Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2013.769917

51 (2013) 7004–7011
November

Taylor & Francis
Taylor & Francis Group

Enhancement of biomass productivity and nutrients removal from pretreated piggery wastewater by mixotrophic cultivation of *Desmodesmus* sp. CHX1

Haixiang Cheng, Guangming Tian*, Junzhi Liu

Department of Environmental Engineering, Zhejiang University, Hangzhou 310058, China
Tel./Fax: +86 571 86971898; email: 10914024@zju.edu.cn

Received 7 November 2012; Accepted 19 January 2013

ABSTRACT

Promoting biomass production of the involved strains simultaneously with efficient nutrients removal from wastewater is realistic significance to ensure successful application of microalgae-based process for wastewater treatment. Enhancement of biomass productivity and nutrients removal by mixotrophic cultivation of *Desmodesmus* sp. CHX1 was conducted in this study when treating aerated piggery wastewater. The results showed that air-stripping might be an effective option as a pre-treatment to remove ammonia nitrogen from piggery wastewater. Mixotrophic cultivation of microalga-bacteria system significantly promoted algal growth and nutrients removal efficiency with the maximal biomass and lipid productivity being $0.869\,g\,l^{-1}\,d^{-1}$ and $118.2\,mg\,l^{-1}\,d^{-1}$ (14.5% of the lipid content), respectively, which were superior to other reported values. Nutrients in the piggery wastewater were also removed efficiently, for example, the removal rates of total nitrogen, ammonia nitrogen and total phosphorus were 87.3%, over 95%, and 93.1%, respectively. This study suggested that mixotrophic cultivation of microalgae-bacteria system might be a practical alternative to efficiently enhance nutrients removal from piggery wastewater coupled with biomass production. A slight reduction of chemical oxygen demand (COD), however, indicate that it cannot replace the traditional biological treatment, and more researches are required to find the optimum balance between reduction in COD value and biomass production.

Keywords: Enhancement; *Desmodesmus* sp. CHX1; Mixotrophic cultivation; Biomass production; Nutrients removal

1. Introduction

Effluents from intensive pig farms often contain high concentrations of nutrients (organic and inorganic), which have been identified as the main causes leading to surface water eutrophication and groundwater pollution [1,2]. Therefore, the wastewater must receive suitable treatment before being discharged into water bodies. Conventional biological treatment processes, including activated sludge process [3] and anaerobic digestion followed by post-treatment in high-rate oxidation ponds [4], exists for the removal of nutrients from wastewater, but these treatment technologies only focused on the innocent treatment and neglect the resourceful utilization of the

*Corresponding author.

wastewater, additionally, lower removal efficiency of nutrients associated with occupying large land area retarded widespread implementation in rural areas. In this context, to develop and implement cost-effective technologies that can reduce export of nutrients into the watershed while increasing farm profits was crucial for the establishment of sustainable farming [5].

Microalgae-based process has been proposed as an alternative biological treatment to solve the problem [6]. In this system, microalgae could serve as a dual role of bioremediation of wastewater as well as generating biomass for high-protein feed supplements or biofuel production with concomitant carbon dioxide sequestration [7–10]. With the above-mentioned merits, many studies have been conducted for treating piggery wastewater, including diluted primary effluent [10,11], secondary effluent treated by anaerobic digestion [4,12] and effluent from wastewater treatment facilities [13,14]. However, the success of the wastewater treatment system heavily depends on the performance of the involved strains. Also, the variations in the composition of wastewater would limit such a notion as only specific strain performs their potential. Many researchers pointed out that algae isolated from local wastewater treatment plant site or real water body can adapt to the practical conditions better and show higher removal efficiency [15–17]. In our previous study, a newly isolated microalga identified as *Desmodesmus* sp. (strain CHX1) has presented fast growth and high nutrients removal efficiency when growing in diluted piggery effluent collected from a local stabilization pond, being considered as a candidate strain involved in the ongoing major collaborative efforts on mass cultivation of algae on piggery wastewater for bioremediation and biomass production between Zhejiang University and the local Government [18]. However, the dilution of wastewater prior to biological decomposition is a serious restriction to be used commercially. To enhance the practical feasibility of using *Desmodesmus* sp. CHX1 for treating piggery wastewater, pretreatment other than dilution is mandatory. Ammonia air-stripping commended by Bonmati and Flotats [19] might be a good option as a pre-treatment, as high concentration of NH_4^+–N was observed in the piggery wastewater.

Recently, industrial cultivation of microalgae has not been realized mainly due to the slow growth and low biomass production of the involved strains. So, improving growth and biomass production of *Desmodesmus* sp. CHX1 is also important for its subsequent industrial cultivation. Wang [11] proposed that mixotrophic cultivation of microalgae could offer the possibility of greatly increasing the cell density and productivity, and was considered to provide a simplified way to produce algal oil and simultaneously treat high organic content wastewater [20–22]. Zhao et al. [23] also suggested that native bacteria in the natural water bodies often contributed to algal growth, and were recognized as an important factor in the physiology and dynamics of algal blooms [24]. Despite the advantages of the native bacteria, wastewaters engaged in the previous studies were always used after being filtered or auto-claved to prevent the strains interaction with other microorganisms. Few investigations on the use of algal-bacterial systems for the treatment of livestock effluents have been reported [25,26]. There is, indeed, a lack of basic research addressing the practical processes treating piggery breeding effluent based on algal-bacterial system under mixotrophic cultivation condition.

Therefore, this study attempted to evaluate the feasibility of air-stripping without adjustment of initial pH as a pre-treatment to reduce NH_4^+–N concentration of piggery wastewater, and preliminarily to investigate the characteristics of biomass production and nutrients removal from piggery wastewater during the treatment of pretreated piggery wastewater based on algal-bacterial system under mixotrophic condition.

2. Methods and materials

2.1. Algal strain and medium for pre-culture

The strain used in this study was isolated from a local pond and identified as *Desmodesmus* sp. CHX1 with assistance of both morphological observation and DNA sequencing (Genebank No.: JX258841). The isolated microalga was maintained in a modified BG-11 medium, which consists of the following components shown in Table 1 and de-ionized water was adopted as the solvent, on an orbital shaker at 150 rpm at 27 ± 2°C with two-compacted fluorescent

Table 1
Components of BG-11 medium

Component	Content ($mg\,l^{-1}$)	Component	Content ($mg\,l^{-1}$)
$NaNO_3$	1,500	Na_2-EDTA	1
$K_2HPO_4{\cdot}3H_2O$	40	H_3BO_3	2.86
$MgSO_4{\cdot}7H_2O$	75	$MnCl_2{\cdot}H_2O$	1.81
$CaCl_2{\cdot}2H_2O$	36	$ZnSO_4{\cdot}7H_2O$	0.222
Na_2CO_3	20	$CuSO_4{\cdot}5H_2O$	0.079
$C_6H_8O_7{\cdot}H_2O$	6	$NaMoO_4{\cdot}2H_2O$	0.39
$Fe(NH_4)_3C_{18}H_{10}O_{14}$	6	$Co(NO_3)_2{\cdot}6H_2O$	0.049

lights (Philips TLD 55 W fluorescent lamp) providing round-the-clock illumination at light intensity of 60 μmol photo $m^{-2} s^{-1}$.

2.2. Piggery wastewater

The wastewater was collected from a stabilization pond located in a piggery farm in Xiaoshan District, Hangzhou City, China, which was mainly composed of urine and flushing water directly discharged from the breeding zones. Wastewaters from different locations around the pond were sampled, mixed and stored in two 25 l buckets. After being sent to the laboratory, portions of the wastewater required for the experiment were transferred into sterile transparent plastic bottles and stored in a refrigeratory maintained at 4℃ to avoid the variation of the composition with collection time. The characteristics of the effluent were summarized in Table 2.

2.3. Pretreatment of the collected piggery wastewater

In the preliminary experiment, the raw wastewater resulted in algal aggregations when directly adopted as the growth medium, showing an inhibitory growth of *Desmodesmus* sp. CHX1. It might be attributed to high concentration of NH_4^+–N in the wastewater [18]. Therefore, we attempted to remove NH_4^+–N from the wastewater by air-stripping prior to the subsequent application for the cultivation of *Desmodesmus* sp. CHX1. The wastewater without adjustment of initial pH was aerated through air that had been sterilized with a 0.2 μm membrane filter at a flow rate of 1,500 ml min^{-1} for a period of 12 h per day at room temperature. After seven-day cycle, pretreated wastewater was prepared as growth medium, and the pretreated wastewater after being auto-claved (121℃ for 30 min) was adopted as the positive control.

Table 2
Characteristics of the piggery wastewater used in the experiment

Item	Concentration (mg l^{-1})	
	Before pretreatment	After pretreatment
pH	7.44 ± 0.01	8.00 ± 0.00
Total N	898.2 ± 3.26	88.0 ± 4.07
Total P	19.6 ± 0.39	7.51 ± 0.11
(NH_4^+–N)	854.3 ± 46.6	86.5 ± 4.74
COD_{cr}	2116.9 ± 139.2	1616.9 ± 80.2
Total Cu (μg l^{-1})	530.9 ± 6.20	492.0 ± 7.81
Total Zn (μg l^{-1})	261.7 ± 6.71	144.5 ± 0.00

2.4. Batch experiments

Treatments, namely as T1, T2, and T3 were adopted in the experiment, and descriptions were shown in Table 3. After being adjusted initial pH to 10.0 by 1.0 M NaOH, 500 ml of above-mentioned growth medium was put in sterile 1,000-ml conical flasks, and algal culture (OD_{690} = 2.08) was inoculated at 10% (v/v). Light was continuously supplied (Philips TLD 55 W fluorescent lamp) at an intensity of 100 μmol photo $m^{-2} s^{-1}$ measured at the surface of the flask with an illuminance meter (ZDS-10, Shanghai Cany Precision Instrument Ltd., China). Batchwise cultivation lasted for eight days at 27 ± 2℃, and then, media were harvested per two days for measurement. No inoculations were served as the corresponding negative control to observe the variation of the composition of the growth media with cultivation time elapse. All experiments were carried out in triplicate and average values with standard deviation were reported.

2.5. Determination of algal growth

Biomass concentration (DCW, dry-weight of the cells in culture medium, g l^{-1}) was estimated by an equation that corroborated the dry-weight determinations [18]:

$$\text{DCW (g l}^{-1}) = 0.9914\text{OD}_{690} - 0.0216, R^2 = 0.9455$$

where OD_{690} was the absorbance measured at 690 nm by a UV–vis spectrophotometer (Thermo Evolution 220, USA).

The specific growth rate was calculated by fitting the cell dry weight:

$$\text{GR (day}^{-1}) = (\ln X - \ln X_0)/t,$$

where t (day) was the time between the two measurements, X and X_0(g l^{-1}) were the concentrations of biomass at day t and t_0, respectively.

Table 3
Description of culturing media

Treatment	Medium
T1	Pretreated wastewater after auto-clave
T2	Pretreated wastewater
T3	Pretreated wastewater bubbled with air containing 5% CO_2 (v:v) that had been sterilized with a 0.2 μm membrane filter at a flow rate of 100 ml min^{-1}

The biomass productivity during the culture period was calculated from the equation:

$$P_{\text{biomass}}(\text{g l}^{-1}\text{day}^{-1}) = (X - X_0)/t,$$

where X was the concentrations of biomass at the end of the cultivation, and $X_0(\text{g l}^{-1})$ was that at the beginning, and t was the duration of cultivation (eight days).

2.6. Measurement of lipid production

Total lipid was determined by the method of Zhou et al. [17]. The cell suspensions were collected and centrifuged at 5,000 rpm for 15 min. Then, the pellets were freeze-dried and stored at −20°C before analysis. The lipid contents were calculated from the equation:

$$C_{\text{lipid}}\ (\text{g g}^{-1}) = W_{\text{L}}/W_{\text{A}}$$

where W_{L} (g) was the weight of the extracted lipids and W_{A} (g) was the dry algal biomass.

The lipid productivity was calculated from the equation:

$$P_{\text{lipid}}(\text{g l}^{-1}\text{day}^{-1}) = (C_t X - C_0 X_0)/t$$

where $C_0(\text{g g}^{-1})$was the content of microalgae lipid at the beginning and $C_t(\text{g g}^{-1})$ was that at the end of the cultivation, X and $X_0(\text{g l}^{-1})$ were the concentrations of biomass at the corresponding end and beginning, respectively, and t was the duration of cultivation (eight days).

2.7. Chemical analysis

Liquid sample for nutrient analysis was collected and filtered through a 0.45 μm pore-size membrane. The filtration was collected and properly diluted for analysis of total nitrogen (TN), ammonium (NH_4^+–N), total phosphorus (TP), chemical oxygen demand (COD). TN, (NH_4^+–N), TP and COD were determined following the standard methods [27].

3. Results and discussion

3.1. Characterization of wastewater before and after pretreatment

The characteristics of the piggery wastewater before and after pre-treatment were shown in Table 1. Wastewater was rich in (NH_4^+–N) with the concentration being as high as 854.3 mg l^{-1}, which was much higher than 750 mg l^{-1} as shown by Tam and Wong [28] who observed a complete inhibition of *Chlorlla vulgaris*. Hereby, pre-treatment with the aim of reducing the (NH_4^+–N) concentration in the wastewater was adopted prior to the cultivation of *Desmodesmus* sp. CHX1, and avoided retarding the algal growth observed in the preliminary experiments. After aeration for seven-day cycle, (NH_4^+–N) concentration was remarkably reduced from 854.3 to 86.5 mg l^{-1}, and a fraction of TN, TP, COD, Cu, and Zn was also significantly reduced. This indicated that air-stripping without adjustment of initial pH could be adopted as an effective pre-treatment method to reducing toxicity of piggery wastewater which contained high concentration of ammonia nitrogen. Generally, (NH_4^+–N) at normal cultivation levels was preferred by many microalgae species [29], and the uptake of ammonium was considered to be important in the removal of nitrogen during wastewater treatment [30].

3.2. Growth of Desmodesmus sp. CHX1 under cultivation conditions

Algal growth, as indicated by biomass concentrations in piggery wastewater under cultivation conditions, was shown in Fig. 1. With no obvious lag phase exhibited, the newly isolated *Desmodesmus* sp. CHX1 survived in all samples of piggery wastewater, which indicated that piggery wastewater after pre-treatment presented less-toxic and valuable compounds required for growth of microalgae and further testified that air-stripping could be adopted as an effective pretreatment process. Fast growth of *Desmodesmus* sp. CHX1 was observed in T3 treatment, followed by in T2

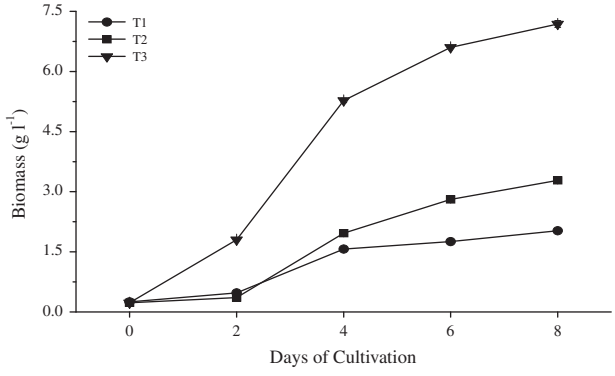

Fig. 1. Growth curves of *Desmodesmus* sp. CHX1 in pretreated wastewater under different cultivation conditions.

treatment, both of which were quite superior to T1 (the control) which was autoclaved for repressing microbial activity. By comparison, there was a significant difference in biomass productivities between the best and the control cultivation, which were 0.869 and $0.222\,g\,l^{-1}\,d^{-1}$, respectively. It indicated that no sterilization of the wastewater simultaneously with CO_2 addition could enhance algal biomass productivity, and further demonstrated that the isolated strain *Desmodesmus* sp. CHX1 is capable of mixotrophic growth. More studies have reported that CO_2 addition augments carbon availability in the medium for algal growth and also serves to mitigate pH inhibition to the native bacteria [26,31]. Bacterial growth in the wastewater in turn could enhance microalgal metabolism by releasing growth-promoting factors [25] or by reducing O_2 concentration in the medium [32,33]. Consequently, the symbiotic microalgal-bacterial relationship under mixotrophic cultivation is shown to be a good strategy to obtain a large biomass and high growth rates [34–36]. For the newly isolated microalga *Desmodesmus* sp. CHX1, no sterilization of the effluent simultaneously with CO_2 addition might be alternatively indeed standard practice in the latter experiments.

3.3. *Nutrients removal efficiencies of* Desmodesmus *sp. CHX1 under cultivation conditions*

Removal efficiencies of (NH_4^+–N), TN, TP and COD during cultivation period by *Desmodesmus* sp. CHX1 were shown in Fig. 2. Most of the nitrogen in the pretreated piggery wastewater was in the form of ammonium nitrogen, which was one of the forms of inorganic nitrogen that could be directly assimilated for all eukaryotic algae, including *Desmodesmus* sp. CHX1, and therefore could be readily available to algae [12]. Results from this experiment showed that

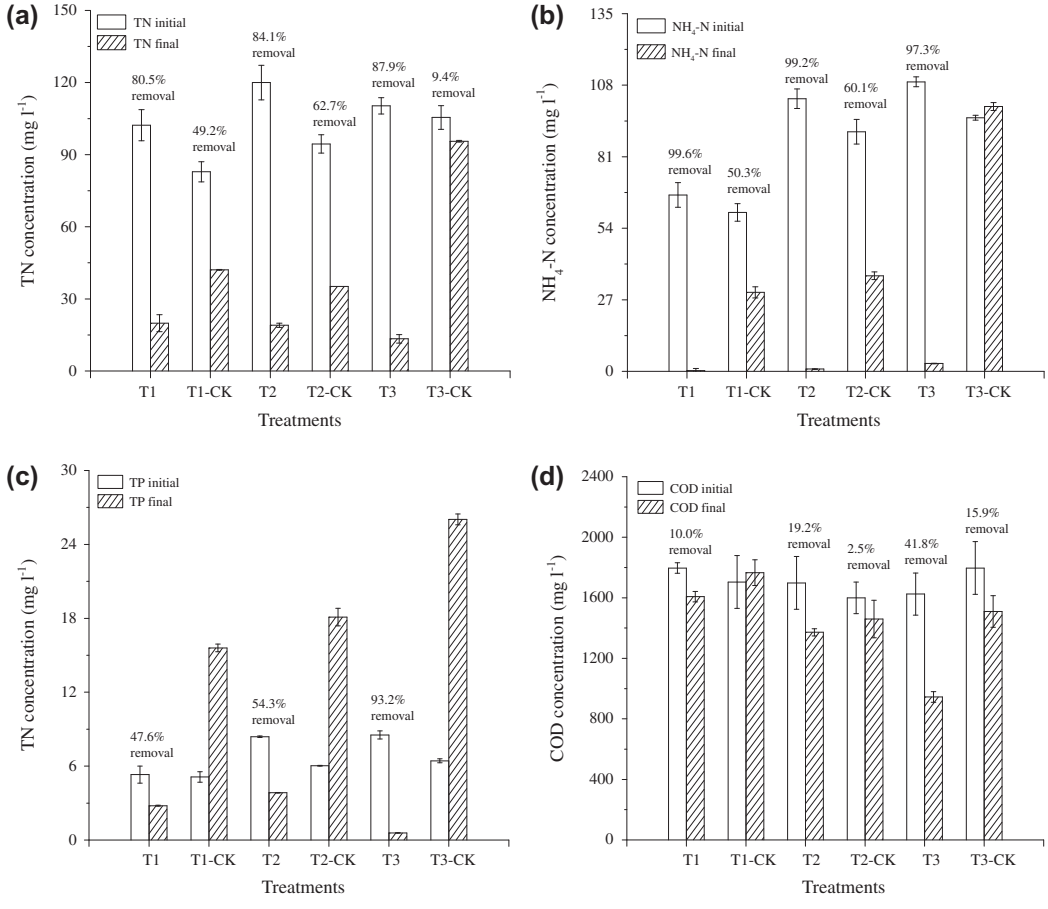

Fig. 2. The removal rates of (a) TN, (b) ammonium nitrogen, (c) TP and (d) COD calculated from their concentrations at the initial and final stages of the cultivation of *Desmodesmus* sp. CHX1 in pretreated wastewater under different cultivation conditions (TN: total nitrogen; (NH_4^+–N): ammonia nitrogen; TP: total phosphorus; COD: chemical oxygen demand).

(NH_4^+–N) removal of over 95% was achieved for all inoculation treatments as indicated in Fig. 2(b). The concentration dropped from 66.6 to 0.3 mg l^{-1} in T1, from 102.9 to 0.86 mg l^{-1} in T2, and from 109.3 to 2.97 mg l^{-1} in T3, respectively. Remarkable reduction in TN and TP were also observed for all inoculation treatments; 80.5–87.9% removal of TN and 47.6–93.1% removal of TP were superior to the published results obtained by Wang et al. [11] who found that *Chlorella pyrenoidosa* cultivated in diluted primary piggery wastewater could provide removal rates of 54.7–74.6% for TN and 31.0–77.7% for TP. Moreover, a slight reduction in COD was observed in the axenic culture condition (T1), indicating *Desmodesmus* sp. CHX1 could utilize organic carbon as a source of energy and a substrate for the cell growth. COD removal was enhanced in T3 when the piggery wastewater was proceeded by algal-bacterial process with the addition of CO_2, which was in agreement with results reported by *de* Godos et al. [37], who observed 76% of COD was removed in piggery wastewater associated with microbe in high rate algal ponds.

Microalgae could assimilate a significant amount of nutrients because they require high amounts of nitrogen and phosphorus for proteins, nucleic acids and phospholipids synthesis. Nutrient removal can also be further increased by NH_3 stripping or P precipitation due to the raise in the pH associated with photosynthesis [15,31,38,39]. In this study, the media pH of T1 and T2 were also significantly ascended during the cultivation period (Fig. 3). Compared with the negative control, the reduction in nutrients was significantly enhanced due to the metabolism of *Desmodesmus* sp. CHX1, which might be reasonable to conclude that the nutrients removal in T1 and T2 treatments was attributed to the conjunction of algal metabolic uptake and phosphates-precipitation as well as NH_3-stripping. Unlike the process of T1 and T2, the medium pH of T3 was around seven with assistance of CO_2 addition, and consequently, the nutrients removal was mainly related to the utilization by the involved algal-bacterial strains, which performed a practical significance of increasing the recovery of nutrients in the wastewater into biomass production. Therefore, efficient nutrients recovery from piggery wastewater coupled with fast growth were propitious to the practical potential of the newly isolated microalga *Desmodesmus* sp. CHX1 for treating piggery wastewater under the condition of mixotrophic cultivation.

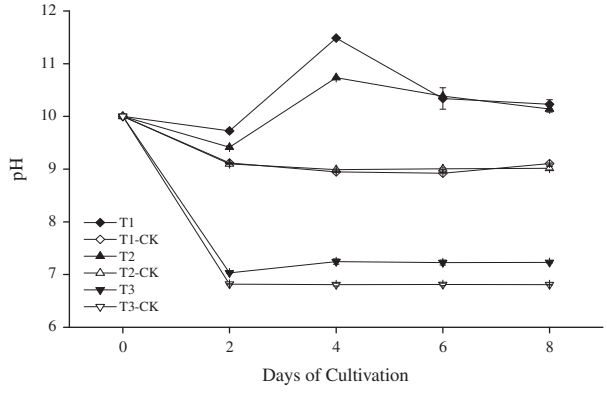

Fig. 3. Changes of media pH during cultivation period in inoculation treatments and the negative controls.

3.4. Lipid content and lipid productivity of Desmodesmus sp. CHX1 under cultivation conditions

Microalgae based on wastewater for biofuel production have received considerable attention [17] and selection of fast-growing and high-lipid-productivity strains is of fundamental importance to the success of commercial applications of microalgae [40]. Therefore, in this study, we also investigate the lipid production performance of the isolated strain *Desmodesmus* sp. CHX1 when grown on pretreated piggery wastewater. The results showed that lipid contents grown in piggery wastewater were roughly at equal level, ranging from 9.90 to 14.5% (Fig. 4), which is similar to those reported in the literatures, showing that in general a moderate lipid content of 10–18% was obtained from *Scenedesmus* sp. [41–43]. But the lipid productivities were evidently different. The highest lipid productivity of 118.2 mg l^{-1} d^{-1} was obtained in T3, followed by 55.3 mg l^{-1} d^{-1} of lipid productivity in T2, and 21.9 mg l^{-1} d^{-1} in T1 (Fig. 4). The lower level of lipid

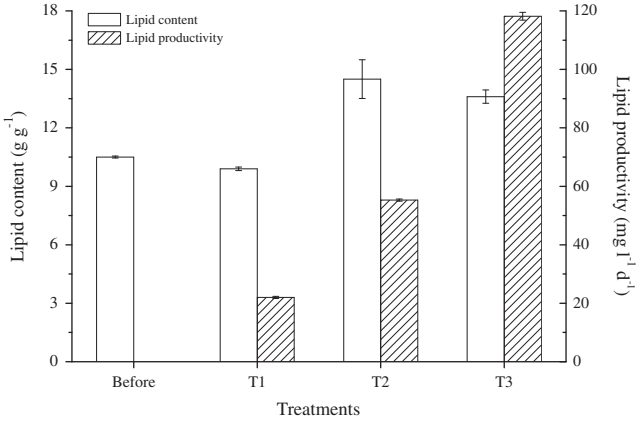

Fig. 4. Lipid content and lipid productivity from *Desmodesmus* sp. CHX1 culture in pretreated wastewater under different cultivation conditions.

productivity in T1 should be due to its lower biomass, which was almost half of that in T3 (Fig. 1).

Griffiths and Harrison [40] have demonstrated a general correlation between biomass productivity and lipid productivity, further indicating that lipid productivity is a key indicator of species for biodiesel production. The biomass and lipid productivity obtained in the present work was superior to those reported elsewhere using different strains of microalgae [11,17,44]. For instance, Wang et al. [11] reported that mixotrophic cultivation of *C. pyrenoidosa* in diluted piggery wastewater had the maximum lipid productivity of $6.3\,mg\,l^{-1}\,d^{-1}$ when the initial COD was $1{,}000\,mg\,l^{-1}$. In Woertz's et al. report, the maximum lipid productivities of *C. vulgaris* achieved for microalgae culture in a semi-continuous mode were $17\,mg\,l^{-1}\,day^{-1}$ on dairy wastewater and $24\,mg\,l^{-1}\,day^{-1}$ on municipal wastewater [44]. This suggests that in continuous culture mode the lipid productivity in piggery wastewater might be improved by continuous supplementation of nutrients such as ammonium, which has a limited concentration in batch cultivation. If this method could be adapted on a commercial scale, transforming piggery wastewater into biodiesel by algae approach with no food competition issues and provide a valuable mode of degrading sewage for environmental protection [11]. More research is required to find the optimum balance between lipid productivity and nutrients recovery.

4. Conclusions

Air-stripping could be adopted as an effective pretreatment method to reduce toxicity of piggery wastewater with a high concentration of ammonia nitrogen prior to its utilization for the cultivation of *Desmodesmus* sp. CHX1, and mixotrophic mode provides an effective method for reducing the values of TN, TP and ammonia nitrogen into a profitable biomass production, further indicating that *Desmodesmus* sp. CHX1 is a strain with potential uses in the development of a large-scale process of piggery wastewater treatment. However, the treatment we proposed is not enough, and is necessary to fit together with conventional treatment as to get a significant reduction of COD. It is highly recommended that more experiments are conducted to find the optimum balance between reduction in COD value and biomass production on a large scale.

Acknowledgements

The study is supported by the Major Project on Control and Management Technology of Water Pollution of China (2009ZX07317-008), the Natural Science Foundation of Zhejiang province, China (NO. Y12E080084) and China Postdoctoral Science Foundation (20110491792). The authors gratefully acknowledge all the reviewers for their great contribution to the improvement of this article.

References

[1] X. Flotats, A. Bonmati, B. Fernandez, A. Magri, Manure treatment technologies: on farm versus centralized strategies, NE Spain as case study, Bioresour. Technol. 100 (2009) 5519–5526.
[2] I.G. Krapaca, W.S. Deya, W.R. Roya, C.A. Smyth, E. Storment, S.L. Sargent, J.D. Steele, Impacts of swine manure pits on groundwater quality, Environ. Pollut. 120 (2002) 475–492.
[3] L. Wang, J. Zhu, C. Miller, Nitrite accumulation from swine wastewater in sequencing batch reactor, Trans. A.S.A.B.E. 52 (2009) 1363–1370.
[4] E.J. Olguin, Phycoremediation: key issues for cost-effective nutrient removal processes, Biotechnol. Adv. 22 (2003) 81–91.
[5] Chesapeake Bay Foundation, A guide to preserving agricultural lands in the Chesapeake Bay region: keeping stewards on the land, 2006, http://www.cbf.org/site/DocServer/CbF-Preserving Farmland_Finalpd f?docID=5934.
[6] I. Rawat, R.R. Kumar, T. Mutanda, F. Bux, Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production, Appl. Energy 88 (2011) 3411–3424.
[7] W. Mulbry, S. Kondrad, C. Pizarro, E. Kebede-Westhead, Treatment of dairy manure effluent using freshwater algae: algal productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers, Bioresour. Technol. 99 (2008) 8137–8142.
[8] J.K. Pittman, A.P. Dean, O. Osundeko, The potential of sustainable algal biofuel production using wastewater resources, Bioresour. Technol. 102 (2011) 17–25.
[9] H.L. Tran, S.J. Hong, C.G. Lee, Evaluation of extraction methods for recovery of fatty acids from *Botryococcus braunii* LB 572 and *Synechocystis sp*. PCC 6803, Biotechnol. Bioproc. E. 14 (2009) 187–192.
[10] L. Travieso, F. Benitez, E. Sanchez, R. Borja, A. Martin, Batch mixed culture of *Chlorlla vulgaris* using settled and diluted piggery waste, Ecol. Eng. 28 (2006) 158–162.
[11] H.Y. Wang, H.R. Xiong, Z.L. Hui, X.B. Zeng, Mixotrophic cultivation of *Chlorella pyrenoidosa* with diluted primary piggery wastewater to produce lipids, Bioresour. Technol. 104 (2012) 215–220.
[12] L. Wang, Y.C. Li, P. Chen, M. Min, Y.F. Chen, J. Zhu, R. Ruan, Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella* sp., Bioresour. Technol. 101 (2010) 2623–2628.
[13] M.K. Kim, J.W. Park, C.S. Park, S.J. Kim, K.H. Jeune, M.U. Chang, J. Acreman, Enhanced production of *Scenedesmus sp*. (green microalgae) using a new medium containing fermented swine wastewater, Bioresour. Technol. 98 (2007) 2220–2228.
[14] M.S. Kumar, Z.H.H. Miao, S.K. Wyatt, Influence of nutrient loads, feeding frequency and inoculum source on growth of *Chlorella vulgaris* in digested piggery effluent culture medium, Bioresour. Technol. 101 (2010) 6012–6018.
[15] X. Li, H.Y. Hu, J. Yang, Lipid accumulation and nutrient removal properties of a newly isolated freshwater microalga *Scenedesmus* sp. LX1, growing in secondary effluent, New Biotechnol. 27 (2010) 59–63.
[16] M.V.J. Perez, P.S. Castillo, O. Romera, D.F. Moreno, C.P. Martinez, Growth and nutrient removal in free and immobilized planktonic green algae isolated from pig manure, Enzyme Microb. Technol. 34 (2004) 392–398.

[17] W.G. Zhou, Y.C. Li, M. Min, B. Hu, P. Chen, R. Ruan, Local bioprospecting for high-lipid producing microalgal strains to be grown on concentrated municipal wastewater for biofuel production, Bioresour. Technol. 102 (2011) 6909–6919.
[18] H.X. Cheng, G.M. Tian, Identification of a newly isolated microalga from a local pond and evaluation of its growth and nutrients removal potential in swine breeding effluent, Desalin. Water Treat. (in press).
[19] A. Bonmati, X. Flotats, Air stripping of ammonia from pig slurry: characterization and feasibility as a pre- or post-treatment to mesophilic anaerobic digestion, Waste Manage. 23 (2003) 261–272.
[20] M.R. Andrade, J.A.V. Costa, Mixotrophic cultivation of microalga *Spirulina platensis* using molasses as organic substrate, Aquacul. 264 (2007) 130–134.
[21] Y.N. Liang, N. Sarkany, Y. Cui, Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions, Biotechnol. Lett. 31 (2009) 1043–1049.
[22] J.M. Lv, L.H. Cheng, X.H. Xu, L. Zhang, H.L. Chen, Enhanced lipid production of *Chlorella vulgaris* by adjustment of cultivation conditions, Bioresour. Technol. 101 (2010) 6797–6804.
[23] G.Y. Zhao, J.J. Du, Y. Jia, Y.N. Lv, G.M. Han, X.J. Tian, The importance of bacteria in promoting algal growth in eutrophic lakes with limited available phosphorus, Ecol. Eng. 42 (2012) 107–111.
[24] J.Q. Liu, A.J. Lewitus, P. Brown, S.B. Wilde, Growth-promoting effects of a bacterium on raphidophytes and other phytoplankton, Harmful Algae 7 (2008) 1–10.
[25] L.E. de Bashan, M. Moreno, J.P. Hernandez, Y. Bashan, Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae *Chlorella vulgaris* coimmobilized in alginate beads with the microalgae growth-promoting bacterium *Azospirillum brasilense*, Water Res. 36 (2002) 2941–2948.
[26] C. Gonzalez, J. Marciniak, S. Villaverde, P.A. Garcia-Encina, R. Munoz, Microalgae-based processes for the biodegradation of pretreated piggery wastewaters, Appl. Microbiol. Biotechnol. 80 (2008) 891–898.
[27] L.S. Clesceri, A.E. Greenberg, A.D. Eaton, Standard Methods for Examination of Water and Wastewater, 20th ed., American Public Health Association Washington, DC, 1998.
[28] N.F.Y. Tam, Y.S. Wong, Effect of ammonia concentrations on growth of *Chlorella vulgaris* and nitrogen removal from media, Bioresour. Technol. 57 (1996) 45–50.
[29] S.R. Josette, Biotreatment of swine manure by production of aquatic valuable biomass, Agr. Ecosyst. Environ. 68 (1998) 177–186.
[30] J.M. Park, H.F. Jin, B.R. Lim, K.Y. Park, K. Lee, Ammonia removal from anaerobic digestion effluent of livestock waste using green alga *Scenedesmus sp*, Bioresour. Technol. 101 (2010) 8649–8657.
[31] R. Muňoz, B. Guieysse, Algal-bacterial processes for the treatment of hazardous contaminants: a review, Water Res. 40 (2006) 2799–2815.
[32] E. Molina, J. Fernandez, G. Acien Fernandez, Y. Chisti, Tubular photobioreactor design for algal cultures, J. Biotechnol. 92 (2001) 113–131.
[33] J.L. Mouget, A. Dakhama, M.C. Lavoie, J. De la Noue, Algal growth enhancement by bacteria: is consumption of photosynthetic oxygen involved? FEMS Microbiol. Ecol. 18 (1995) 35–43.
[34] A.P. Abreu, B. Fernandes, A.A. Vicente, J. Teixeira, G. Dragone, Mixotrophic cultivation of *Chlorella vulgaris* using industrial dairy waste as organic carbon source, Bioresour. Technol. 118 (2012) 61–66.
[35] T. Heredia-Arroyo, W. Wei, R. Ruan, B. Hu, Mixotrophic cultivation of *Chlorella vulgaris* and its potential application for the oil accumulation from non-sugar materials, Biomass Bioenerg. 35 (2011) 2245–2253.
[36] W.B. Kong, H. Song, S.F. Hua, H. Yang, Q. Yang, C.G. Xia, Enhancement of biomass and hydrocarbon productivities of *Botryococcus braunii* by mixotrophic cultivation and its application in brewery wastewater treatment, Afr. J. Microbiol. Res. 6 (2012) 1489–1496.
[37] I. de Godos, S. Bianco, P.A. Garcia-Encina, E. Becares, R. Munoz, Long-term operation of high rate algal ponds for the bioremediation of piggery wastewaters at high loading rates, Bioresour. Technol. 100 (2009) 4332–4339.
[38] S.B. He, G. Xue, Algal-based immobilization process to treatment the effluent from a secondary wastewater treatment plant (WWTP), J. Hazard. Mater. 178 (2010) 895–899.
[39] Y.C. Li, Y.F. Chen, P. Chen, M. Min, W.G. Zhou, B. Martinez, J. Zhu, R. Ruan, Characterization of a microalga *Chlorella sp.* well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production, Bioresour. Technol. 102 (2011) 5138–5144.
[40] M.J. Griffiths, S.T.L. Harrison, Lipid productivity as a key characteristic for choosing algal species for biodiesel production, J. Appl. Phycol. 21 (2009) 493–507.
[41] L. Gouveia, A.C. Oliveira, Microalgae as a raw material for biofuels production, J. Int. Microbiol. Biotechnol. 36 (2009) 269–274.
[42] S.H. Ho, W.M. Chen, J.S. Chang, *Scenedesmus obliquus* CNWN as a potential candidate for CO_2 mitigation and biodiesel production, Bioresour. Technol. 101 (2010) 8752–8760.
[43] C. Yoo, S.Y. Jun, J.Y. Lee, C.Y. Ahn, H.M. Oh, Selection of microalgae for lipid production under high levels carbon dioxide, Bioresour. Technol. 101 (2010) S71–74.
[44] I. Woertz, A. Feffer, T. Lundquist, Y. Nelson, Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock, J. Environ. Eng.-ASCE 135 (2009) 1115–1122.