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Cultivation of *Chlorella vulgaris* in Dairy Wastewater Pretreated by UV Irradiation and Sodium Hypochlorite

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Abstract There is potential in the utilization of microalgae for the purification of wastewater as well as recycling the resource in the wastewater to produce biodiesel. The large-scale cultivation of microalgae requires pretreatment of the wastewater to eliminate bacteria and protozoa. This procedure is costly and complex. In this study, two methods of pretreatment, UV irradiation, and sodium hypochlorite (NaClO), in various doses and concentrations, were tested in the dairy wastewater. Combining the efficiency of biodiesel production, we proposed to treat the dairy wastewater with NaClO in the concentration of 30 ppm. In this condition, The highest biomass productivity and lipid productivity of *Chlorella vulgaris* reached 0.450 g L^{-1} day^{-1} and 51 mg L^{-1} day^{-1} after a 4-day cultivation in the dairy wastewater, respectively.

Keywords Dairy Wastewater · *Chlorella vulgaris* · UV Irradiation · Sodium Hypochlorite Pretreatment · Large-Scale Cultivation

Introduction

The increasing consumption of energy with the improving of living standards means that the world supply of energy resources will reach its limit more rapidly, and the resulting prospect of an energy shortage becomes one of our most serious problems [1]. Microalgae are considered to be potential feedstock candidates for biofuels for their high photosynthetic efficiencies, high-growth rates, and low land requirements [2, 3]. But the high cost would seriously limit the development of sustainable microalgae biofuels technology [4, 5]. Except

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for power consumption, carbon dioxide, and water, fertilizers contribute 14.8 % of raw materials and utility costs of microalgae biomass [6].

For animal wastewater rich in nitrogen and phosphorus, the direct emissions would have unfriendly effects to the environment [7]. With the development of dairy industries, treatment of dairy wastewater becomes an important environmental challenge in many countries. An effective and widely used method of treating animal waste involves anaerobic treatment followed by post-treatment in high-rate oxidation ponds [8]. Although this method can solve the pollution problem, the wastewater has not been utilized as resources. Thus, it's necessary to link pollution control and resource utilization. Contrary to conventional wastewater treatment, bio-utilization of the nutrients for microalgal production has been regarded as a very promising strategy which can simultaneously satisfy both energy needs and wastewater treatment [9–11].

Microalgae can effectively grow and utilize nutrients and metals in the wastewater, providing an attractive means to wastewater treatment in a sustainable manner with low cost [12, 13]. However, the growth of microalgae is strongly restricted by the presentation of bacteria and protozoa in the wastewater. In previous studies, wastewater is mainly pretreated through autoclaving or filtration [14, 15]. Due to time consumption, energy, and complexity perspective, such pretreatments may not be feasible for large-scale cultivation [16, 17]. Ultraviolet (UV) disinfection is used in air and water purification, sewage treatment, protection of food and beverages, and many other disinfection and sterilization applications [18]. Chlorination is widely used in wastewater treatment. Bleach (sodium hypochlorite (NaClO)) is frequently used in the aquaculture hatcheries [19]. Post-chlorinated domestic wastewater can support microalgal growth. And the pre-chlorinated wastewater could be attributed to the presence of protozoans and rotifers which are known and documented as microalgal predators [20].

In this study, we compared the sterilization effect of UV irrigation and NaClO in dairy wastewater. Then, we comprehensively evaluated the feasibility of using the dairy wastewater as a growth medium for biodiesel production with *Chlorella vulgaris*. The nutrient removal through the *C. vulgaris* cultivating in the dairy wastewater was also investigated in this paper.

Materials and Methods

Algae Strain and Culture Condition

The *C. vulgaris* strain used in the study was maintained in BG-11 medium [21]. All cultivations of *C. vulgaris* was performed in 1.3-L glass photobioreactors (PBRs) (Φ=6× 70 cm) containing 500 ml of liquid media and grown at 25±1 °C and illuminated with white fluorescent lamps at the single side of the PBRs (light intensity of 300±10 μmol photons m^{-2} s^{-1}) continuously.

Characteristics of Wastewater and Experiment Layout

The dairy wastewater was collected from the Aochun Dairy Co., Ltd. of Foshan, Guangdong, China. Large solid particles were removed by sedimentation and filtration with filter paper (Wypall X70, Kimberly–Clark Professional). After filtration, the nutrient composition of the dairy wastewater was determined following the Hach DR 2800 Spectrophotometer Manual.

The experiments in this project were carried out with two pretreatments of dairy wastewater. Ultraviolet C (UVC) light-emitting diodes with peak irradiance at 253.7 nm were used as the sterilization device whose UVC intensity was 117 μW cm^{-2}. In all cases, the light was switched on for 30 min before the start of the reaction to stabilize the emission power and

spectrum. Clean enamel plate (30×40 cm) with 500-ml filtrated dairy wastewater placed under ultraviolet light. The distance between the UVC light and the surface of the dairy wastewater was 100 cm. UV irradiation was performed in a dark room at 25 °C for 5, 10, and 15 min, respectively. Subsequently, the pretreated dairy wastewater was transferred into sterilized glass PBRs. In the Sodium hypochlorite treatment time, 500-ml filtrated dairy wastewater was transferred into sterilized glass PBRs. Then, a certain amount of sodium hypochlorite (terminal available chlorine at 10, 30, 50, 70, and 90 ppm) was respectively added into PBRs. Sodium hypochlorite treatment was performed in a dark room for 12 h and then placed under sun exposure for 1 day to remove the oxidation activity. And ultimately, sodium thiosulfate solution (1 mol L^{-1}) was used to neutralize residual chlorine.

The initial optical density (OD_{680}) in all cases was 0.27. Each treatment was aerated with air supplemented with 5 % CO_2 at a rate of 1 vvm.

Sampling and Nutrients Analysis

Samples after UV and sodium hypochlorite treatment and liquid samples at the end of the culture were collected for nutrient analysis. The whole experiment ended after 4 days. Samples were centrifuged at 5,000 rpm for 5 min and supernatants were collected for analyses of ammonium (NH_3-N), total Kjeldahl nitrogen (TKN), total phosphorus (TP), and chemical oxygen demand (COD). Those characteristics of the dairy wastewater were determined using Hach DR2700 spectrophotometer (Hach Co., Loveland, CO, USA) following the manufacturer's manual. Removals for those parameters were calculated by dividing the difference between concentrations after filtration and final of the experiment concentrations by concentration after filtration, and then multiplied by 100.

Algal Growth Determination

The biomass of microalgae at the beginning and end of the cultivation was harvested by centrifugation at 5,000 rpm for 5 min, and then washed twice with distilled water. The collected biomass was dried by lyophilization.

The biomass concentrations and productivities during the culture period were calculated from the equations as follows:

$$C_B\left(g\,L^{-1}\right) = X / V$$
$$P_{biomass}\left(g\,L^{-1}day^{-1}\right) = (X - X_0)/T$$

Where X and X_0 were the concentrations of dried biomass at the end and beginning of the cultivation, respectively; V was the cultivation volume; and T was the culture time.

Lipid Content and Fatty Acid Analysis

Total lipids were determined by the method of Bigogno et al. [22] with slight modification.

The lipid contents were calculated from the equation

$$C_L\left(g\,g^{-1}\right) = W_L/W_A$$

Where W_L (gram) was the weight of the extracted lipids and W_A (gram) was the dry algae biomass.

The lipid productivities of the batch cultivation were calculated from the equation

$$P_{\text{lipids}}\left(\text{g L}^{-1}\text{day}^{-1}\right) = W_G \times C_L/V \times T$$

Where W_G was the cumulative algae biomass production, V was the cultivation volume, and T was the cultivation time.

The lipid composition was determined as fatty acid methyl esters (FAMEs) through the direct transesterification method according the procedure of Slover and Lanza with modification [23]. Twenty milligrams of freeze-dried sample were put into a capped test tube and extracted three times with 2.5 ml of H_2SO_4-CH_3OH (v/v=2:98) under stirring at 80 °C for 2.5 h. After the suspension cooled, 1 ml of hexane and 1 ml of saturated NaCl solution were added to form separated layers in the tube. The upper clear layer of alkane was pipetted out for analysis by a gas chromatograph (Shimadzu GC-2010, Kyoto, Japan) that was equipped with a flame ionization detector. To each specimen, 0.1 mg of an internal standard (methyl heptadecanoate, Fluka, Sweden) was added. Separation was achieved on a HP-5 capillary column (30 m×0.25 mm×0.25 μm; Hewlett-Packard, USA) with N_2 as the carrier gas at a flow speed of 1 ml min^{-1}. One microliter of the sample solution was injected in split mode (split ratio=1:30) for each analysis. The injector temperature of the machine was set at 250 °C and the column temperature program was set as follows: the initial temperature was 195 °C and maintained for 12 min; then it rose to 230 °C at a rate of 10 °C min^{-1} and maintained for 15 min. Identification of the components was preceded by comparing the retention times and fragmentation pattern with a Supelco 37-Component FAME Mix (Sigma, USA).

Results and Discussion

Growth Assessment of *Chlorella vulgaris*

The results of batch cultivation experiments obtained by different pretreatments were shown in Table 1. Although *C. vulgaris* could survive in all cases including in the untreated wastewater, the biomass concentration and productivity of *C. vulgaris* culture in untreated wastewater were obviously lower than pretreated samples. In fact, the microscopic examination showed many species of bacteria, green algae, diatoms, and two species of freshwater protozoa in the untreated wastewater. These original biological contaminants limited and threatened the growth of *C. vulgaris* and limited the lipid yield of *C. vulgaris*.

The treatments of sodium hypochlorite and UV irradiation could inactivate or kill these original microorganisms effectively. And the sterilization results were confirmed with microscope. All sterilization means could not eliminate 100 % of the contaminants from the cultivation medium. But the relatively pure culture of *C. vulgaris* after these pretreatments showed significant improvement in both biomass concentration and biomass productivity. And the NaClO pretreatment obtained better results than the UV irradiation treatment. Among the serial treatment tests, the highest biomass concentration and biomass productivity of *C. vulgaris* were 1.870 and 0.450 g L^{-1} day^{-1} when the concentration of available chlorine was 70 ppm. The biomass concentration was comparable to the biomass concentrations of *Nannochloropsis salina* cultured in bubbling system under night–day cycle and different cycles of CO_2 supplying [24].

Table 1 Biomass concentration and biomass productivities of different samples (mean ± SD)

		Biomass concentration (g L^{-1})	Biomass productivity (g L^{-1} day^{-1})
Untreated		0.861±0.066	0.198±0.017
UV	5 min	0.935±0.001	0.216±0.001
	10 min	0.967±0.076	0.224±0.019
	15 min	1.218±0.036	0.287±0.009
Available chlorine	10 ppm	1.339±0.145	0.317±0.036
	30 ppm	1.815±0.098	0.436±0.025
	50 ppm	1.814±0.003	0.435±0.001
	70 ppm	1.870±0.118	0.450±0.030
	90 ppm	1.835±0.048	0.441±0.012

Nutrient Removal Efficiencies

Dairy Wastewater Characteristics Before and After Pretreatment

Results of the wastewater characteristics experiments showed that different treatments and doses lead to varying degrees of change (Table 2). The COD, TKN, and TP have slight reduction while the NH_3-N displayed a certain degree of increase after ultraviolet treatments. And the reduction of COD, TKN, TP and the increase of NH_3-N were more obvious under sodium hypochlorite treatments.

Dairy Wastewater Nutrients Removal by Algae Growth

The variation in COD, TKN, TP, and NH_3-N of samples after different pretreatments for the 4-day batch culture was depicted in Fig. 1a–d, respectively.

TKN was greatly reduced by 86.5–95.4 and 63.6–87.3 % under UV and sodium hypochlorite treatments, respectively (Fig. 1b). Unexpectedly, a significant reduction (94.4–94.8 and 91.6–99.7 %) of TP was found in all of the samples (Fig. 1c), which was higher than the results by Woertz [25] who achieved around 76 % phosphate reduction when treating

Table 2 Characteristics of the dairy wastewater before and after pretreatment (means ± SD)

Parameters		COD (mg L^{-1})	TKN (mg N L^{-1})	TP (mg PO_4^{-3} L^{-1})	NH_3-N (mg N L^{-1})
Before pretreated		1,560	97.0	63.1	51.9
After UV irradiation pretreated	5 min	1,530±14	92.5±0.7	59.7±0.4	60.6±0.0
	10 min	1,535±21	95.5±1.5	62.1±0.4	61.5±0.4
	15 min	1,505±36	92.5±3.5	62.9±0.1	62.0±1.9
After sodium hypochlorite pretreated	10 ppm	1,273±33	85.5±5.5	59.4±0.8	70.8±0.6
	30 ppm	1,338±12	65.0±1.0	60.0±0.5	70.6±1.0
	50 ppm	1,353±29	64.0±3.0	59.3±0.1	68.9±0.1
	70 ppm	1,352±4	70.5±2.5	60.6±0.5	70.3±0.5
	90 ppm	1,364±24	59.0±4.0	58.3±0.2	68.1±0.7

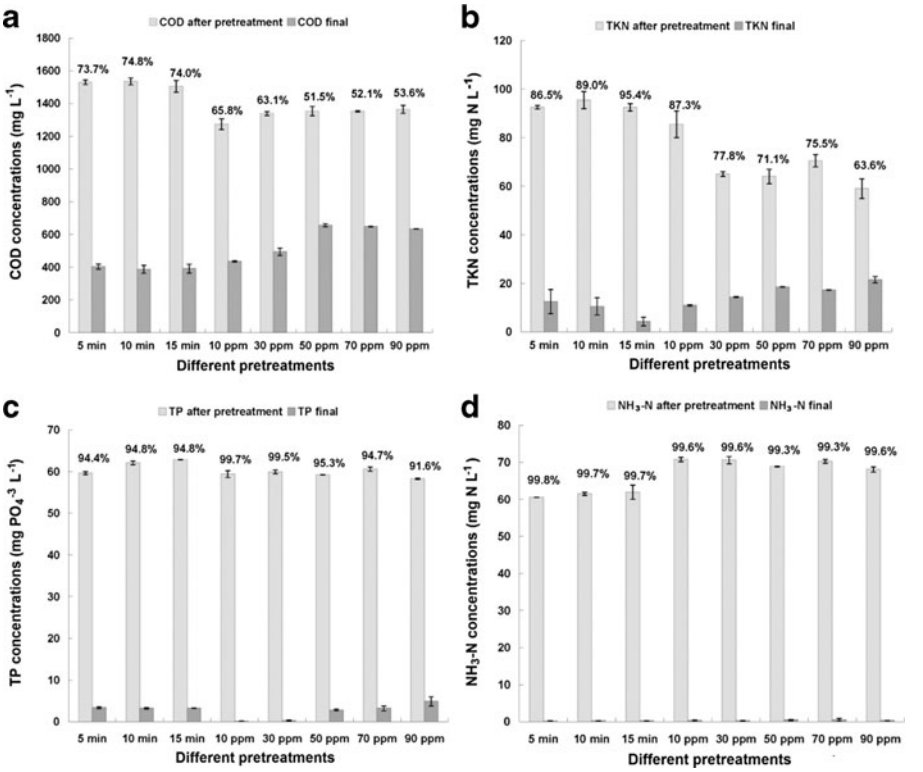

Fig. 1 Characteristics of the dairy wastewater before and after 4-day cultivation (**a**, **b**, **c**, **d** refer to COD, TKN, TP, NH3-N, respectively). The *values above the columns* refer to the removal rate of each pretreatment

digested dairy manure with algae at a lower starting concentration of 7.7 mg PO_4^{3-} L^{-1}. These results are also better than the results reported by Wang et al. [26] who found that a wild-type *Chlorella* sp. cultivated in 10- to 25-fold diluted manure could provide removal rates of 75.7–82.5 % for TKN and 62.5–74.7 % for TP. The unexpected high TP removal might be caused by the pH around 8.0 at the end of experiment which could contribute to coagulation and adsorption of inorganic phosphates [27].

Ammonium was almost completely removed within the 4-day growth period in all samples regardless of the initial concentration (60.6–70.8 mg L^{-1}) was (Fig. 1d). The result was in accordance with the results using agro-industrial wastewater and synthetic wastewater as culture media [28, 29]. Although not as efficient as the nitrogen and phosphorus removal (Fig. 1b, c), the removals of COD in the dairy wastewater after different pretreatments were 73.7–74.8 and 51.5–65.8 %, respectively (Fig. 1a). A previous study reported that the maximum of COD removal with 73.18 % was achieved when *Arthrospira* (*Sprirulina*) *platensis* was cultivated in 25 % olive oil mill wastewater pretreated by 12.5 g L^{-1} NaClO [30].

These results showed that removal rates of COD and TKN after sodium hypochlorite treatments were lower than those after UV treatments, although the biomass concentrations of algae under sodium hypochlorite treatments were higher. This may be due to a wide range of reasons. As we talked about before, the relatively pure culture of *C. vulgaris* after these pretreatments showed significant improvement in both biomass concentration and biomass

productivity, but no sterilization methods could kill all contaminants from the culture medium. Microscopic examination showed several microorganisms (mainly bacteria) had grown. Thus, we assumed the organic compound was partly biodegraded by the bacteria. The biodegradation was enhanced by the continuously provided air. The air enriched the medium with oxygen, which was utilized by the bacteria to perform an enzymatic attack and to biodegrade organic compound [30, 31].

Algal Lipid Content and Fat Acids Composition

Table 3 shows lipid content and lipid productivity of *C. vulgaris* cultivated in the pretreated dairy wastewater. Lipid contents of *C. vulgaris* in dairy wastewater after sodium hypochlorite pretreatment were roughly at the same level, but the lipid productivities were evidently different. The highest lipid content was 14.38 % under 15-min UV pretreatment. However, the lipid productivity was 44 mg L^{-1} day^{-1} in this condition. This value was lower than the productivity of all sodium hypochlorite treatment except the 10 ppm one. The highest lipid productivity was 51 mg L^{-1} day^{-1} when concentration of available chlorine was 30 ppm.

The lipid content of *C. vulgaris* coincide with former reports which showed 14.7 % of the lipid content and 20.22 mg L^{-1} day^{-1} of lipid productivity of *C. vulgaris* cultivated in Bold's basal medium at 25 °C[32]. The highest lipid yield of microalgae reported currently were 17 mg L^{-1} day^{-1} on dairy wastewater and 24 mg L^{-1} day^{-1} on municipal wastewater [33].

Table 4 shows the fatty acid profiles derived from triacylglycerol, phospholipid and free fatty acids in *C. vulgaris* cultivated under different conditions in our research. Hexadecanoic acid (C 16:0), linoleic acid (C 18:2), and linolenic acid (C 18:3) were the abundant fatty acids. Hexadecanoic acid(C 16:0) was the most abundant fatty acid in *C. vulgaris* under all treatments, ranging from 36.85 to 45.30 % of the total fatty acids. The proportion of octadecadienoic acid (C 18:2) and linolenic acid (C 18:3) were different but not obvious, 18.80–23.40 and 16.90–24.20 %, respectively, which were obviously lower than the contents of hexadecanoic acid (C 16:0). Our results were in agreement with a former report [14] which showed that hexadecanoic acid (C 16:0), linoleic acid (C 18:2), and linolenic acid (C 18:3) were the abundant fatty acids in *Chlorella pyrenoidosa* cultivated with Bristol's solution and the diluted sample with 500 mg COD L^{-1}. Compared to Bristol's solution, there were remarkable reductions in the relative content of hexadecadienoic acid (C 16:2) and oleinic acid (C 18:1) while an increase exist in hexadecanoic acid (C 16:0) for the diluted piggery waste samples.

Table 3 Lipid contents and lipid productivities of different samples (mean ± SD)

		Lipid content (%)	Lipid productivity (mg L^{-1} day^{-1})
UV	5 min	11.43±2.02	27±5
	10 min	13.35±0.14	32±2
	15 min	14.38±0.04	44±1
Available chlorine	10 ppm	11.05±1.13	37±8
	30 ppm	11.28±0.04	51±3
	50 ppm	11.08±0.32	50±2
	70 ppm	10.30±0.71	48±1
	90 ppm	10.50±0.78	48±2

Table 4 Summary of FAME profiles of *C. vulgaris* cultivated with different pretreatments

Fatty acid	UV			Available chlorine				
	5 min	10 min	15 min	10 ppm	30 ppm	50 ppm	70 ppm	90 ppm
Saturated fatty acids (% of total fatty acids)	45.90	50.55	50.45	47.90	50.30	46.30	45.75	54.80
C14:0	1.60	1.50	1.35	2.65	2.50	4.85	3.90	4.55
C16:0	40.80	45.00	44.70	41.65	44.20	36.85	37.15	45.30
C18:0	3.20	3.85	4.00	3.60	3.30	4.20	4.70	4.65
C24:0	0.30	0.20	0.40	0.00	0.30	0.40	0.00	0.30
Monounsaturated fatty acids (% of total fatty acids)	10.50	5.55	6.40	11.00	11.60	9.55	6.70	9.55
C14:1	0.00	0.30	0.45	1.40	2.90	2.65	2.50	3.00
C16:1	5.15	2.10	2.45	4.75	3.40	1.25	0.00	0.55
C18:1	1.00	0.60	0.00	1.60	1.80	0.85	0.00	0.00
C20:1	3.80	2.55	2.80	2.45	3.50	3.90	2.80	4.80
C24:1	0.55	0.00	0.70	0.80	0.00	0.90	1.40	1.20
Polyunsaturated fatty acids (% of total fatty acids)	46.55	44.05	43.15	41.20	38.00	45.15	47.60	35.70
C18:2	23.30	22.20	22.25	20.90	19.90	23.10	23.40	18.80
C18:3	23.25	21.85	20.90	20.30	18.10	22.05	24.20	16.90

According to the European Standard EN 14214, the content of linolenic acid in biodiesel for vehicle is limited and additional treatment, like hydrogenation, is required to meet the standard [34, 35]. In conclusion, taking lipid productivities, removal rates of nutrients, and hexadecanoic acid proportion of the total fatty acids into consideration, we suggest using 30 ppm available chlorine pretreatment for *C. vulgaris* cultivation with dairy wastewater to get the desired lipid production.

Conclusion

The direct emission of dairy wastewater would have unfriendly effect on the environment. New techniques which can link pollution control and resource utilization are necessary. In this study, we investigated the pretreatment of dairy wastewater by UV and NaClO which were feasible for large-scale cultivation. The highest biomass productivity and lipid productivity of *C. vulgaris* could reach 0.450 g L^{-1} day^{-1} and 51 mg L^{-1} day^{-1}, respectively. And the ideal nutrition removals were obtained in the end of cultivations. Those findings in our research could provide a potential solution which combined pollution control and resource utilization with low cost and high feasibility for further scale-up microalgal biodiesel production.

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References

1. Du, Y., Wang, Y., Peng, G., Su, Z., Xu, M., Feng, W., et al. (2011). Reducing COD and BOD, as well as producing triacylglycerol by LDS5 grown in CTMP effluent. *Bioresources, 6*(3), 3505–3514.
2. Markou, G., Angelidaki, I., & Georgakakis, D. (2012). Microalgal carbohydrates: an overview of the factors influencing carbohydrates production, and of main bioconversion technologies for production of biofuels. *Applied Microbiology and Biotechnology, 96*(3), 631–645.
3. Ghasemi, Y., Rasoul-Amini, S., Naseri, A. T., Montazeri-Najafabady, N., Mobasher, M. A., & Dabbagh, F. (2012). Microalgae biofuel potentials (review). *Prikladnaia Biokhimiia i Mikrobiologiia, 48*(2), 150–168.
4. Brennan, L., & Owende, P. (2010). Biofuels from microalgae-a review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews, 14*(2), 557–577.
5. Bhola, V., Desikan, R., Santosh, S. K., Subburamu, K., Sanniyasi, E., & Bux, F. (2010). Effects of parameters affecting biomass yield and thermal behaviour of *Chlorella vulgaris*. *Journal of Bioscience and Bioengineering, 111*(3), 377–382.
6. Acien, F. G., Fernandez, J. M., Magan, J. J., & Molina, E. (2012). Production cost of a real microalgae production plant and strategies to reduce it. *Biotechnology Advances, 30*(6), 1344–1353.
7. Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P., et al. (2013). Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. *Bioresource Technology, 47*(13), 4294–4302.
8. Olguín, E. J., Galicia, S., Mercado, G., & Pérez, T. (2003). Annual productivity of *Spirulina* (*Arthrospira*) and nutrient removal in a pig wastewater recycling process under tropical conditions. *Journal of Applied Phycology, 15*(2–3), 249–257.
9. Pittman, J. K., Dean, A. P., & Osundeko, O. (2011). The potential of sustainable algal biofuel production using wastewater resources. *Bioresource Technology, 102*(1), 17–25.
10. Samorì, G., Samorì, C., Guerrini, F., & Pistocchi, R. (2012). Growth and nitrogen removal capacity of *Desmodesmus* communis and of a natural microalgae consortium in a batch culture system in view of urban wastewater treatment: part I. *Water Research, 47*(2), 791–801.
11. Dalrymple, O. K., Halfhide, T., Udom, I., Gilles, B., Wolan, J., Zhang, Q., et al. (2013). Wastewater use in algae production for generation of renewable resources: a review and preliminary results. *Aquatic Biosystem, 9*(1), 2.
12. de-Bashan, L. E., & Bashan, Y. (2010). Immobilized microalgae for removing pollutants: review of practical aspects. *Bioresource Technology, 101*(6), 1611–1627.
13. Mallick, N. (2002). Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. *BioMetals, 15*(4), 377–390.
14. Wang, H., Xiong, H., Hui, Z., & Zeng, X. (2012). Mixotrophic cultivation of *Chlorella pyrenoidosa* with diluted primary piggery wastewater to produce lipids. *Bioresource Technology, 104*, 215–220.
15. Ji, M. K., Kim, H. C., Sapireddy, V. R., Yun, H. S., Abou-Shanab, R. A. I., Choi, J., et al. (2012). Simultaneous nutrient removal and lipid production from pretreated piggery wastewater by *Chlorella vulgaris* YSW-04. *Applied Microbiology and Biotechnology, 97*(6), 2701–2710.
16. Prajapati, S. K., Kaushik, P., Malik, A., & Vijay, V. K. (2013). Phycoremediation and biogas potential of native algal isolates from soil and wastewater. *Bioresource Technology, 135*, 23–28.
17. Prajapati, S. K., Kaushik, P., Malik, A., & Vijay, V. K. (2013). Phycoremediation coupled production of algal biomass, harvesting and anaerobic digestion: possibilities and challenges. *Biotechnology Advances*. doi:10.1016/j.biotechadv.2013.06.005.
18. Bintsis, T., Litopoulou-Tzanetaki, E., & Robinson, R. K. (2000). Existing and potential applications of ultraviolet light in the food industry—a critical review. *Journal of the Science of Food and Agriculture, 80*(6), 637–645.
19. Kawachi, M., & Noël, M. H. (2005). *Sterilization and sterile technique in algal culturing techniques*. Amsterdam: Elsevier Academic Press.
20. Mutanda, T., Karthikeyan, S., & Bux, F. (2011). The utilization of post-chlorinated municipal domestic wastewater for biomass and lipid production by *Chlorella* spp. under batch conditions. *Applied Biochemistry and Biotechnology, 164*(7), 1126–1138.
21. Rippka, R., Deruelles, J., Waterbury, J., Herdman, M., & Stanier, R. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology, 111*(1), 1–61.
22. Bigogno, C., Khozin-Goldberg, I., Boussiba, S., Vonshak, A., & Cohen, Z. (2002). Lipid and fatty acid composition of the green oleaginous alga *Parietochloris incisa*, the richest plant source of arachidonic acid. *Phytochemistry, 60*(5), 497–503.
23. Slover, H. T., & Lanza, E. (1979). Quantitative analysis of food fatty acids by capillary gas chromatography. *Journal of the American Oil Chemists' Society, 56*(12), 933–943.

24. Sforza, E., Cipriani, R., Morosinotto, T., Bertucco, A., & Giacometti, G. M. (2012). Excess CO_2 supply inhibits mixotrophic growth of *Chlorella protothecoides* and *Nannochloropsis salina*. *Bioresource Technology, 104*, 523–529.
25. Woertz, I. (2007). Lipid productivity of Algae grown on dairy wastewater as a possible feedstock for Biodiesel. California Polytechnic University pp. 57–58.
26. Wang, L., Li, Y. C., Chen, P., Min, M., Chen, Y. F., Zhu, J., et al. (2010). Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. *Bioresource Technology, 101*(8), 2623–2628.
27. Song, Y., Hahn, H. H., & Hoffmann, E. (2002). Effect of solution conditions on the precipitation of phosphate for recovery: a thermal dynamic evaluation. *Chemosphere, 48*(10), 1029–1034.
28. Gonzalez, L. E., Canizares, R. O., & Baena, S. (1997). Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*. *Bioresource Technology, 60*(3), 259–262.
29. Peng, Y. J., Li, C., & Zhang, D. W. (2011). Lipid production of *Chlorella vulgaris* cultured in artificial wastewater medium. *Bioresource Technology, 102*(1), 101–105.
30. Markou, G., Chatzipavlidis, I., & Georgakakis, D. (2012). Cultivation of *Arthrospira* (*Spirulina*) *platensis* in olive-oil mill wastewater treated with sodium hypochlorite. *Bioresource Technology, 112*, 234–241.
31. Lika, K., & Papadakis, I. A. (2009). Modeling the biodegradation of phenolic compounds by microalgae. *Journal of Sea Research, 62*(2–3), 135–146.
32. Converti, A., Casazza, A. A., Ortiz, E. Y., Perego, P., & Del Borghi, M. (2009). Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chemical Engineering and Processing Process Intensification, 48*(6), 1146–1151.
33. Woertz, I., Feffer, A., Lundquist, T., & Nelson, Y. (2009). Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. *Journal of Environmental Engineering, 135*(11), 1115–1122.
34. Dijkstra, A. J. (2006). Revisiting the formation of trans isomers during partial hydrogenation of triacylglycerol oils. *European Journal of Lipid Science and Technology, 108*(3), 249–264.
35. Knothe, G. (2006). Analyzing biodiesel: standards and other methods. *Journal of the American Oil Chemists' Society, 83*(10), 823–833.