Pilot-scale cultivation of microalgae Chlorella sp. in municipal wastewater using an open pond raceway

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Abstract
Microalgae is one of the prominent sources for biodiesel production. Cultivation of a specific microalgae strain (*chlorella sp.)* in wastewater using a pilot-scale open pond raceway was studied. Daily measurement of the raceway parameters such as pH, optical density and dissolved oxygen indicated that growth phase prolonged for 15 days and pH of the culture stayed on 9.4 for the most part of the period. Dissloved oxygen initially decreased sharply and then rised to approximately 8 mg/l during cultivation. The results showed that municipal wastewater is a suitable culture medium for algal cultivation and is comparable with synthetic cultures in terms of main parameters of growth.

Keywords: Pilot-scale cultivation, Microalgae, open pond raceway.

Introduction
Burgeoning concerns about the greenhouse gas emissions and the global warming has drawn researcher's attention to the development of biofuels as an alternative to fossil fuels.
Among various resources for biodiesel production, microalgae, which almost cover 75% of algae species, are promising choice due to their rapid growth rate, high lipid contents and easier cultivation conditions [1, 2]. The microalgae growth requires light energy, CO_2, necessary nutrients and ambient temperature between 20 to 30 °C [3] as well as a suitable photobioreactor.
Open pond raceways are currently one of the most common open system photobioreactors to cultivate algae on the commercial scale [4]. While CO_2 consumption for both closed system PBRs and open pond raceways is almost the same [5], raceways are economically more favorable than closed system PBRs. Raceways also have lower capital costs and energy requirements [6].
In the present study microalgae *Chlorella sp.* was investigated for pilot-scale cultivation in wastewater using a raceway pond and then examination of variations of physical properties of culture during cultivation period and photosynthesis.

Experimental Algal Strain

The microalgae *Chlorella sp.* was isolated from aerobic pond of Parkandabad wastewater treatment facilities in Mashhad, Iran [7].

Culture Conditions and Inoculations

Due to the high volume of inoculant required for the raceway (50 liters), the inoculant was prepared through a three-step process of successive cultivations. First, 500 ml algal solution was cultured seeding from 50 ml of a stock algal solution. In the second step, the prepared 500 ml solution was used as inoculant to cultivate 5 liters of algal solution. For these two steps 1L glass PBRs were used and air flow consisting of 6% CO_2 at flow rate of 1vvm was sparged into the PBRs. Fluorescent lamps with the intensity of 9000 lux were used as the light source with 16:8 light/dark cycles. In the 3rd step, the prepared 5 liter algal solution was inoculated into a 50-liter flat-plate PBR in which air containing 6% CO_2 was bubbled via a sparger at a flowrate of 0.5 vvm. Illumination was supplied through a set of LEDs with the intensity of 11000 lux for empty vessle and 16:8 light/dark cycles. BG11 Synthetic culture medium was used through all the steps of the inoculant preparation. The 50-liter prepared algal solution was used for inoculation of raceway pond.

Open pond raceway architecture

The open pond raceway used in this study was a stainless steel vessel with the total volume of 500 l. A plexyglass sheet separated the two lanes from each other and mixing and circulation in the raceway were enforced by a motor-driven paddlewheel with 7.5 rpm. The average liquid surface velocity was 15 cm/s. Light energy was supplied through a set of LEDs mounted on a structure that was illuminating from the top. The light intensity was 11000 lux at the distance of 10 cm from the liquid surface with 16:8 light/dark cycles.

Open pond raceway operation

The raceway was used to culture algal strain in municipal wastewater from Parkandabad wastewater treatment facility, Mashhad, Iran. The raceway was filled with 400 l of wastewater and 50 l of algal solution as inoculum. Sodium biocarbonate was used as the carbon source with the final concentration of 4 g/l in a fed-batch mode. Also, the evaporated water was compensated on a daily basis in order to preserve the initial liquid volume of the 450 l. Optical density, dissolved oxygen, and pH of the culture was monitored daily throughout the growth period.

Analysis methods

Optical density was measured at wavelength of 680 nm using a spectrophotometer (UV/VIS-2100, Unico, U.S.A) as the algal biomass growth indicator. The pH value was measured using a pH meter (827 AutoLab, Switzerland). Dissolved oxygen was measured using a standard DO probe (Milwaukee, USA) as a measure of photosynthesis activity.

Results and Discussion

Pilot-scale cultivation of an unidentified strain of *Chlorella sp.* in municipal wastewater was studied aiming at growth bahavior of the species. Algal growth is heavily relied on photosynthesis reaction that converts carbon source, mainly CO_2, to carbohydrate using light energy. As the photosynthesis reaction proceeds, the inorganic carbon source is consumed and the appropriate from of inorganic carbon avaiable in the solution is dictated by pH since

carbon source also plays role of bufferring system. Therefore, pH has a great deal of effect on cultivation condition. Figure 1a demonstrates the pH variation during algal growth. The initial pH value of raw wastewater was 7.66 and after inoculation increased to 7.8. Adding carbon source to the solution, pH value sharply increased to 8.9 and the process started at this value. During the first day of cultivation pH decreased to 8.5 and continued to rise until reached 9.2 after a week. The pH value stayed approximately constant for the rest of cultivation period to day 18. Figure 1b indicates the growth phase of algal strain with two days lag phase at the beginning. Some flactuations are visible on days 10 and 12, but these defficacies might be due to erronuous measurements. The Figure indicates that growth phase was completed on day 15. Figure 1c clearly shows the variation of dissolved oxygen (DO) that is an indication of photosynthsis acitivity. During the first day, DO decreased sharply and at the same time pH dropprd. Raw wastewater has a high value of DO beacause because the swage stream passes through a long canals to reach the facility. Oxygen consumption produce NADH and H^+ resulting in low pH. Due to the surface turbulancy caused by paddlewheel rotaion DO increases, but photosynthesis actvity specifically in dark cycles, keeps the DO at an approximate value between 8 to 10 mg/l.

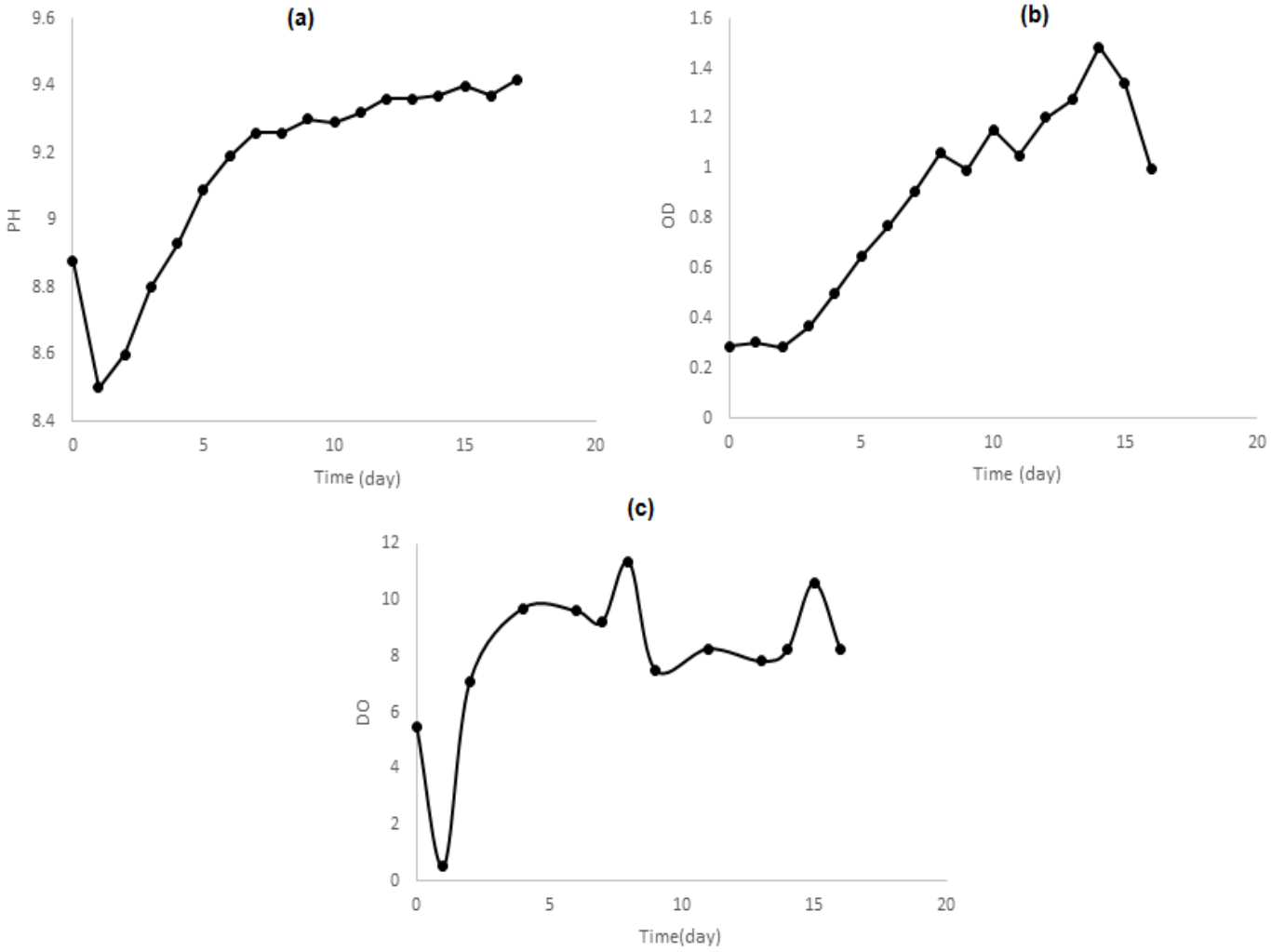

Fig. 1. Daily measurement of raceway parameters: a) pH, b) OD, c) DO

Conclusions

Cultivation of a *Chlorella sp.* in municipal wastewater using a pilot-scale open pond raceway was studied. The results indicated that in pilot raceway growth phase continues on 15 days that is normally longer than growth phase in lab-scale. Also in wastewater, lag phase is expectedly longer due to the complex analysis of the medium. The most important physical parameter i.e. pH changes at the beginning of the process and becomes constant for the most part of cultivation period on 9.4 when bicarbonate is used as carbon source. Also, DO as a measure of photosynthesis changes slightly over time mostly duirng light or dark cycles. The average DO during cultivation was 8 mg/l.

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