Effects of Urea and Light Intensity on the Growth of *Chlorella* sp.

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**Abstract**

The cultivation of *Chlorella* sp., isolated from natural pond using urea as a nitrogen source in glass column and fermenter was investigated. The cultivation in modified N–8 medium containing 1000 mgL$^{-1}$ urea at a light intensity of 5000 lux supported good growth of *Chlorella* sp. E1708. The results showed that the maximum cell concentration and cell dry weight were $3.88 \times 10^8$ cells.mL$^{-1}$ and 19.82 gL$^{-1}$ respectively on day 8 of cultivation in a 300 mL glass column. *Chlorella* sp. E1708 exhibited the highest yield, showing maximum cell concentration and cell dry weight of $2.96 \times 10^8$ cells.mL$^{-1}$ and 18.37 gL$^{-1}$, respectively on day 5 of cultivation in the modified medium in a 5 L-fermenter at 100 rpm agitation and 3 Lmin$^{-1}$ aeration.

**Key words**: *Chlorella* sp., urea, light intensity, cultivation, stirred tank reactor

1. Introduction

Microalgae are the primary food source for larval and juvenile bivalves and for the larvae of some crustaceans and fish species in aquaculture. Several species of microalgae have been shown to produce high-value products such as protein [1] polyunsaturated fatty acid [2, 3, 4] antioxidants [5, 6] vitamins [6] bioactive compounds [7, 8, 9, 10] and natural colorants [11, 12, 13, 14]. They represent a valuable source of aquaculture feed. However, large scale cultivation of algae is labor intensive and also associated with various technical difficulties such as open systems and closed systems [15].

The generation of biomass by photosynthethic microalgae cultures varies depending on a number of environmental factors, including temperature, nitrogen concentration, light intensity, etc. in most species [2, 16, 17]. Nitrogen is one of the most important limiting nutrients in the marine environment [18] and nitrogen control is critical for the intensive cultivation of algae due to its role in growth, and regulation of metabolism [19].

Urea as a source of nitrogen is cheaper than nitrate and has no effect on the final chlorophyll content of the cultures [20, 21]. Previous work have demonstrated that *Spirulina platensis* could utilize urea in fed-batch and batch culture [22, 21]. The use of urea for marine microalg *Isochrysis galbana* resulted in higher fatty acid contents than the use of nitrate and nitrite [23] similarly the growth rate and total lipid content of *Chlorella* sp. also varied with the level of urea concentration in during the cultivation [24].

However, among the organic nitrogen sources, urea is the best nitrogen source for culturing *Chlorella* [25] and human urine has be used for production of algae [26].

The aims of this study were to assess the effects of urea concentration and light intensity, under optimized conditions on cell density, dry weight productivity and specific growth rate of *Chlorella* sp. in glass column and the stirred tank reactor.

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2. Materials and Methods

2.1 Microorganisms

Three strains of unicellular green algae, *Chlorella* sp. A0505, *Chlorella* sp. D1708 and *Chlorella* sp. E1708 were isolated from natural fresh water ponds in Bangkok, Thailand. N-8 medium [27] containing 1000 mgL⁻¹ KNO₃ as a nitrogen source was used to maintain the algae to prepare the inoculum.

2.2 Culture methods

The algae cultures were cultivated in 300 ml capacity glass columns each containing 200 mL of N-8 medium. Cultures were stirred by bubbling air at room temperature and kept under continuous illumination of 4000 lux with cool-white fluorescent tubes for 7 days. The highest growth strain of *Chlorella* sp. was selected and maintained in modified N-8 medium with KNO₃ being replaced by urea at 800, 1000, 1200 and 1400 mgL⁻¹ under the same condition were used to investigate the effect of the initial urea concentrations on biomass production. Using the optimal urea concentration in modified N-8 medium, the three light intensities at 3000, 4000 and 5000 lux were used to investigate the effect of light intensity on biomass production.

For the fermenter study, the strain of *Chlorella* sp. was inoculated in a 5 L stirred tank reactor with a working volume of 3 L N-8 medium containing urea as a nitrogen source as described above with an inoculation ratio of 10% (v/v). Agitation was provided by two six-flat-blade impellers located at 3.5 and 8.5 cm, respectively, above the bottom of the vessel. The impeller speeds were 100 and 200 rpm, respectively. The aeration rates were 1 and 2 vvm. (3, 6 Lmin⁻¹, respectively) under continuous illumination (using the light intensity producing the highest biomass production) and a temperature of 25ºC. For all experiments, the pH was initially adjusted to 6.8.

2.3 Growth measurements

Productivity of biomass was calculated by measuring cell density, dry weight and specific growth rate. The cell density was determined using an improved Neubauer haemacytometer and 40 x magnification power of the light microscope. For dry weight determination, culture samples (5 ml) taken at different times were centrifuged at 5000 rpm for 5 min. After rinsing twice with distilled water, the pellets were dried overnight in an oven at 70ºC and then cooled down in a desiccator before weighing.

3. Results and Discussion

Algal growth

Fig.1 shows the growth curves of *Chlorella* sp. strains A0505, D1708 and E1708 in 200 ml cultures. It was evident that under the same cultural conditions among different strains, the cell density and the growth yield of *Chlorella* sp. E 1708 was significantly higher than those of *Chlorella* sp. strains A.0505 and D1708 and *Chlorella* sp. E1708 was thus selected for further studies.
Fig. 1 Growth curves of *Chlorella* sp. strains A0505, D1708 and E1708 cultivated in glass columns using N-8 medium (KNO$_3$ concentration 1000 mgL$^{-1}$) and continuous illumination (light intensity of 4000 lux): (A) cell density; (B) cell dry weight.

**Effect of urea**

The experiments were performed using autotrophic, batch cultures of *Chlorella* sp. E. 1708 in modified N-8 medium; the initial urea concentrations were adjusted to 800, 1000, 1200 and 1400 mgL$^{-1}$. Autotrophic growth of *Chlorella* sp. E 1708 in the various media containing 800 – 1400 mgL$^{-1}$ urea was achieved and the results are shown in Fig. 2. *Chlorella* sp E 1708
Fig. 2 Effect of initial urea concentration on *Chlorella* sp. E1708 cultivated in glass columns under continuous illumination (light intensity of 4000 lux): (A) cell density; (B) cell dry weight.

Exhibited the highest cell density of $3.57 \times 10^8$ cells mL$^{-1}$ and the highest growth yield of 19.09 gL$^{-1}$ at 1000 mgL$^{-1}$ urea concentration on day 8 at the light intensity of 4000 lux. At day 8 the cell density and the growth yield of *Chlorella* sp. E1708 at the urea concentrations of 1200 and 1400 mgL$^{-1}$ were less than those at the urea concentrations 1000 and 800 mgL$^{-1}$. At urea concentrations of 1200 and 1400 mgL$^{-1}$ the biomass obtained was 4.78 and 2.50 gL$^{-1}$ respectively.

Urea concentration had also a strong influence on cell division. Maximal cell density increased with increasing nitrogen concentration up to 1000 mgL$^{-1}$. From Fig.2 it looks as if cell densities were beginning to decline after day 5 for urea concentrations 1200 and 1400 mgL$^{-1}$. According to literature reports, urea is quickly converted to ammonia, the problem of ammonia toxicity at high concentrations of urea may explain the results seen in Fig.2 [20]. This finding is similar to the result previously shown for *Dunaliella viridis* [17] and *Spirulina platensis* [22]. The same behavior has been reported for the cell growth of *Chlorella vulgaris* at higher nitrate concentrations [28]. Adamsson [26] suggested that diluted human urine (2%) could be used as a growth medium for *Scenedesmus acuminatus*.

Effect of light intensity

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Batch cultures of *Chlorella* sp. E 1708 were grown in modified N-8 medium containing 1000 mg L\(^{-1}\) urea under various light intensities. After 8 days of cultivation at 5000 lux, cell dry weight and cell density increased up to 19.82 g L\(^{-1}\) and 3.88 x 10\(^8\) cells mL\(^{-1}\), respectively (Fig. 3). Light intensity is an important factor for the maximal conversion of incident light energy to algal biomass [18]. Light adsorption by unicellular algae is a function of the size, shape and pigment content [29]. Cells grown under saturated light conditions accumulate carbohydrate and triacylglycerals as storage materials [3], resulting in high content of biomass. Similarly, Hu et al. [16] showed increasing cell density of *Chlorococcum littorale* with increasing irradiance using a flat–plate photobioreactor.

Fig. 3 Effect of light intensity on the growth of *Chlorella* sp. E 1708 in glass columns (urea concentration of 1000 mg L\(^{-1}\)) : (A) cell density ; (B) cell dry weight

**Effect of the aeration rate and agitation speed**

The effect of aeration rate and agitation speed on the growth of *Chlorella* sp. E 1708 are shown in Fig. 4. The cultures were grown in a stirred tank reactor (urea concentration of 1000 mg L\(^{-1}\)) at the aeration rate of 1 vvm and 2 vvm and the agitation speed of 100 and 200.
rpm and light intensity of 5000 lux. The highest cell density of $2.96 \times 10^8$ cells mL$^{-1}$ and the dry weight of 18.37 mgL$^{-1}$ were obtained at the lowest aeration rate (1 vvm) and the lowest agitation speed (100 rpm) at day 5. On the other hand, increasing of the aeration rate (2 vvm, 100 rpm) and the impeller speed (1 vvm, 200 rpm) resulted in a decrease of the biomass concentration. This may be explained by the fact that a high aeration rate or a high agitation speed were a limiting factors in stirred tank reactor. Agitation creates shear forces, which affect microorganism such as variation in their growth , product formation and may be damaging to the cell structure [30].

4. Conclusions

The green microalgae Chlorella sp. isolated from natural pond, Chlorella sp.E1708 was demonstrated to posses the ability to grow autotrophically in modified N-8 medium containing urea as a source of nitrogen. In particular, whereas the growth of Chlorella sp.E1708 was significantly influenced by a light intensity of 5000 lux, urea concentration of 1000 mgL$^{-1}$ at 100 rpm agitation and 3 Lmin$^{-1}$ aeration. in a 5 L-fermenter which was a little lower than that in the glass column. These results indicate that Chlorella sp.E1708 is a strain with potential uses the development of a large-scale process that can be coupled with primary food source and urea migration in wastewater.

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References


