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Effects of harvesting cell density, medium depth and environmental factors on biomass and lipid productivities of *Chlorella vulgaris* grown in swine wastewater

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HIGHLIGHTS

- Kinetics, light and heat transfers were integrated to predict algal growth in ORPs.
- Optimum condition for *C. vulgaris* was 24 °C, 230 μE m^{-2} s^{-1} light intensity and pH 7.4.
- *C. vulgaris* yields 0.160 g/day in 1 l swine wastewater with 102 mg N and 76 mg P.
- *C. vulgaris* yields 0.191 g/day in 1 l Bold's medium with 100 mg N and 53 mg P.
- Medium depth and cell density in ORPs significantly affect the algal productivity.

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ABSTRACT

A regression model was developed to determine the growth rate of *Chlorella vulgaris* that is affected by the environmental factors of temperature, light intensity and pH value. The optimum environmental condition for growing *C. vulgaris* was experimentally determined at light intensity of 240 μE m^{-2} s^{-1}, 24 °C and pH of 7.4. At the optimum environmental condition, the growth rate of *C. vulgaris* in swine wastewater with 102 mg N/l and 76 mg P/l was 0.160 g/l/day, compared to 0.191 g/l/day for its growth on a modified Bold's medium with 100 mg N/l and 53 mg P/l. The regression model was further integrated with a light and heat transfer model to estimate the biomass productivity of *C. vulgaris* grown on the swine wastewater in an open raceway pond (ORP) with different medium depths and harvesting cell densities under the weather condition in North Carolina yearly around. At 20 cm medium depth, the highest growth rate was 0.162 g/l/day, which was obtained at 0.1 g/l harvesting cell density, 24 °C and 1350 μE m^{-2} s^{-1} solar irradiance in August. If the medium depth increased to 30 cm, the highest growth rate at 0.1 g/l harvesting cell density was 0.156 g/l/day, which was obtained at 23 °C and 1500 μE m^{-2} s^{-1} in June. If the harvesting cell density increased to 0.4 g/l, the highest growth rate decreased significantly to 0.033 and 0.02 g/l/day for 20 cm and 30 cm medium depths, respectively. At 0.1 g/l harvesting cell density, the yearly algal productivity was 80 and 59 t/ha at 30 cm and 20 cm medium depths, respectively. At the average 25% lipid content of *C. vulgaris* grown in swine wastewater, the highest lipid yield was 20 and 14.75 t/ha/year at 30 cm and 20 cm medium depths, respectively.

1. Introduction

There is an increasing interest in the growth of microalgae for the production of biofuels such as biodiesel, bioethanol and biohydrogen (Singh and Gu, 2010). It is well established that microalgal-derived biofuels have a potential to make a significant contribution to the US fuel market, due to several unique characteristics inherent to algae (Davis et al., 2011). But biofuels from microalgae are not yet feasible because of their high cost (Mehrabadi et al., 2014). One of the major costs for algal production includes

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fertilizer and chemical expenses (Mehrabadi et al., 2014). Algae can be grown in wastewater that may be unfit for crop irrigation or municipal use (Sander and Murthy, 2010). Wastewater derived from municipal, agricultural, and industrial activities is a source of nutrients for microalgal cultivation that could significantly reduce the operational costs of algal production systems (Abou-Shanab et al., 2013). Efficient growth of microalgae in wastewater depends on environmental variables and the concentrations of essential nutrients such as N and P (Pittman et al., 2011; Abou-Shanab et al., 2013). The optimal temperature, light intensity and pH for maximum algal growth rate vary among algal species (Park et al., 2011; Rawat et al., 2013). Deviation from the optimum growth condition subsequently leads to the reduction in biomass yield (Rawat et al., 2013). Therefore, the microalgal biomass production in wastewater can be increased by optimizing the environmental factors, and manipulating rate-limiting nutrients.

ORPs have been intensively developed since the 1950s in order to provide an industrial-scale solution for microalgal culture (Farooq et al., 2014). Individual pond areas are up to 1 ha, with an average depth of 20–30 cm (Andersen, 2005). The biggest advantage of these open ponds is their simplicity (Singh and Gu, 2010), and compared with conventional mechanical wastewater treatment technologies, open raceways have lower capital and operating costs (Muga and Mihelcic, 2008). However, the growing season is largely dependent on location and, aside from tropical areas, is limited to the warmer months. Bad weather can often stunt algal growth (Singh and Gu, 2010). Energy production in ORPs is a function of several factors that can be classified into three categories: environmental, operational and biological. Besides the environmental conditions, the operating conditions of the medium depth (Stephen and Yebo, 2015) and harvesting cell density (Christenson and Sims, 2011; Park et al., 2013) also significantly affect the algal productivity of an open pond due to their effects on light distribution and utilization (Rawat et al., 2013; Andersen, 2005). One strategy to optimize light utilization is to reduce the harvesting culture cell concentration. At large scale, harvesting of 25–33% of the culture volume may be required daily to keep the operating culture cell concentration at a low level for viable productivity by continuously harvesting algae and adding fresh medium to enhance the light distribution in the medium (Rawat et al., 2013). Pond depth ranges between 20 and 30 cm (Andersen, 2005), where depths less than 20 cm increase the head loss and so the energy consumption per unit volume (Norsker et al., 2011; Andersen, 2005), and depths over 30 cm decrease the production due to light limitations (Andersen, 2005).

Chlorella vulgaris has been identified as a potential candidate to be grown in wastewater for biofuel production and wastewater treatment (Packer et al., 2011; Xie et al., 2012; Pittman et al., 2011; Ruiz-Marin et al., 2010; Park et al., 2011). Studies have been conducted on piggery wastewater treatment using microalgae (Abou-Shanab et al., 2013). However, no research was found to analyze the effects of harvesting cell density, medium depth and environmental factors of the growth of *C. vulgaris* in swine wastewater. The objective of this study was to generate a regression model to predict the maximum growth *C. vulgaris* and integrate it with a light and heat transfer model to predict the productivity of *C. vulgaris* in ORPs using swine wastewater at different harvest cell densities, medium depths and environmental conditions. A case study was conducted to use the integrated model to predict the annual algal biomass production in Greensboro, North Carolina, using the weather temperature and the average sunlight intensity for each month during a year.

2. Materials and methods

2.1. Microalgae cultivation

2.1.1. Microalgae and inoculum preparation

C. vulgaris (UTEX 2714) was purchased from the UTEX culture collection of algae at the University of Texas at Austin. The algal seed was cultivated using the autoclaved Bold's medium. The seed tubes were incubated in an incubated shaker (AlgaeTron AG 130-ECO, Quibit systems Inc, Canada) at a temperature of 24 °C and light intensity of 100 $\mu E\ m^{-2}\ s^{-1}$ to prepare the pre-culture as recommended by the supplier.

2.1.2. Bold's medium and swine wastewater

For the optimization of the growth environmental condition, the traditional Bold's medium was modified to minimize the effects of nutrients on the algal growth, and to simulate the wastewater nutrient level by increasing the nitrogen concentration from 42 to 100 mg/l. The phosphorus concentration of Bold's medium was not changed due to its high amount at 53.2 mg/l. The modified Bold's medium was thus composed of 25 mg/l $CaCl_2 \cdot 2H_2O$, 75 mg/l $MgSO_4 \cdot 7\ H_2O$, 610 mg/l $NaNO_3$ (250 mg/l originally), 25 mg/l NaCl, 75 mg/l K_2HPO_4, 175 mg/l KH_2PO_4, 8.82 mg/l $ZnSO_4 \cdot 7H_2O$, 1.44 mg/l $MnCL_2 \cdot 4H_2O$, 0.71 mg/l MoO_3, 1.57 mg/l $CuSO_4 \cdot 5H_2O$, 0.49 mg/l $Co(NO_3)_2 \cdot 6H_2O$, 11.42 mg/l H_3BO_3, 50.0 mg/l EDTA, 31.0 mg/l KOH, 4.98 mg/l FeSO4 · $7H_2O$ and 1.0 ml/l H_2SO_4.

Swine wastewater was collected from a lagoon located on the North Carolina A&T State University's farm in Greensboro, North Carolina. The wastewater was then stored in a refrigerator at 4 °C. During the experiments, the wastewater was filtered to remove solid particles, and autoclaved at 121 °C for 20 min. The nutrients of the wastewater were characterized using a colorimeter (LaMotte SMART3) and given in Table 1. The chemical components of nitrogen and phosphorus in the wastewater were expressed in the forms of ammonia-N, nitrate-N and phosphate-P. The other components (Fe, Cu and sulfate) were determined in the previous work (Zhang et al., 2014).

2.1.3. Experimental design

150 ml petri dishes with 1 cm liquid medium depth were used to culture the algae to generate the regression model that is a function of the growth environmental parameters of temperature, light intensity and pH value. The petri dishes with a large open surface and shallow depth of the algal medium were used to minimize the effects of gradients of light, temperature, CO_2 and O_2 on the regression model that may occur in large-scale closed photobioreactors. The sterile dishes were inoculated in a thermostatic incubator at a shaking rate of 50 rounds per minute. A light controller was placed on the top of the shaker-incubator and set at 12 h:12 h light/dark cycle for 10 days. The 5-level-3-factor central composite design (CCD) given in Table 2 was used to analyze the environmental factors of temperature, light intensity and pH value on the regression model. The pH was controlled at a specific value in the range of 6.8–8.0 using the HEPES buffer.

Table 1
Characteristics of the swine wastewater.

Component	Concentration (ppm)	Reference
NH_3-N	22	
NO_3-N	80	
PO_4-P	82	
Fe	2.4	Zhang et al. (2014)
Cu	0.4	Zhang et al. (2014)
sulfate	240	Zhang et al. (2014)