

**ALGAL SINGLE CELL PROTEIN FROM EXTRACT OF COW MANURE ENRICHED WITH DIFFERENT NITROGEN SOURCES.**

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**ABSTRACT**

Water extract of cow manure waste enriched with some nitrogen sources was used as culture media for *Chlorella pyrenoidosa*. The lowest concentration (0.0125%) of the nitrogen sources added to the cow manure extract affected the highest growth rate. Urea proved to be the best nitrogen source that could be added at low concentration to the water extract of cow manure waste for single cell protein production by *Chlorella pyrenoidosa*. At higher concentrations, urea proved to be more toxic than both nitrate or ammonia.

**INTRODUCTION**

Recycling waste material not only minimizes the problem of pollution but also is used in the production of single cell protein. With a view to the increasing universal problem of food storage, the cultivation of unicellular algae, both fresh water and marine species, has been conducted successfully in waste, liquid and water extracts of sewage sludge (Ludwig and Oswald, 1952; Dunstan and Menzel, 1971; Ryther et al., 1972; Goldman and Ryther, 1975; Wong, 1977 a & b; Wong and Ho, 1977; Wong et al., 1977 and Yip and Wong, 1978), in barley and soybean wastes (Wong, 1977 b) as well as in chicken manure and blood waste (Wong, 1981).

*Chlorella* sp. is rich in vitamins, fats and proteins (Provaside, 1974). Its efficiency in photosynthesis and the conversion of nitrogen to protein is greater than that of higher plants (Wassink et al., 1964). It has autotrophic, heterotrophic and mixotrophic modes of nutrition.

The present work is an attempt to study the effect of different additional nitrogen sources to the water extract of cow manure waste on the efficiency of single cell protein production by *Chlorella pyrenoidosa*.

**MATERIALS AND METHODS**

Cow manure waste was air dried at  $22 \pm 3^\circ$  C for about two weeks. Water extracts were prepared by boiling the waste with distilled water for 20 minutes. The suspension was centrifuged for 15 minutes at 15,000 r.p.m. and the supernatant collected was diluted with distilled water to a

final concentration of 1.5% concerning the solid waste. Preliminary experiments have been carried out by employing a series concentrations. One and half percent found to be the optimum concentration.

Culturing was carried out in sterilized 100 cm<sup>3</sup> Erlenmeyer flasks containing each, 30 cm<sup>3</sup> of the medium. Culture media composed of the water extract to which four concentration of the tested nitrogen sources were added in the following order: 0.0125, 0.050 and 0.1%. The different nitrogen sources used were urea, ammonium sulphate and potassium nitrate. Waste-water extract alone was as a control medium. Each experiment was performed in triplicate. In all treatment, the initial pH was adjusted to 7. To each culture flask an inoculum of *Chlorella pyrenoidosa* was added in a concentration of  $2 \times 10^6$  cells/ml. Culture flasks were incubated in a growth chamber for 7 days at  $25 \pm 3^\circ$  C with a 16 h light (2500 lux) 8 h dark cycle. The optimum incubation period was determined experimentally and it was found to be 7 days. Using the microkjeldahl, the total nitrogen of the air-dried waste was found to be 3.8%.

At the end of the culture period the final pH was determined and the cell suspensions had been thoroughly stirred before the determination of the algal cell counts using a haemocytometer. The algal cells were separated by centrifugation and analyzed for their content by a spectrophotometric method (Kalb and Bernlohr, 1977) and chlorophyll a content (Jespersen & Christoffersen, 1987).

## RESULTS AND DISCUSSION

The effect of water extract of cow manure enriched with different nitrogen sources on the growth of *Chlorella pyrenoidosa* is shown in Figs. 1 & 2. It is clear from these results that *Chlorella* is able to utilize a wide range of nitrogenous compounds as a source of nitrogen for growth in the light. The utilization of urea by *Chlorella* is in the line with pattern reported for other algae (Syrett, 1962; Thomas, 1968; Naylor, 1970; Leftley & Syrett, 1973 and Bekheet and Syrett 1977. Highest growth rate values were obtained at the concentration 0.0125% for all the tested nitrogen sources. Any further increase above this level did not result in any further increase in growth. The percent of decrease in both nitrate or ammonia was gradual, but in case of urea it was rapid. At concentration 0.0125%, urea gave the highest growth rate (45% and 80% increase in both number of cells and protein content, respectively) when compared to nitrate and ammonia (Fig. 2). Although ammonia is toxic, plants can detoxify it by forming ammonium salts of organic acids or from the amides glutamine or asparagine (Webster, 1959). The rapid decrease in the growth rate of *Chlorella* at urea concentrations above 0.0125% indicates that urea is

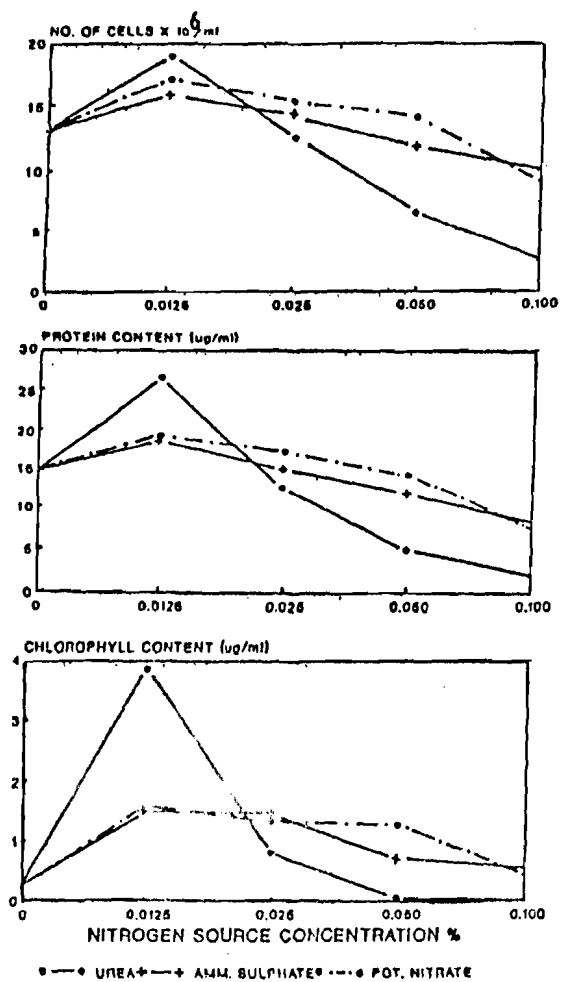


FIG. 1  
Effect of cow manure extract enriched with different nitrogen sources on the growth of *Chlorella pyrenoidosa*.

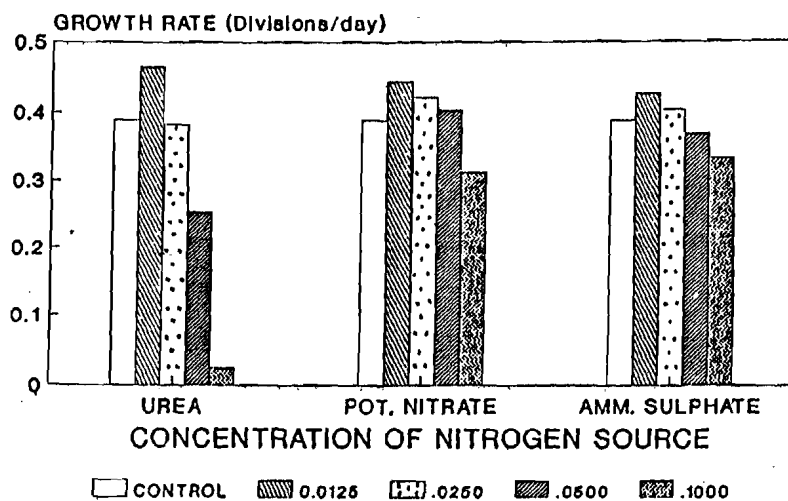


FIG. 2.  
Growth rate of *C. pyrenoidosa* grown on cow-manure  
extract enriched with different nitrogen sources.

more toxic than both ammonia or nitrate. It was reported that urea stimulates the plant to synthesize and accumulate a certain nucleotide which can "switch off" the synthesis of macromolecules in the cell (Lau et al., 1977; Ownby et al., 1979; and Friga, 1980). Such a "switch off" may be considered as a logical reason for the decrease in the growth rate of *Chlorella pyrenoidosa* at higher concentrations of urea.

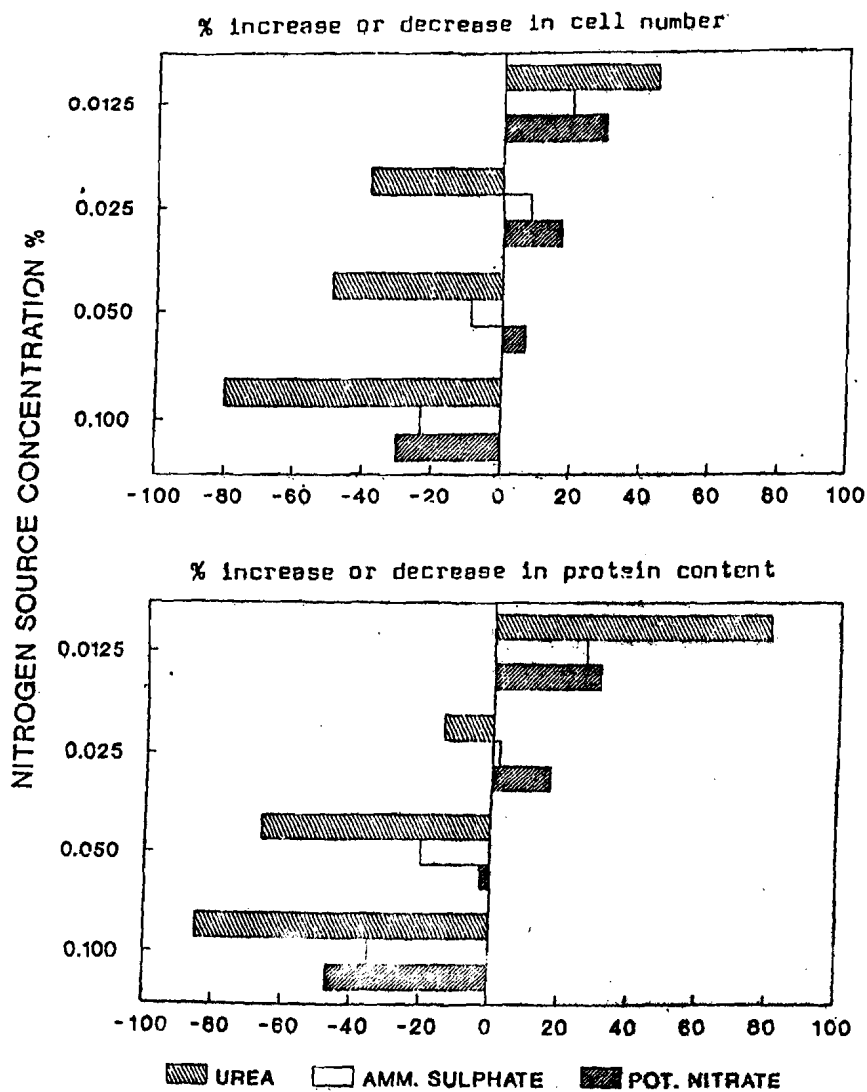


FIG. 3  
Percent increase or decrease in cell number  
and protein content from the blank.

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