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The comparison of three common microalgae for treating piggery wastewater

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ABSTRACT

To better remove the nitrogen and phosphorus in piggery wastewater, more and more attention has been paid to microalgae-based process. The success of this process primarily depends on the involved microalgae, and thus screening suitable microalgae is very important. In this study, the potential of three microalgae species, including *Chlorella vulgaris*, *Botryococcus braunii* and *Desmodesmus* sp., was compared based on their growth rates, nutrients removal efficiency and the contents of valuable substances. Results showed that the biomass, the removal efficiency of NH_4^+-N and TP of *Desmodesmus* sp. CHXl were 0.88 g·L^{-1}, 78.5% and 91.7%, respectively, and the contents of chlorophyll, carotenoid, protein and lipid of *Desmodesmus* sp. CHXl were 8.89 mg·L^{-1}, 3.19 mg·L^{-1}, 0.51 g·L^{-1} and 0.095 g·L^{-1}, respectively, which were significantly higher than those of *Chlorella vulgaris* 1068 and *Botryococcus braunii* 765. Results indicated that the local specie of *Desmodesmus* sp. CHXl is advantageous for purification of piggery wastewater and also as nutritional resources, and it is more reliable to screen the involved microalgae from the local habitat.

Keywords: Three common microalgae; Piggery wastewater; Biomass; Nutrients removal efficiency; Valuable substances' contents; Comparison

1. Introduction

In recent years, the swine industry of China has developed rapidly to satisfy the supply of meat in urban and rural areas; however, it also brings a serious impact on the local ecological environment, especially the impact from the arbitrary discharge of breeding effluent. Effluents from pig farms often contain high concentrations of nutrients (organic and inorganic), which have been identified as the main causes of surface water eutrophication. Therefore, the wastewater must receive suitable treatment before being discharged into water bodies [1]. Existing conventional biological treatment processes for the removal of nutrients from piggery wastewater include activated sludge process [2], anaerobic digestion [3], and constructed wetland [4]. But these treatment technologies only focused on the treatment with lower removal efficiency of nutrients and the occupancy of large land area which can retard widespread implementation in rural areas. In this context, it is crucial for the establishment of sustainable farming to develop and implement cost-effective technologies that can reduce export of nutrients into the watershed and at the same time increase farm profits [1].

Microalgae-based wastewater treatment has attracted increasingly global attention as its dual roles of bioremediation of wastewater and generating biomass for biofuel production or high-protein feed supplements with concomitant carbon dioxide sequestration [5–9]. Many studies have been conducted for treating piggery wastewater, including diluted primary effluent [10,11], secondary effluent, which was treated by anaerobic digestion compost waste [12]. How-

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ever, the success of the wastewater treatment system mainly depends on the performance of the involved strains. Also, the variations in the composition of wastewater would limit such a notion as only specific strains perform their potentials. Although many algal species such as *Chlorella* sp. [13,14], *Botryococcus braunii* [15], and *Scenedesmus* sp. [16] have been proven their potentials of piggery wastewater treatment, more species should be screened, especially the algae isolated from local wastewater treatment plant site or real water body [17,18]. In our previous study, a newly isolated microalga identified as *Desmodesmus* sp. (strain CHX1) showed fast growth rate and high nutrients removal efficiency when growing in diluted piggery effluent collected from a local stabilization pond, and has been considered as a good candidate strain for bioremediation and biomass production [1]. However, comparative studies with other microalgae have not been performed synchronously. Further to verify the reliability of the local screening for piggery wastewater, this study was conducted to compare the potential of two common algal species of *Chlorella vulgaris* and *Botryococcus braunii*, and the isolated local specie *Desmodesmus* sp.

2. Materials and methods

2.1. Algal species and pre-culture

The algal species of *Chlorella vulgaris* 1068, *Botryococcus braunii* 765 and *Desmodesmus* sp. CHXl were selected in this study. Among them, *Chlorella vulgaris* 1068 and *Botryococcus braunii* 765 were purchased from institution of hydrobiology, Chinese Academy of Sciences. *Desmodesmus* sp. CHXl was isolated from local piggery wastewater collected from a stabilization pond that located in a piggery farm in Xiaoshan District, Hangzhou City, China, and identified as *Desmodesmus* sp. CHX1 with assistance of both morphological observation and DNA sequencing (Genebank NO.: JX258841) [19].

All algal cells were preserved in 50 ml flasks containing 25 ml BG-11 medium, which consists of the components shown in Table 1, and de-ionized water was used as the solvent. All the flasks were placed on an orbital shaker at 120 rpm at 25 ± 2°C with two-compacted fluorescent lights (Philips TLD 55W fluorescent lamp) providing round-the-clock illumination at light intensity of 60 μmol photo·m^{-2}·s^{-1}. When the color of algal cells changed from yellow green to dark green, 20 ml of the culture was transferred into flasks with 150 ml BG-11 medium, and gradually to flasks with 1000 ml BG-11 medium for amplification cultivation. Before the following experiments, the culture was centrifuged (6000 rpm, 5 min), and the cells were suspended by de-ionized water, and centrifuged again (6000 rpm, 5 min) to obtain the inoculum in sequences.

Table 1
Components of BG-11 medium

Component	Content (mg·L^{-1})	Component	Content (mg·L^{-1})
$NaNO_3$	1500	Na_2–EDTA	1
$K_2HPO_4·3H_2O$	40	H_3BO_3	2.86
$MgSO_4·7H_2O$	75	$MnCl_2·H_2O$	1.81
$CaCl_2·2H_2O$	36	$ZnSO_4·7H_2O$	0.222
Na_2CO_3	20	$CuSO_4·5H_2O$	0.079
$C_6H_8O_7·H_2O$	6	$NaMoO_4·2H_2O$	0.39
$Fe(NH_4)_3C_{18}H_{10}O_{14}$	6	$Co(NO_3)_2·6H_2O$	0.049

2.2. Pretreatment of the piggery wastewater

The piggery wastewater was collected from a stabilization pond located in a piggery farm in Xiaoshan District, Hangzhou City, China. In the preliminary experiment, algal aggregations occurred when the raw wastewater was used as the growth medium directly, suggesting an inhibitory growth of the microalgae species. It might be attributed to high concentration of NH_4^+-N in the wastewater [18]. Therefore, NH_4^+-N was removed from the wastewater by air stripping before the next experiment. The wastewater without adjustment of initial pH was aerated through air and sterilized with a 0.2 μm membrane filter at a flow rate of 1500 ml·min^{-1} for a period of 42 h at room temperature. After pretreatment, the piggery wastewater was applied as growth medium after being filtered by 0.45 μm membrane and then being auto-claved (121°C for 30 min). The characteristics of the wastewater after pretreatment were summarized in Table 2.

2.3. Experimental designs

Pretreated wastewater (600 ml) was stored in sterile 1000 ml conical flasks, and then concentrated algae of *Botryococcus braunii* 765, *Chlorella vulgaris* 1068 and *Desmodesmus* sp. CHXl were inoculated with the density of 0.1 g·L^{-1}. Light was continuously supplied (Philips TLD 55 W fluorescent lamp) at an intensity of 100 μmol photo. m^{-2}·s^{-1} that was measured at the surface of the flask by an illuminance meter (ZDS-10, Shanghai Cany Precision Instrument Ltd., China). Batch experiment cultivation lasted for 7 days at 27 ± 2°C, and then media were harvested per day for the measurement of algal parameters including the biomass, chlorophyll and carotenoid content, and also the wastewater parameters including NH_4^+-N and TP. After 7-d cultivation, the microalga was harvested for the determination of protein and lipid contents. No inoculations were served as the corresponding negative controls to observe the variation of the composition of the growth media with cultivation time. All experiments were carried out in triplicate and the average values with standard deviation were reported.

Table 2
Characteristics of the pretreated piggery wastewater

Item	Concentration (mg·L^{-1})
pH	7.89
COD	224
Total N	128.2
Total P	3.1
NO_3^--N	2.9
NH_4^+-N	112.1

2.4. Parametric determination

2.4.1. Determination of the biomass

According to the preliminary experiment, the biomass (dry-weight) of *Chlorella vulgaris* 1068, *Botryococcus braunii* 765 and *Desmodesmus* sp. CHXl have good correlations with the absorbance measured at 680 nm, 680 nm and 690 nm, respectively, and so, the microalgal dry-weight was estimated by Eqns. (1), (2) and (3), respectively:

$$YB = 1.2153A_{680} - 0.0020, R^2 = 0.9987 \quad (1)$$

$$YC = 0.2434A_{680} - 0.0020, R^2 = 0.9912 \quad (2)$$

$$YD = 0.9946A_{690} - 0.0170, R^2 = 0.9982 \quad (3)$$

where *YB*, *YC* and *YD* (g·L^{-1}) represent the biomass of *Botryococcus braunii* 765, *Chlorella vulgaris* 1068 and *Desmodesmus* sp. CHXl, respectively; A_{690} is the absorbance of *Desmodesmus* sp. CHXl that was measured at 690 nm by a UV-Vis spectrophotometer; A_{680} is the absorbance of *Chlorella vulgaris* 1068 or *Botryococcus braunii* 765 that was measured at 680 nm by a UV-Vis spectrophotometer (Thermo Evolution 220, USA).

The specific growth rate was calculated by Eq. (4):

$$GR = \frac{(lnx - lnx_0)}{t} \quad (4)$$

where *GR* represents the specific growth rate; *t* (day) is the time between the two measurements, *x* and x_0 (g·L^{-1}) are the concentrations of biomass at day *t* and t_0, respectively.

The biomass productivity during the culture period was calculated based on the following equation:

$$P\left(mg \cdot l^{-1} \cdot d^{-1}\right) = \frac{(x - x_0)}{t} \quad (5)$$

where *P* represents the biomass productivity; *x* and x_0 (g·L^{-1}) are the concentrations of biomass at day *t* and t_0, respectively, and *t* (day) is the time between the two measurements.

2.4.2. Determination of chlorophyll and carotenoid content

The amount of 10 ml algal liquid was filtered with a 0.45 μm membrane, and then placed the residue into a 25 ml centrifuge tube with 10 ml anhydrous alcohol, sealed in 4°C in the refrigerator. After 24 h extraction, the tubes were centrifuged (12000 rpm, 5 min, 4°C), and the obtained suspensions were measured at the wavelength of 470 nm, 645 nm, 663 nm, respectively. The contents of chlorophyll and carotenoid were calculated by the following equations [20]:

$$C_{a+b} = 20.2 \times A_{645} + 8.02 \times A_{663} \quad (6)$$

$$C_a = 12.21 \times A_{663} - 2.81 \times A_{645} \quad (7)$$

$$C_b = 20.13 \times A_{645} - 5.03 \times A_{663} \quad (8)$$

$$C_{x-c} = \frac{\left(1000 \times A_{470} - 3.27 \times C_a \quad 104 \times C_b\right)}{229} \quad (9)$$

where A_{470}, A_{645} and A_{663} are the values of absorbance measured at 470 nm, 645 nm and 663 nm, respectively, and C_{a+b}, C_a, C_b and C_{x-c} (mg·L^{-1}) are the contents of total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid, respectively.

2.4.3. Determination of protein and lipid content

The protein was determined by ultraviolet spectrophotometer method [21], and the content of protein was calculated by the following equation (10):

$$PC = 1.45A_{280} - 0.74A_{260} \quad (10)$$

where A_{280} and A_{260} are the absorbance values that were measured at 280 nm and 260 nm, respectively, and *PC* (g·L^{-1}) represents the content of protein.

Total lipid was determined by the method reported by Zhou et al. [18]. Freeze-dried samples of algal powder were sonicated for 0.5 h in 10 ml methanol–chloroform (2:1, v/v). After filtered, 0.9% NaCl was added and centrifuged at 1000 rpm for 10 min. The chloroform layer was transferred into a glass tube, evaporated to dryness under vacuum, and weighted as the total lipid.

The lipid content was calculated by the following equation (11):

$$C_{lipid}\left(g \cdot g^{-1}\right) = \frac{W_L}{W_A} \quad (11)$$

where W_L (g) is the weight of the extracted lipids and W_A (g) is the dry algal biomass.

2.4.4. Chemical analysis

Liquid sample for nutrient analyses was collected and filtered through a 0.45 μm pore-size membrane. The filtration was collected and properly diluted for analyses of total nitrogen (TN), ammonium (NH_4^+-N), nitrate nitrogen (NO_3^--N), total phosphorus (TP), chemical oxygen demand (COD). TN, NH_4^+-N, NO_3^--N, TP and COD were determined by the standard methods [22].

2.5. Statistical analysis

All data were expressed as mean ± SE and performed by software Excel. One-way analysis of variance (ANOVA) was used to evaluate differences between the three microalgae, in which $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Algal growth under cultivation conditions

Algal growth of *Chlorella vulgaris* 1068, *Botryococcus braunii* 765 and *Desmodesmus* sp. CHXl as indicated by biomass concentrations in piggery wastewater under cultivation conditions, was shown in Fig. 1. With no obvious lag phase,

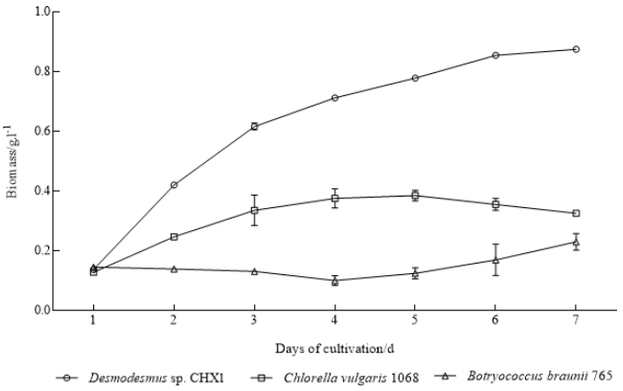

Fig. 1. Growth curves of *Desmodesmus* sp. CHXl, *Chlorella vulgaris* 1068 and *Botryococcus braunii* 765 in piggery wastewater during cultivation period.

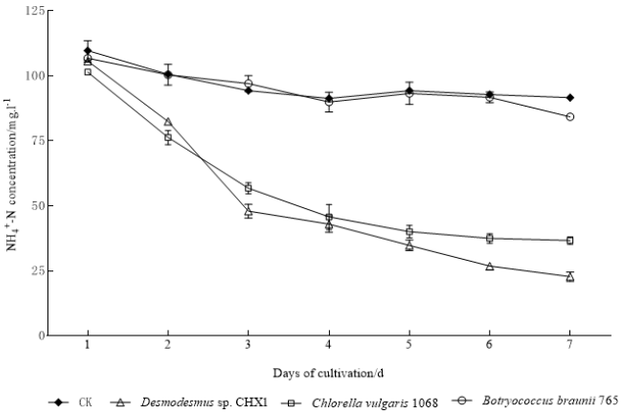

Fig. 2. Changes of NH_4^+-N concentration during cultivation period in inoculation treatments and the control.

algal growth of *Chlorella vulgaris* 1068 and *Desmodesmus* sp. CHXl performed well in the piggery wastewater. However, no significant increase in biomass of *Botryococcus braunii* 765 can be observed during the early five days. After 7-days' cultivation, the biomass of *Desmodesmus* sp. CHXl was 0.88 g·L^{-1}, which was significantly higher than those of *Chlorella vulgaris* 1068 (0.33 g·L^{-1}) and *Botryococcus braunii* 765 (0.23 g·L^{-1}) ($p < 0.05$). The data indicated that *Desmodesmus* sp. CHXl is more suitable for growth in piggery wastewater than *Chlorella vulgaris* 1068 and *Botryococcus braunii* 765**.**

Being consistent with the biomass, the specific growth rate and biomass production rate of *Desmodesmus* sp. CHXl, which were up to 1.847 mg·L^{-1}·d^{-1} and 124.8 mg·L^{-1}·d^{-1}, respectively, as shown in Table 3 ($p < 0.05$), were also significantly higher than those of *Chlorella vulgaris* 1068 and *Botryococcus braunii* 765.

3.2. Nutrients removal efficiencies under cultivation conditions

3.2.1. NH_4^+-N removal efficiency under cultivation conditions

Removal efficiency of NH_4^+-N during cultivation period by *Chlorella vulgaris* 1068, *Botryococcus braunii* 765 and *Desmodesmus* sp. CHX1 can be found in Fig. 2. After 7 days' cultivation, NH_4^+-N concentration decreased from 105.6 mg·L^{-1} to 22.8 mg·L^{-1}, from 101.4 mg·L^{-1} to 36.3 mg·L^{-1}, and from 106.7 mg·L^{-1} to 84.2 mg·L^{-1}, respectively, when *Desmodesmus* sp. CHX1, *Chlorella vulgaris* 1068 and *Botryococcus braunii* 765 were inoculated in the piggery wastewater, and the corresponding NH_4^+-N removal efficiencies were 78.5%, 63.8% and 24.2%, respectively. Meanwhile, NH_4^+-N concentration decreased from 109.6 mg·L^{-1} to 91.6 mg·L^{-1} in the control, and the NH_4^+-N removal efficiency was 16.4%. Results showed that *Desmodesmus* sp. CHXl showed the highest removal efficiency of NH_4^+-N, followed by *Chlorella vulgaris* 1068 and *Botryococcus braunii* 765.

Table 3
Specific growth rate and biomass production rate of *Chlorella vulgaris* 1068, *Botryococcus braunii* 765 and *Desmodesmus* sp. CHXl

	Chlorella vulgaris 1068	*Desmodesmus* sp. CHXl	*Botryococcus braunii* 765
Specific growth rate	0.935 ± 0.041	1.847 ± 0.004	0.654 ± 0.132
Biomass productivity (mg·L^{-1}·d^{-1})	46.5 ± 1.762	124.8 ± 1.165	32.6 ± 2.827

3.2.2. TP removal efficiency under cultivation conditions

Removal efficiency of TP during cultivation period by *Chlorella vulgaris* 1068, *Botryococcus braunii* 765 and *Desmodesmus* sp. CHX1 is shown in Fig. 3. Results showed that *Chlorella vulgaris* 1068 and *Desmodesmus* sp. CHX1 could rapidly and effectively remove phosphorus in the piggery wastewater. During the first three days, TP concentration of piggery wastewater with *Chlorella vulgaris* 1068 decreased from 3.16 mg·L^{-1} to 1.331 mg·L^{-1}, and then kept stable until the seventh day. For the *Desmodesmus* sp. CHXl, TP concentration of piggery wastewater decreased from 3.14 mg·L^{-1} to 0.25 mg·L^{-1} during the first four days, and then kept stable until the end of cultivation. TP concentration of piggery wastewater with *Botryococcus brau-*

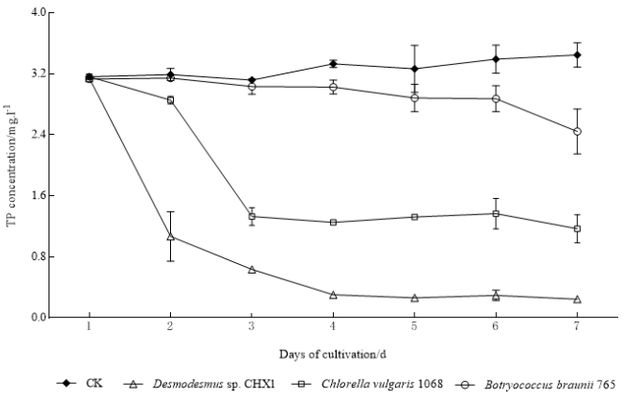

Fig. 3. Changes of TP concentration during cultivation period in inoculation treatments and the control.

nii 765 decreased slowly during the first six days, but decreased dramatically from 3.13 mg·L^{-1} to 2.44 mg·L^{-1} on the seventh day. The 92% TN removal efficiency with *Desmodesmus* sp. CHXl was significantly higher than 62.9% with *Chlorella vulgaris* 1068 and 22.0% with *Botryococcus braunii* 765.

3.3. The contents of chlorophyll and carotenoid

Changes of chlorophyll and carotenoid contents in *Chlorella vulgaris* 1068, *Botryococcus braunii* 765 and *Desmodesmus* sp. CHX1 during the cultivation period are displayed in Figs. 4 and 5, respectively. The chlorophyll and carotenoid contents of *Chlorella vulgaris* 1068 and *Desmodesmus* sp. CHXl increased first and then decreased steadily, while those of *Botryococcus braunii* 765 only changed slightly. After 7 days' cultivation, the chlorophyll and carotenoid contents of *Desmodesmus* sp. CHXl were 8.89 mg·L^{-1} and 3.19 mg·L^{-1}, accounting for about 1.02% and 0.36% of the biomass, respectively, however,

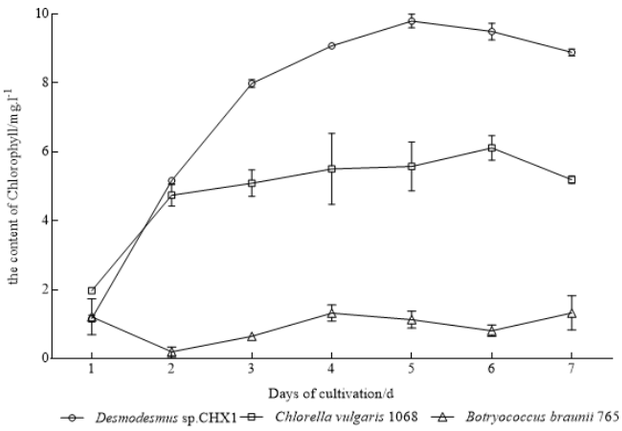

Fig. 4. Changes of chlorophyll contents in *Desmodesmus* sp. CHXl, *Chlorella vulgaris* 1068 and *Botryococcusbraunii* 765 during cultivation period.

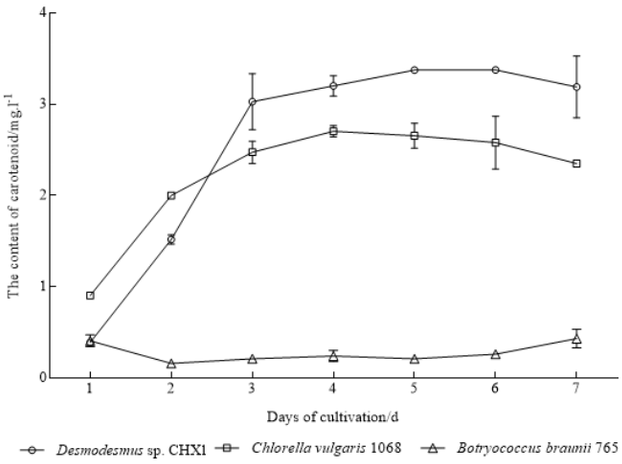

Fig. 5. Changes of carotenoid contents in *Desmodesmus* sp. CHXl, *Chlorella vulgaris* 1068 and *Botryococcusbraunii* 765 during cultivation period.

the chlorophyll and carotenoid contents of *Chlorella vulgaris* 1068 were 5.19 mg·L^{-1} and 2.35 mg·L^{-1}, and those of *Botryococcus braunii* 765 were 1.33 mg·L^{-1} and 0.43 mg·L^{-1}, both of which were significantly lower than those of *Desmodesmus* sp. CHXl.

3.4. The contents of protein and lipid

The contents of protein and lipid of *Desmodesmus* sp. CHXl, *Chlorella vulgaris* 1068 and *Botryococcus braunii* 765 under cultivation conditions are shown in Table 4. The contents of protein and lipid of *Desmodesmus* sp. CHXl were the highest, followed by those of *Chlorella vulgaris* 1068, and the lowest were those of *Botryococcus braunii* 765. After 7 days' cultivation, the protein and lipid contents of *Desmodesmus* sp. CHXl were 58.3% and 10.8%, respectively, with 0.51 g·L^{-1} and 0.095 g·L^{-1} of the yield production, respectively, while contents of *Chlorella vulgaris* 1068 were 44.6% and 10.7%, respectively, with 0.39 g·L^{-1} and 0.094 g·L^{-1} of the yield production. For the *Botryococcus braunii* 765, the protein and lipid contents were 26.1% and 9.56%, respectively, with 0.23 g·L^{-1} and 0.08 g·L^{-1} of the yield production.

4. Discussion

Screening suitable algae species is the key to promote the technology for piggery wastewater treatment based on microalgae, and it further ensures the practicality of the technology that the obtained algal cells have the great value for resource utilization [23]. For example, microalgae can be used as a renewable, alternative and sustainable source for the production of biodiesel [6]. This study compared the potential of three common microalgae for piggery wastewater treatment, including *Chlorella vulgaris*, *Botryococcus braunii* and *Desmodesmus* sp., based on their growth, NH_4^+-N and TP removal efficiency and resource utilization. Results showed that *Desmodesmus* sp. CHX1 presented the best performance with faster growth, stronger purifying ability, and higher content of valuable substances than those of the other two species. During the whole cultivation period, both *Desmodesmus* sp. CHX1 and *Chlorella vulgaris* 1068 showed good ability of adaptability and competition, while *Botryococcus braunii* 765 showed lower growth, even the division and death of algal cells, which might be related to different optimum conditions for different microalgae species, such as the optimum growth conditions of *Botryococcus braunii* were at a temperature of 23°C, a light intensity of 60 W·m² and

Table 4
The contents of protein and lipid of *Desmodesmus* sp. CHXl, *Chlorella vulgaris* 1068 and *Botryococcus braunii* 765

Items	*Desmodesmus* sp. CHX1	*Chlorella vulgaris* 1068	*Botryococcus braunii* 765
Protein content %	58.3 ± 1.34	44.6 ± 0.89	26.1 ± 0.45
Lipid content %	10.8 ± 0.08	10.7 ± 0.08	9.56 ± 0.04

a light period of 12 h per day [24]. Zhu et al. [13] pointed out that the livestock waste compost medium with 2000 mg·L^{-1} COD provided an optimal nutrient concentration for *Chlorella* sp. cultivation, where the highest productivities of biomass(288.84 mg·L^{-1}·d^{-1}) and lipid (104.89 mg·L^{-1}·d^{-1}) were presented. As far as the conditions were concerned in the study, microalgae selected from the local wastewater treatment ponds might be more suitable for piggery wastewater treatment, which further illustrated that algae isolated from local wastewater treatment plant site or real water body can better adapt to the practical conditions and show higher nutrient removal efficiency [17,18,25]. However, it should be pointed out that the removal efficiency of other nutrients, such as COD, should be also determined in the further researches to obtain comprehensive evaluation of the microalgal potential involved in microalgae-based system for purifying the piggery wastewater.

5. Conclusions

This study compared the potential of three microalgae species, including *Chlorella vulgaris* 1068, *Botryococcus braunii* 756 and *Desmodesmus* sp. CHX1 based on the biomass, removal efficiencies of TP and NH_4^+-N, and the contents of chlorophyll, carotenoid, proteinand lipid. Results showed that the biomass and the removal efficiencies of NH_4^+-N and TP of *Desmodesmus* sp. CHXl were 0.88 g·L^{-1}, 78.5% and 91.7%, respectively. The contents of chlorophyll, carotenoid, protein and lipid of *Desmodesmus* sp. CHXl were 8.89 mg·L^{-1}, 3.19 mg·L^{-1}, 0.51 g·L^{-1} and 0.095 g·L^{-1}, respectively, which were significantly higher than those of *Chlorella vulgaris* 1068 and *Botryococcus braunii* 765. Superior performances of local microalgae specie of *Desmodesmus* sp. CHXl were obtained based on the purification efficiency and resource utilization, further indicating that microalgae should be screened from local wastewater treatment plant site or real water body for wastewater treatment.

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