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Comparative Study of the Effects of Bean Extract and Ammonium Chloride Solution as Nitrogen Sources on the Growth of *Euglena gracilis* Z.

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ABSTRACT

The effects of two nitrogen sources, bean extract and ammonium chloride solution, on the growth of *Euglena gracilis Z* were compared using photoheterotrophic batch culture method. The bean extract was prepared by boiling 50 g of bean in 250 ml of distilled water for one hour, while ammonium chloride solution was prepared by dissolving 1g in 250 ml of distilled water. The effects of graded volumes (0, 10, 20, 30 and 40 ml) of each of the nitrogen sources on the growth of Euglena cells were also investigated. The result showed that bean extract supported higher cell growth ($2.69 \pm 0.27 \times 10^6$ cells/ml) than ammonium chloride solution ($1.25 \pm 0.00 \times 10^6$ cells/ml) after 7 days of cultivation and that 10 ml of each of the nitrogen sources per 200 ml of the basic medium gave the highest cell growth, while higher amounts (30 and 40 ml) decreased cell growth. This suggests that bean extract, which is often thrown away as waste during bean processing, can be used to enhance biomass production.

Keywords: *Euglena gracilis,* nitrogen sources, bean extract, ammonium chloride, biomass production.

INTRODUCTION

Euglena is a unicellular organism classified into the kingdom prostista, and phylum euglenophyta. All *Euglena* cells have chloroplast and can make their own food by photosynthesis (autotrophy). They also act as a heterotrophic microorganism and usually live in quiet ponds or puddle [1]. In most developing countries like Nigeria, there is high level of malnutrition among the poor resulting from deficiency of protein in their diets. Although, the conventional protein foods such as beans and soya bean may be available, they tend to be very expensive and unaffordable to the poor [2]. Again, food crops take longer time to grow into maturity and also seasonal, which means that they will be scarce at some periods of the year [4]. Therefore, there is a need for alternative sources of protein to supplement the inadequate conventional sources. *Euglena gracilis Z* has a very high potential as protein source for human and as animal feed supplement. The main importance of this microalga lies on its high proteins contents (essential amino acids), vitamins A and E and polyunsaturated fatty acid (linoleic acid) [5,6] Consequently, it is used as health food and animals feed in developed countries. *Euglena gracilis Z* is among the photosynthetic microorganisms employed for production of single cell protein (SCP) a new food source used as an alternative source of protein in developed countries like America and Europe [7]. Although many higher plants are known to produce these vitamins, their production in photosynthetic microorganisms is much higher owing to higher growth rates and absence of seasonal influences, which make it possible for the cells to be cultivated all year round [8].

The role of *Euglena gracilis Z* as a good source of nutritionally and medically important substances has called for more research on how to maximize its production. Reports abound that it grows well in analytical grade glucose and sucrose as carbon and energy sources [1], but these substrates are expensive and their use for biomass production is not economical. To produce Euglena and other microbial biomass economically, there is a need to develop a very cheap medium [4].

Protein resources remain the most expensive food component in Nigeria, where single cell protein (SCP) technology has either not been adopted or developed to supplement the conventional sources such as fishes, eggs, meat and legumes [7]. Thus, this study was intended to contribute to strategies towards reducing the problem of protein inadequacy in the diets of the poor.

MATERIALS AND METHODS

Microorganism

Euglena gracilis Z stock culture used for this study was obtained from the algae collection centre of the Institute of Applied Microbiology, University of Tokyo, Japan. It was maintained at OGB Biotechnology Research and Development Centre, Enugu.

Media Preparation

The media were of two types, one contained bean extract and the other was added ammonium chloride solution, in graded amounts, as nitrogen sources. The basic medium was composed of 1.0 g analytical grade glucose and 1.0 g of chemical fertilizer (NPK 15:15) in 200ml of distilled water. The media were sterilized by autoclaving at 121^{o}C for 15 minutes and allowed to cool to room temperature before inoculation with the stock of *Euglena gracilis*.

Cell Cultivation

The stock culture (1ml) was inoculated into each of the 10 conical flasks (500ml) using a sterile syringe and needle in a clean inoculation hood. Five of the flasks were used for different volumes of the bean extract (0, 10, 20, 30 and 40 ml) and the other five for the same volumes of ammonium chloride solution. The initial cell density of the batch culture was determined immediately after inoculation using haemocytometer and light microscope. The cultures were shaken manually for 2-3 minutes at least 5 times daily to enhance mass transfer and prevent cell sedimentation. Illumination of cultures was done with daylight fluorescent tubes (16 Watts, 200-220V) placed such that the flasks were between parallel fluorescent tubes to ensure efficient and uniform light distribution in the broth. Each batch lasted for a period of 10- days.

Determination of cell concentration

Total cell counts were microscopically determined with a Neubauer counting chamber (depth = 0. 1mm; area = 1/400mm^{2}) (Weber England). The cell concentration was calculated using the formula, Number of cell/ml = n x 4x 10^{6} cell/ml, where n = average number of cells per well.

RESULTS AND DISCUSSION

This study compared the effects of two different nitrogen sources; bean extract (an organic nitrogen source) and ammonium chloride solution (a chemical source) on the growth of *Euglena gracilis* Z and examined the effect of graded concentrations of the nitrogen sources. The result showed that there was higher cell growth in bean extract than in ammonium chloride solution (Fig.3) and that 10 ml of each of the nitrogen sources per 200 ml of the basic medium gave the highest cell growth, whereas higher concentrations decreased growth rate of the cells. This suggests that 30 ml of the nitrogen sources and above were inhibitory to the cells and therefore, decreased cell growth. The inhibitory effects of higher concentrations of the nitrogen source may be due to direct toxic effect of the substrate or indirectly through its effects on pH, water activity and other properties of the broth. This agreed with a report by [7] that there are minimum and maximum substrate concentrations for the growth of *Euglena gracilis Z* and microbial cells in general. The dependency of cell growth on limiting substrate concentration was described in Monod equation and its modifications [6].

Organic nitrogen sources (bean extract) are known to also contain other substances that promote cell growth such as minerals, organic nutrients, growth factors etc. [8] while chemical nitrogen sources (ammonium chloride solution) on the other hand, lack these additional growth supporting substances. This may be the reason why higher cell growth was obtained with the bean extract than with ammonium chloride solution. Therefore, bean extract that is usually discarded as waste during bean processing can be used for production of microbial biomass.

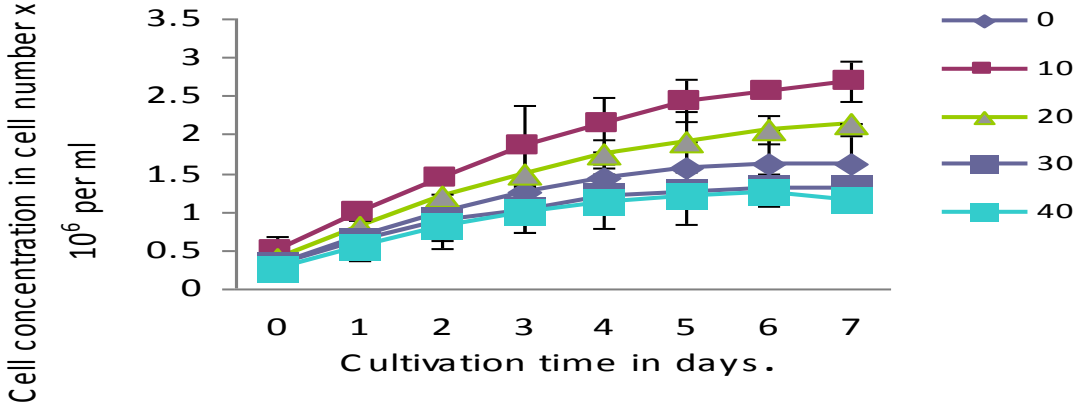

Fig.1 Effect of bean extract on the growth of *Euglena gracilis Z*

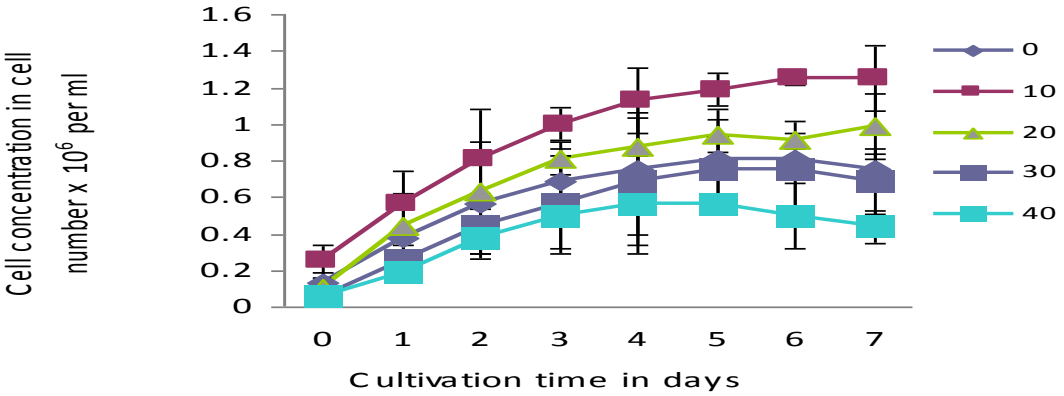

Fig 2 Effect of ammonium chloride solution on the growth of *Euglena gracilis Z*

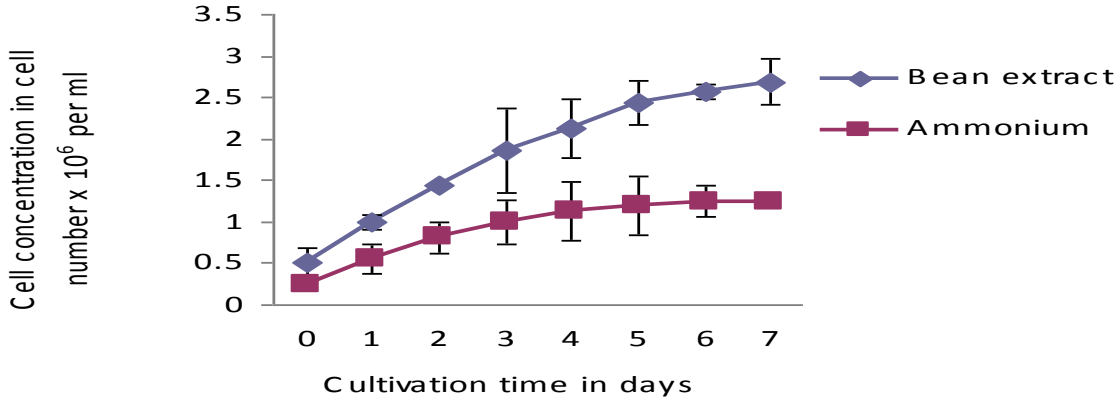

Fig. 3 Comparison of the effects of bean extract and ammonium chloride solution as nitrogen sources on the growth of *Euglena gracilis Z*.

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