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

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10 **Abstract**

11 A regression model was developed to determine the growth rate of *C.vulgaris*
12 that is affected by the environmental factors of temperature, light intensity
13 and pH value. The optimum environmental condition for growing *C.vulgaris*
14 was experimentally determined at light intensity of $240 \mu Em^{-2}s^{-1}$, 24°C and
15 pH of 7.4. At the optimum environmental condition, the growth rate of
16 *C.vulgaris* in swine wastewater with 102 mg N/l and 76 mg P/l was 0.160 g/l/day,
17 compared to 0.191 g/l/day for its growth on a modified Bold's medium with
18 100 mg N/l and 53 mg P/l. The regression model was further integrated
19 with a light and heat transfer model to estimate the biomass productiv-
20 ity of *C.vulgaris* grown on the swine wastewater in an open raceway pond

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(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 1,350 $\mu Em^{-2}s^{-1}$ 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 1,500 $\mu Em^{-2}s^{-1}$ 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 ton/hectare 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 ton/hectare/year at 30 cm and 20 cm medium depths, respectively.

Keywords: *Chlorella vulgaris*; growth condition optimization; swine wastewater; culture medium depth; open raceway pond; cell density

1. Introduction

There is an increasing interest in the growth of microalgae for the production of biofuels such as biodiesel, bioethanol and biohydrogen [1]. It is

39 well-established that microalgal-derived biofuels have a potential to make a
40 significant contribution to the US fuel market, due to several unique charac-
41 teristics inherent to algae [2]. But biofuels from microalgae are not yet feasi-
42 ble because of their high cost [3]. One of the major costs for algal production
43 includes fertilizer and chemical expenses [3]. Algae can be grown in wastew-
44 ater that may be unfit for crop irrigation or municipal use [4]. Wastewater
45 derived from municipal, agricultural, and industrial activities is a source of
46 nutrients for microalgal cultivation that could significantly reduce the oper-
47 ational costs of algal production systems [5]. Efficient growth of microalgae
48 in wastewater depends on environmental variables and the concentrations of
49 essential nutrients such as N and P [5, 6]. The optimal temperature, light
50 intensity and pH for maximum algal growth rate vary among algal species
51 [7, 8]. Deviation from the optimum growth condition subsequently leads to
52 the reduction in biomass yield [8]. Therefore, the microalgal biomass produc-
53 tion in wastewater can be increased by optimizing the environmental factors,
54 and manipulating rate-limiting nutrients.

55 ORPs have been intensively developed since the 1950s in order to provide
56 an industrial-scale solution for microalgal culture [9]. Individual pond areas
57 are up to 1 hectare, with an average depth of 20 to 30 cm [10]. The biggest

58 advantage of these open ponds is their simplicity [1], and compared with
 59 conventional mechanical wastewater treatment technologies, open raceways
 60 have lower capital and operating costs [11]. However, the growing season is
 61 largely dependent on location and, aside from tropical areas, is limited to
 62 the warmer months. Bad weather can often stunt algal growth [1]. Energy
 63 production in ORPs is a function of several factors that can be classified
 64 in three categories: environmental, operational and biological. Besides the
 65 environmental conditions, the operating conditions of the medium depth [12]
 66 and harvesting cell density [13, 14] also significantly affect the algal produc-
 67 tivity of an open pond due to their effects on light distribution and utilization
 68 [8, 10] One strategy to optimize light utilization is to reduce the harvesting
 69 culture cell concentration. At large scale, harvesting of 25-33% of the culture
 70 volume may be required daily to keep the operating culture cell concentra-
 71 tion at a low level for viable productivity by continuously harvesting algae
 72 and adding fresh medium to enhance the light distribution in the medium [8].
 73 Pond depth ranges between 20-30 cm [10], where depths less than 20 cm in-
 74 crease the head loss and so the energy consumption per unit volume [10, 15],
 75 and depths over 30 cm decrease the production due to light limitations [10].
 76 *C.vulgaris* has been identified as a potential candidate to be grown in wastew-

77 ater for biofuel production and wastewater treatment [6, 7, 16–18]. Studies
78 have been conducted on piggery wastewater treatment using microalgae [5].
79 However, no research was found to analyze the effects of harvesting cell den-
80 sity, medium depth and environmental factors of the growth of *C.vulgaris* in
81 swine wastewater. The objective of this study was to generate a regression
82 model to predict the maximum growth *C.vulgaris* and integrate it with a light
83 and heat transfer model to predict the productivity of *C.vulgaris* in ORPs
84 using swine wastewater at different harvest cell densities, medium depths and
85 environmental conditions. A case study was conducted to use the integrated
86 model to predict the annual algal biomass production in Greensboro, North
87 Carolina, using the weather temperature and the average sunlight intensity
88 for each month during a year.

89 2. Materials and methods

90 2.1. Microalgae cultivation

91 2.1.1. Microalgae and inoculum preparation

92 *C.vulgaris*(UTEX 2714) was purchased from the UTEX culture collection
93 of algae at the University of Texas at Austin. The algal seed was cultivated
94 using the autoclaved Bold’s medium. The seed tubes were incubated in an

95 incubated shaker (AlgaeTron AG 130-ECO, Quibit systems Inc, Canada) at
96 a temperature of 24°C and light intensity of $100 \mu Em^{-2}s^{-1}$ to prepare the
97 pre-culture as recommended by the supplier.

98 2.1.2. *Bold's medium and swine wastewater*

99 For the optimization of the growth environmental condition, the tradi-
100 tional Bold's medium was modified to minimize the effects of nutrients on
101 the algal growth, and to simulate the wastewater nutrient level by increas-
102 ing the nitrogen concentration from 42 to 100 mg/l. The phosphorus con-
103 centration of the Bold's medium was not changed due to its high amount
104 at 53.2 mg/l. The modified Bold's medium was thus composed of 25 mg/l
105 $CaCl_2 \cdot 2H_2O$, 75 mg/l $MgSO_4 \cdot 7H_2O$, 610 mg/l $NaNO_3$ (250 mg/l originally),
106 25 mg/l $NaCl$, 75 mg/l K_2HPO_4 , 175 mg/l KH_2PO_4 , 8.82 mg/l $ZnSO_4 \cdot 7H_2O$,
107 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
108 $Co(NO_3)_2 \cdot 6H_2O$, 11.42 mg/l H_3BO_3 , 50.0 mg/l EDTA, 31.0 mg/l KOH , 4.98
109 mg/l $FeSO_4 \cdot 7H_2O$ and 1.0 ml/l H_2SO_4 .

110 Swine wastewater was collected from a lagoon located on the North Car-
111 olina A&T State University's farm in Greensboro, North Carolina. The
112 wastewater was then stored in a refrigerator at 4°C. During the experi-
113 ments, the wastewater was filtered to remove solid particles, and autoclaved

114 at 121 °C for 20 min. The nutrients of the wastewater were characterized
115 using a colorimeter (LaMotte SMART3) and given in Table 1. The chemical
116 components of nitrogen and phosphorus in the wastewater were expressed in
117 the forms of ammonia-N, nitrate-N and phosphate-P. The other components
118 (Fe, Cu and sulfate) were determined in the previous work [19].

119 2.1.3. *Experimental design*

120 150 ml petri dishes with 1 cm liquid medium depth were used to culture
121 the algae to generate the regression model that is a function of the growth
122 environmental parameters of temperature, light intensity and pH value. The
123 petri dishes with a large open surface and shallow depth of the algal medium
124 were used to minimize the effects of gradients of light, temperature, CO₂
125 and O₂ on the regression model that may occur in large-scale closed photo-
126 bioreactors. The sterile dishes were inoculated in a thermostatic incubator
127 at a shaking rate of 50 rounds per minute. A light controller was placed on
128 the top of the shaker-incubator and set at 12 hour:12 hour light/dark cycle
129 for ten days. The 5-level-3-factor central composite design (CCD) given in
130 Table 2 was used to analyze the environmental factors of temperature, light
131 intensity and pH value on the regression model. The pH was controlled at a
132 specific value in the range of 6.8-8.0 using the HEPES buffer. HEPES buffer

133 was successfully utilized for microalgal culture by several authors [20, 21].

134 The maximum and minimum levels of each environmental factor were
135 chosen based on the data reported in the literature for temperature [17, 22,
136 23], light intensity [24–26] and pH [10]. The DesignExpert (trial version 7.0)
137 was used to design the experiments and analyze the data.

138 The response of the biomass concentration after 10 days as a function
139 of the environmental factors was approximated by the polynomial quadratic
140 regression model, which was given by:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i x_i^2 + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i=1}^k \sum_{j=1}^k \beta_{ij} x_i \beta_{ii} x_j + \varepsilon \quad (1)$$

141 where Y is the biomass concentration in a 10-day period, β_0 is the offset term,
142 β_i is the linear effect, β_{ii} is the squared effect, β_{ij} is the interaction effect, x_i
143 is the i th independent variable and x_j is the j th independent variable [27].

144 2.1.4. Cultivation of *C.vulgaris* in swine wastewater

145 The nitrogen concentration of swine wastewater was as the same level
146 as the modified Bold's medium used for the optimization experiments. The
147 swine wastewater also had a high phosphorus content. Thus, it was assumed
148 that the regression model obtained using the experimental data collected in

149 the modified Bold's medium could be modified with a correction factor and
150 used for estimating the algal production rate in swine wastewater.

151 *C.vulgaris* was grown in the wastewater and modified Bold's medium at
152 the optimum environmental conditions, using a tubular bioreactor including
153 eight tubes, each 2.5 cm in diameter and 80 ml in volume(Z160 MultiCultiva-
154 tor, Qubit systems). The optimum condition which was determined by RSM
155 included light intensity at $240 \mu Em^{-2}s^{-1}$, temperature at 24°C and pH at
156 7.4 which was controlled by injecting CO₂ once a day. The biomass concen-
157 tration of *C.vulgaris* in the Bold's medium and the swine wastewater after
158 10 days were then compared to determine a correction factor for the regres-
159 sion model to predict the algal growth in swine wastewater. This factor was
160 obtained by dividing the biomass yield produced in the swine wastewater by
161 the biomass concentration in the Bold's medium.

162 2.1.5. Analysis of nutrients in growth media and measurement of biomass

163 concentration and lipid content

164 Samples were taken from the growing medium and centrifuged at an ac-
165 celeration of 36 g for 10 minutes to separate the algal cells from the liquid
166 medium. The concentrations of NH₃-N, NO₃-N and PO₄-P in the liquid
167 medium, which were considered as the main forms of nitrogen and phospho-

rus to be assimilated by algal cells, were then measured using a colorimeter
(LaMotte Smart3, Lamotte company, Chestertown, MD, USA).

After the stationary phase was observed, the biomass was separated from
the medium by centrifuging under 36 g acceleration and 25 °C for 10 minutes.
To measure the dry weight of algal biomass, the wastewater was dumped from
the top of the centrifuged samples. The biomass was then sucked up using a
1000 μ l micro pipet and dried at 50 °C in an incubator over night. Biomass
dry weight was calculated by subtracting the total mass of dishes with dry
biomass and the mass of empty dishes. Lipid content of the dry algal biomass
was then measured using the traditional Bligh and Dyer method [28].

2.2. Mathematical modeling of the light and temperature distribution in algal culture medium at different cell densities and medium depth

There is a large variation in the local growth condition in a large ORP.
The light transfer in a culture medium generates a gradient of light intensity
in the culture medium, which decreases from the surface irradiance with the
increase of the medium depth and algal cell concentration. The medium tem-
perature in an ORP is usually different from the temperature of surrounding
air, which is caused by the solar radiative heat absorbed by the medium
heat loss due to water evaporation from the pond, heat loss from the pond

187 wall and convective heat exchange between medium and air. Therefore, it is
188 necessary to develop an integrated regression model, light transfer and heat
189 exchange model to predict the algal productivity in an ORP.

190 *2.2.1. Modeling of light distribution in growth medium*

191 The solar irradiance data set provided by National Renewable Energy
192 Laboratory (NREL) (Figure 1a), represents the light intensity only on the
193 surface of the medium. The light intensity decreases through the medium
194 depth because of the absorption by the microalgae cells. Thus, the light
195 intensity would be lower at deeper levels of the medium in the open pond's
196 channels.

197 Two of the major factors that must be considered in estimation of the
198 algal biomass production using ORPs, are the medium depth and the har-
199 vesting cell density, which affect the light distribution in the open ponds.
200 The delivery and the distribution of the light into the culture medium can
201 be calculated using Beer-Lambert's law (Equation 2), which was usually used
202 to estimate local light intensity within a culture medium [29]:

$$I = I_0 e^{-(k_c + k_m)z} \quad (2)$$

203 where I is the light intensity at a given depth of z , I_0 is the light intensity at
 204 the culture surface, c is the cell density, k_c is the extinction coefficients for
 205 algae cells suspended in swine wastewater, and k_m is the medium extinction
 206 coefficient. k_c and k_m were measured at 2.0 and 0.08 l cm⁻¹ g⁻¹ respectively,
 207 using a spectrophotometer for *C.vulgaris* suspended in the swine wastewater.

208 The average light intensity in the microalgal medium in each divided
 209 zone along the medium depth was used to calculate the algal growth rate
 210 in each divided zone. This method takes into account the shading effects of
 211 microalgae cells in ORP's channels. According to Equation 2, the average
 212 light intensity in a culture medium with a given cell concentration and a
 213 given medium depth can be calculated by [30]:

$$\begin{aligned}
 \bar{I}_n &= \frac{1}{\Delta z} \int_{z_{n-1}}^{z_n} I_{n-1} e^{-(k_c c + k_m)z} dz \\
 &= \frac{I_{n-1} - I_n}{\ln I_{n-1} - \ln I_n}
 \end{aligned}
 \tag{3}$$

217 where $\bar{I}_n$ is the average light intensity at zone n , and Δz is the zone height
 218 $(z_n - z_{n-1})$.

219 *2.2.2. Modeling of temperature distribution in growth medium*

220 As a ORP for algal cultivation is usually a shallow pond, it was assumed
 221 that there was no temperature gradient in the culture medium. The pond
 222 wall was assumed to be well insulated and the heat loss through the pond
 223 wall, Q_w was thus zero. Effects of radiation, evaporation and convection on
 224 the medium temperature were analyzed by the heat balance equation of the
 225 culture medium in an ORP, which can be expressed as:

$$226 \quad \rho c_p A X \frac{dT}{dt} = Q_r - Q_c - Q_e - Q_w \quad (4)$$

227 where ρ is the density (kg/m^3), c_p is the heat capacity ($J/kg K$), A is the
 228 area size of the pond (m^2), X is the depth of the pond (m), T is temperature
 229 ($^{\circ}C$), t is time (s), Q_r is the net solar radiation that is absorbed by the
 230 medium (W) which is the difference between the incident solar radiation on
 231 the pond's surface, $Q_{r\,in}$ and the solar radiation which comes out of the pond,
 232 $Q_{r\,out}$:

$$233 \quad Q_r = Q_{r\,in} - Q_{r\,out} \quad (5)$$

234 Q_c is the convective heat loss from the surface (W), Q_e is the evaporative
 235 heat loss from the surface (W), and Q_w is the heat loss through walls of the

236 pond (W).

237 The fractions of solar radiation on the surface that reaches the depth of
238 x in water is given as [31]:

$$239 \quad h_x = H(0.36 - 0.08 \ln x) \quad (6)$$

240 where x is the thickness of water layer in meters, h_x is the solar radiation
241 in any depth of water (W/m^2), and H is the solar radiation on the surface
242 (W/m^2). Equation 6 is valid from 0.01 to 10 m water depth. Therefore, the
243 amount of solar radiation that is absorbed at a given medium depth can be
244 calculated by:

$$245 \quad Q_{rin} = HA(1 - 0.36 + 0.08 \ln x) \quad (7)$$

246 where x is the depth of pond.

247 Radiation heat loss on the surface can be calculated as:

$$248 \quad Q_{rout} = \sigma \epsilon A (T^4 - T_k^4) \quad (8)$$

249 where σ is the Stefan-Boltzmann's constant ($5.673 \times 10^{-8} W/m^2 K^4$), ϵ is
250 the emissivity of water =0.83, and T_k is the sky temperature (K), that is

251 calculated as an air temperature by:

$$252 \quad T_k = 0.0552T_a^{1.5} \quad (9)$$

253 The convective heat exchange between the medium and air was determined
254 by:

$$255 \quad Q_c = h_c A [T - T_a] \quad (10)$$

256 In equation 10, h_c is the heat transfer coefficient from the water surface to
257 the air (W/m^2) which was calculated as a fraction of the monthly average
258 wind speed, v (m/s):

$$259 \quad h_c = 5.7 + 3.8v \quad (11)$$

260 The heat loss from the surface by water evaporation (Q_e) was given by:

$$261 \quad Q_e = \left(\frac{\lambda h_c (p - p_a)}{1.6 C_s p_{atm}} \right) A \quad (12)$$

262 where λ is the latent heat of vaporization ($334 kJ/kg$ for water), h_c is the
263 convective heat transfer to water, and C_s is the humid heat capacity of air
264 ($kJ/kg.K$).

265 The humid heat capacity of air was determined by:

$$266 \quad C_s = 1.005 + 1.82\gamma_h \quad (13)$$

267 where γ_h is the relative humidity (Figure 1c).

268 The saturated water vapor pressure at the water temperature, p (*mm* Hg),
269 was calculated as:

$$270 \quad p = \exp[18.403 - 3885/(T + 230)] \quad (14)$$

271 The partial pressure of water vapor in the ambient air, p_a (*mm* Hg), was
272 calculated as:

$$273 \quad p_a = \gamma_h \exp[18.403 - 3885/(T_a + 230)] \quad (15)$$

274 where the unit of temperature was in °C.

275 The heat balance equation (Equation 4), was solved using a MATLAB
276 code. The density and specific heat capacity of the culture medium were
277 assumed to be constant at $\rho=1000$ kg/m³ and $c_p=4180$ J/kgK for water. It
278 was assumed that there was no change in medium depth, X , during algal
279 cultivation. The heat balance analysis was conducted for an ORP with a

280 surface area of 1 hectare ($A=1$ hectare= 10000m^2).

281 2.3. Calculation of algal production rate in an ORP

282 It was assumed that the microalgal medium was well mixed, so the ef-
283 fects of cell distribution on local light intensity at a given medium level was
284 neglected. The culture medium in an ORP was divided into N zones along
285 the medium depth. The local algal biomass concentration in each zone was
286 then calculated by the regression model equation (generated from Equation
287 1) using the local light intensity calculated by Equation 3, and the medium
288 temperature calculated by Equation 4. The total algal biomass concentration
289 will be the average of sum of the algal biomass concentration for a ten day
290 period in each divided zone, which was expressed as:

$$291 \quad Y = \frac{\sum_{n=1}^N Y_n}{N} \quad (16)$$

292 where Y_n is the local biomass concentration at zone n .

293 *2.4. Estimation of algal biomass and lipid productivity in North Carolina*
294 *using the integrated regression model, light transfer and heat transfer*
295 *model*

296 The regression model was integrated with the light and heat transfer mod-
297 els to estimate the potential of growing algae on swine wastewater in ORPs in
298 North Carolina yearly around. Solar irradiance and temperature determine
299 the length of the growing period and directly affect algae productivity in a
300 specific region. Figure 1a and 1b show the monthly surface sunlight intensity
301 and temperature in Greensboro, North Carolina, respectively, which were
302 reported by NREL [32].

303 The monthly average solar irradiation, I_0 , and temperature, T_a , in Greens-
304 boro, North Carolina were used as the boundary conditions of the light
305 transfer and heat transfer models to calculate the light intensity and tem-
306 perature profiles in the growth medium at different harvesting cell densities
307 and depths, respectively. These light intensity and temperature profiles were
308 further used in the regression model to calculate the monthly algal biomass
309 productivity. The pH value in the regression model was kept at its optimum
310 value of 7.4, determined by the kinetic model.

311 The potential of algal biomass production in an ORP with two culture

depths of 20 and 30 cm has been estimated. Four different harvesting cell concentrations of 0.1, 0.2, 0.3 and 0.4 g/l were used to estimate the annual biomass production using swine wastewater as the culture medium.

3. Results and discussion

3.1. Growth regression model and optimum growth conditions

Table 3 shows the experimental conditions, and the predicted and experimental responses. Table 4 gives the the results of the Analysis of Variance (ANOVA) for the regression. The regression model is significant with the F -value of 162.11 and the P -value of <0.0001 . The R^2 is 0.9932, which implies this response model could explain 99.32% of the variability. Equation 17 is the generated regression model obtained with RSM analysis:

$$\begin{aligned}
 Y = & -156.77 + 0.21T + .023L + 41.54pH \\
 & + 3.39 \times 10^{-6}T.L + .022T.pH + 1.81 \times 10^{-5}L.pH \\
 & - 8.63 \times 10^{-3}T^2 - 5.09 \times 10^{-5}L^2 - 2.84pH^2
 \end{aligned} \tag{17}$$

The average algal biomass production rate was calculated as:

$$Y(g/l/d) = Y/t \quad t = 10 \text{ days} \tag{18}$$

3.1.1. Light intensity

Figures 2a, 2b show the effect of light intensity on the algal biomass production. As it can be seen, the biomass concentration in a 10-day culture period, increased with the light intensity to a maximum value and then decreased with the further increase of light intensity. Therefore, there is an optimum light intensity for algal growth, which has been confirmed by the well-known Photosynthesis-Irradiance (P-I) curve for analyzing the response of photosynthetically grown cells to light [33]. According to the P-I curve, cell growth increases with light intensity in the light limited region and then decreases with the further increase of the light intensity in the photoinhibited region [33]. The optimum light intensity for *C.vulgaris* which was determined by the regression model was $240 \mu E.m^{-2}s^{-1}$. This value is close to the value previously reported in the literature [34]. Eliminating the effects of self shading of microalgae cells due to shallow culture medium depth used in the experiments, resulted in a maximum light intensity of $400 \mu E.m^{-2}s^{-1}$. Therefore, the regression model is an independent function of local microalgae cell positions, thus the shading effects, and depends on the local average light intensity at different culture medium depths in the pond's channels.

347 3.1.2. Temperature

348 The effects of temperature on the algal biomass production is presented
349 in Figures 2c, 2d. The algal biomass production decreased with very low and
350 high temperatures. As the increase of temperature generally accelerates the
351 metabolic rate of microalgae, very low temperature could inhibit the algal
352 growth. However, CO₂ solubility in water decreases with the temperature,
353 thus decreasing the algal growth rate. Therefore, it is very important to keep
354 the temperature close to an optimum value. The optimum temperature for
355 *C.vulgaris* that was determined by the regression model was 24°C.

356 3.1.3. pH

357 Figures 2e, 2f show the effect of pH on the algal biomass production. It
358 can be seen that the algal biomass production was very low at a very low or
359 high pH value. As the pH value of algal medium tends to increase during the
360 light period, the injection of a proper amount of CO₂ into the algal medium
361 decreases the pH back to the optimum level, as well as increasing the carbon
362 supply, which leads to an increase in the algal biomass production. The
363 optimum pH value for *C.vulgaris* was determined at 7.4 by the regression
364 model.

365 3.2. Cultivation of *C.vulgaris* in swine wastewater

366 *C.vulgaris* was grown in swine wastewater at the optimum environmental
367 condition. The optimum condition was previously determined at the tem-
368 perature of 24°C, light intensity of $240 \mu Em^{-2}s^{-1}$ and pH value of 7.4. Cul-
369 tivations were stopped after a 10-day period as there was no further increase
370 in the algal biomass. The biomass production obtained in swine wastewater
371 was then compared to the algal growth results in the Bold's and modified
372 Bold's medium, obtained at the optimum growth condition.

373 3.2.1. Biomass concentration

374 Figure 3 shows the biomass production of *C.vulgaris* growth in different
375 microalgal media. The microalgae grown in swine wastewater with 102 mg/l
376 nitrogen and 76 mg/l phosphorus at the optimum condition for 10 days
377 achieved its highest biomass production at 1.6 g/l. The biomass produc-
378 tion of the algae grown in the Bold's medium with 42 mg/l nitrogen and
379 53 mg/l phosphorus was 0.86 g/l, whilst the biomass production in the mod-
380 ified Bold's medium with 100 mg/l nitrogen and 53 mg/l phosphorus at the
381 optimum condition was 1.91 g/l. Therefore, at the optimum environmental
382 condition, the biomass production obtained in the swine wastewater was 84%

of that obtained in modified Bold's medium which had similar amounts of N and P. A correction factor of 0.84 was then used to modify the regression model so that the modified regression model can be used to predict the growth of *C.vulgaris* in swine wastewater at different environmental conditions. The decrease of the growth rate in the swine wastewater at the nutrient levels as modified Bold's medium might be caused by the turbidity of the wastewater that decreased the light intensity, the absence of some micronutrients, and the presence of some algal growth inhibitors.

3.2.2. Nutrient removal

The final nutrient concentrations in terms of nitrate-nitrogen ($\text{NO}_3\text{-N}$) and phosphate-phosphorous ($\text{PO}_4\text{-P}$) were analyzed after the cultivation was stopped. *C.vulgaris* could remove 99.6% of nitrogen and 32.6% of phosphorus in the swine wastewater at the optimum growth condition after 10-day growth. Therefore, *C.vulgaris* can significantly utilize the nutrients in the swine wastewater. The swine wastewater had a large excess amount of phosphorus that was needed by the algae.

399 3.3. Temperature distribution in pond

400 Figure 4 shows the profiles of medium average temperature in a pond
401 operating at the annual weather condition given in Figure 1. The difference
402 between the ambient and medium temperatures steadily increases from 0°C
403 in January to 3°C in July and decreases to 0°C in December.

404 3.4. Estimation of algal biomass and lipid productivities in North Carolina

405 The algal biomass and lipid productivities in North Carolina were calcu-
406 lated based on a continuous culture with harvesting cell densities of 0.1, 0.2,
407 0.3 and 0.4 g/l, for two different culture depths of 20 and 30 cm.

408 3.4.1. Biomass productivity

409 As seen in Figures 5a to 5d, the growth rate on a volume basis increases
410 with the increase of light intensity and temperature in the culture medium.
411 The mean light intensity in the growth medium will increase with the decrease
412 of the medium depth and harvesting cell density at a given solar irradiation.
413 The local temperature and light intensity in May, June and July, as shown in
414 Figures 1b and 1a, are close to the optimum environmental growth conditions
415 for *C.vulgaris* ($240 \mu Em^{-2}s^{-1}$, 24°C and pH of 7.4). As it can be seen in
416 Figure 5a, the growth rate between November to February is close to zero for

417 the harvesting cell density of 0.1 g/l. However the number of months with
418 negligible growth increased with the increase of the harvesting cell density.
419 As it can be seen from Figure 5d, at the harvesting cell density of 0.4 g/l, the
420 growth was close to zero from October to March. The highest production
421 rates were 0.162 g/l/day for 20 cm and 0.156 g/l/day for 30 cm medium depths
422 obtained at 0.1 g/l harvesting cell density in May-September (23-25°C and
423 1400-1500 $\mu Em^{-2}s^{-1}$), compared to 0.058 g/l/day for 20 cm and 0.02 g/l/day
424 for 30 cm at 0.4 g/l harvesting cell density.

425 The growth rate on a surface area basis can be calculated by multiplying
426 the productivity on a volume basis by medium depth. At the 0.1 g/l har-
427 vesting cell density, the growth rate on a surface area basis in the open
428 pond with 30 cm depth was 85 ton/hectare/year, which was higher than
429 65 ton/hectare/year for the 20 cm depth. By increasing the harvesting cell
430 density to 0.4 g/l, the growth rate on a surface area basis in the open pond
431 with 20 cm depth was 14 ton/hectare/year, which was higher than 5 ton/hectare/year
432 for the 30 cm depth.

433 Figure 6 shows the monthly biomass yield for one hectare surface area,
434 at both 20 and 30 cm medium depths. The highest monthly biomass yield
435 in the open pond with the 30 cm medium depth was 14.1 ton/hectare ob-

436 tained at 0.1 g/l harvesting cell density in June-August, which was higher
437 than 9.6 ton/hectare for the 20 cm medium depth. Without manipulating the
438 growth environmental conditions, the yearly biomass yields for one hectare
439 surface area were 59 tons for 20 cm and 80 tons for 30 cm medium depths. The
440 major challenge in designing a microalgal production plant in this region is
441 the high variation in light irradiance and temperature between summer and
442 winter. Since swine wastewater can provide sufficient nutrients through fed-
443 batch operation, low light intensity and temperature are the two barriers to
444 achieve high algal biomass production from November to February.

445 3.4.2. *Lipid production*

446 An average total lipid content of 25% were used to calculate the total
447 lipid production for *C.vulgaris* grown in swine wastewater. Similar values
448 have been reported for small scale systems by other authors [35]. In this
449 work, it was assumed that the value of 25% is valid for all the growth con-
450 ditions yearly around. Based on these data, the highest lipid yield is ob-
451 tained for the system with the harvesting cell concentration of 0.1 g/l and the
452 medium depth of 30 cm which was calculated at 20 ton/hectare/year (out of
453 80 ton/hectare/year biomass). The lipid yield for the same condition for the
454 system with a medium depth of 20 cm is calculated at 14.75 ton/hectare/year

455 (out of 59 ton/hectare/year biomass).

456 4. Conclusion

457 The main environmental growth factors including temperature, light in-
458 tensity and pH for *C.vulgaris* were analyzed by utilizing RSM. The optimum
459 growth condition was found at $240 \mu Em^{-2}s^{-1}$, 24°C and pH of 7.4, using
460 the modified Bold's medium with 100 mg/l nitrogen and 53 mg/l phospho-
461 rus. The experimental data were used to generate a regression model with
462 a R^2 of 0.9932 for the predict the biomass productivity as a function of
463 growth environmental condition. The microalgae grown in swine wastewa-
464 ter with 102 mg/l nitrogen and 76 mg/l phosphorus at the optimum condition
465 achieved the average growth rate at 0.160 g/l/day, compared to 0.191 g/l/day
466 for the of the algae grown in the modified Bold's medium with 100 mg/l nitro-
467 gen and 53 mg/l phosphorus at the same condition. Therefore, a correction
468 factor of 0.84, which was the ratio of 0.160 g/l/day and 0.191 g/l/day growth
469 rates of the algae in swine wastewater and modified Bolds medium at the
470 optimum environmental condition, respectively, was used in the regression
471 model that was integrated with a light and heat transfer model to estimate
472 the algal productivity in swine wastewater. In an ORP, the growth rate

473 on a volume basis increased with the increase of light intensity and tem-
 474 perature in the culture medium. The mean light intensity in the growth
 475 medium also increased with the decrease of the medium depth and harvest-
 476 ing cell density at a given solar irradiation. The highest production rates
 477 were 0.162 g/l/day for 20 cm and 0.156 g/l/day for 30 cm medium depths
 478 obtained at 0.1 g/l harvesting cell density in North Carolina in Summer with
 479 a temperature from 23-25°C and solar irradiance of 1400-1500 $\mu Em^{-2}s^{-1}$.
 480 However, if the harvesting cell density increased to 0.4 g/l, the highest pro-
 481 duction rates decreased to 0.058 g/l/day for the 20 cm medium depth and
 482 0.02 g/l/day for 30 cm medium depth at the same location. The algal produc-
 483 tivity was 80 ton/hectare/year in the open pond with 30 cm medium depth,
 484 which was higher than 59 ton/hectare/year for the open pond with 20 cm
 485 depth. At the harvesting cell density of 0.4 g/l, the annual algal biomass
 486 productivities were only 14 ton/hectare/year for the 20 cm medium depth,
 487 which was higher than 5 ton/hectare/year for the 30 cm depth. By assuming
 488 a 25% lipid content in *C.vulgaris* biomass, the highest lipid productivities
 489 were 20 ton/hectare/year and 4.75 ton/hectare/year for the medium depths
 490 of 30 cm and 20 cm at the harvesting cell density of 0.1 g/l, respectively.

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Figure 1: The annual weather data in Greensboro, North Carolina; (a) Average monthly solar irradiance ($\mu Em^{-2}s^{-1}$) (Reported by NREL); (b) Average daily temperature for each month ($^{\circ}C$) (Reported by NREL); (c) Average relative humidity yearly around, reported by University of North Carolina at Chapel Hill (UNC Chapel Hill); (d) Average annually wind speed (Reported by UNC Chapel Hill)

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Figure 6: Monthly productivity for one hectare surface area

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	[19]
Cu	0.4	[19]
sulfate	240	[19]

Table 2: Independent variables and levels used for response surface design

Independent variables	Symbols	Levels				
		-1.68	-1	0	1	1.68
Temperature ($^{\circ}\text{C}$)	x_1	10	16	24	32	38
Light intensity ($\mu\text{E}/\text{m}^2.\text{s}$)	x_2	40	120	240	350	430
pH	x_3	6.8	7.1	7.4	7.7	8.0

Table 3: Experimental and predicted algal biomass concentration over a 10-day growth period

Experiment	Coded independent variable levels			Actual values of variables			Biomass concentration (g/l)	
	x ₁	x ₂	x ₃	Temperature (°C)	Light intensity $\mu E/m^2s$	pH	Experimental	Predicted
1	-1	-1	-1	16	120	7.1	0.80	0.76
2	+1	-1	-1	32	120	7.1	0.08	0.08
3	-1	+1	-1	16	350	7.1	0.84	0.74
4	+1	+1	-1	32	350	7.1	0.01	0.08
5	-1	-1	+1	16	120	7.7	0.72	0.664
6	+1	-1	+1	32	120	7.7	0.095	0.20
7	-1	+1	+1	16	350	7.7	0.64	0.65
8	+1	+1	+1	32	350	7.7	0.15	0.20
9	-1.68	0	0	10	240	7.4	0.7	0.70
10	+1.68	0	0	38	240	7.4	0.01	-0.27
11	0	-1.68	0	24	40	7.4	0.014	-0.02
12	0	+1.68	0	24	430	7.4	0.01	-0.03
13	0	0	-1.68	24	240	6.8	1.13	0.87
14	0	0	+1.68	24	240	8.0	1.26	0.89
15	0	0	0	24	240	7.4	1.92	1.91
16	0	0	0	24	240	7.4	1.9	1.91
17	0	0	0	24	240	7.4	1.94	1.91
18	0	0	0	24	240	7.4	1.85	1.91
19	0	0	0	24	240	7.4	1.85	1.91
20	0	0	0	24	240	7.4	1.93	1.91

Table 4: ANOVA for the fitted quadratic polynomial model for optimization of microalgae biomass concentration

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value (prob>F)
Model	13.97	9	1.55	89.23	<0.0001 (significant)
x_1 (Temperature)	1.07	1	1.0	61.53	<0.0001
x_2 (Light intensity)	2.61E-4	1	2.61E-4	0.015	0.9049
x_3 (pH)	6.69E-4	1	6.69E-4	0.038	0.8484
$x_1 x_2$	9.11E-5	1	9.11E-5	5.23E-3	0.9437
$x_1 x_3$	0.024	1	0.024	1.37	0.2666
$x_2 x_3$	6.12E-6	1	6.12E-6	3.52E-4	0.9854
x_1^2	5.55	1	5.55	319.04	<0.0001
x_2^2	7.93	1	7.93	455.91	<0.0001
x_3^2	1.51	1	1.51	86.74	<0.0001
Residual	0.17	10	0.17		
Lack of fit	0.072	5	0.072	0.7	0.6474 (not significant)
Pure error	0.10	5	0.02		
cor Total	14.14	19			
R ² =.9877					