Domestic wastewater treatment and biofuel production by using microalga *Scenedesmus* sp. ZTY1

Tian-Yuan Zhang, Yin-Hu Wu and Hong-Ying Hu

ABSTRACT

Cultivation of microalgae for biomass production is a promising way to dispose of wastewater and recover nutrients simultaneously. The properties of nutrient removal and biomass production in domestic wastewater of a newly isolated microalga *Scenedesmus* sp. ZTY1 were investigated in this study. *Scenedesmus* sp. ZTY1, which was isolated from a wastewater treatment plant in Beijing, grew well in both the primary and secondary effluents of a wastewater treatment plant during the 21-day cultivation, with a maximal algal density of 3.6×10^6 and 1.9×10^6 cells $\cdot$ mL^{-1}, respectively. The total phosphorus concentrations in both effluents could be efficiently removed by over 97% after the cultivation. A high removal rate (over 90%) of total nitrogen (TN) was also observed. After cultivation in primary effluent for 21 days, the lipid content of *Scenedesmus* sp. ZTY1 in dry weight had reached about 32.2%. The lipid and triacylglycerol (TAG) production of *Scenedesmus* sp. ZTY1 was increased significantly with the extension of cultivation time. The TAG production of *Scenedesmus* sp. ZTY1 increased from 32 mg L^{-1} at 21 d to 148 mg L^{-1} at 45 d in primary effluent. All the experiments were carried out in non-sterilized domestic wastewater and *Scenedesmus* sp. ZTY1 showed good adaptability to the domestic wastewater environment.

Key words | biodiesel, lipid, microalgae, *Scenedesmus* sp., wastewater treatment

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INTRODUCTION

The excessive consumption of fossil fuel is one of the largest problems for humans in the 21st century. In order to reduce the risk of energy shortage (Salameh 2003) and global warming (Brennan & Owende 2010), which were heightened by the consumption of fossil fuel, the development of biodiesel has been seriously considered and supported in recent years. The Energy Independence and Security Act (EISA) was passed by the federal government of the USA in 2007 to increase the production of renewable fuels to 36 billion (10^9) gallons per year by 2022 (Congress 2007). Microalgae biomass, which is capable of meeting the large demand of transport fuels, is a very promising source for biodiesel production (Chisti 2007).

However, the large-scale cultivation of microalgae biomass would consume a huge amount of water and nutrient. Through life-cycle analysis, Yang *et al.* (2011) reported that to reach the EISA goal of biodiesel production in 2022, the demand for water and phosphorus would account for over 85% and 103% of the total national usage of the USA in 2010, respectively. This enormous cost of water and nutrient is unaffordable and severely limits the commercial use of microalgal biofuel (Behzadi & Farid 2007).

As domestic wastewater is a stable, nearly zero-cost source of huge amounts of water and nutrients, a feasible way to significantly reduce the cost of microalgae-based biodiesel is to couple biomass production with the wastewater treatment process (Li *et al.* 2010). Through economic analysis, Lundquist *et al.* (2010) reported that after taking the profit from the wastewater disposal fee into account, the overall cost of microalgae-based biodiesel could be minimized to $28/bbl, which was much cheaper than the general cost of fossil diesel. Beside the extraction of lipids, the residual microalgal biomass is also considered as a healthy feed for animals due to its high protein content (Bassi *et al.* 2014). The N and P assimilated by microalgae can be reused by using the microalgal biomass as animal feed. Compared with the activated sludge process, which just focuses on the disposal of contaminants and fails to recover the nutrients in wastewater, microalgal technology can convert the nitrogen and phosphorus in wastewater

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into valuable biomass and is more meaningful for sustainable development.

However, domestic wastewater is an adverse environment for microalgae. As numerous contaminants and predators exist in wastewater, microalgal cells can be preyed upon or disrupted. Li *et al.* (2010) tested the survivability of 11 species of high-lipid microalgae obtained from the algae bank and reported that none of these species could grow well in real secondary effluent. The isolation and selection of a suitable microalgal strain is the foundation of researching the coupled technology of wastewater treatment and biofuel production. The selected microalgal strain should be able to grow well in real domestic wastewater (without sterilization or adding any other nutrients) with a high lipid content and contaminant removal rate.

Scenedesmus sp. ZTY1 (patent no. CGMCC 7059 in the China General Microbiological Culture Collection Center) was isolated by us from a wastewater treatment plant in Beijing. Previous research showed that this algae strain grows well in real domestic wastewater. In this work, we focused on the growth, lipid production and nutrient removal properties of this newly isolated microalga in real primary and secondary effluents of a wastewater treatment plant.

MATERIALS AND METHODS

Microalga and medium

The *Scenedesmus* sp. ZTY1 used in this study was isolated from a wastewater treatment plant in Beijing. The strain was kept in BG11 medium. Nitrate and phosphate were used as the nitrogen and phosphorus sources, respectively. The growth medium BG11 contained the following: 1,500 mg L^{-1} $NaNO_3$, 40 mg L^{-1} $K_2HPO_4 \cdot 3H_2O$, 75 mg L^{-1} $MgSO4 \cdot 7H_2O$, 36 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 6 mg L^{-1} citric acid, 6 mg L^{-1} ferric ammonium citrate, 1 mg L^{-1} EDTA, 20 mg L^{-1} Na_2CO_3, and 1.0 mg L^{-1} A5+ Co solution. The A5+ Co solution contained 2.86 g L^{-1} H_3BO_3, 1.81 g L^{-1} $MnCl_2 \cdot H_2O$, 222 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, 79 mg L^{-1} $CuSO_4 \cdot 5H_2O$, 390 mg L^{-1} $Na_2MoO_4 \cdot 2H_2O$, and 49 mg L^{-1} $Co(NO_3)_2 \cdot 6H_2O$. The stock conditions were: light intensity 55–60 lmol $\cdot$ photon $\cdot$ m^{-2} s^{-1}, light/dark periods of 14/10 h, relative humidity 75%, and temperature 25 °C.

Experimental set up

The domestic wastewater used in this study was acquired from a wastewater treatment plant (WWTP) in Beijing. The primary treatment in this WWTP was a sedimentation process and the secondary treatment was an A_2O process. The primary effluent and the secondary effluent were collected after the primary settling pond and the secondary settling pond, respectively. All of the wastewater had undergone a long-time sedimentation to remove the suspended solids in the wastewater completely. No sterilization process was carried out and no exogenous nutrient was added to the wastewater before or during the cultivation. The water quality of the domestic wastewater used in this study is reported in Table 1.

The microalga was cultured in 200 mL realistic domestic wastewater in 500 mL Erlenmeyer flasks. *Scenedesmus* sp. ZTY1 cells were inoculated at 2.5% (v/v) into the wastewater. A sample of precultivated microalgae was centrifuged (10,000 rpm $\times$ 10 min at 4 °C). The pelleted microalgal cells were washed twice with 15 mg L^{-1} $NaHCO_3$ solution and re-suspended in $NaHCO_3$ solution for inoculation into the wastewater. The initial microalgal density was about 7.5×10^5 cells $\cdot$ mL^{-1}. The cultivation conditions were the same as the stock condition in the BG11 medium. All tests were carried out in triplicate.

Analytical methods

For the measurement of water quality, the microalgal culture was centrifuged (10,000 rpm $\times$ 10 min at 4 °C) and the supernatant was filtered through a 0.45 μm membrane for use in the determination of dissolved organic carbon (DOC), total nitrogen (TN) and total phosphorus (TP). The measurements were conducted according to the *Monitoring Method of Water and Wastewater* (Chinese state standard monitoring methods) (Administration 2002).

The algal density was measured by cell counting using a blood cell counting chamber and the dry weight of algal

Table 1 | Water quality of the primary and secondary effluents used in this study

Parameters	Primary effluent	Secondary effluent
DOC/mg L^{-1}	112 ± 0.9	28 ± 0.5
COD/mg L^{-1}	235 ± 0.8	41 ± 0.8
TN/mg L^{-1}	41 ± 0.8	11 ± 0.4
NH_4^+-N/mg L^{-1}	32.7 ± 2.2	0.14 ± 0.02
TP/mg L^{-1}	8.4 ± 0.3	1.9 ± 0.1
pH	7.4	7.1
SS/mg L^{-1}	162 ± 8	7 ± 2

DOC: dissolved organic carbon; COD: chemical oxygen demand; TN: total nitrogen; TP: total phosphorus; SS: suspended solids.

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biomass was determined using the method of suspended solids (SS) measurement according to Chinese state standard testing methods (Administration 2002).

The total lipids were extracted with a chloroform/methanol solution (1/1, v/v) and were quantified gravimetrically (Bligh & Dyer 1959). Triacylglycerols (TAGs) are the main raw material in biodiesel production (Schenk *et al.* 2008) and an important index to indicate the lipid content in algae cells. After the measurement of total lipids, the dried lipids were dissolved in 0.8 mL of isopropyl alcohol. Then the TAGs were estimated by an enzymatic colorimetric method using a commercial kit from Beijing BHKT Clinical Reagent Co., Ltd, No. 2400076. Before the measurement of lipids, TAGs and dry weight, the algal cells had been washed twice with pure water to eliminate the effect of the original contents of the wastewater.

RESULTS AND DISCUSSION

Growth properties of *Scenedesmus* sp. ZTY1 in primary and secondary effluents

The growth curves of *Scenedesmus* sp. ZTY1 in primary and secondary effluents are shown in Figure 1. Results showed that *Scenedesmus* sp. ZTY1 was capable of significant growth in both effluents. The short adaptive phases and the stable exponential phases illustrated that the strain adapted to the wastewater condition well. In secondary effluent, *Scenedesmus* sp. ZTY1 entered into the stationary phases in about 5 days, with a maximal algae cell density of 1.9×10^6 cells $\cdot$ mL^{-1}. Before the microalga cultivated in the secondary effluent entered into the stationary phase, no significant difference between the two growth curves was observed. Compared with the secondary effluent, the primary effluent could support twice the maximal algal density, reaching 3.8×10^6 cells $\cdot$ mL^{-1}.

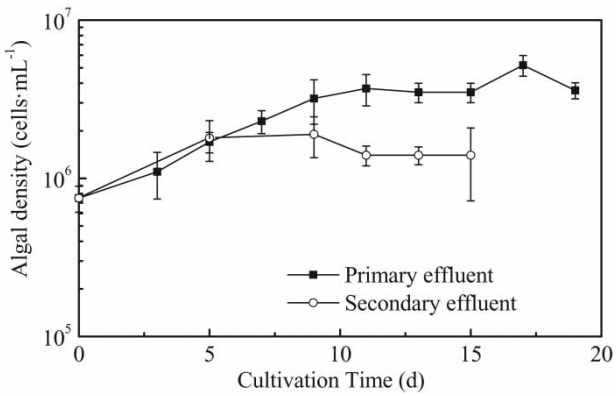

Figure 1 | Growth curves of *Scenedesmus* sp. ZTY1 in primary and secondary effluents of the wastewater treatment plant.

During the exponential phases and stationary phases, microscopic examination found that the microalgal cells were healthy and they had not been contaminated by pollutants or microorganisms. However, after 10 d cultivation in secondary effluent, the algal density of *Scenedesmus* sp. ZTY1 decreased with the disruption of cells, probably because of the depletion of nutrient in the medium. *Scenedesmus* sp. ZTY1 could healthily grow in the primary effluent for more than 45 days. The results showed that the primary effluent was more suitable than the secondary effluent for cultivation of *Scenedesmus* sp. ZTY1.

Nutrient removal properties of *Scenedesmus* sp. ZTY1 in primary and secondary effluents

The nutrient removal properties of *Scenedesmus* sp. ZTY1 are shown in Table 2. Before the growth condition of *Scenedesmus* sp. ZTY1 deteriorated, the nutrient concentrations in the primary effluent and secondary effluent were determined after 21 and 13 d cultivations of *Scenedesmus* sp. ZTY1, respectively. Results showed that *Scenedesmus* sp. ZTY1 could remove the nutrients in primary and secondary effluents effectively. After 13 days of cultivation, TP and TN in the secondary effluent reduced to 0.05 and 1.1 mg L^{-1}, with removal ratios of 97.4% and 90.0%, respectively. After 21 days of cultivation, TP and TN in the primary effluent reduced to 0.07 mg L^{-1} and 2.9 mg L^{-1}, with removal ratios of 99.2% and 92.9%, respectively. In addition, the DOC in the wastewater could also be removed by over 60% because of the combined action from bacteria and microalgae. As some microalgal strains have the ability to assimilate organic carbons in wastewater (Schenk *et al.* 2008), possibly part of the DOC was removed by microalga *Scenedesmus* sp. ZTY1.

Table 2 | Nutrient removal properties of *Scenedesmus* sp. ZTY1 in primary and secondary effluents

	Primary effluent after cultivation		Secondary effluent after cultivation	
Parameters	Concentration (mg L^{-1})	Removal ratio (%)	Concentration (mg L^{-1})	Removal ratio (%)
DOC	31.3	72.1	10.6	62.1
TN	2.9	92.9	1.1	90.0
TP	0.07	99.2	0.05	97.4

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During the experiment, the average removal rates of TN and TP in the primary effluent were 1.8 mg · (L d)$^{-1}$ and 0.4 mg · (L d)$^{-1}$, respectively. In this research, as the maximum dry weight during the cultivation was only 478 mg L^{-1}, and much less than the common sludge concentration in the domestic wastewater treatment plant, the removal rates of TN and TP were not as high as those in the A^{2}O process. Increasing the microalgal concentration in wastewater may increase the removal rates of nutrients. In addition, considering the low content of TN and TP in effluents after the cultivation, the use of the microalgal process for the advanced treatment of domestic wastewater is very promising (Li *et al.* 2010).

Amount of lipid produced by *Scenedesmus* sp. ZTY1 in primary effluent

As the primary effluent has the ability to support more algal cells than the secondary, the primary effluent has been chosen as the medium to cultivate *Scenedesmus* sp. ZTY1 for biomass production. The lipid and TAG production properties of *Scenedesmus* sp. ZTY1 in primary effluent have been investigated and are shown in Figure 2.

After 21 d cultivation, *Scenedesmus* sp. ZTY1 had entered into the stationary phase, the amounts of lipid and TAG in the primary effluent were 102 and 32.2 mg L^{-1}, respectively. At this time, the dry weight of *Scenedesmus* sp. ZTY1 in wastewater was 478 mg L^{-1}, which means the lipid content in dry weight had reached about 32.2%. This lipid content is comparable with the performance of some high lipid content species (lipid content varing from 20% to 50%) cultivated in artificial medium under sterile conditions (Chisti 2008; Demirbaş 2008; Maharajh & Lalloo 2008).

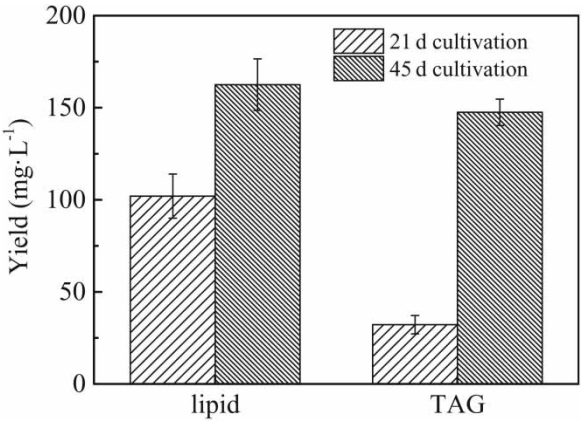

Figure 2 | Lipid and triacylglycerol (TAG) yield of *Scenedesmus* sp. ZTY1 in primary effluents of domestic wastewater.

The lipid and TAG amounts in wastewater (mg L^{-1}) were increased significantly with the extending of the cultivation time. After a 45 d cultivation in real domestic wastewater, the lipid and TAG amounts of *Scenedesmus* sp. ZTY1 had significantly increased to 162.5 and 147.5 mg L^{-1}, respectively. The content of TAGs in lipid almost tripled from 31.6% to 90.7%. This phenomenon demonstrates that *Scenedesmus* sp. ZTY1 is capable of accumulating lipids and TAGs in cells with the extending of cultivation time in primary effluent. Some references reported that the nutrient deficiency could enhance lipid accumulation in microalgae cells (Illman *et al.* 2000; Takagi *et al.* 2000; Rodolfi *et al.* 2009). As the nutrient in the primary effluent was nearly depleted after a 21 d cultivation (TP: 0.07 mg L^{-1}, TN: 2.9 mg L^{-1}), the deficiency of the nutrient may be the reason for the increased lipid and TAG content from the 21 d to the 45 d cultivations. After the cultivation, due to the high content of lipids and TAGs, the microalgal biomass is a valuable feedstock for biodiesel production. The residual biomass can also be dried and utilized as animal feed to realize the recovery of the nutrients in the wastewater.

CONCLUSION

The newly isolated microalga *Scenedesmus* sp. ZTY1 demonstrated good performance in lipid production and nutrient removal in real domestic wastewater.

Compared with the secondary effluent, the primary effluent can support twice the maximum algal density of *Scenedesmus* sp. ZTY1, reaching 3.8×10^6 cells · mL^{-1}. *Scenedesmus* sp. ZTY1 could remove over 97% of TP and 90% of TN in the primary and secondary effluents.

After cultivation in the primary effluent for 21 days, the lipid content of *Scenedesmus* sp. ZTY1 reached 32.2%. Lipid and TAG content significantly increased with the extension of the cultivation time from 21 days to 45 days. After the 45 d cultivation in the primary effluent, the lipid and TAG yields reached 162.5 and 147.5 mg L^{-1}, respectively.

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