are non-motile colonial green microalgae consisting of cells aligned in a flat plate. The size of this species is

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about 8.5 µm in width and 13.5 µm in length. *Scenedesmus* sp. has been cultured for live feed aquatic organisms.[4] Optimal media provide the minimum quantity of nutrients to support maximum growth of algae. Nutrient deficiency generally induces decreased cell growth rates and as a result decreases the total chlorophyll content. The extremely low synthesis rate of chlorophyll ultimately inhibits the cell growth. Because the consumption of nutrients synchronizes with cell growth, it is necessary to continuously monitor the composition of the medium and continuously replace nutrients.[5]

The aim of the present work is to study the effect of the absence of nitrogen and phosphorus sources in the growth rate and total chlorophyll content in the microalgae *Scenedesmus* sp.

Materials and Methods

Green microalgae were collected from the lake Tamdil. Isolation of *Scenedesmus* sp. was done in the laboratory and grown in modified Chu-10 medium (with complete nutrient supplements, without nitrogen supplement and lastly without phosphorus supplement) illuminated by fluorescent tubes under 8 hour photoperiod at pH 7.5. The cultures were hand shaken at least 2 -3 times daily. Cultures were maintained at a temperature of 25°C and the initial pH of the medium were adjusted to 7.5 using digital pH meter 335 (Systronics). Growth was determined by measuring the optical density of microalgae culture at the wavelength of 440 nm in a UV/ VIS spectrophotometer (Systronics, India) on alternate days up to the 15th day by using reference blank of basal culture medium. The specific growth rate (μd^{-1}), based on absorbance was calculated for control and treatment after 96 h, using the equation:

$$\mu = [\ln (n_2/n_1)]/ [t_2 - t_1]$$ [6]

Where µ stands for specific growth rate and n_1 and n_2 are absorbance of culture suspension at the beginning (t_1) and the end (t_2) of the selected time interval. For total chlorophyll estimation, 5 ml of fresh algal cells from the culture were centrifuged at 12000 rpm for 15 minutes. To the pellet, 5 ml of 80% acetone was added and left for 12 hours at 4°C. This was again centrifuged and the optical density of the supernatant was taken at 645 and 663 nm for quantifying total chlorophyll content by using the equation: [7]

$$Chl\ (mg/l) = 8.02 \times OD_{663} + 20.21 \times OD_{645}.$$

Statistical analysis

All values are presented as the mean ± SD of the replicates.

Results and Discussion

The growth behavior of *Scenedesmus* sp. under three different modified Chu-10 medium is shown in Figure 1. Cultures stressed with nitrogen deficiency showed no growth and eventually started to decline from the tenth day of the experiment. On contrary, cultures grown in the medium with complete nutrient supplements i.e. control condition show considerable increase in growth. The cultures stressed with phosphorus deficiency behaved in a similar growth pattern with the cultures grown in the control condition

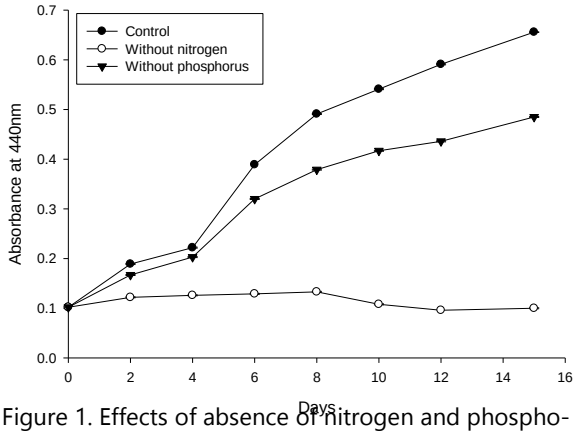

Figure 1. Effects of absence of nitrogen and phosphorus sources on the growth pattern of *Scenedesmus* sp. Values are means of three replicates.

but the increased level is lesser and slower in comparison with the control condition. The control culture attained the highest optical density on the fifteenth day of the experiment while the culture stressed with nitrogen deficiency show the lowest optical density on the last day of the experiment.

Table 1. Effects of the absence of nitrogen and phosphorus sources on percentage growth and inhibition of *Scenedesmus* sp. after 96h of treatment.

Culture medium condition	μd^{-1}	% growth	% inhibition
Chu-10 medium with complete nutrient supplements	0.1944 ± 0.0011251	100	0
Chu-10 medium without nitrogen source	0.0528 ± 0.009751	27.16	72.84
Chu-10 medium without phosphorus source	0.1745 ± 0.0031419	89.76	10.24

The specific growth rate of the algae grown under control conditions i.e. with complete nutrient supplements was 0.1944 ± 0.0011251 after 96 hour of culture is shown in Table 1. The algal growth rate was significantly decreased when it was cultured under nitrogen deficient medium. The algal growth rate was reduced to 0.0528 ± 0.009751 after 96 hour of culture in nitrogen deficient medium. The specific growth rate for stress condition under phosphorus deficiency was found to be 0.1745 ± 0.0031419 after 96 hour of culture.

The complete Chu-10 medium allows the highest cellular duplication rate bringing highest growth rate. This result is consistent with the total chlorophyll accumulation under the same condition as shown in Figure 2. For the total chlorophyll content, culture stressed with nitrogen deficiency shows great reduction with 87.42% after 96 hour of culture (Fig. 2). There is no reduction in the total chlorophyll content of

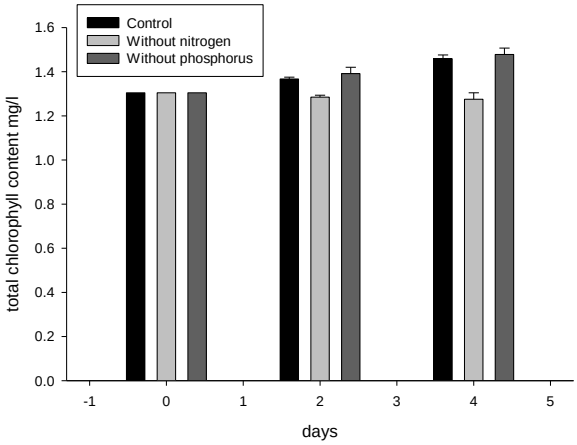

Figure 2. Effect of the absence of nitrogen and phosphorus sources on total chlorophyll content of *Scenedesmus* sp after 96h treatment. Values are means of three replicates.

the culture grown under phosphorus deficient medium (Fig. 2).

In the absence of nitrogen source, no growth was observed and the cells appeared bleached (Fig. 1). Nitrogen is important macronutrients for growth and metabolism of algal cells. Mandal and Mallick[8] have also reported decreased growth pattern in *Scenedesmus obliquus,* under nitrogen deficient conditions.[9] The slow but continual growth in nitrogen deficient cultures is due to both sharing of nitrogen by new cells and to storage of photosynthate. About 10% of the cell nitrogen is lost during the first 12 hours but is reabsorbed during the next 12 hours. This loss and uptake is coupled with the decreased growth rate. At a decreasing concentration of nitrogen sources there was decreased growth, chlorophyll and biomass. Nitrogen starvation also triggered a rapid decline in nitrogen containing compound such as photosynthetic pigments causing complete loss of photosynthetic efficiency, because the consumption of nutrients synchronizes with cell growth and chlorophyll content. Phosphorus is an element required for microalgal growth, especially for generating and transforming metabolic energy.[10] Phosphorus is present in phosphorus-rich ribosome where photosynthetic

linked in proteins are synthesized.[11] Therefore limiting phosphate availability may reduce photosynthetic activity resulting in a lowered growth rate.[12] Since phosphorus, in the form of phosphate, is involved in numerous metabolic processes, it is required in relatively high amounts by all organisms and is one of the key nutrients for biomass production. Surprisingly, elimination of phosphate from the medium had negligible effect on the growth. This is likely to be due to the presence of intracellular stores of phosphate in the cells that were used to initiate the cultures.[13] In view of the fact that phosphorus is an essential nutrient that by spending its own reserves the alga can grow in the absence of external phosphorus. This situation has been discussed by various authors. Rhee[14] observed exponential growth of *Scenedesmus* sp. after eliminating phosphorus from the culture medium, suggesting that growth depended on the internal phosphorus content, such as polyphosphate, and ceased when this nutrient decreased to a critical value. Nevertheless, Curtis *et al.*[15] developed a model for the culture of plant cells, applicable to algae, which explained linear growth in the absence of phosphorus from the nutrient medium. In general, the specific growth rate of microalgae depends on the internal content of nutrients.

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