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# A Novel Open Raceway Pond Design for Microalgae Growth and Nutrients Removal from Treated Slaughterhouse Wastewater

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**ABSTRACT:** The present work investigates nitrate and phosphate removal from synthetic treated slaughterhouse wastewater in a novel open raceway pond with sedimentation zone. For this purpose, microalgae *Chlorella salina* has been cultivated in synthetic wastewater and sedimentation zone has been added to enhance both algae separation in the system and nutrient removal. The effectiveness of *Chlorella salina* to treat nitrate and phosphate has been tested in open raceway ponds with harvest system. It has been found that Biomass concentration of the *Chlorella salina* is 1.35 g/L during 11 days of experiment. Also, maximum specific growth rate of the species in the pond has been 0.74 day$^{-1}$. Throughout the cultivation period, nitrate and phosphate have been analyzed to show that their average removal efficiencies were 100% and 45%, respectively. It can be concluded that the growth of *Chlorella salina* in novel open pond system is an effective way to reduce nitrate and phosphate levels in slaughterhouse synthetic wastewater. Also, wastewater is suitable for algal growth.

**Keywords:** Nitrate; Phosphate; Sedimentation zone; *Chlorella salina*; Algal growth

## INTRODUCTION

Population growth and ever increasing utilization of resources have caused humans to release more waste products and wastewater, thus rendering wastewater along with its excretion a human challenge in the new era (Park et al., 2010).

Among industrial wastewaters, slaughterhouses wastewater has high pollution. Due to the presence of fat, manure, undigested stomach contact, blood, and intestinal contents, the significant water consumption of slaughterhouses results in large volumes of wastewater (Mittal, 2006), which has high pollution load as well as concentration of organic matter and is suitable for heterotrophic cultivation of the microalgae. Nitrogen (N) and phosphate (P) are usually found in these materials, in favorable ratios of carbon to nitrogen and nitrogen to phosphorous, suggestive of microalgal growth. Also the wastewater shows to contain assimilated compounds, which --in most cases-- there are no toxic compounds or growth inhibitors (Queiroz et al., 2013; Merzouki et al., 2007).

Reports on the use of microalgal-based systems for treatment of agro-industrial wastewater mainly emphasize the influence of operating conditions on the efficiency of

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organic matter and nutrient removal (Chinnasamy et al., 2010; Kuei-Ling et al., 2010; Markou et al., 2011; Riano et al., 2011). However, photobioreactor efficiencies and type of photobioreactor should be assessed.

Tubular photo-bioreactor and raceway pond are two methods for large-scale production (Janssen et al., 2003), with closed photo-bioreactors being more important than open systems, as they allow better controlling the cultivation conditions. Also, closed photo-bioreactors have higher biomass productivities and lower contamination possibility, whereas open raceway ponds have easier operation and construction, even though they are limited in controlling culture conditions. Raceway ponds are the most profitable methods, being very commercial for mass cultivation of microalgae (Ugwu et al., 2008).

Since a large part of the nutrients in algal wastewater treatment systems will end up as biomass, harvesting is crucial in order to separate both nitrate and phosphate from the water; however, due to the small size and slow settling velocity of microalgae, separation of algae from culture medium is a costly part of algal production (Brennan and Owende, 2010; de la Noue et al., 1992). One can both harvest and thicken microalgal by means of several techniques including coagulation–flocculation and sedimentation (Smith and Davis, 2012), flotation, centrifugation, magnetic separation, and electrophoresis (Salim et al., 2011; Danguah et al., 2009; de Godos et al., 2011; Granados et al., 2012). However, in the context of wastewater treatment, only low-cost techniques capable of managing large volumes of water and biomass can be applied, such as sedimentation followed by solid/liquid separation (Milledge and Heaven, 2013). Indeed, sedimentation without adding chemicals is the most common method in full-scale facilities (Garcia et al., 2000), which may lead to a solid concentration in microalgal biomass from 1 to 5% w/w (Smith and Davis, 2012), and is appropriate for downstream processes such as biomass extraction.

The objective of the present study is to evaluate nitrate and phosphate removal from synthetic wastewater in a novel open pond, for which a sedimentation system was constructed and applied in the open pond. Also, microalgae *Chlorella salina* was selected to study nutrients removal from the wastewater in novel open raceway pond system.

## MATERIAL AND METHODS

The microalgae *Chlorella salina* was provided by the University of Agricultural Sciences and Natural Resources, Institute of Genetics and Biotechnology Tabarestan (Sari, Iran). It was cultivated in the BG11 medium (Zamani et al., 2011). The pilot plant has no temperature control system; therefore, the culture temperature will be the laboratory one (25±2 ºC). An important reason to select *Chlorella salina* in this work was its optimal temperature, ranging between 22 and 25 ºC (Akhtar et al., 2008).

The present work used treated slaughterhouse wastewater (Gurel and Buyukgungor, 2011). The composition of the synthetic secondary wastewater was based on the recipe of the BG 11 culture medium modified. The composition of BG 11 medium consists of 0.14 (g/L) $NaNO_3$, 0.024 (g/L) $K_2HPO_4.3H_2O$, 0.075 (g/L) $MgSO_4.7H_2O$, 0.036 (g/L) $CaCl_2.H_2O$, 0.02 (g/L) $Na_2CO_3$, 0.006 (g/L) Citric acid, 0.001 (g/L) EDTA, and 1mL of trace elements solution with the following composition: 2.86 (g/L) $H_3BO_3$; 1.81 (g/L) $MnCl_2.4H_2O$; 0.222 (g/L) $ZnSO_4.7H_2O$; 0.39 (g/L) $NaMoO_4.2H_2O$; 0.079 (g/L) $CuSO_4.5H_2O$; and 0.0494 (g/L) $Co(NO_3)_2.6H_2O$. Table 1 shows the concentration of organic load, nitrate, and phosphate.

**Table 1. Concentration of nitrate, phosphate, and organic load in synthetic wastewater**

| Nutrient | Nitrate | Phosphate | Organic load |
|---|---|---|---|
| Concentration (mg L$^{-1}$) | 100 | 10 | 30 |

The raceway ponds are extensive farming systems that require large sunny tracts, so these plants occupy large areas. The ponds were made of Plexiglas acrylic sheets, a transparent plastic material 0.6 cm wide and easy to use. The ponds were assembled over one wood structure. In this way, raceway pond consists of 4 corridors. Fig. 1 shows the pond size. The illuminated area of culture is 0.383 m$^2$.

In Fig. 2 presents a real image of open race way pond with the sedimentation (right image in Fig. 2) zone. The sedimentation process was activated in the 6th day of the experiment (second cycle of experiment).

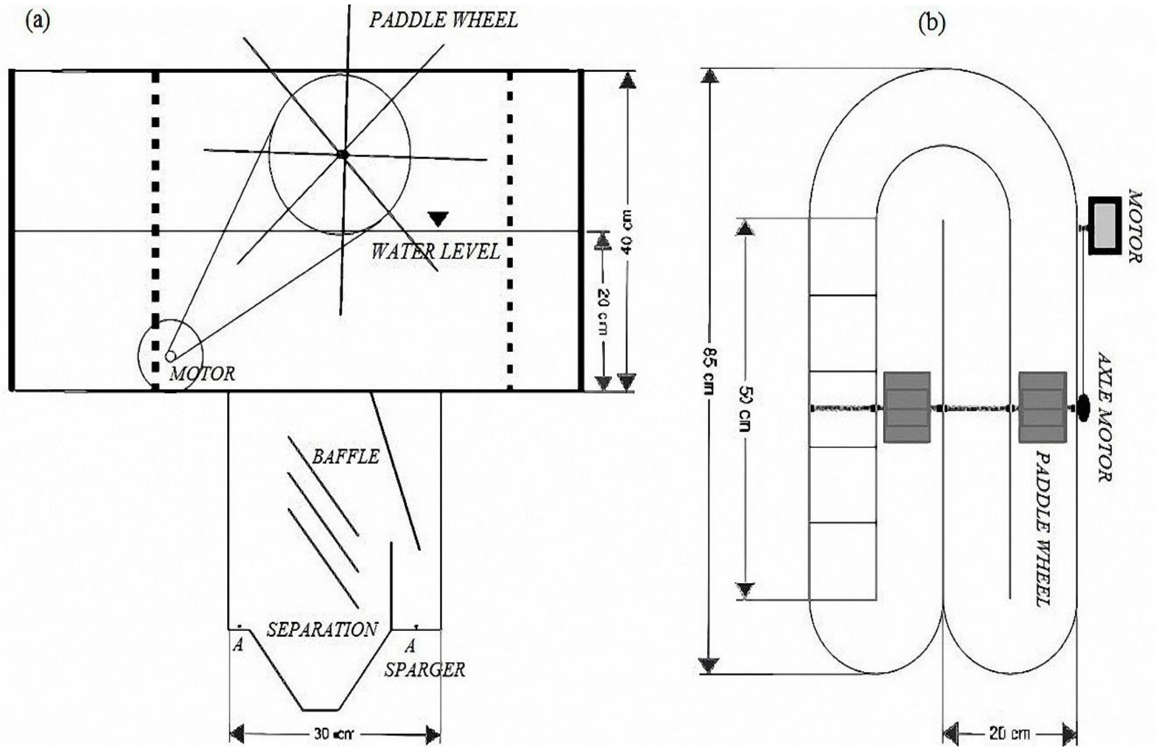

Fig. 1. Schematic diagram of the raceway pond; (a) side view, (b) top view

Fig. 2. Left: open raceway pond; Right: sedimentation zone

To set the depth, it is important to avoid the shadowing effect where the microalgae cells do not allow getting into the culture, in which case the cells in the bottom of the water column do not get well illuminated (Qiang et al., 1996). Therefore, the depth of 30 cm was chosen (Chisti, 2007). Furthermore, working volume of the pond was about 72 L.

The purpose of the lighting system was to simulate the sunlight in outdoor cultures. For this reason, the lamps must offer intensity and color temperature, similar to daylight. This system consists of two lamps with cool white fluorescent lamp (Pars, 36W, FPL) with light intensity being 28 ± 2 μmol photons $m^{-2}s^{-1}$ under 16:8 light-dark cycles (Dayananda et al., 2007).

In order to mix and recirculate the culture, the ponds ought to get stirred constantly, since it is necessary to avoid concentration gradients. Stirring system provides light to the microalgae homogenously. The goal is that the other solution would reduce the shadowing effect, since stirring causes movement into all water columns. Stirring is produced by a couple of Plexiglas paddle wheels; both joined to the same main axle (Fig. 3).

The axle leant on two bearings in the structure. The movement was produced by an electric motor (70 W, 12 V and 5 A) and the speed was kept between 17 and 20 rpm, being transmitted from the motor to the axle by a drive belt. Out of pilot plant in the culture tests, an air compressor supplied $O_2$ and the air was supplied by plastic pipes with a regulator (valve).

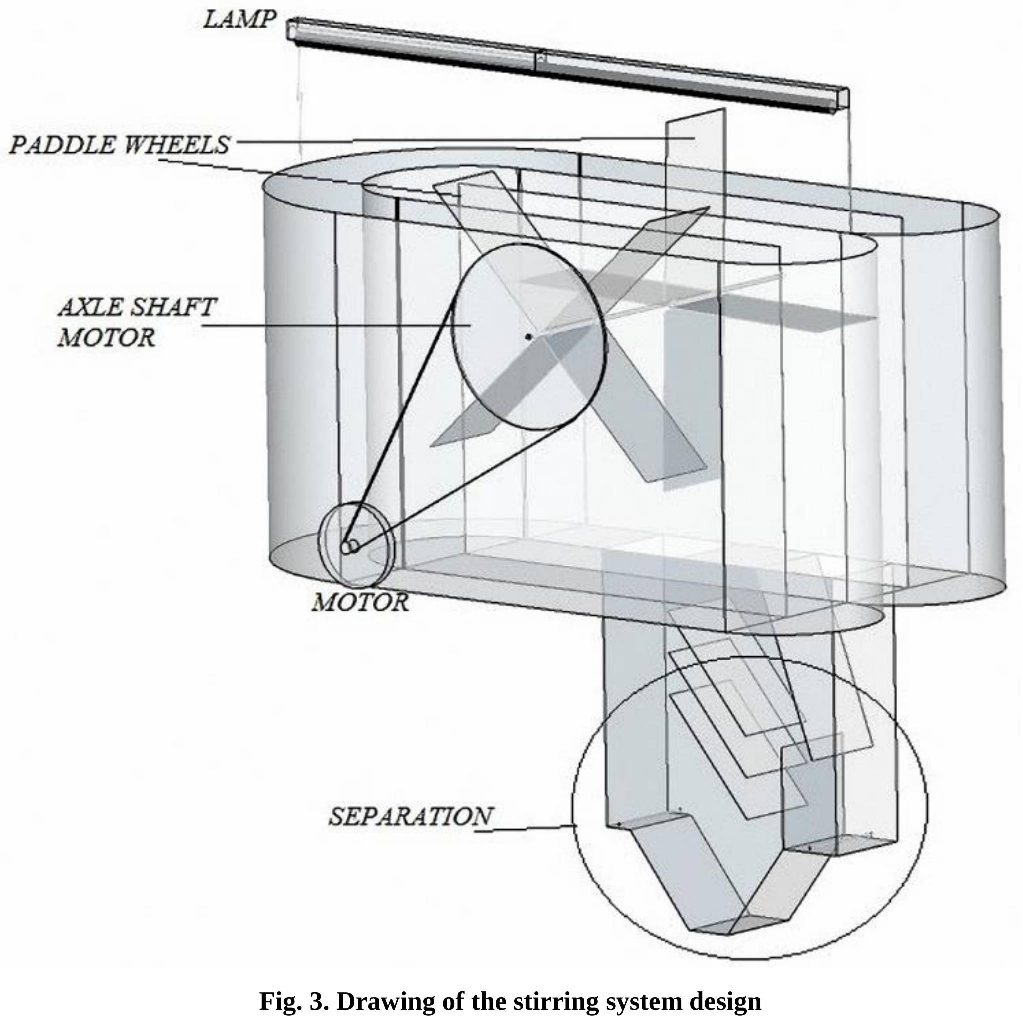

**Fig. 3. Drawing of the stirring system design**

All tests were performed at a temperature of 25±2 °C and the pH was recorded daily. The Optical Density (OD) of the broth was determined by measuring the absorbance at 550 nm (Delavari et al., 2014), in a double beam UV/Vis spectrophotometer (1800, Shimadzu, Japan). To measure biomass dry weight, a 50-mL sample of algal suspension was filtered through a pre-dried and pre-weighed 47 mm Whatman paper filter (GF/F, nominal pore size 0.7 µm) and washed twice with 20 mL distilled water (Delavari et al., 2014). To determine residual nitrate and phosphate, the microalgae was separated by centrifugation (2500g, 10 min), and the resultant material was passed through a membrane filter with a pore size of 0.45 µm and a diameter of 0.0254 m (Shabani et al., 2016). The concentration of nitrate and phosphate was measured with UV-1800 spectrophotometer (Shimadzu, Japan) and wavelength phosphate and nitrate were 690 nm and 220 nm, respectively, in accordance with the standard methods (APHA, 2000).

In order to calculate the specific growth rate, based on the dry weight, Eq. (1) can be used (Tang et al., 2011):

$$\mu = \frac{\ln\left(\frac{X_t}{X_0}\right)}{t} \tag{1}$$

where µ represents the specific rate of growth (day$^{-1}$), while $X_0$ is primary biomass concentration (g L$^{-1}$) and $X_t$, biomass concentration at time t.

## RESULTS AND DISCUSSION

According to the results from Table 2, which were calculated with Eq. (1), the growth rate increased by reducing the concentration of nitrate and phosphate in the synthetic wastewater of the treatment slaughterhouse. As demonstrated, the highest specific growth rate of *Chlorella salina* was 0.74 (day$^{-1}$) in the treatment.

**Table 2. Mean specific growth rate trend in the treatment of *Chlorella salina*.**

| Time (day) | 1-3 | 3-5 | 5-7 | 7-9 | 9-11 |
|---|---|---|---|---|---|
| µ (day$^{-1}$) | 0.74 | 0.53 | 0.15 | 0.19 | 0.04 |

Growth rate of microalgae depend on nutrient concentration and light intensity in the system. Using sedimentation zone enhanced microalgae precipitation; as a result, there was sufficient light for a longer period of time in the system. This phenomenon increased nutrient removal. As can be seen in Table 2, by activating the sedimentation process after the 6th day, the specific growth rate of *Chlorella salina* significantly ascended in synthetic wastewater, and the presence of the sedimentation zone increased the percentage of nitrate removal, causing higher growth of *Chlorella salina* in days 7 to 9. In a study, conducted by Mousavi et al. (2009) on the growth of *Chlorella vulgaris* in different cell densities, it was revealed that less phytoplankton density resulted in lower nitrogen removal efficiency. Also, very high densities of algae in the treatments reduced light penetration, increasing shade effects, which in turn limited the growth and metabolic activities in the phytoplankton cells.

The pilot-scale open raceway pond with sedimentation was tested for slaughterhouse synthetic wastewater. Fig. 4 and 5 show the removal of nitrate and phosphate by *Chlorella salina*, obtained during the experiments with synthetic wastewater. The growth rate increased with the nitrate concentrations. To illustrate this with an example, the dry weight reached 1.35 g/L when supplied with 100 mg/L Nitrate. Within 11 days of exposure, nitrate was almost completely removed from the synthetic wastewater by *Chlorella salina* (Fig. 4). Nitrate-N concentrations in day 5 were 45 mg L$^{-1}$. In a second cycle of exposure (after activation of the sedimentation process in the 6th day), with sedimentation of microalgae inside the deposition section, system turbidity decreased and exposure to the environment increased, and –based on Table 2—specific growth rate rose. Because of this, as shown in Fig. 4, nitrate removal was increased. High removal efficiency of nitrate can be explained by the fact that nitrogen could be used as an energy source for the growth of *Chlorella salina*.

The removal of phosphate from slaughterhouse synthetic wastewater during the 11 days experimental course was tested at different time periods, viz. every other day. Fig. 5 represents the phosphate removal at various time intervals in *Chlorella salina*. As the figure indicates, with the passage of time, the phosphate concentration in the aqueous solution reduced and the lowest phosphate concentration was reported at a concentration of 5.5 mg/L, thereby indicating 45% removal by microalgae *Chlorella salina*.

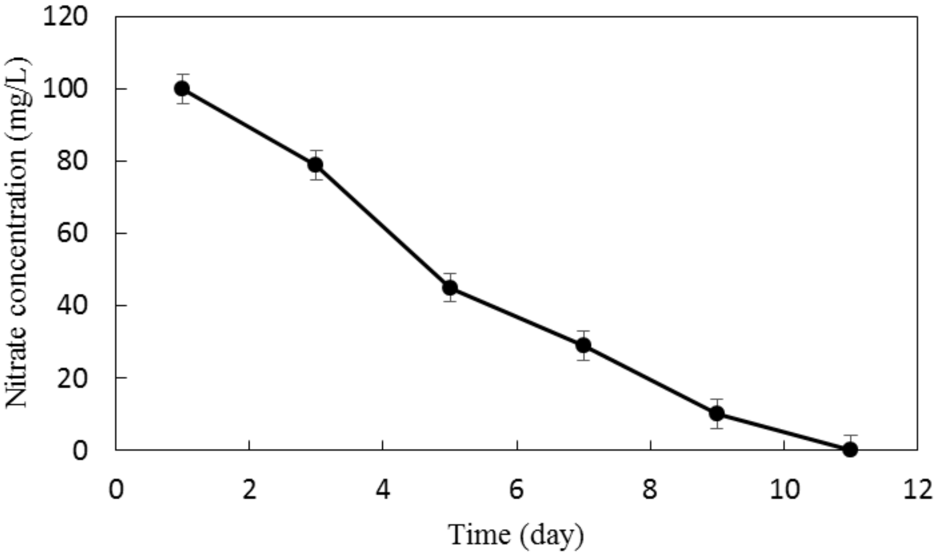

**Fig. 4. Nitrate concentration in open raceway pond**

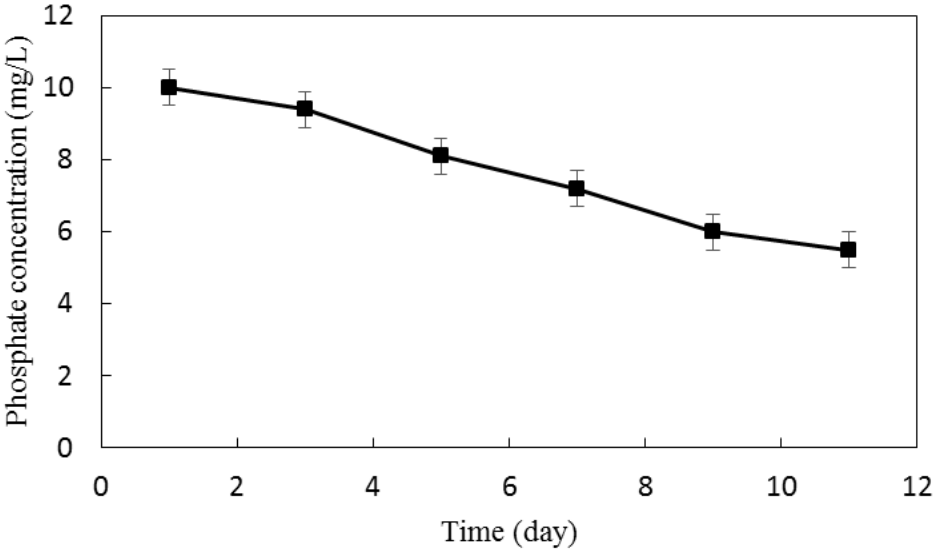

**Fig. 5. Phosphate concentration in open raceway pond**

The production of *Chlorella salina* cells required abundance of such nutrients as carbon, nitrate, and phosphate. Hongyang et al. (2011) reported 77.8%, 88.8%, and 70.3% COD, nitrogen, and phosphorus removal, respectively from soybean processing wastewater via cultivation of microalgae *Chlorella pyrenoidosa*. In another report by Usha et al. (2016), *Scenedesmus sp.* removed 65% nitrate and 71.29% phosphate from pulp and paper mill effluent. This may be attributed to the difference in microalgae species and the wastewater considered.

## CONCLUSION

The new open raceway pond for cultivation of microalgae not only offers an efficient method but also improves efficient utilization of sedimentation box for optimum biomass extraction. The maximum volumetric productivity of *Chlorella salina*, recorded under batch culturing, was 1.35 g/L from slaughterhouse synthetic wastewater.

Results indicate that *Chlorella salina* was potential to completely remove the high concentrations of nitrate and phosphate with an increase in biomass production; however, there is a linear relation between biomass production and nitrate concentration. Moreover, *Chlorella salina* can be safely considered a viable option for tertiary treatment of synthetic treated slaughterhouse wastewater.

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