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Optimization of environmental factors affecting tertiary treatment of municipal wastewater by *Chlorella protothecoides* in a lab scale photobioreactor

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ABSTRACT

In the present study, a lab scale externally illuminated photobioreactor was used to study the potential of a freshwater microalga *Chlorella protothecoides* (SAG-211-10C) in tertiary treatment of municipal wastewater. The effect of parameters such as: light intensity, photoperiod, pH, concentration of carbon dioxide in air, temperature and aeration rate on nutrient removal efficiencies of wastewater was analyzed. Low irradiance and low photoperiods (2 klux and 8 h:12 h) resulted in poor treatment efficiency (17.97% Chemical oxygen demand,29.44% total nitrogen, 23.91% total phosphorous). Alkaline pH (8,9 and 10) reduced the COD and TN removal efficiency, but improved total phosphorous removal rates. Higher carbon dioxide concentration in air adversely affected COD removal rates (27.97% at 8% CO_2 v/s 69.95% at 2% CO_2). The nutrient removal efficiencies could not be increased by operating the PBR under a non-optimum lower temperature(20°C) or higher temperature (30°C). Under optimum conditions (Light intensity-6 klux, photoperiod–16 h:8 h, pH-6.8, concentration of carbon dioxide in air −6%, temperature–25 °C and aeration rate-3 lpm), highest removal of COD (78.03% on 10th day), 100% removal of TN (on 7th day) and 100% removal of TP (on 6th day) was observed. The highest biomass concentration under optimum conditions was 1.96 g/L.

1. Introduction

The increasing population density in major cities around the world has provided significant pressure on the water treatment facilities having to treat increasing amounts of wastewater. An excessive discharge wastes into water bodies have contributed to eutrophication [1,2]. Eutrophication is a serious environmental problem and has become more widespread since the mid-20th century [3]. Conventional wastewater treatment method provides satisfactory levels of carbon, nitrogen and phosphorous removal but at the expense of high energy consumption and nutrient loss. Data from Aqualia, the third largest wastewater treatment company in Europe, which processes up to 500 Mm^3/year of urban wastewater, indicate that conventional wastewater treatment involves an average energy consumption of 0.5 kWh/m^3, costing 0.2 €/m^3–50% of the cost corresponding to the energy consumed. This company alone removes up to 25,000 t/year of nitrogen and 5.000 t/year of phosphorous to the environment. Utilizing this large amount of nitrogen and phosphorous to produce microalgae would allow the production of up to 0.5 Mt./year of biomass, 20 times higher than the present worldwide production of microalgae biomass [4]. Thus, “Microalgal based wastewater treatment” is an eco-friendly and sustainable option over conventional technologies. However, the C:N:P ratio in wastewater (around 20:8:1) is much lower than algal biomass (around 106:16:1). Hence, addition of CO_2 is one of the effective methods to overcome this limitation and to stimulate algae growth [5].

Several reports have successfully demonstrated this strategy, including cultivation in photobioreactors (PBRs) [6,7] as well as High rate algal ponds (HRAPs) [8,9]. Open ponds culture systems provide an advantage of cheaper construction cost and operation. However, they suffer with disadvantages of low biomass productivity (typical biomass productivity of 4–21 g m^{-2} day^{-1}) [10]. The limitations include temperature fluctuation, low CO_2 transfer, limited light transmission and contamination with other organisms such as protozoa. In contrast, closed culture systems (engineered photobioreactors) have been developed to overcome the limitations of open culture systems. There is less of a contamination problem. Thus, it has higher productivity compared to the open system. For instance, tubular and flat panel photobioreactors have shown the areal biomass productivity of 13–47.7 g m^{-2} day^{-1} for the tubular PBR and 10.2–22.8 g m^{-2} day^{-1}

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Fig. 1. Pictorial view of photobioreactor used in present study.

for the flat panel PBR [11,12]. However, the performance of PBRs is significantly affected by environmental factors such as: light intensity, photoperiod, temperature, concentration of carbon dioxide in air, pH and aeration rate. Adequate optimization of these variables will lead to high performance of PBR in terms of biomass production and nutrient removal capability.

In this regard, in the present work, a lab scale externally illuminated PBR was used to evaluate the nutrient removal performance of microalga *Chlorella protothecoides* (SAG-211-10C) when cultivated in secondary domestic wastewater and supplemented with Carbon dioxide. The influence of aforementioned environmental factors on removal of COD, Total nitrogen (TN), Total phosphorous (TP) was studied and optimum operating conditions were evaluated.

2. Materials and methods

2.1. Construction of photobioreactor

The pictorial view and Process and instrumentation diagram (PID) of Photobioreactor (PBR) used in the present study are shown in Fig. 1 and Fig. 2 respectively. The set up consists of a 5 l borosilicate vessel connected to a panel of straight glass tubes through a diaphragm pump. The panel of straight glass tubes consists of 6 glass tubes (1 inch diameter, 80 cm long) connected to each other through bends, flexible couplings and O-rings. The media from 5 l vessel is circulated through straight glass tubes and returned to the vessel by diaphragm pump. The vessel is equipped with pH, temperature and light intensity controllers. pH electrode (Mettler Tolerdo) and Pt-100 sensor have been used to measure pH and temperature of media in reactor. LED panels are arranged around 5 l vessel and straight glass tubes. The light intensity (0–10 klux) was adjusted from control panel. Two rotameters are provided for controlling flow rate of Carbon dioxide and air (Range of rotameter for air: 1 LPM- 5 LPM, range of rotameter for CO_2: 1 mlPM to 50 mlPM). The two gases are mixed in a mixing module and the CO_2 enriched air was passed through 0.2 µm filter and bubbled through culture in 5 l vessel. The outlet gas from vessel was also passed through 0.2 µm filter before venting to avoid contamination of culture. The desired concentration of CO_2 in air was obtained by adjusting the flow rate of CO_2.

2.2. Collection of algae strains and culture condition

The original strains of *Chlorella protothecoides* (SAG211-10C) was obtained from Sammulung von Algenkulturen (SAG), Germany. It was maintained on agar slants containing BG11 medium [13]. The pH of the medium was adjusted to 6.8. For the preparation of inoculum, 100 ml of sterilized liquid medium was inoculated with cells from slants and the alga was grown in 250 ml flasks at 25°C for 4 days with orbital shaking at 150 rpm. The illumination was provided by 18 W cool white fluorescent light at 2 klux.

2.3. Source and pretreatment of wastewater

Secondary domestic effluent was collected from sewage treatment plant of our institution and filtered with 0.2 µm nylon microfilter to remove microorganisms and fine suspended particles. It was stored at −4 °C until used to avoid contamination. The characteristics of pretreated effluent were determined by standard procedures described in Hach DR 5000 Spectrophotometer Manual [14] and are depicted in Table 1.

2.4. Acclimatization of cells to CO_2 and wastewater

For the adaptation of microalgal cells to high concentrations of wastewater and carbon dioxide, 100 ml of pretreated secondary effluent was inoculated at 10% concentration ($V_{inoculum}/V_{Secondary\ effluent}$), and the initial biomass concentration was adjusted at 0.1 g/L. The cells were incubated at room temperature, aerated with 5% CO_2-air mixture and grown under a light intensity of 5 klux, 12 h:12 h photoperiod, for 8 days. After five subcultures, the cells were fully adopted to CO_2 and full strength wastewater as demonstrated by repeatable nature of growth rate curves.

2.5. Cultivation of algal strains in photobioreactor

2.5.1. Optimization of parameters in PBR

In order to optimize the operating conditions of PBR, various runs were conducted. The ranges of parameters studied are given in Table 2. In a given run, the acclimatized cells of inoculum were aseptically transferred to PBR containing sterilized secondary effluent and the initial biomass concentration was adjusted at 0.1 g/L. The operating conditions of PBR were set in control panel and CO_2 enriched air of known concentration was continuously bubbled through medium. Samples were collected each day and analyzed for biomass, TN, TP, and COD. The run was continued for sufficiently longer time of 10 days to understand the wastewater treatment efficacy of *Chlorella protothe-*