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STUDY ON THE GROWTH ENHANCEMENT ACTIVITY OF HUMIC ACID ON MICROALGAE BIOMASS

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ABSTRACT

Photosynthetic microalgae are attracting significant attention as they can serve as important source for cosmetic, food and pharmaceutical products and also as biofuels. Currently, the rate of microalgae production is very low, which in turn affects the large scale production. To increase algal productivity, the effect of growth stimulator - humic acid that had been extracted from lignite was analyzed in this study. Batch culture experiments of microalgae treated with various concentrations (0.01, 0.05, and 0.1 %) of humic acid was used to evaluate the growth enhancement activity such as the growth (biomass concentration), pigment production (chlorophyll), Nitrogen (N), Phosphorus (P) and Potassium (K) range. Results demonstrated a highly significant positive effect of humic acid on biomass, phytochrome production and also in Nitrogen, Phosphorous and Potassium range. The response of microalgae to humic acid was seen at an optimal range of concentration studied and is low-cost and yields high productivity. In conclusion the humic acid is found to be supportive in enhancement of algal growth.

Key words: growth, humic acid, microalgae, pigment.

INTRODUCTION

Micro-algae are large and diverse group of aquatic organisms that lack complex cell structures like in higher plants. They are found in diverse environments, some species are found thriving in freshwater and others in saline conditions and also sea water (1). Humic acid and humic matter are growth stimulators and are resistant towards adverse weather conditions, most particularly during the drought condition. When in favorable condition it acts upon bio-chemical process and helps in growth. The absorbed nutrients are slowly released to the plant, which is not seen in soluble chemical fertilizer those without humic acid (2, 3). In many places humic acid is found to be used directly as fertilizer, soil conditioner, pest controller and also as micronutrient carrier (4). Humus releases nutrient gradually throughout the growing season that is right from sprout formation (5). Humic matter are the most wide spread natural products on earth surface, they

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are found in soil, lakes, rivers and seas (1). However, despite their broad pressure in nature little is known about their origin, formation chemical structure etc. Coal and lignite contain humic matter and lignite is found to be a richer source of humic acid (1, 2).

MATERIALS AND METHODS

Extraction of Humic acid

Lignite with very low ash content is best suited for the production of humic acid. Lignite was digested with known quality of caustic soda for about two hours and allowed for cooling, after which it was diluted and filtered using cartridge filter. The obtained filtrate contains sodium bromate which is then precipitated as humic acid by neutralizing it with hydrochloric acid. The precipitated form contains chlorides which is removed by washing and is filtered under pressure of about 5 to 6 kg/cm2. Thick cakes of humic acid are obtained which are then sun-dried, powdered and packed.

Microalgae and culture media

The microalgae culture was obtained from the Centre for Applied Research and Development (NLC). The cultures were grown in Bristol medium as previously described by James (1978). A stock of humic acid was added to the culture medium to yield the final concentration: 0.05 %, 0.01 % and 0.1 % respectively. All cultures were grown in 1 L flask equipped with inlet and outlet tubes for aeration and cultures were continuously agitated by bubbling with air and sunlight exposure.

Biomass recovery and estimation

The exponential phase cultures were subjected to the following analysis. The algae samples were then filtered and the residues were collected. The residue was kept in hot air driers at 70 °C for three hours. The concentration of biomass calculated as follows:

Net weight = final weight of the filter paper - initial weight of the filter paper

Chlorophyll

The spectrophotometric method of Hansmann (1973) was used to estimate the chlorophyll a content in the algal cells. The optical density of the suspension was determined at 665 nm using a Perkin Elmer Lambda Spectrophotometer. The concentration of chlorophyll a (mg) was calculated as follows:

A665/8.4 x Volume of ethanol = mg chlorophyll

Phycobilins

The optical density of the suspension was determined at 562, 615 and 652 nm wavelength using a Spectrophotometer. The concentration of total phycobilins was calculated as follows:

Phycobilins [mg/mL] = C x Ve / Vc

Nitrogen, phosphorus and potassium

The harvested cells were subjected to distillation and titration. Colorimetric and flame photometric methods were used for the estimation of nitrogen, phosphorus and potassium concentration.

RESULTS

The growth of microalgae (Fig.1) is expressed as biomass concentration (dL) and positive effect is seen after addition of the humic acid at various concentrations. The biomass concentration has also gradually increased in the 0.01, 0.05, and 0.1% humic acid treated cells up to the 20th day. The highest value (80 mg/dL) was observed in cells which were grown at 0.01 % HA on day 20. Humic acid treated cells were found to grow well than those cells that were not treated with humic acid (control).

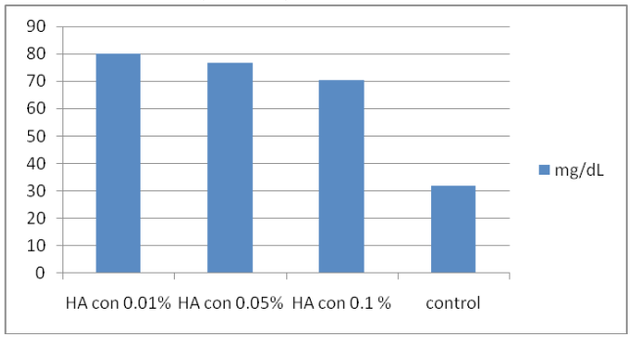

Figure 1.

Effect of different concentration of HA in microalgae biomass

The chlorophyll content of the microalgae was detected after humic acid treatment, where the pigment content was found increasing gradually 160 mg/100g (Fig. 2). A maximum carotenoid content (Fig. 3) of 120.42 mg/100g dry weight was detected in algal cells that grew at 0.01 % humic acid concentration.

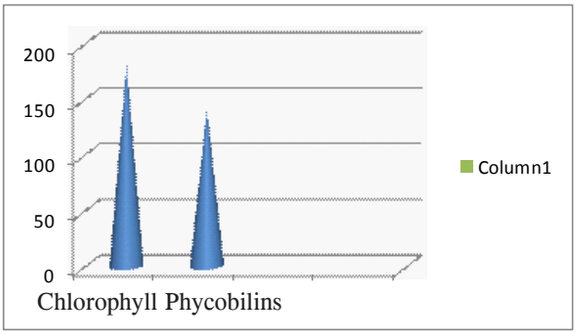

Figure 2. Effect of Humic acid in pigment development

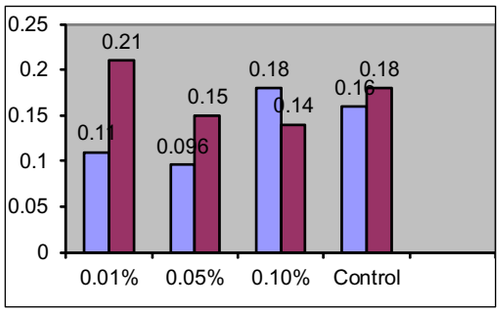

Figure 3. Growth performances of micro algae in humic acid

The nitrogen content was found to be increased 2.98 % g/100g dry weight at 0.01 % humic acid. Phosphorus content in microalgae increased to about 2.90 ppm after the addition of 0.01 % of humic acid when compared to that of the control. Potassium content was also relatively high as 3.80 % in 0.01 % humic acid concentration. The maximum value (8 %) was observed in cells grown in 0.05 % acid concentration.

DISCUSSION

The microalgae cultures are able to grow on different concentrations of humic acid and produce useful products such as phycobilins and other phytochrome. This study is highly essential as the humic acid is found to improve the nutritional quality of microalgae. Based on concentration of humic acid treatment on algal culture, the production of algal mass is found increasing significantly. The stimulatory capacity of humic acid on algal growth has been studied by many authors (6, 7, 8).

Our results demonstrated a positive effect on pigment production in microalgae. At 0.01 % concentration, humic acid has shown maximum chlorophyll a in microalgae species. Further, the investigated species are characterized by high phycobilins content even at control condition (9, 10). Studies have demonstrated that microalgae pigments transferred to zooplankton may contribute to their nutritional value (11).

The high ash content found in cultures grown under control conditions was consistent with other studies on marine microalgae (2, 12) and differs from that of the fresh water algae, Scnedesmus and Spirulina (5). The addition of a low concentration of humic acid caused a decrease in ash content in both microalgae species (13).

In conclusion, our major objective was to evaluate the growth enhancement effect of humic acid on the microalgae biomass, pigment production, and nitrogen, phosphorus, potassium content. The result of this present study demonstrates the potential effect of humic acid in increase good yield. The response of the investigated microalgae to various humic acid concentrations showed the optimal effects in the lower concentrations, i.e., low-cost and high-yield. Thus humic acid is useful for scale up production to increase the yield. The biomass which is grown on lignite by-products can be used for enormous purposes like bio-diesel production, biofertilizer, SCP and waste reclamation like reduction of CO_2 emission and wastewater treatments.

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