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Effect of Tris-(hydroxymethyl)-amino methane on microalgae biomass growth in a photobioreactor

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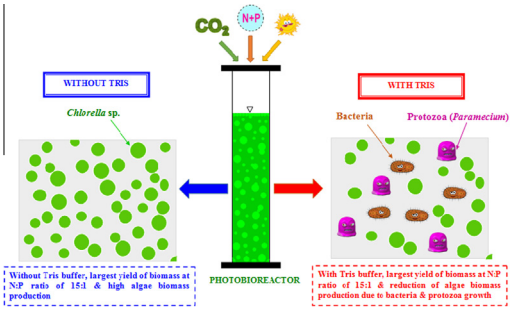
HIGHLIGHTS

- Effect of Tris on microalgae growth was investigated at different *N:P* ratios.
- *Chlorella* sp. performed well in both conditions with and without Tris at N/P of 15.
- Dry microalgae biomass without Tris was 3-fold higher than that with Tris.
- Tris can determine the presence of *Paramecium* in the cultivation of *Chlorella* sp.

GRAPHICAL ABSTRACT



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ABSTRACT

One of the buffers namely Tris (Tris-(hydroxymethyl)-amino methane) was used to increase the growth of microalgae by stabilizing the pH value in microalgae cultures. The objective of this research is to determine the growth rate and biomass productivity of *Chlorella* sp. with and without Tris addition. Both conditions function at various *N:P* ratios cultured in photobioreactors (carbon dioxide of 5% (v/v), light intensity of 3.3 Klux). Daily variations in nutrient removal (nitrogen and phosphorus), cell concentration, DO, temperature and pH were measured for data analysis. The results show that the largest yield of biomass was achieved at the *N:P* ratio of 15:1 with and without Tris. After cultivation lasting 92 h, the algae concentration at this ratio was 1250 mg L^{-1} and 3568 mg L^{-1} with and without Tris, respectively. This indicates that adding Tris to the photobioreactor greatly reduces algae biomass due to bacterial competition.

1. Introduction

The rising global demand for energy is a serious issue with respect to fossil fuel depletion and carbon dioxide emissions linked to global greenhouse scenarios. Carbon dioxide is the most important anthropogenic greenhouse gas. Biofixation is the only economically feasible and environmentally sustainable technology in the long-term (Kumar et al., 2010). This biomass which is produced by converting carbon dioxide can be used to create products of high value, such as fatty acids, biodiesel, biogas, ethanol and organic fertilizers (Lopes et al., 2008; Giordano et al., 2014). It is worth noting that microalgae with 70% oil by weight can produce 23 times more oil compared to oil palm, the current major biofuel producer (Wang et al., 2009). The principle of biological fixation is that carbon dioxide is used to provide the carbon source for microalgae in the photobioreactor. Microalgae are known to contain large amounts of lipids within their cell structure, and so they are increasingly attracting interest as a

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biofuel feedstock. Microalgae photosynthesis will not produce any additional carbon dioxide while energy production and nutrient utilization for their growth can be achieved sustainably (Kumar et al., 2010; Pittman et al., 2011).

Chlorella sp., a genus of green unicellular microalgae which is highly efficiency at removing the nutrient from wastewater (Shi et al., 2007; Imaizumi et al., 2014). It is spherical in shape, about 2–9 μm in diameter and without flagella. Although no useful microalgae have been found to be useful for carbon dioxide sequestration, *Chlorella* sp. has good commercial values and can grow under a high carbon dioxide concentration of 40% (Sakai et al., 1995). It is regarded as one of the most important energy microalgae due to its high protein accumulation, high lipid content and product variation (Das et al., 2011). The lipid content of *Chlorella* sp. is about 32 ± 34% of the dry weight (Chiu et al., 2008). In addition, the carbon dioxide fixation rate is 0.68 mg L^{-1} day^{-1} (David and Prabakaran, 2012). They produce approximately half of the atmospheric oxygen and when used simultaneously the greenhouse gas carbon dioxide can grow photoautotrophically (Melanie, 2013). Indeed, *Chlorella* sp. is suitable microalgae for CO_2 mitigation and biodiesel production.

The process of microalgae cultivation is influenced by many factors. Nitrogen and phosphorous are two of the key factors in algae growth. The relative amounts of such essential nutrients required for growth and reproduce differ among algae species. Furthermore, the type of cultivation conditions for microalgae also need to be considered significantly. Phototrophic cultivation is the most commonly used cultivation condition for microalgae growth (Yoo et al., 2010). Namely photobioreactor is a closed system which is used to cultivate a single-species culture of microalgae for prolonged duration. It produces a large amount of microalgae biomass, which is about 13 times as concentrated as the biomass found in a raceway pond (Chisti, 2007). Bubble column reactors owe their many uses to their excellent mass and heat transfer characteristics. It is easy to construct and operate and has high surface area to volume ratio (Ugwu et al., 2008), and requires only low maintenance costs. However, there may be significant phased back-mixing occurring. It is difficult to scale-up due to the complex interaction between the faces. The sole source of agitation is provided by the isothermal expansion of sparged gas (Chisti et al., 2006).

In previous studies, the cultivation of microalgae has incorporated the use of organic buffers to increase microalgae growth. Tris (Tris-(hydroxymethyl)-amino methane) is a buffer used to stabilize pH in microalgae cultures (Suzana et al., 2008). However, Tris is very controversial because the efficiency of pH stabilization has not been clearly demonstrated (Fabregas et al., 1993). In addition, the harmful effects of Tris have been observed in some phytoplankton species and freshwater algae (Harrison et al., 1980). Previous studies have indicated that Tris impacts on photosynthesis by inhibiting mechanisms such as the transportation of HCO_3^- across the plasma membrane (Axelsson et al., 2000). This buffer also stimulates the growth of bacteria, leading to cultivation being severely curtailed (Fabregas et al., 1993). Nonetheless, it is questionable whether the beneficial effects of Tris are more or less than its effects on photosynthesis. Thus, this research aims to determine the growth rate and biomass productivity of *Chlorella* sp. with and without Tris. Both conditions are operated at various *N*:*P* ratios cultured in a photobioreactor.

2. Methods

2.1. Microalgae strain and culture medium

The microalgae strain used in this study was *Chlorella* sp. which was supplied by The Research Center of Aquaculture II. Ruan et al. (2011) cultivated *Chlorella* sp. in the culture medium with the following solid ingredients: 100 mg L^{-1} $MgSO_4{\cdot}7H_2O$; 50 mg L^{-1} $CaCl_2{\cdot}2H_2O$. The liquid chemicals include: 1 mL L^{-1} glacial acetic acid; 1 mL L^{-1} trace elements solution consisted of 50 g L^{-1} Na_2EDTA; 22 g L^{-1} $ZnSO_4{\cdot}7H_2O$; 0.05 g L^{-1} $CaCl_2{\cdot}2H_2O$; 11.4 g L^{-1} H_3BO_3; 5.06 g L^{-1} $MnCl_2{\cdot}4H_2O$; 4.99 g L^{-1} $FeSO_4{\cdot}7H_2O$; 1.61 g L^{-1} $CoCl_2{\cdot}6H_2O$; 1.57 g L^{-1} $CuSO_4{\cdot}5H_2O$; 1.10 g L^{-1} $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ and 16 g L^{-1} KOH.

2.2. Mass ratio adjustment

In this study involving an experiment to produce a *Chlorella* sp. biomass, the concentration of Tris and NH_4Cl was adjusted to suit the different *N*:*P* ratios (10:1, 15:1, 20:1, 25:1). The remaining chemical component remains unchanged. The concentration of Tris and NH_4Cl was altered but not the concentration of K_2HPO_4 and KH_2PO_4 in the culture medium. The final concentrations of Tris, NH_4Cl, K_2HPO_4, and KH_2PO_4 in the medium are presented in Table 1.

2.3. Bubble column photobioreactor

A diagram of the experimental pilot used in this study is illustrated in Fig. 1. The photobioreactor was covered with a thick wood cover (5 mm) to retain a constant temperature and prevent outside light from affecting it, and to concentrate the light illuminated by three 18 W lamps which were set up in the box. The microalgae were cultivated in two identical columns – scale photobioreactors with a diameter of 100 mm and a height of 600 mm. The working volume in the photobioreactor column was 4000 mL. The aeration system for the reactor consisted of a 20 mm diameter air diffuser which was located at the bottom of the column. The system was operated under the following conditions: temperature of 29 ± 2 °C, 3 Klux of light intensity and 24:0 light-dark cycles (continuous illumination provided by three cool white lamps). Air mixture flow into the photobioreactor was provided via an air pump and a pure carbon dioxide tank through a 6 mm gas tube. With three rotameters which measured the air's flow (from the air pump), the carbon dioxide gas and gas mixture, respectively. The carbon dioxide/air mixture at 2.0 L min^{-1} flow rate was adjusted to achieve an air stream with 5% (v/v) of carbon dioxide. All experiments were carried out in batch mode.

2.4. Relationship between cells density and dry mass

By using both methods together, cells density of *Chlorella* sp. was measured with a counting method and dry biomass was measured by filtering a known volume of culture medium through a 0.45 micrometer filter. It was then dried at 60 °C for 24 h, and the standard curve of cell density and dry biomass were done.

Table 1
Components of synthetic medium.

Components	*N*:*P* ratio			
	10:1	15:1	20:1	25:1
	Without Tris			
NH_4Cl (mg L^{-1})	1339.9	2009.4	2679.3	3349.1
K_2HPO_4 (mg L^{-1})	120			
KH_2PO_4 (mg L^{-1})	60			
	With Tris			
Tris-$H_2NC(CH_2OH)_3$	1346.3	2019.4	2692.5	3365.6
NH_4Cl (mg L^{-1})	744.3	1116.5	1488.7	1860.9
K_2HPO_4 (mg L^{-1})	120			
KH_2PO_4 (mg L^{-1})	60			