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Bioremediation of Swine Wastewater and Biofuel Potential by using *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, and *Chlamydomonas debaryana*

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Abstract

Four commercial microalgae strains of *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, *Scenedesmus dimorphus* and *Neochloris oleoabundans*, and a local strain of *Chlamydomonas debaryana* were studied in this research. It's found that *S. dimorphus* and *N. oleoabundans* were unable to grow on the swine wastewater. The optimal culture conditions for *C. vulgaris* and *C. reinhardtii* were found to be (600 μ mol $m^{-2}s^{-1}$ and 25 ℃) and (300 μ mol $m^{-2}s^{-1}$ and 20 ℃), respectively. The growth kinetics was determined using optical density (OD) method and flow cytometry. Under the optimal culture conditions, the highest specific growth rates were found to be 1.336 day^{-1} and 1.286 day^{-1} for *C. vulgaris* and *C. reinhardtii*, respectively. The removal efficiency of nutrients from the wastewater is a function of microalgae growth. When comparing a local *C. debaryana* strain with these two commercial strains, the final biomass yields and lipid contents were 1.25 g/L and 15.2% (of the total cell dry weight), 0.86 g/L and 19.7%, and 0.73 g/L and 21.7% for *C. vulgaris, C. debaryana* and *C. reinhardtii*, respectively. In addition, the lipids from these microalgal strains contain a variety of fatty acids, which are suitable for the biofuel production.

Keywords: Microalgae; Bioremediation; Swine wastewater; Growth rate; Lipid

Introduction

Nowadays, it is truism to recognize that the pollution problem is a major concern of the society. Environmental laws are given general applicability and their enforcement has been gradually stricter [1]. The strategic importance of fresh water and air is universally recognized more than ever before. Issues concerning sustainable water management and air pollution can be found almost in every agenda all over the world. There are few things invented which can be used to mitigate both water and air pollution. Microalgal technology is one of them which can be used to reduce these crises as it ensures sustainable management of both air and water.

Microalgae are a diverse group of prokaryotic and eukaryotic photosynthetic microorganisms, typically found in freshwater and marine systems [2]. Algae are essential to global carbon, nitrogen and sulfur cycling. Approximately 45% of photosynthetic carbon assimilation is achieved by algae [3]. In addition, microalgae are considered as a promising feedstock for biofuel production. The oil yield of some algal stains is 1,000-4,000 gallons of oil/acre/yr whereas soybeans and other oil crops can generate 48 gallons of oil/acre/yr approximately. As an energy crop, microalgae do not compete with grain crops for limited arable lands because they may grow in water.

Bioremediation of wastewater using algal strains such as *Chlorella* and *Dunaliella*, spans over 75 years [3]. Algae bioremediation was applied for a range of purposes, some of which are used for the removal of coliform bacteria, reduction of chemical and biochemical oxygen demand (COD, BOD), removal of N and/or P, and also for the removal of heavy metals [1]. A number of microalgal strains including *Ourococcus multisporus*, *Nitzschia cf. pusilla*, *Chlamydomonas mexicana*, *Scenedesmus obliquus*, *Chlorella vulgaris*, *Chlorella zofingiensis*, and *Micractinium reisseri* had been used to treat municipal wastewater treatment effluents, livestock wastewater, agro-industrial wastewater and industrial wastewater [4-6]. Growing algae requires the consideration of three primary nutrients: carbon, nitrogen, and phosphorus. Micronutrients required in traceable amounts include silica, calcium, magnesium, potassium, iron, manganese, sulfur, zinc, copper, and cobalt, although the supply of these essential micronutrients rarely limits algal growth when wastewater is used.

Therefore, the ultimate objective of this research is to optimize the growth

conditions and determine growth kinetics of selected microalgal strains to assimilate nutrients in wastewater for swine wastewater treatment and bioenergy production. The growth characteristics and composition of microalgae are known to significantly depend on the cultivation conditions, such as light intensity, photoperiod, temperature, CO_2 flow rate, nutrient composition, etc [7]. In order to optimize the growth and lipid content of microalgae, it is required to combine all growth parameters in a systemic way so that the efficiency of an algal growth system can be maximized. In this study, the effects of cultivation temperature, light intensity, and nutrient removal on microalgae growth were observed.

Materials and Method

Microalgae strains and pre-culture conditions

Four microalgae strains of *Chlorella vulgaris* (UTEX 2714), *Chlamydomonas reinhardtii* (UTEX 90), *Scenedesmus dimorphus* (UTEX 1237) and *Neochloris oleoabundans* (UTEX 1185) were obtained from the Culture Collection of Algae at the University of Texas at Austin (UTEX). *Chlamydomonas debaryana* AT24 was isolated from the lagoon at the farm of the North Carolina A&T State University (NC A&T). *C. vulgaris, S. dimorphus,* and *C. debaryana* were preserved in the Proteose medium. *C. reinhardtii* and *N. oleoabundans* were preserved in the Soil Extract medium. Proteose medium consists of the following ingredients: $NaNO_3$ (2.94 mM), $CaCl_2.2H_2O$ (0.17 mM), $MgSO_4.7H_2O$ (0.3 mM), K_2HPO_4 (0.43 mM), KH_2PO_4 (1.29 mM), NaCl (0.43 mM), and Proteose Peptone (1g L^{-1}). Soil extract medium consists of the following ingredients: $NaNO_3$ (2.94 mM), $CaCl_2.2H_2O$ (0.17 mM), $MgSO_4.7H_2O$ (0.3 mM), K_2HPO_4 (0.43 mM), KH_2PO_4 (1.29 mM), NaCl (0.43 mM), and soil water (40ml L^{-1}).

Culture of microalgae with swine wastewater

Swine wastewater was collected from a swine growing facility at the NC A&T farm, and used as the substrate to cultivate microalgae. Pretreatment of swine wastewater was carried out by sedimentation and filtration with a Whatman quantitative filter paper (8 μm pore) to remove large, non-soluble particulate solids. After filtration the wastewater was autoclaved for 15 min at 121 ℃.

In order to investigate the effect of environmental factors on microalgae growth, a AlgaeTron Multi-Cultivator MC 1000 photobioreactor (Brno, Czech Republic) was used. It consists of eight 100 ml tubular bioreactors. The temperature of the bioreactors is controlled centrally, and each bioreactor is independently illuminated by an array of white LEDs. Air is bubbled through each tube at the flow rate of 100 ml/min.

The autoclaved wastewater was used accordingly as medium for algal cultivation. Subsequently, 72 ml of swine wastewater and 8 ml of seed microalgae suspension were introduced into each photobioreactor. In all cases, microalgae were cultured in the swine wastewater for 15 days varying light intensity (300, 600 and 900 μmol $m^{-2}s^{-1}$) and temperature (20, 25 and 30 ℃). The experimental designs are shown in Table 1. All experiments and analyses were performed in triplicate or duplicate.

Nutrients analysis

After 15-day cultivation, microalgal cells from each photobioreactor were collected and centrifuged at 4000 × G and 20 ℃ for 15 min. Supernatants from the centrifuge were separated to check the nutrient removal from the wastewater. Supernatants were filtered using a 0.45 mm nylon membrane filter. Then, the filtrates were appropriately diluted and analyzed for COD, ammonia nitrogen and total phosphorus according to the Lamotte Smart 3 colorimeter manual.

Nutrient removal ratio was defined as following:

$$Removal\ ratio\ (\%) = \frac{\text{Nutrient content in the wastewater after algae cultivation}}{\text{Nutrient content in the original wastewater}} \times 100\% \quad (1)$$

Determination of microalgae growth

Microalgae growth was monitored by measuring the optical density at 680 nm every other day. A correlation between the optical density of microalgae at 680 nm and the cell number was determined by using a Guava easyCyte HT 5 Flow cytometer (Billerica, Massachusetts) and Spectrophotometer.

The specific growth rate μ in the exponential phase of algal growth was calculated by using equation (2) [8]:

$$\mu = \frac{\ln (N_2 - N_1)}{t_2 - t_1} \quad (2)$$

where, N_1 and N_2 are defined as the cell number concentration (cell/ml) at time t_1 and t_2, respectively. The time required to duplicate the cell number: division rate (k), was calculated according to the equation (3):

$$k = \frac{\mu}{\ln 2} \quad (3)$$

Lipid extraction

A modified Christie method [9] was applied to quantify the amount of total lipid content. Approximately 0.1-0.2 g dried microalgae were weighed into clean screw-top glass tubes and 5 ml of a 2:1 chloroform-methanol (v/v) mixture was added. The tubes were incubated at 65 ℃ for 1 hr. Then the mixture was centrifuged at 4000 × G for 10 min, after which the supernatant was collected, and the leftover was re-extracted following the same procedures until the supernatant became colorless. All supernatants were pooled, and then evaporated by blowing nitrogen gas through the tube. The lipid was gravimetrically quantified.

Fatty acid methyl ester (FAME) content analysis

Extracted algal lipids (~20 mg) were weighed into clean, 20 ml screw-top glass tubes, to which 4 ml fresh solution of a mixture of methanol, concentrated sulfuric acid, and chloroform (1.7:0.3:2.0 v/v/v) was added. The tubes were closed tightly with Teflon tape to avoid leakage, and then weighed. For transesterification, tubes were placed inside a heating block at temperatures of 90 ℃ and heated for 60 min. On completion of the reaction, the tubes were cooled down to room temperature and weighed again to dismiss leaking samples. Then, 1 ml distilled water was added into the mixture, and the mixture was thoroughly vortexed for 1 min. After the formation of two phases, the lower phase containing FAME was transferred to a 1.5 ml GC vial. Samples were stored in the freezer (-20 ℃) until GC-MS analysis [10].

Chemical compositions of the liquid products were identified using a GC-MS (Agilent 7890A gas chromatography with a HP-5MS capillary column /5975c mass spectrometer). The GC was programmed at 60 ℃ for 4 min and then increased at

10 ℃/min to 280 ℃, and finally held with an isothermal for 5 min. The injector temperature was 250°C, and the injection size was 1 μl. The flow rate of the carrier gas (helium) was 1 ml/min. The ion source temperature was 230 ℃ for the mass selective detector. The compounds were identified by comparison with the NIST Mass Spectral Database [11].

Elemental and compositional analysis of microalgae

A Perkin-Elmer 2400 Series II CHNS/O elemental analyzer (Waltham, Massachusetts) was used to analyze the elemental compositions of microalgal biomass. The protein content of microalgae was determined by Dumas method [12]. The moisture content of the biomass was determined by the method of LAP #001, which is the laboratory analytical procedures (LAPs) developed by the National Renewable Energy Laboratory[13, 14]. The ash content of the biomass was determined by the method of LAP #005 [15].

Statistical Analysis

The statistical significance of the cultivation temperature and light intensity on the algal cell density was assessed using ANOVA F-test. The experimental data measured under each condition were pooled together. The significance test was done for each factor using one-way ANOVA. The null hypothesis is that the population means measured at different levels of the experimental factor are all equal. The alternative is that they are not all equal, or the factor is significant. The statistical significance level cut-off was chosen as $P = 0.05$.

Results

Microalgae growth

During the preliminary tests of four commercial microalgae strains, it's found that *Scenedesmus dimorphus* (UTEX 1237) and *Neochloris oleoabundans* (UTEX 1185) were unable to grow on the swine wastewater. So *Chlorella vulgaris* (UTEX 2714) and *Chlamydomonas reinhardtii* (UTEX 90) were further investigated for the bioremediation ability. Correlations between the optical density of *C. vulgaris* and *C. reinhardtii* at 680

nm and the cell number were pre-determined. These correlations are shown as the equations 5 and 6, which were used to determine the growth kinetics of both species under different conditions.

The correlation for *C. vulgaris* is

$$\text{Cell number (cell/ml)} = 8\times10^{6} OD_{680} + 425897 \qquad R^2 = 0.9588 \qquad (5)$$

The correlation for *C. reinhardtii* is

$$\text{Cell number (cell/ml)} = 7\times10^{6} OD_{680} + 800979 \qquad R^2 = 0.9591 \qquad (6)$$

The kinetics parameters for the growth of *C. vulgaris* and *C. reinhardtii* under different conditions are summarized in Table 1.

The effect of temperature

The temperature range of 20-30 ℃ was chosen, because it is easy to achieve in North Carolina. It was found that the optimal growth temperatures for two strains are different. A cultivation temperature of 25^{o}C gave the highest specific growth rates for *C. vulgaris*. The highest specific growth rates and division rates were found to be 1.336 day^{-1} and 1.928 day^{-1}, respectively, which were obtained at 25°C and 600 μmol m^{-2}s^{-1}. At 20 ℃ the specific growth rates and division rates obtained were 1.287-1.317 day^{-1} and 1.856-1.9 day^{-1}, respectively. The lowest growth rates of 1.199-1.214 day^{-1} were found at 30 ℃.

For *C. reinhardtii*, the highest specific growth rate and division rate were found to be 1.286 day^{-1} and 1.854 day^{-1}, respectively, which were obtained at 20 ℃ and 300 μmol m^{-2}s^{-1}. At 25 ℃ the specific growth rates and division rates obtained were 1.173-1.272 day^{-1} and 1.692-1.839 day^{-1}, respectively. The lowest growth rates of 1.141-1.184 day^{-1} were found at 30 ℃ and 25 ℃/900 μmol m^{-2}s^{-1}.

The effect of light intensity

The reason for the selection of light intensities of 300-900 μmol m^{-2}s^{-1} is the same as the temperature selection. Moreover, the light intensities of 600 μmol m^{-2}s^{-1} and 900 μmol m^{-2}s^{-1} were used to study the effect of photoinhibition on microalgae growth.

Fig. 1 shows the growth curves of *C. vulgaris* and *C. reinhardtii* at 20 ℃ under different light intensities. While microalgae were grown under 300 μmol m^{-2}s^{-1}, the

growth curves revealed different patterns for *C. vulgaris* and *C. reinhardtii*. The lag phase was short for both species according to the curve patterns in this phase. The difference was seen in the exponential phase after day 5. The cell density of *C. vulgaris* increased more rapidly in this phase than that of *C. reinhardtii*. After day 13, *C. reinhardtii* showed a comparatively high growth rate in this experiment. The cell densities reached the maximum value on day 15 for both species, and the curves were still upslope on day 15, indicating that the growth was still in the exponential phase. The experiments were stopped on day 15 for both species.

When the light intensity was increased from 300 μmol $m^{-2}s^{-1}$ to 600 or 900 μmol $m^{-2}s^{-1}$, the growth patterns in lag phases were quite similar, and *C. vulgaris* had higher growth rate than *C. reinhardtii*. When the microalgae cultivation was conducted at 25 ℃ or 30 ℃, the growth patterns of these two microalgal strains showed the similar trends.

Both the growth curves (Fig. 1) and the specific growth rates (Table 1) show that a higher light intensity led to a significantly reduced growth rate. The lowest growth rates of both species were found at the highest light intensity of 900 μmol $m^{-2}s^{-1}$ and 25 ℃.

Statistical Analysis

The P-values for the cultivation temperature and light intensity were 0.0375 and 0.530, respectively. If a statistical significance level cut-off is chosen as $P = 0.05$, it is concluded that the cultivation temperature was a significant factor, while the light intensity was not significant at a level of $P < 0.05$.

Removal of COD, ammonia nitrogen, and total phosphorus from swine wastewater

In this study, the autoclaved wastewater was used as medium for algal cultivation. To provide the mass transfer and CO_2, air is bubbled through each bioreactor at the flow rate of 100 ml/min during the 15-day culture. Table 2 summarizes the contents of COD, ammonia nitrogen, and total phosphorus in the swine wastewater before and after autoclave, and after 15-day air bubbling. By bubbling air through the wastewater, almost all nutrient contents were reduced. In order to calculate the nutrient removal rate, the data of the autoclaved wastewater bubbled with air was used as the reference.

The results indicated that the removal efficiency of nutrients is a function of

microalgae growth (Table 3). For both microalgal species, the cultivation conditions for the highest growth rates also resulted in the highest nutrient removal efficiencies. The highest removal efficiencies of ammonia nitrogen, COD, and total phosphorus for *C. vulgaris* were 92.5%, 58.9% and 74.3%, respectively, which was achieved at the temperature of 25 ℃ and light intensity of 600 μmol $m^{-2}s^{-1}$. For *C. reinhardtii*, the highest removal efficiencies of ammonia nitrogen, COD, and total phosphorus were 89.2%, 46.0% and 48.8%, respectively, which was achieved at the temperature of 20 ℃ and light intensity of 300 μmol $m^{-2}s^{-1}$. *C. vulgaris* often had higher growth rate and removed more nutrients than *C. reinhardtii* under the same cultivation conditions.

Comparison of *Chlamydomonas debaryana* with *C. vulgaris* and *C. reinhardtii*

C. debaryana AT24 is a North Carolina native green microalgal species (unpublished data), which served as a benchmark for the bioremediation ability of the native algal strains. Three strains were cultivated at 25 ℃ and light intensity of 300 μmol $m^{-2}s^{-1}$. The results of elemental and composition analysis are listed in Table 4. The protein contents in all microalgal species are significantly high, representing 60-72 % of total dry weight. The ash contents are between 7.9-11.3% of total dry weight. The final biomass yields were 1.25 g/L, 0.86 g/L and 0.73 g/L for *C. vulgaris*, *C. debaryana* and *C. reinhardtii*, respectively. *C. debaryana* was able to remove 88.1% ammonia nitrogen, 53.6% total phosphorus, and around 47% COD from the swine wastewater. *C. vulgaris* gave the highest removal efficiency among three strains, and *C. debaryana* showed better performance than *C. reinhardtii*.

Lipid extraction and characterization

Lipids of microalgae were extracted and characterized according to the methods described in the Materials and Method section. Lipid contents from *C. vulgaris*, *C. reinhardtii* and *C. debaryana* are approximately 15.2%, 21.7%, and 19.7% of the total cell dry weight, respectively.

A GC/MS analysis of microalgal oils is presented in Table 5. Hexadecanoic Acid (C16) and three C18 fatty acids (Linoleic, Linolenic, and Octadecanoic acid) were found in all three microalgal oils. Moreover, the microalgal oil from *C. debaryana* also contains

C12, C14, C15 and C17 fatty acids.

Discussion

Microalgal technology may combine bioremediation of swine wastewater and biofuel production in a more effective and sustainable way. However, the swine wastewater contains high doses of nutrients, which limited the growth of all microalgal species tested in this study.

As indicated by the statistical analysis, the effect of the light intensity on the algal cell density was not significant, but different microalgae species response to the light intensity differently. When the light intensity increased from 600 to 900 μmol $m^{-2}s^{-1}$, the growth rate of *C reinhardtii* decreased much more than that of *C. vulgaris*, which may indicate that *C. reinhardtii* is more sensitive to photoinhibition.

C. debaryana AT24 was isolated from the lagoon at a NC A&T's farm. As a native green microalgal species, *C. debaryana* may have a competitive advantage under the local geographical, climatic and ecological conditions. Application of native microalgal species will also reduce the risk of invasive algae species. In addition, a variety of fatty acids was detected in the microalgal oil from *C. debaryana*. These medium-chain-length fatty acids are good sources for the production of bio-jet fuel.

Conclusion

This study describes a systemic optimization of microalgae growth for bioremediation of swine wastewater and the potential for biofuel production. It's found that *Scenedesmus dimorphus* and *Neochloris oleoabundans* were unable to grow on the swine wastewater. The optimal culture conditions for *Chlorella vulgaris* and *Chlamydomonas reinhardtii* were found to be (600 μ mol $m^{-2}s^{-1}$ and 25 ℃) and (300 μ mol $m^{-2}s^{-1}$ and 20 ℃), respectively. The growth kinetics was determined using optical density (OD) method and flow cytometry. Under the optimal culture conditions, the highest specific growth rates were found to be 1.336 day^{-1} and 1.286 day^{-1} for *C. vulgaris* and *C. reinhardtii*, respectively. Three microalgal strains of *C. vulgaris*, *C. debaryana* AT24 and *C. reinhardtii* were able to effectively treat swine wastewater, and the relationships between microalgae growth and the removal efficiency of nutrients were established. The

final biomass yields and lipid contents were 1.25 g/L and 15.2% (of the total cell dry weight), 0.86 g/L and 19.7%, and 0.73 g/L and 21.7% for *C. vulgaris, C. debaryana* and *C. reinhardtii*, respectively. The potentials of using these microalgal biomass for biodiesel production were also addressed.

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Table 1 Experimental design and kinetics parameters for microalgae growth

Experimental design number	Temperature (℃)	Light intensity (μmol $m^{-2}s^{-1}$)	*C. vulgaris*			*C. reinhardtii*		
			Final cell density, (cell/ml)	Specific growth rate, μ (day^{-1})	Division rate, k (day^{-1})	Final cell density, (cell/ml)	Specific growth rate, μ (day^{-1})	Division rate, k (day^{-1})
1	20	300	9.5×10^6	1.317	1.900	7.9×10^6	1.286	1.854
2		600	7.6×10^6	1.297	1.872	7.1×10^6	1.271	1.834
3		900	6.7×10^6	1.287	1.856	6.9×10^6	1.249	1.802
4	25	300	1.1×10^7	1.332	1.921	7.2×10^6	1.272	1.836
5		600	1.2×10^7	1.336	1.928	7.3×10^6	1.275	1.839
6		900	9.2×10^6	1.223	1.764	5.6×10^6	1.173	1.692
7	30	300	9.1×10^6	1.214	1.752	5.9×10^6	1.184	1.708
8		600	8.9×10^6	1.211	1.747	5.8×10^6	1.182	1.706
9		900	8.9×10^6	1.199	1.730	5.0×10^6	1.143	1.649

Table 2 Properties and nutrient contents of swine wastewater

Nutrients	Original swine wastewater	Autoclaved Wastewater	Autoclaved wastewater bubbled with air
COD (mg/L)	2140	2060	1630
Ammonia Nitrogen (mg/L)[a]	86.6	81.5	41.2
Total Phosphorous(mg/L)	182.7	172.6	144.6
pH	8.37	8.75	8.13

a: The total nitrate content in the swine wastewater is negligible.

Table 3 Removal ratios (% of the reference) of ammonia nitrogen, COD, and total phosphorous after 15 days culture. The autoclaved swine wastewater that was bubbled with air for 15 days was used as the reference. The reference contains ammonia nitrogen of 41.2 mg/L, COD of 1630 mg/L, and phosphorous of 144.6 mg/L.

Experimental design number	*C. vulgaris*			*C. reinhardtii*		
	Ammonia	COD	Phosphorous	Ammonia	COD	Phosphorous
1	91.5±1.0	53.7±2.2	61.7±1.3	89.2±0.9	46.0±0.4	48.8±1.2
2	88.4±0.3	46.3±2.3	55.4±0.8	86.9±0.7	37.4±0.9	39.8±3.2
3	83.7±0.7	43.9±2.2	49.6±2.2	81.2±0.2	31.6±1.3	23.7±0.8
4	90.7±0.5	55.2±2.6	67.5±0.4	84.0±1.0	41.7±2.6	44.7±1.5
5	92.5±0.3	58.9±1.7	74.3±1.8	89.0±0.9	40.5±0.9	46.8±0.3
6	89.4±0.2	54.6±2.6	61.1±2.3	85.9±0.3	35.3±1.3	32.9±1.2
7	87.1±0.3	50.9±0.9	60.7±0.4	83.5±0.3	44.5±2.2	44.4±0.9
8	86.9±0.3	47.2±1.7	57.4±0.5	84.5±0.7	40.8±1.3	45.9±2.4
9	86.0±0.2	47.2±2.6	53.6±2.0	80.6±0.3	29.8±1.3	29.7±0.7

Table 4 Elemental and composition analysis of microalgae (Moisture free basis, % by weight)

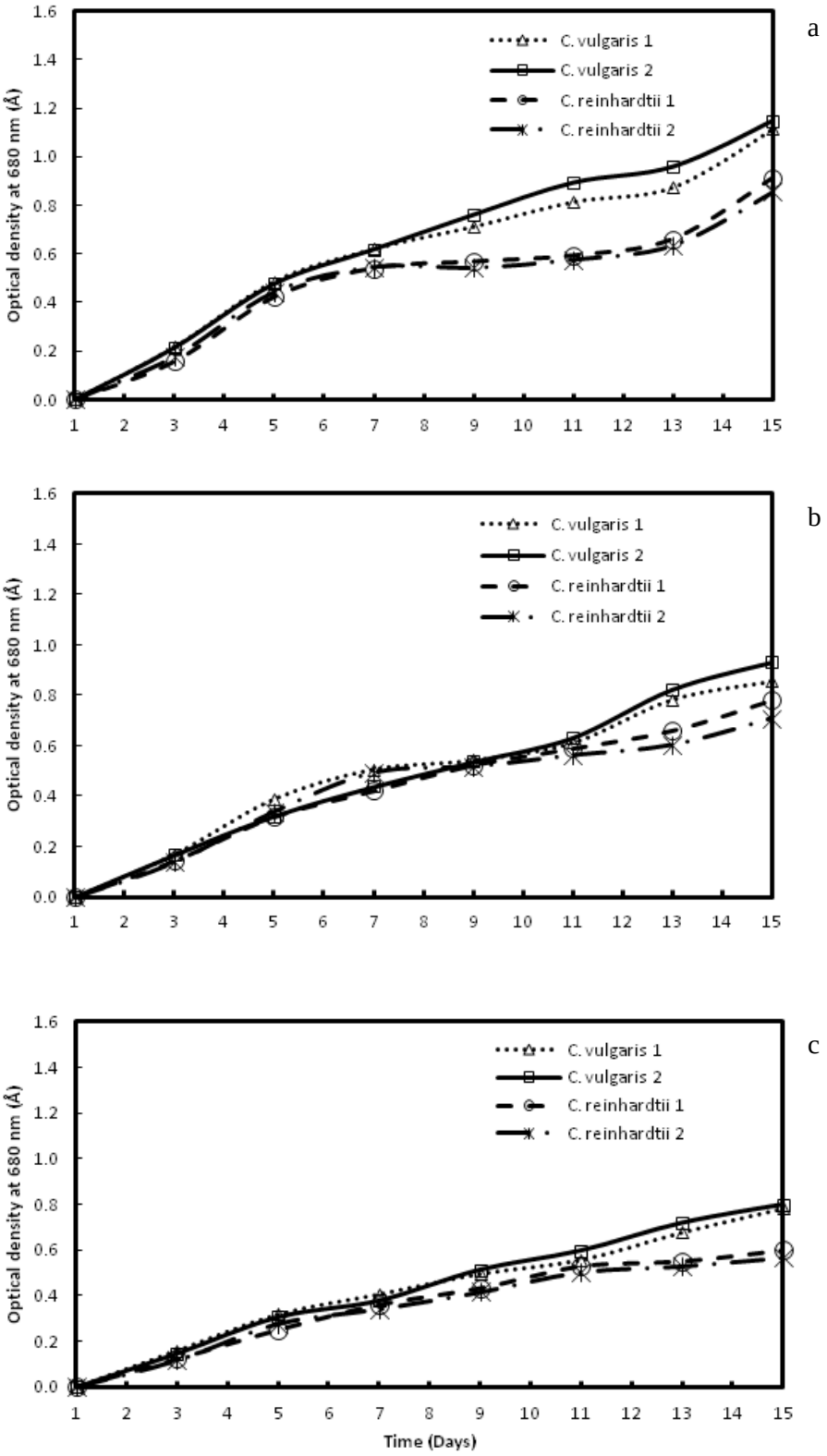
Composition (% wt)	*C. vulgaris*	*C. reinhardtii*	*C. debaryana* AT24
C	45.9	48.9	51.2
H	6.4	6.9	7.2
N	11.6	10.3	9.5
S	1.4	1.4	1.1
ash	11.3	10.9	7.9
protein	72.3	64.4	59.4

Table 5 GC/MS analysis and chemical identification of microalgal oils

Retention time (min)	Possible Formula	Possible chemical structure	*C. vulgaris*	*C. reinhardtii*	*C. debaryana* AT24
15.2	$C_{13}H_{26}O_2$	Dadecanoic acid, methyl ester	-	-	+
17.6	$C_{15}H_{30}O_2$	Methyl tetradecanoate	-	-	+
18.3	$C_{16}H_{32}O_2$	Tetradecanoic acid, 12-methyl-methyl ester	-	-	+
19.4	$C_{17}H_{30}O_2$	7,10-Hexadecandienoic acid, methyl ester	-	-	+
19.7	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	+	+	+
20.3	$C_{18}H_{36}O_2$	Hexadecanoic acid, 15-methyl-methyl ester	-	-	+
21.3	$C_{19}H_{34}O_2$	Linoleic acid, methyl ester	+	+	+
21.4	$C_{19}H_{32}O_2$	Linolenic acid, methyl ester	+	+	+
21.6	$C_{19}H_{38}O_2$	Octadecanoic acid, methyl ester	+	+	+

+: present, -: not detectable

Fig. 1 Growth curves of *C. vulgaris* and *C. reinhardtii* grown at 20 ℃ and a) 300 μmol $m^{-2}s^{-1}$, b) 600 μmol $m^{-2}s^{-1}$, and c) 900 μmol $m^{-2}s^{-1}$



Corrigenda to the original version:

Hasan R, Zhang B, Wang L, Shahbazi A (2014) Bioremediation of Swine Wastewater and Biofuel Potential by using Chlorella vulgaris, Chlamydomonas reinhardtii, and Chlamydomonas debaryana. J Pet Environ Biotechnol 5:175. doi: 10.4172/2157-7463.1000175

1. The first sentence of the 3rd paragraph in the introduction section, "such as *Chlorella* and *Dunaliella*": these two words should be italic.

2. The 3rd sentence of the 3rd paragraph in the introduction section, "*Ourococcus multisporus*, *Nitzschia cf. pusilla*," should be italic.

3. Page 2, the formula #1, there are some letters in different format.

4. Page 2, the formula #2, i should be μ.

5. Page 2, the formula #3, i should be μ.

6. Page 4, in Statistical Analysis section, "The P-values for the cultivation temperature and light intensity were 0.375 and 0.530, respectively": "0.375" should be "0.0375", it was a typo.

7. Page 5, the 2rd sentence of the Discussion section, "However, Swine wastewater" should be "However, the swine wastewater".

8. Page 5, the Acknowledgment section, please use the following statement:

"This publication was made possible by Grant Number NC.X-269-5-12-130-1 and NC.X2013-38821-21141 from the National Institute of Food and Agriculture (NIFA) which is an agency within the U.S. Department of Agriculture (USDA). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute of Food and Agriculture."