# A PRELIMINARY STUDY ON THE EFFECT OF MEDIA CONCENTRATION ON THE GROWTH EFFICIENCY AND THE BIOCHEMICAL COMPOSITION OF FRESHWATER MICROALGA *SCENEDESMUS* SP.

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## Introduction

Like many other organisms, the availability of nutrients is one of the primary factors regulating the growth, reproduction and biochemistry of phytoplankton. Nitrogen has often been cited to be the primary nutrient limiting phytoplankton production in the world's oceans. It has been reported that cells can live in high nitrate concentrations with no lethal effect but showed a decrease in biomass and growth *Isochrysis galbana*, for example, can double the fatty acid content in nitrate-grown cells but contains lower concentration and only few amino acids (Flynn et al. 1992). Many researchers have studied the effect of limiting nitrate concentration on algal growth by altering the molarity of certain nitrate sources in the growth media. In this paper we examine the effect of media concentration on the growth and the biochemical contents in *Scenedesmus* sp.

## Materials and Methods

Algal culture *Scenedesmus* sp. used in this study was obtained from the COMAS Culture Collection of microalgae. Axenic batch cultures were grown in 5 liters flasks at 22 ± 2°C on 24:0 (light: dark) cycle with Triton™ coolwhite fluorescent light which delivered 90μmolm$^2$s$^1$ irradiance. Cultures were maintained in distilled water enriched with BBM growth media (Nichols, 1973). The growth media were prepared in two different concentrations; one at full concentration and the other at half concentration. After 10 days, the cells were harvested by centrifugation at 5000 rpm for 3 min. The pellets of the cells obtained were combined and transferred into cryovials and frozen at -80°C prior to freeze-drying. For chlorophyll$_a$ determination, 10 ml cell suspensions were harvested daily and filtered onto cellulose membrane filter. The filter paper containing cells was homogenized and extracted with acetone: DMSO (5:1) mixture (v/v) and stored for 24 h in dark refrigerator for pigment extraction. The extracts were centrifuged at 3000 rpm for 3 min and Chlorophyll$_a$ was determined spectrophotometrically. Total protein was determined using the dye binding methods About 3-5 mg freeze dried samples were homogenized in 0.5N NaOH after 5 min sonication in DMSO. The homogenates were kept for 24 h at room temperature for complete extraction. The extracts were finally assayed with alkaline copper reagent to get the colour reaction. The carbohydrates were analysed by the colorimetric phenol-sulphuric acid method after hydrolysis in 2N HCl for 1 h at 80°C. For lipid extraction, a 30-40 mg sample was extracted using $CHCl_3$: MeOH:H2O (1:1:0.9 v/v/v) and determined gravimetrically.

## Results and Discussion

The chlorophyll$_a$ contents were measured daily for 10 days to observe cell growth. The results obtained indicated that there was no lag phase observed because of the high inoculation rate (20%). Cells grown at full concentration yielded the highest chlorophyll$_a$ on the 9th day and started to decrease on the 10th day. However, the cells grown in low media concentration exhibited a faster growth but the growth lasted only for 5 days. The biochemical contents also changed with changes in the media concentration. The reduction in cell nitrogen at low media concentration led to a decrease in chlorophyll$_a$ content which indicated a reduction in the protein-rich chloroplast apparatus as well as the lipids in the chloroplast membranes. The total proteins and lipids diminished as the media concentration decreased (from 29.40 ± 0.69% to 19.91 ± 0.06% and from 33.02 ± 0.51 to 22.03 ± 0.54% respectively). However, total carbohydrate production was higher in low media concentration (34.80 ± 0.37%) compared to full concentration (28.77 ± 1.07%).

The media concentration can be manipulated to affect the growth rate and formation of major biochemical constituents. As reported previously, high nitrate concentration in the cells leads to a decrease in the cell density and photosynthetic pigment content (Jimenez and Niell, 1991). From the results we observed that at low nitrate concentrations cells grew faster but lasted only for 5 days after which the growth rate slowed down approaching stationary phase. Under half strength media, we also observed a high percentage of carbohydrate. Probably in the late stationary stage mature cells contain more carbohydrate. It has been reported that in a batch mode, microalga can produce high levels of protein and low carbohydrate and lipid during the logarithmic phase, but contains more carbohydrate at the stationary stage (Brown et al. 1993).

## Conclusions

Our study indicated that the biochemical compositions can vary if the cells are harvested at different growth phases. Further studies to harvest the cells at the 5th day and 9th day of cultivation of cultures under half strength and full strength media, respectively are being planned.

## References

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