Comparative study on cultivation of microalgae for nutrient removal and lipid production in different artificial wastewaters

Hao Rui, Yu Zhen, Li Jinchen, Gao Min, Ma Weiling, Zhu Yi*

(*College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 10083, China*)

Abstract: Wastewater contains high concentration of nutrients, like nitrogen and phosphorus, which have been identified as the main reasons for water eutrophication and serious ecological issues. Therefore, cultivating a tolerant and adaptive microalgae strain in wastewater is considered as a promising approach for sustainable biomass/ lipid production. The potential usages of *Desmodesmus* sp. for biomass and lipid production within different artificial wastewater (AW) were investigated and the removal efficiencies of nutrient were compared. The maximum removal rate of chemical oxygen demand, ammonia nitrogen, nitrate nitrogen and phosphate were 272 mg/(L·d), 14.021 mg/(L·d), 7.774 mg/(L·d) and 3.347 mg/(L·d), respectively in AW2, AW3, AW5 and AW2. Maximum biomass (1.159 g/L) and lipid (280 mg/L) productions were observed in AW5, while the highest lipid content achieved was 37.42% in AW1. Fatty acid analysis showed that lipids extracted from AW-cultivated *Desmodesmus* sp. contained 59.57%-77.79% polyunsaturated fatty acids (30.6%-44.47% was linoleic acid).

Keywords: microalgae, *Desmodesmus* sp., artificial wastewater, nutrient removal, biomass production, lipid production

DOI: 10.3965/j.ijabe.20171001.2273

Citation: Hao R, Yu Z, Li J C, Gao M, Ma W L, Zhu Y. Comparative study on cultivation of microalgae for nutrient removal and lipid production in different artificial wastewaters. Int J Agric & Biol Eng, 2017; 10(1): 107–114.

1 Introduction

Recently, with the development of industrial and agricultural productions, as well as the increase of population, large quantities of wastewater and domestic sewage are produced and become one of the major environmental concerns. A major requirement of wastewater treatment is to remove high concentration of nutrients (e.g. organic carbon, nitrogen and phosphate), which would otherwise threaten the environment and human health if they were accumulated in rivers and lakes[1]. Microalgae, as one of the most potentially valuable energy sources, could be used as an alternative renewable source of fuel to replace fossil fuels in the future[2,3]. It can utilize organic carbon, inorganic nitrogen and phosphate contents in wastewater[4,5] to generate biomass and oil that are suitable for biodiesel conversion[6]. Therefore, the fundamentals and key points in algae field throughout the world[7] are the high lipid accumulation and environmental regulation mechanism of microalgae cultivated in organic wastewater, and saving water.

In most cases, microalgae has lower biomass productivity of 6.0-345.6 mg/L·d in wastewater, which is due to the low nitrogen and phosphorus concentrations, high concentrations of toxic elements therein and competitive effects of indigenous bacteria and protozoa[1,8]. The removal rates of nutrients from real wastewater by microalgae are equivalent to those obtained from artificial wastewater[9]. The presence of toxic dyes and heavy metals in microalgae might affect

Received date: 2015-12-15 **Accepted date:** 2016-03-23

Biographies: Hao Rui, PhD, research interest: algae resource biotechnology and utilization, Email: hr911119@cau.edu.cn; **Yu Zhen,** PhD candidate, research interest: antagonism between heavy metal and metal nanoparticles of algae, Email: zyu1987@163.com; **Li Jinchen,** Graduate student, research interest: algae's metal nanoparticles stress, Email: tlchen818@ gmail.com; **Gao Min,** Graduate student, research interest: autophagy of algae, Email: minruku@hotmail.com; **Ma Weiling,** Master candidate, research interest: heavy metal adsorption of algae cell wall, Email: maweiling18@163.com.

***Corresponding author: Zhu Yi,** PhD, Associate Professor, research interest: algae resource biotechnology and utilization, Tel/Fax: +86-10-62737538; Email: zhuyi@cau.edu.cn.

its applications in animal feeding stuff[10]. On the other hand, microalgae-based biodiesel production is not economically feasible due to its high unit cost[11]. Thus, more investigation into microalgae cultivation in wastewater for biodiesel production still needs to be focused since more cost effective medium can be obtained and the treatment of wastewater could be achieved more economically.

The chemical oxygen demand (COD), nitrogen and phosphorus concentrations of wastewater are significantly different depending on the wastewater category (Table 1). In most cases, domestic wastewater contains very low concentration of nutrients, which can be directly used for microalgae cultivation. In the meanwhile, the livestock breeding and agricultural wastewater contained extremely high concentration of nutrients such as anaerobic digestion effluent and dairy manure wastewater, which need to be diluted before using in microalgae cultivation[12]. Although wastewater could provide essential nutrients for microalgae cultivation, the performance of different microalgae strains in wastewater in terms of lipid content was in the range of 10%-30%[12]. Furthermore, the productivity of biomass and lipid are also different within the same strain in different types of wastewater[13]. Also, microalgae biomass and lipid production are directly affected by the availability of nutrient sources, light supply, pH, temperature and salinity[14]. Thus, the selection of high environmental-compatible microalgae strains tolerant to various culturing conditions plays an essential role. It is mentioned that *Desmodesmus* sp., a green microalgae, might be one of the suitable strains that contains high lipid concentration (typically more than 50%)[15], which can survive and reproduce itself rapidly in wastewater[16]. It has also been observed to be thermo-tolerant[15], not sensitive to pH change (varied from 5 to10)[17] and posses self-protective aggregations form[12], furthermore, a better nutrients removal efficiency (almost 100%) was reported with this strain cultured in wastewater[18].

The current study focused on evaluating the effect of different artificial wastewater (AW) on the cultivation of *Deamodesmus* sp. The capability of *Deamodesmus* sp. in removing COD, nitrogen and phosphorus as well as its biomass and lipid production in different types of AWs were investigated experimentally.

Table 1 Physicochemical characteristics of different wastewaters and major compositions of AWs

No.	Wastewater category	Characteristics of wastewater/mg·L^{-1}				Major compositions of AWs /g·L^{-1}				References
		COD	NH_4-N	NO_3-N	PO_4-P	Glucose	NH_4Cl	$NaNO_3$	K_2HPO_4	
AW1	Textile wastewater[a]	610.84	25.65	3.42	2.04	0.572	0.098	0.020	0.010	[19]
AW2	Municipal wastewater	783	49.4	13.4	9.5	0.734	0.189	0.082	0.042	[20]
AW3	Anaerobic digestion wastewater (10× dilution)	690	82.4	8.4	4.0	0.647	0.315	0.051	0.018	[21]
AW4	Carpet mill wastewater[b] (2× dilution)	706	10.86	7.09	13.85	0.662	0.042	0.043	0.061	[22]
AW5	piggery wastewater (5× dilution)	281.8[c]	14	70.4	25.8	0.705	0.054	0.428	0.113	[23]

Note: [a] Estimated from 231.67-990 mg/L COD, 0.47-50.83 mg/L NH_4-N, 1.23-5.60 mg/L NO_3-N and 0.07-4.01 mg/L PO_4-P.

[b] Estimated from 1412 mg/L COD, 17.58-25.85 mg/L NH_4-N, 0.21-28.13 mg/L NO_3-N and 20.31-35.10 mg/L PO_4-P.

[c] TOC.

2 Materials and methods

2.1 Microalgae strain and pre-culture conditions

Desmodesmus sp. EJ15-2 was obtained from the BioEnergy Engineering and Low Carbon Technology Laboratory of China Agricultural University, Beijing, China[17]. The strain was preserved in the BG-11 medium containing the following chemicals: 1500 mg/L $NaNO_3$, 40 mg/L K_2HPO_4, 75 mg/L $MgSO_4·7H_2O$, 36 mg/L $CaCl_2·2H_2O$, 6 mg/L citric acid, 6 mg/L ferric ammonium citrate, 1 mg/L $EDTANa_2$, 20 mg/L Na_2CO_3 and 1 mL/L A5 trace metal solution (2.86 g/L H_3BO_3, 1.86 g/L $MnCl_2·4H_2O$, 0.22 g/L $ZnSO_4·7H_2O$, 0.39 g/L $Na_2MoO_4·2H_2O$, 0.08 g/L $CuSO_4·5H_2O$, and 0.05 g/L $Co(NO_3)_2·6H_2O$). The initial pH value of the medium was titrated to 7.0 with 1 mol/L HCl.

Desmodesmus sp. was inoculated with autoclaved BG-11 medium (121°C, 20 min) in 100 mL Erlenmeyer flasks containing 60 mL of the medium. The culture flasks were incubated under the optimized conditions at (30±1)°C with (98±2) μmol/m^2/s continuous photon flux density by cool-white fluorescent light illumination with

light/dark cycles (L:D) of 14: 10 in a growth chamber. All flasks were shaken three times per day periodically to avoid sedimentation.

2.2 Microalgae growth in AWs

The major compositions of AWs were listed in Table 1 to simulate several typical wastewater; while other chemicals in AWs consisted of the following components: 75 mg/L $MgSO_4 \cdot 7H_2O$, 36 mg/L $CaCl_2 \cdot 2H_2O$, 5 mg/L $FeSO_4 \cdot 7H_2O$, 1 mg/L $EDTANa_2$ and 1 mL/L A5 trace metal solution, and the pH of AWs was adjusted to 7.0 with 1 mol/L NaOH.

Batch experiments were performed for 10 d to evaluate the growth and lipid characteristics of *Desmodesmus* sp. and the removal of COD, ammonia nitrogen (NH_4-N), nitrate nitrogen (NO_3-N) and phosphate (PO_4-P) in AWs. The culture conditions were prepared as mentioned above in Section 2.1. All experiments were conducted in at least triplicates and average values were statistically calculated.

2.3 Analytical procedures

2.3.1 Determination of nutrients concentration and removal efficiency

Liquid samples were filtered using 0.45 μm glass microfiber filters (Whatman, USA), the filtrate was used for measuring COD, NH_4-N and PO_4-P by the spectrophotometric method (National Standard Method of China)[24] and the Hach DR 2700 Spectrophotometer Manual (Hach Company, USA). The NO_3-N concentration was analyzed using a continuous flow injection analyzer (AA3, Seal Analytical, UK).

Nutrient removal efficiencies (R_i) were obtained according to Equation (1):

$$R_i = (S_{i0} - S_{it}) / S_{i0} \times 100\% \quad (1)$$

where, S_{i0} and S_{it} represents the mean values of substrate i (COD, NH_4-N, NO_3-N or PO_4-P) concentration at the initial time t_0 and time t_i, respectively.

The rate of nutrient removal: (r_i) was determined by Equation (2):

$$r_i\,(\text{g/L/d}) = (S_{i0} - S_{it}) / (t_i - t_0) \quad (2)$$

where, S_{i0} is the initial substrate concentrations and S_{it} is the corresponding substrate concentration at time t_i; t is the time interval (days) between S_{i0} and S_{it}.

2.3.2 Analysis of biomass concentration

Optical density of microalgae was measured at 680 nm (OD_{680}) daily as the cell density using a spectrophotometer (UV-7504PC, Xinmao Instrument, Shanghai, China). The linear correlation between OD_{680} (x) and dry cell weight (DCW, y_{biomass}) was determined previously for this strain:

$$y_{\text{biomass}}\,(\text{g/L}) = 0.3021x - 0.0221 \ (R^2 = 0.998, p < 0.05) \quad (3)$$

The biomass productivity (P_{biomass}) was calculated as given in Equation (4):

$$P_{\text{biomass}}\,(\text{g/L/d}) = (y_i - y_0) / (t_i - t_0) \quad (4)$$

where, y_i and y_0 are DCW (g/L) at time t_i and t_0 (initial time), respectively.

The initial OD_{680} for all experimental variations was found at an absorbance of 0.1.

2.3.3 Lipid extraction

Samples were first centrifuged at 5000 r/min, 4°C for 10 min, washed three times with distilled water and then freeze-dried under −80°C for 48 h using a freeze dryer (FD-1B-05, Boyikang Instrument, Beijing, China) prior to lipid analysis. Total lipid extractions were performed as described by Bligh and Dyer[25]. Approximately 50 mg of lyophilized algae biomass was homogenized by tube disperser (T10 Basic, IKA-Werke GmbH & Co., Germany) at 25 000 r/min for 2 min. The lipid was extracted with 3 mL chloroform/methanol (2:1, v/v), centrifuged at 6000 r/min for 2 min, and the liquid phase was then transferred into a fresh tube. Approximately 3 mL chloroform/methanol had been added into the initial tube and extracted three times, and further mixed with the additional methanol and water with a final solvent ratio of 1:1:0.9 (chloroform/methanol/water, v/v/v). The extracted lipids were collected from the chloroform layer; lipid content (y_{lipid}) was calculated according to Equation (5):

$$y_{\text{lipid}}\,(\%) = p/y \times 100\% \quad (5)$$

where, p is lipid weight, g; y is DCW, g.

The lipid productivity (P_{lipid}) was calculated as follows:

$$P_{\text{lipid}}\,(\text{g/L/d}) = y_{\text{lipid}} \times y_{\text{biomass}} / t \quad (6)$$

where, y_{lipid} is lipid content, %; y_{biomass} is DCW, g/L; t is the time interval.

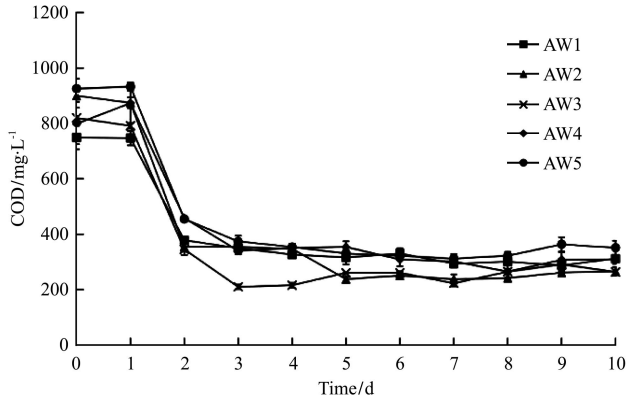
2.3.4 Fatty acid methyl ester (FAME) content

FAME was prepared according to Indarti et al.[26] using one-step method of extraction-transesterification. The FAMEs (100 mg freeze-dried algae) were extracted with 10 mL mixture of methanol, concentrated sulfuric acid, and chloroform (4.25/0.75/5, v/v/v) at 90°C water batch for 90 min. After extraction with chloroform, the obtained FAME was analyzed by a gas chromatography spectrometer (GC-2010 Plus, Shimadzu, Japan), equipped with a HP-Wax capillary column (30 m × 0.32 mm × 0.25 μm, Agilent Technologies, USA). The injector temperatures was set at 220°C, the temperature gradient was programmed at 100°C for 3 min, ramped to 200°C with an increase of 4°C/min, held at 200°C for 5 min followed by a rise to 250°C with 3°C/min and then the temperature was fixed at 250°C for 10 min. The carrier gas (pure nitrogen, 99.9992%, Beijing AP BAIF Gases Industry Co., Ltd., China) was controlled at 2.0 mL/min. The detected peak signals were matched with standards, and their respective relative areas were converted into the proportion of total peak areas, which defined the content of individual FAME compounds.

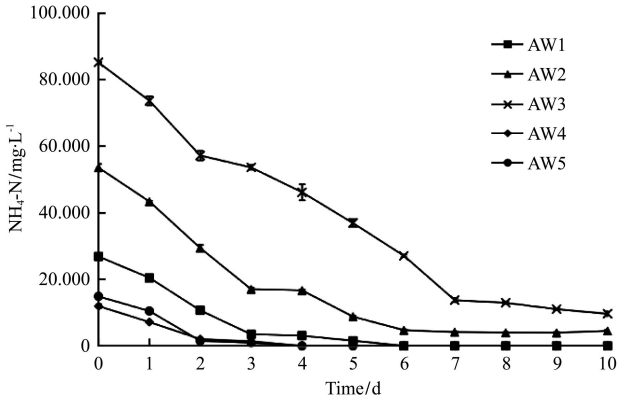
3 Results and discussion

3.1 Nutrient removal

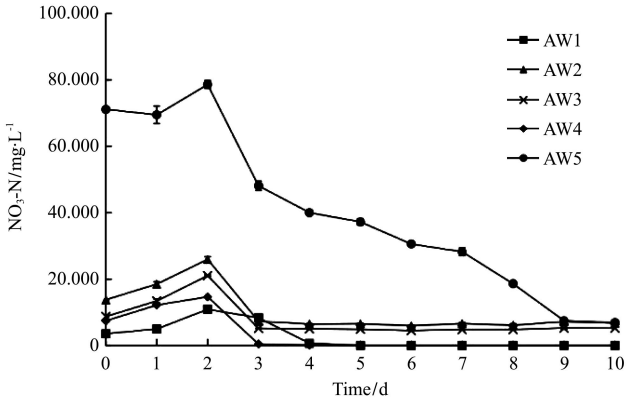
The variations in COD, NH_4-N, NO_3-N and PO_4-P removal with time in different AWs for 10-days batch culture are exhibited in Figure 1. The initial COD value did not change during the first day, but after an initial decrease on day 2, it then remained almost constant for the rest of the cultivation period (Figure 1a). In comparison, there was a gradual decrease in NH_4-N and PO_4-P concentrations throughout this study in different AWs (Figures 1b and 1d). The NH_4-N in five different AWs showed similar trend by continually decreasing and therefore nearly all of the PO_4-P was removed within 3 d in AW1, AW2 and AW3. For nitrate, the NO_3-N increased from initial concentration to a peak value of 10.901 mg/L, 25.931 mg/L, 21.058 mg/L, 14.664 mg/L and 78.616 mg/L after two days and decreased to 0, 6.742 mg/L, 5.221 mg/L, 0 and 6.936 mg/L, at the end of the experiment in AW1 to AW5, respectively (Figure 1c).



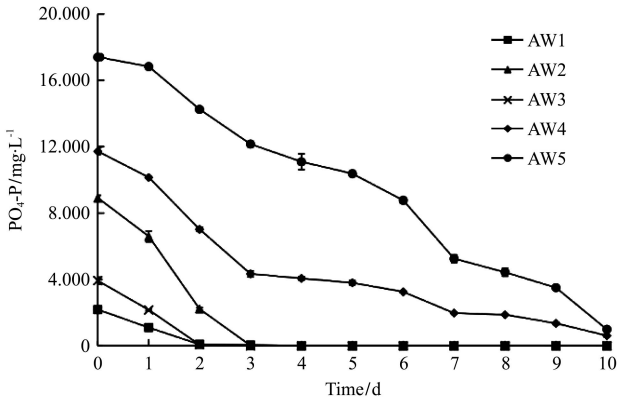
a. COD concentration profile



b. NH_4-N concentration profile



c. NO_3-N concentration profile



d. PO_4-P concentration profile

Figure 1 Nutrients concentration by *Desmodesmus* sp. grown under five different AWs within 10 days

The composition of initial nutrients in AWs and removal efficiency by microalgae were investigated, as shown in Tables 2 and 3, the removal efficiency of COD consumption were 58.34%-70.43%; and the maximum removal rate was 272 mg/(L·d) in AW2. Comparing with the COD, nitrogen and phosphorus were much easier to remove in AWs, the removal rates of NH_4-N were 91.59% and 83.78% from AW2 and AW3, while 100.00% removal from other AWs was observed; and the NO_3-N in the AWs could not be removed completely except in AW1 and AW4. The *Desmodesmus* sp. was able to achieve an almost complete phosphorus removal from different AWs containing 2.186-17.396 mg/L PO_4-P within 10 days.

Table 2 Composition of the different AWs before algae cultivation and biomass production

	Initial concentration/mg·L^{-1}				DCW/g·L^{-1}
	COD	NH_4-N	NO_3-N	PO_4-P	
AW1	749±43	26.875±0.656	3.523±0.111	2.186±0.128	0.546±0.015
AW2	900±22	53.601±1.057	13.773±0.070	8.913±0.154	0.632±0.007
AW3	820±95	85.242±0.193	8.719±0.113	3.930±0.209	0.637±0.014
AW4	798±58	11.971±0.627	7.418±0.074	11.722±0.197	0.801±0.017
AW5	925±36	14.894±0.537	71.128±0.312	17.396±0.136	1.159±0.021

Table 3 Nutrient removal performance in different AWs within 10-days cultivation.

	Removal efficiency				Maximum removal rate/mg·(L·d)$^{-1}$			
	COD	NH_4-N	NO_3-N	PO_4-P	COD	NH_4-N	NO_3-N	PO_4-P
AW1	58.34%	100.00%	100.00%	100.00%	185	8.095	0.712	1.048
AW2	70.43%	91.59%	51.05%	100.00%	272	12.185	2.156	3.347
AW3	67.79%	83.78%	40.11%	100.00%	237	14.021	1.190	1.931
AW4	61.29%	100.00%	100.00%	94.78%	170	4.963	2.325	2.461
AW5	62.03%	100.00%	90.25%	94.30%	235	6.673	7.774	1.746

Carbon is a macronutrient necessary for algae growth, in this study the fluctuation in COD removal was leveled off after two days of cultivation which might have resulted from COD uptake by new cells and organic carbon decomposed and released from insoluble organic matters[27]. Apart from hydrogen and oxygen, nitrogen is quantitatively the most important element after carbon, which contributes to 1%-10% dry weight of microalgae cells[28]. Ammonium is the most preferred nitrogen source for algae, although high levels of NH_4-N are known to inhibit the growth of *Desmodesmus* sp.[21] However, the AWs that was used in the current study contained 11.971-85.242 mg/L initial NH_4-N concentration (Table 2), which were unlikely to inhibit the growth of algae. The NH_4-N decreased rapidly in the first two days while NO_3-N increased, which showed that algae utilize ammonium first when ammonium and nitrate were available together[28]; and most of the removed NH_4-N was transformed into NO_3-N by nitrification process[29]. Phosphorus is required for growth by all organisms, algae included. It is an essential component of nucleotides, which serve as energy storage within cells (ATP) or when linked together, form the nucleic acids DNA and RNA[30]. Among all nutrient reduction parameters, reduction of PO_4-P was the greatest. It might be attributed to high phosphorus adsorption potential of this algae strain combined with phosphates precipitation caused by the pH increment (> 9)[9]. The maximum COD, NH_4-N, NO_3-N and PO_4-P removal ranged between 170.0-272.0 mg/(L·d), 4.963-14.021 mg/(L·d), 0.712-7.774 mg/(L·d) and 1.048-3.347 mg/(L·d), respectively (Table 3), which indicated that *Desmodesmus* sp. could remove more nutrients from AWs in comparison with reported values from similar studies of freshwater chlorophytes[29].

Table 2 showed the relationship between initial nutrients in AWs and DCW, with AW1, AW2 and AW3 containing less PO_4-P and as a result, produced less biomass, Figure 1d also showed that PO_4-P were completely consumed in 2 d for AW1 and AW2, and in 3 d for AW3, regardless of remaining nitrogenous nutrients, indicating that phosphorous was the limiting

factor. Otherwise, Figures 1b and 1c indicated that nitrogen was an inhibiting nutrient in AW1 and AW4.

3.2 Microalgae growth

The growth of *Desmodesmus* sp. in five different AWs is shown in Figure 2. The biomass productivity achieved at 0.124 g/(L·d), 0.151 g/(L·d), 0.143 g/(L·d), 0.167 g/(L·d) and 0.179 g/(L·d) in culture with AW1-AW5 at the Day 3, Day 3, Day 3, Day 2 and Day 3, respectively. The maximum DCW of 1.159 g/L was obtained in the culture within AW5, while the minimum DCW of 0.546 g/L was recorded in AW1 after 10 d of cultivation. There were no significant lag phases in all AWs and the growing trends did not show significant differences during the first few days, suggesting that this strain had a greater adaptability and viability in different kinds of wastewater. After Day 5, the growth of microalgae in AW1, AW2 and AW3 moved into a stationary phase while AW4 and AW5 still grew exponentially up to the end of experiment.

The biomass production and initial nutrients concentrations in different AWs are compared in Table 3. In this study, those AWs were shown to contain enough N and P to support algae growth; and there were no inhibitory effects on algae growth during the first few days. The AW5 cultures had a maximum biomass production since it could supply more nutrients than the other AWs for algae to produce biomass. Wu et al.[31] has reported that algae could exhibit significantly high biomass production in wastewater with high concentration of nutrients (below inhibitory levels). Similar results were also observed by Wang and Lan[32].

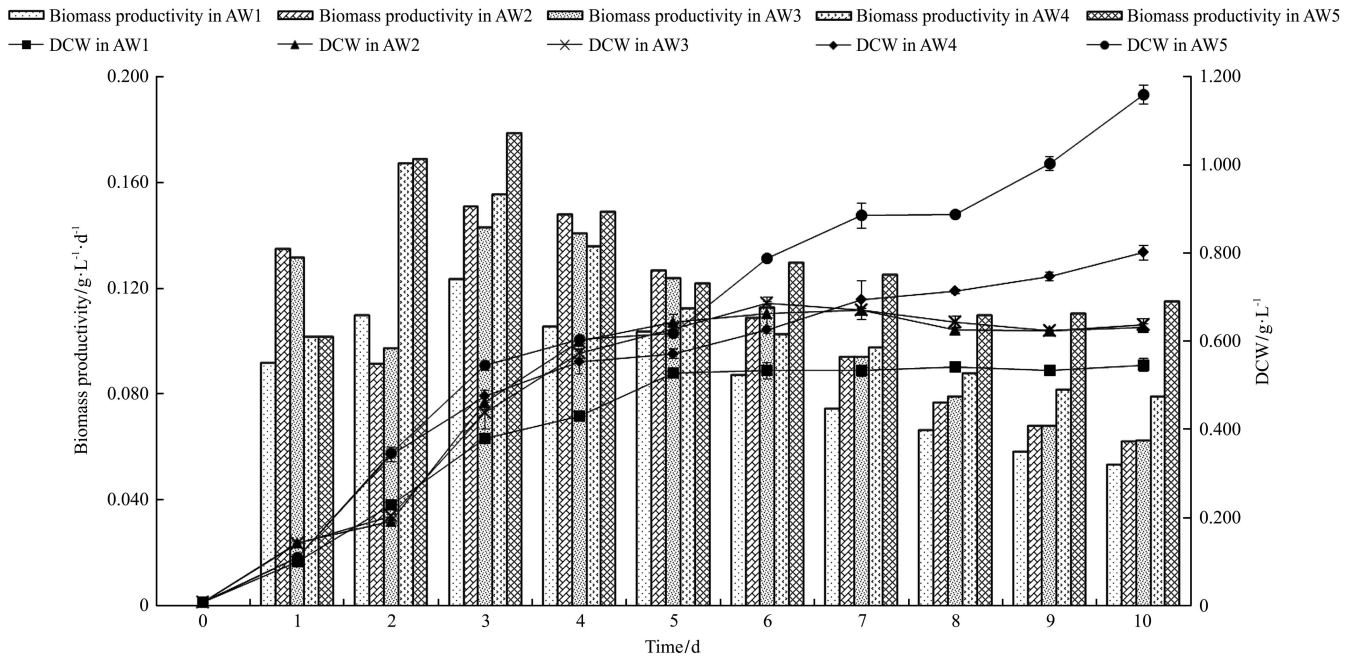


Figure 2 Biomass productivity and DCW of *Desmodesmus* sp. culture in the different AWs

3.3 Lipid productivity and FAME composition

As shown in Figure 3, the percentages of total lipids content of dry weight for cultures in AW1-AW5 were 37.42%, 26.15%, 26.54%, 31.84% and 24.12%, respectively, while the total lipid production range after 10 d of cultivation was 165-280 mg/L. According to previous studies, higher lipid content is expected in nitrogen-limiting conditions[4]. The algae cells cultivated in AW1 and AW4 contained higher lipid (more than 30%) because almost all nitrogen in AW1 and AW4 could be removed by *Desmodesmus* sp. at the Day 5 (Figure 2), then microalgae cells incubated in nitrogen-deficient medium accumulated substantial amount of lipids. This observation proves that algae cultures produce less biomass due to nitrogen limitation, but on the other hand, nitrogen deficiency coupled with high light intensity results in lipid accumulation within the algae cells. Similar results were obtained from Zhu et al.[27], who promoted cellular lipid storage by decreasing the cell density.

Table 4 indicated the fatty acid (FA) profiles derived from triacylglycerol, phospholipid and free fatty acids in

Desmodesmus sp. cultivated in different AWs after 10 d. Overall, linoleic acid (C18:2n-6) was the most abundant FA (30.6%-44.47%). This was probably attributed to lacking of nutrient (e.g. lacking of N) culturing environment, which might restrict the biomass accumulation within microalgae cells. The FA compositions showed that the algae cultivated in AW2-AW4 were very similar, with 77.18%-77.79% polyunsaturated fatty acids and 12.56%-15.86% saturated fatty acids, respectively. Whereas, algae in AW1 contained 59.57% polyunsaturated fatty acids and 29.82% saturated fatty acids, respectively. The cause of such phenomenon could be the influences of changing medium composition and light intensities.

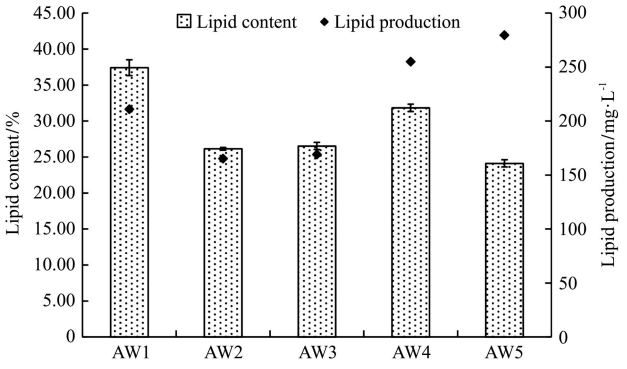


Figure 3 Comparison of lipid content and production of *Desmodesmus* sp. after 10 days batch cultivation on different AWs

Table 4 FA profiles derived from triacylglycerol, phospholipid and free fatty acids in *Desmodesmus* sp.

	AW1	AW2	AW3	AW4	AW5
SFA (% of FA)	29.82	14.52	15.86	12.56	13.13
C14:0	10.91	6.16	5.67	3.29	4.54
C16:0	3.31	1.25	1.46	0.97	0.78
C18:0	5.11	3.68	4.42	2.62	2.70
Others	10.49	3.43	4.31	5.68	5.11
MUFA (% of FA)	10.60	7.69	6.96	13.60	9.87
C16:1	0.85	0.32	0.22	0.11	0.16
C18:1n-9	2.77	3.45	3.14	10.20	7.86
C20:1	5.48	3.92	3.49	2.83	1.60
Others	1.50	n.a.	0.1	0.46	0.25
PUFA (% of FA)	59.57	77.79	77.18	73.84	76.98
C18:2n-6	30.60	44.47	42.86	35.05	43.73
C18:3n-3	25.76	32.58	33.39	37.32	32.00
Others	3.21	0.74	0.93	1.47	1.25

Note: SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; n.a.: not available.

4 Conclusions

The results demonstrated the feasibility of cultivating *Desmodesmus* sp. in five different AWs for nutrients removal, biomass and lipid production. *Desmodesmus* sp. can adapt well in all AWs, removing 58.34%-70.43% COD, 83.78%-100% NH_4-N, 40.11%-100% NO_3-N and 94.30%-100% PO_4-P as well as accumulating the lipid up to 24.12%-37.42%. The major FA extracted from lipids was PUFA (59.57%-77.79%) which contained 30.6%-44.47% of linoleic acid. Based on the current experimental results, the *Desmodesmus* sp. EJ15-2 can be treated as a promising microalgae species in waste-to-biodiesel process, which could benefit the industrial area of both wastewater treatment and biodiesel productions.

Acknowledgments

This work was financially supported by the National Key Technology R&D Program of China (Grant No. 2012BAD47B03).

[References]

[1] Chen P, Min M, Chen Y F, Wang L, Li Y, Chen Q, et al. Review of biological and engineering aspects of algae to fuels approach. Int J Agric & Biol Eng, 2010; 2(4): 1–30.

[2] Wijffels R H, Barbosa M J. An outlook on microalgal biofuels. Science, 2010; 329: 796–799.

[3] Dassey A J, Hall S G, Theegala C S. An analysis of energy consumption for algal biodiesel production: Comparing the literature with current estimates. Algal Res., 2014; 4: 89–95.

[4] Ji F, Wang Y, Li G, Zhou Y, Dong R. Isolation of microalgae with growth restriction and nutrient removal from alkaline wastewater. Int J Agric & Biol Eng, 2015; 8(6), 62–68.

[5] Unnithana V V, Uncb A, Smitha G B. Mini-review: A priori considerations for bacteria-algae interactions in algal biofuel systems receiving municipal wastewaters. Algal Res., 2014; 4: 35–40.

[6] Brennan L, Owende P. Biofuels from microalgae: a review of technologies for production, processing, and extractions of biofuels and co-products. Renew. Sust. Energ. Rev., 2010; 14: 557–577.

[7] DOE. National algal biofuels technology roadmap. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program, USA, 2010.

[8] de-Bashan L E, Bashan Y. Immobilized microalgae for removing pollutants: review of practical aspects. Bioresour. Technol., 2010; 101: 1611–1627.

[9] Ruiz-Marin A, Mendoza-Espinosa L G, Stephenson T.

Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresour. Technol., 2010; 101: 58–64.

[10] Huang G, Chen F, Wei D, Zhang X, Chen G. Biodiesel production by microalgal biotechnology. Appl. Energ., 2010; 87: 38–46.

[11] Williams P J L B, Laurens L M. Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics. Energy Environ. Sci., 2010; 3: 554–590.

[12] Wu Y, Hu H, Yu Y, Zhang T, Zhu S, Zhuang L, et al. Microalgal species for sustainable biomass/lipid production using wastewater as resource: A review. Renew, Sust. Energ. Rev., 2014; 33: 675–688.

[13] Cabanelas I T D, Ruiz J, Arbib Z, Chinalia F A, Garrido-Pérez C, Rogalla F, et al, Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. Bioresour. Technol., 2013; 131: 429–436.

[14] Pandey A, Lee D J, Chisti Y, Soccol C R. Biofuels from Algae. Newnes, 2013.

[15] Pan Y Y, Wang S T, Chuang L T, Chang Y W, Chen C N N. Isolation of thermo-tolerant and high lipid content green microalgae: Oil accumulation is predominantly controlled by photosystem efficiency during stress treatments in *Desmodesmus*. Bioresour. Technol., 2011; 102: 10510–10517.

[16] Li G, Ji F, Zhou Y, Dong R. Life cycle assessment of pyrolysis process of *Desmodesmus* sp. Int J Agric & Biol Eng, 2015; 8(5): 105–112.

[17] Ji F, Hao R, Liu Y, Li G, Zhou Y, Dong R. Isolation of a novel microalgae strain *Desmodesmus* sp. and optimization of environmental factors for its biomass production. Bioresour. Technol., 2013; 148: 249–254.

[18] Samorì G, Samorì C, Guerrini F, Pistocchi R. 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 Res., 2012; 47: 791–801.

[19] Lim S, Chu W, Phang S. Use of *Chlorella vulgaris* for bioremediation of textile wastewater. Bioresour. Technol., 2010; 101: 7314–7322.

[20] Sacristán de Alva M, Luna-Pabello V M, Cadena E, Ortíz E. Green microalga *Scenedesmus acutus* grown on municipal wastewater to couple nutrient removal with lipid accumulation for biodiesel production. Bioresour. Technol., 2013; 146: 744–748.

[21] Ji F, Liu Y, Hao R, Li G, Zhou Y, Dong R. Biomass production and nutrients removal by a new microalgae strain *Desmodesmus* sp. in anaerobic digestion wastewater. Bioresour. Technol., 2014; 161: 200–207.

[22] Chinnasamy S, Bhatnagar A, Hunt R W, Das K C. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresour. Technol., 2010; 101: 3097–3105.

[23] Depraetere O, Foubert I, Muylaert K. Decolorisation of piggery wastewater to stimulate the production of *Arthrospira platensis*. Bioresour. Technol., 2013; 148: 366–372.

[24] Wei F, Qi W, Bi T, Sun Z, Huang Y, Shen Y. Water and wastewater monitoring and analysis method (4th Ed.). China Environmental Science Press, Beijing, 2002. (in Chinese)

[25] Bligh E G, Dyer W J. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 1959; 37: 911–917.

[26] Indarti E, Majid M I A, Hashim R, Chong A. Direct FAME synthesis for rapid total lipid analysis from fish oil and cod liver oil. J. Food Compos. Anal., 2005; 18: 161–170.

[27] Zhu L, Wang Z, Shu Q, Takala J, Hiltunen E, Feng P, et al., Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. Water Res., 2013; 47: 4294–4302.

[28] Perez-Garcia O, Escalante F M, de-Bashan L E, Bashan Y. Heterotrophic cultures of microalgae: metabolism and potential products. Water Res., 2011; 45: 11–36.

[29] Su Y, Mennerich A, Urban B. Comparison of nutrient removal capacity and biomass settle ability of four high-potential microalgal species. Bioresour. Technol., 2012; 124: 157–162.

[30] Peccia J, Haznedaroglu B, Gutierrez J, Zimmerman J B. Nitrogen supply is an important driver of sustainable microalgae biofuel production. Trends Biotech., 2013; 31: 134–138.

[31] Wu P F, Teng J C, Lin Y H, Hwang S C J. Increasing algal biofuel production using *Nannocholropsis oculata* cultivated with anaerobically and aerobically treated swine wastewater. Bioresour. Technol., 2013; 133: 102–108.

[32] Wang B, Lan C Q. Biomass production and nitrogen and phosphorus removal by the green alga *Neochloris oleoabundans* in simulated wastewater and secondary municipal wastewater effluent. Bioresour. Technol., 2011; 102: 5639–5644.