



Fed-batch Mixotrophic Production of *Euglena gracilis* Biomass using NPK 15:15:15 Fertilizer

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ABSTRACT

Mixotrophic cultivation of *Euglena gracilis* results in high algal biomass with high intracellular contents. However, the cost of production is very high due to the high cost of media components. In this study, production of *Euglena gracilis* biomass using a cheap and easily available media was investigated in a fed-batch culture. The growth of cells in NPK 15:15:15, with peptone and ethanol as nitrogen and carbon sources were compared using varying ethanol feed rates of 0.5 mL L⁻¹, 1.0 mL L⁻¹ and 2.0 mL L⁻¹ in 24 h and light intensities of 2570 Lux, 1642 Lux and 851 Lux, respectively. The growth rates of *E. gracilis* under various ethanol feed rates and light intensities were ranked as 1.0 mL L⁻¹ > 0.5 mL L⁻¹ > 2.0 mL L⁻¹ and 2570 Lux > 1642 Lux > 851 Lux, respectively. Peptone did not significantly increase the growth of *E. gracilis*.

Keywords: Mixotrophic, *Euglena gracilis* Biomass

Introduction

Microalgae are photosynthetic cell with the capacity to sequester carbon dioxide in the environment. Microalgae require light, carbon dioxide, and dissolved nutrients (Nitrogen, Phosphorus, trace metals, and vitamins) for growth. In a cell or tissue culture, the microalgal cell mass grows freely suspended in liquid medium. Microalgal biomass is a rich source of some functional nutrients such as β -carotene, α -tocopherol, ω_3 and ω_6 fatty acids, and essential amino acids (Herrero *et al.*, 2006). In recent years, there has been a growing interest in so-called functional foods because they can provide physiological benefits in addition to serving as nutrient and energy sources. For instance, some microalgae biomass are antihypertensive, antioxidant, anti-inflammatory or hypocholesterolemic (Eze *et al.*, 2016; Goldberg, 1996; Herrero *et al.*, 2006). The major components of media used for photoautotrophic cultivation of microalgae include inorganic nitrogen sources and both macro and micro elements (Nwuche *et al.*, 2014).

Among the various microalgae, a species of *Euglena gracilis* was selected as it has such advantages as high protein content and high digestibility for animal feed (Chae *et al.*, 2005). For effective culture of *E. gracilis*, the most desirable initial pH, temperature, CO₂ concentration are 3.5, 27°C, and 10%, respectively (Chae *et al.*, 2005). *Euglena gracilis* biomass has been reportedly cultivated using various cultivation methods. For instance, Ogbonna *et al.* (1998) and Takeyama *et al.* (1997) reported

Photoautotrophic cultivation of *Euglena gracilis* with resultant high α -tocopherol content and low growth rate, and heterotrophic cultivation of *Euglena gracilis* using various organic substrates with resultant lower intracellular tocopherol contents but very high cell concentrations. With ethanol as the organic carbon source and optimizing other culture conditions, a fed-batch heterotrophic culture can be used to obtain high *Euglena* cell concentration with relatively high tocopherol content (Ogbonna *et al.*, 1998). Furthermore, an advantage of using ethanol as the organic carbon source is that it reduces the risk of contamination when strict sterile conditions are not maintained during the cultivation. Also, the ability of *Euglena gracilis* to utilize ethanol as energy source is important because it can minimize the effects of seasonal and diurnal light limitation on growth in outdoor cultures (Eze *et al.*, 2017). However, some of the major limitations of heterotrophic culture include high risk of contamination and high rate of consumption of organic carbon source both of which are cost intensive thereby making the process uneconomical. In the present study, the optimum concentration of NPK fertilizer as cheap basal medium for biomass accumulation of *E. gracilis* biomass under mixotrophic culture condition has been investigated. The effect of different ethanol feed rates as the organic carbon source on the biomass accumulation as well as the effect of peptone nitrogen source and light intensity on cell concentration and growth rate were also investigated.

Materials and Methods

Collection of microorganisms and media composition

Euglena gracilis Z was obtained from the Culture Collection Centre of the Institute of Applied Microbiology, University of Tokyo, Japan and sub cultured at OGB Biotechnology Research and Development Centre Enugu, Nigeria. NPK15:15:15 (composed of 15% nitrogen, 15% phosphorus and 15% potassium) was used as the basal growth medium. Ethanol as a carbon source, and peptone as nitrogen source were added to the basal medium.

Optimization of media for cell growth

Erlenmeyer flasks (500 mL) containing 400 mL of 1.25 g/L, 2.5 g/L, 5.0 g/L and 10.0 g/L of NPK15:15:15 fertilizer were used for the cultures in four replications. They were equipped with a high quality foam plug and narrow heat resistant plastic tube passed into the medium through the plug and extended into the outside for aseptic collection of samples for analysis. The exterior opening of the narrow tube was sealed with cotton wool. The media was sterilized by autoclaving at 121°C for 15 min. Approximately 10% of a pure culture of *E. gracilis* containing 0.5×10^7 cells/mL was aseptically transferred into the medium and then incubated in doors at room temperature ($25^\circ\text{C} \pm 0.5$) between two parallel daylight fluorescent tubes at a light intensity of 2570 Lux. Cell growth was measured daily for a period of one week by counting the cell number with microscope and Neubaur counting chamber. Effect of media concentration on the growth of *E. gracilis* was determined. Growth of the cells was determined in the replicates.

Effects of ethanol concentration on the growth of *E. gracilis*

Erlenmyer flask (500 mL) containing 400 mL of 5.0 g/L NPK15:15:15 basal medium was used for the cultures. The experiment was set up as described previously in four replications. To each of the replicates, 0.0 mL/L, 0.5 mL/L, 1.4 mL/L, or 2.0 mL/L of ethanol was added twice a day. The cell growth was measured daily for a period of one week as described in the preceding section.

Optimization of nitrogen source for cell growth

Erlenmeyer flask (500 mL) containing 400 mL of 5.0 g/L NPK15:15:15 basal medium and 0.0 g/L, 0.5 g/L, 1.25 g/L, or 2.5 g/L of peptone were used for the cultures. The experiment was set up as described previously in four replications. To each of the replicates, 0.4 mL ethanol was added intermittently per day (i.e., 0.2 mL twice per day) and the cell growth measured everyday.

Optimization of light intensity for cell growth

Erlenmyer flask (500 mL) containing 400 mL of 5.0 g/L NPK15:15:15 basal medium and 0.5 g/L peptone was used for the culture. The experiment was set up in four replications as described earlier. To each of the replicates, 0.4 mL ethanol was added intermittently per day (i.e., 0.2 mL twice per day). The replicates were kept between two parallel daylight fluorescent tubes at light intensities of 2570 Lux, 1642 Lux, 851 Lux, respectively and the cell growth determined.

Results

Effect of concentrations of NPK 15:15:15 on the growth of *E. gracilis*

The effect of different concentrations of NPK15:15:15 fertilizer on the growth of *E. gracilis* is shown in Figure 1. Amongst the concentrations of NPK 15:15:15, 5.0 g/L gave the highest algal cell concentration with an average algal concentration of $26.4 \pm 0.6 \times 10^7$ cell/mL. It was also observed that the cell concentration increased continuously throughout the period of cultivation for each concentration of NPK 15:15:15 tested.

Effects of intermittent addition of different concentrations of ethanol on the growth of *E. gracilis* .

The effect of different concentrations of ethanol on the growth of *E. gracilis* is shown in Figure 2. It was observed that a concentration of 1.0 mL/L of ethanol yielded significantly ($p < 0.05$) the highest algal concentration (26.85×10^7 cell/mL) in seven days. The photoautotrophic culture (0.0 mL/L ethanol addition) yielded the least algal concentration. The cells continued to grow with all the concentrations of ethanol listed until the 7th day of cultivation.

Effect of nitrogen source on the growth of *E. gracilis*

The effect of different concentrations of peptone as nitrogen source is depicted in Figure 3. It is evident that 0.5 g/L of peptone yielded an average cell concentration of $28.5 \pm 2.84 \times 10^7$ cells/mL as against $26.85 \pm 0.62 \times 10^7$ cells/mL in the absence of peptone. Increase in growth of *E. gracilis* as a result of peptone addition was found to be statistically non significant ($p < 0.05$). The growth of *E. gracilis* per day in peptone showed a progressive increase in cell concentration in all the peptone concentrations listed up to the 7th day.

Effect of light intensity on the growth of *E. gracilis*.

The light intensity of 2570 Lux gave the highest concentration of algal cells as shown in Figure 4. Light intensities of 1642 Lux and 851 Lux yielded lower cell concentrations. The effectiveness of the light intensities in supporting the growth of *E. gracilis* are ranked in the following order of 2570 lux = 1642 lux > 851 Lux.

Discussions

The results show that each of the concentrations of NPK basal medium yielded different concentrations of *E. gracilis*. The reasons for the differences in the abilities of the NPK fertilizer concentrations to support the growth of *E. gracilis* are not known as this fertilizer is more appropriate for different types of crops. However, the differences in the relative concentrations of nitrogen, phosphorus and potassium in the basal medium may have been a contributing factor. For instance, NPK15:15:15 (0.5 g/L) contains 0.063 g/L of nitrogen, 0.12 g/L of phosphorus, and 0.24 g/L of potassium. The concentrations of each of these nutrients varies as the concentrations of NPK 15:15:15 varied in the culture medium. Other factors such as their availability (solubility), concentrations of other elements, and the nature of the filler material may affect the growth of *E. gracilis*.

Addition of 2.0 ml/L ethanol gave the least growth rate. Ogbonna *et al.*, (1998) reported that 5 g L⁻¹ ethanol in a batch heterotrophic culture increased *E. gracilis* cell concentration. The reason for the conflicting result could be as a result of the difference in the culture methods. Although high ethanol concentration is known to be inhibitory to cells, this concentration (2.0 ml/L) is below the inhibitory concentration for some cells such as *Saccharomyces cerevisiae*. The increase in cell concentration with addition of ethanol when compared to the control (Figure 2) suggests that cultivation of *E. gracilis* under photoheterotrophic condition yields much greater cell concentration than under photoautotrophic condition. In the effect of nitrogen sources, it was found that peptone had no statistically significant ($p < 0.05$) effect on cell growth. This could be due to the colour of peptone, which affected light penetration into the algal culture broth. Furthermore, the basal medium NPK 15:15:15 already contained nitrogen. The result showed that light intensity of 2570 Lux gave the highest growth rate of *E. gracilis*. This may be attributed to light intensity as photoautotrophic and heterotrophic activities occur independently and maximally. Hence, the growth rate of *E. gracilis* was probably an additive effect of photosynthesis and organic carbon assimilation. This seems to agree with the report that at low light supply coefficient, the photoheterotrophic growth rate of *E. gracilis* was about equal to the sum of the heterotrophic and photoautotrophic growth rates (Ogbonna *et al.*, 2002). Light intensity of 851 Lux gave the least growth rate. The effectiveness of the light intensity in supporting the growth of *E. gracilis* can be ranked as 2570 Lux = 1642 Lux > 851 Lux. However, there is need to investigate the effect of higher light intensities.

Conclusion

NPK15:15:15 can serve as a cheap basal medium for the production of *E. gracilis* biomass. The light intensity of 1642 Lux and intermittent addition of 1.0 mL of ethanol significantly increased the growth of *E. gracilis* in NPK15:15:15 basal medium. There is however, a need to supplement other components.

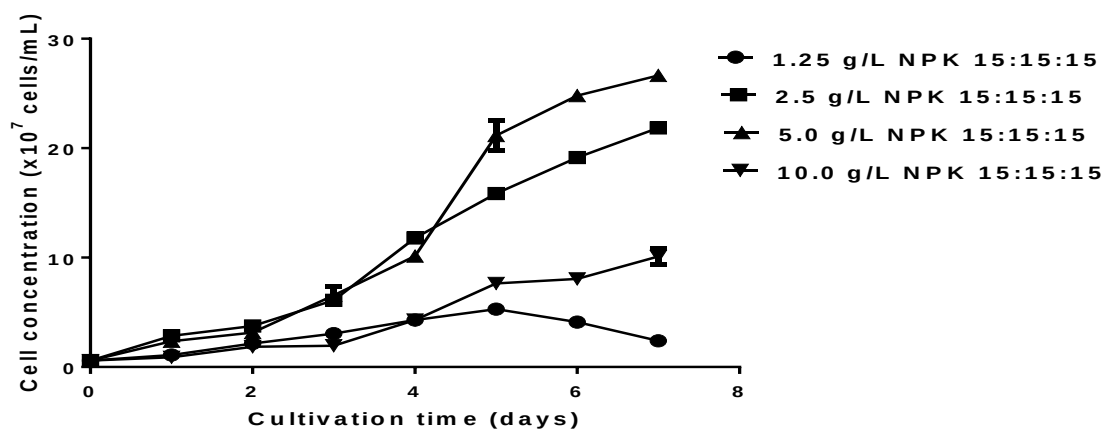


Fig.1: Time courses of *E. gracilis* growth in different concentrations of NPK 15:15:15 at 2570 Lux. control = 5.0 g/L NPK 15:15:15 with ethanol.

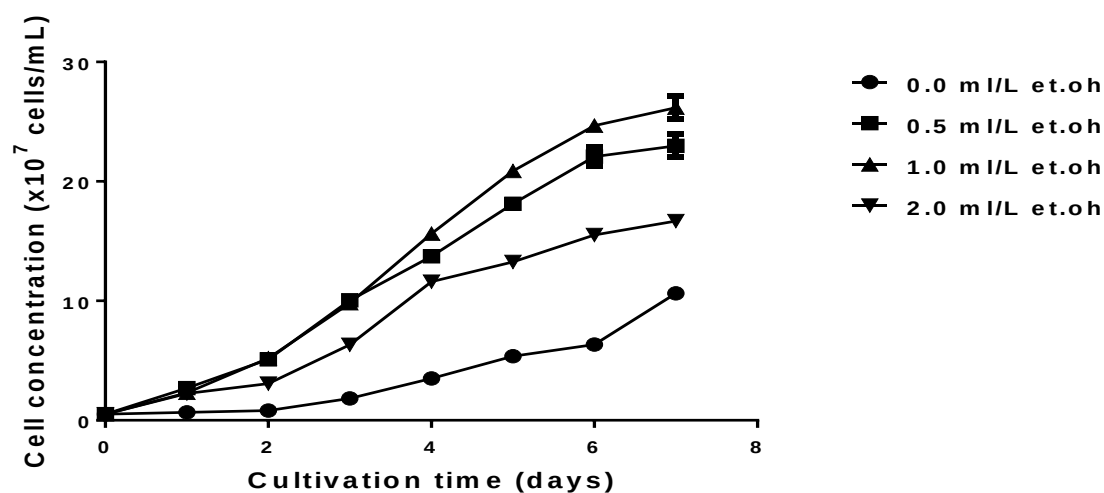


Fig.2: Time courses of *E. gracilis* growth on different concentrations of ethanol addition using NPK15:15:15 as basal medium at 2570 lux

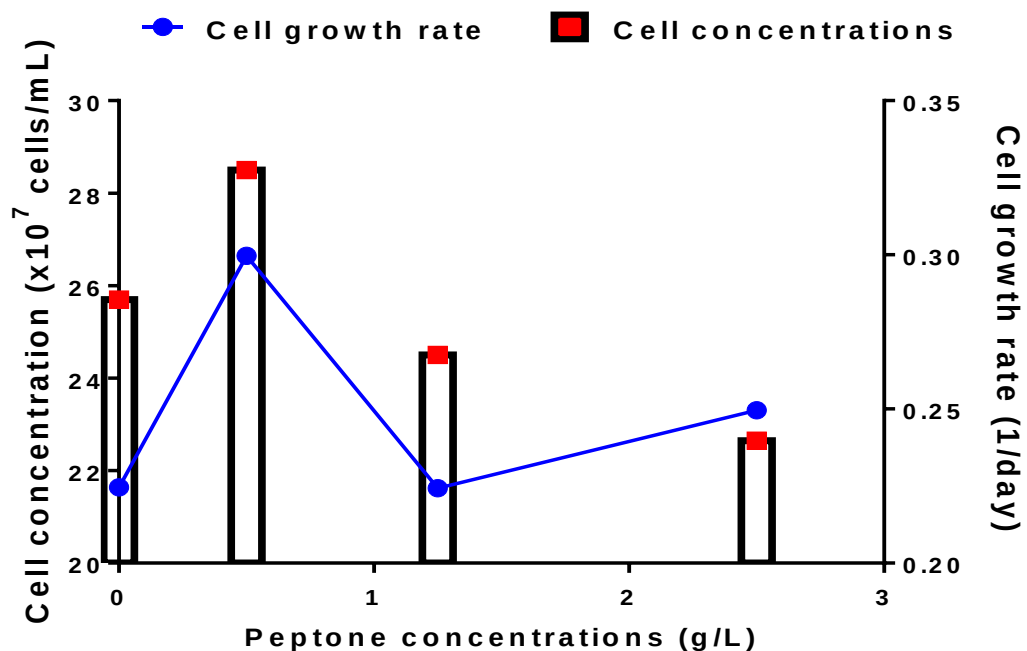


Fig. 3: Effect of peptone concentrations on the growth of *E. gracilis* at 2570 Lux using NPK 15: 15: 15 as the basal medium.

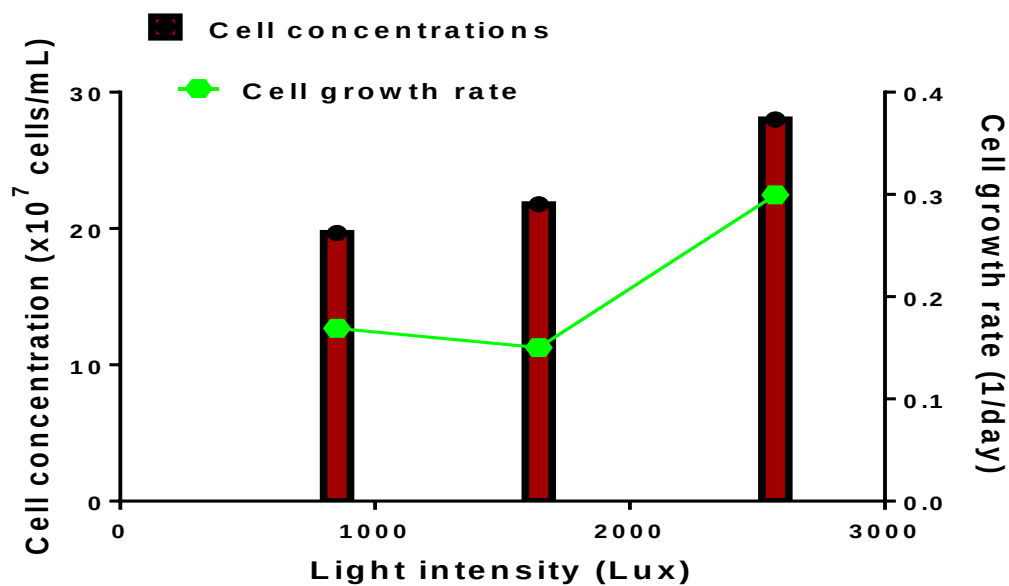


Fig. 4: Effect of light intensity on the growth of *E. gracilis* using NPK 15: 15: 15 as basal medium

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