DOI:10.11835/j.issn.2096-6717.2020.160 开放科学(资源服务)标识码(OSID):

Mechanisms of ammonium removal from digested piggery wastewater by *Oedogonium sp.* assessed via isotope mass balance analysis

WANG Haiping[1,2], ZHENG Liguo[1], SHE Jiarong[3], CAO Qun[1]

(1. Changsha Environmental Protection College, Changsha 410004, P. R. China; 2. Key Laboratory of Agro-ecological Processes in Subtropical Regions, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, P. R. China; 3. School of Materials Science and Engineering, Central South University of Forestry and Technology, Changsha 410004, P. R. China)

Abstract: Nutrient removal from digested piggery wastewater (DPW) using microalgae is of increasing interest as a secondary treatment prior to discharge to avoid environmental contamination. In this study, we investigated the main mechanisms involved in ammonium removal from DPW by *Oedogonium sp.* using the ^{15}N mass balance approach with a focus on the relationship between algal growth and ammonium removal and the dominant ammonium removal pathway. We noted 96.2% ammonium removal and 0.04～0.15 specific growth rate of *Oedogonium sp.* in the diluted autoclaved DPW during the incubation period and 94.1 % ammonium removal and −0.14～0.13 specific growth rate in the diluted raw DPW. Aeration provided a significant benefit to ammonium removal via the stripping effect, which was favored by the high pH in the experimental conditions. Isotope mass balance analysis indicated that bacteria present in the initial DPW had little effect on ammonium removal in the experiment. Algal uptake and gaseous loss were the dominant pathways for NH_4—N removal from the diluted DPW using *Oedogonium sp.* cultures and accounted for 40.97% and 32.59% of the total ^{15}N amount, respectively. Regression and path analyses between NH_4—N removal and its main influencing factors indicated that to improve NH_4—N removal efficiency, the levels of *Oedogonium sp.* and dissolved oxygen (DO) should be increased under weakly alkaline conditions.

Keywords: piggery wastewater; *Oedogonium sp.*; ammonium removal; mass balance; algal uptake

基于^{15}N质量平衡法研究养殖消化废水中氨氮的去除机理

王海萍[1,2],郑立国[1],佘佳荣[3],曹群[1]

(1.长沙环境保护职业技术学院,长沙 410004;2.中国科学院亚热带农业生态研究所 亚热带农业生态过程重点实验室,长沙 410125;3.中南林业科技大学 材料科学与工程学院,长沙 410004)

摘　要:为避免养猪场消化废水对环境造成的污染,利用微藻去除消化废水中营养物质的二次处理方法受到了广泛关注。采用^{15}N质量平衡法研究了鞘藻去除氨氮的主要机理,重点研究了鞘藻生长与氨氮去除的关系以及氨氮去除的主要途径。经高压灭菌后的消化废水在鞘藻培养期的氨氮去除率为96.2%,鞘藻特定生长率为0.04～0.15;稀释后的原消化废水氨氮的去除率为94.1%,鞘藻

Received: 2020-02-28

Foundation items: Research Project of Education Department of Hunan Province (No. 19C0026); Natural Science Foundation of Hunan Province (No. 2017JJ5061)

Author brief: WANG Haiping (1983-), PhD, main research interest: wastewater treatment, E-mail: 2450485691@qq.com.

特定生长率为－0.14～0.13。通过曝气的汽提效应对氨的去除有显著的促进作用，尤其在高 pH 值试验条件下更有利于脱氨。^{15}N 同位素质量平衡分析表明，原始消化废水中存在的细菌对氨氮的去除影响小，在鞘藻培养期去除原消化废水中氨氮的主要途径是鞘藻的吸收和气体的损失，分别占总氮量的 40.97％和 32.59％。氨氮的去除与主要影响因素间的回归和通径分析表明，要提高氨氮去除率，需要提高鞘藻 Chl-a 含量和 DO 浓度，同时限制或保持 pH 值在弱碱性状态。

关键词：养殖废水；鞘藻；氨氮去除；质量平衡；藻类吸收

中图分类号：X703.1　　**文献标志码：**A　　**文章编号：**2096-6717(2021)01-0176-08

Anaerobic digestion by mesophilic or thermophilic bacteria is traditionally used as a primary treatment for piggery wastewater to reduce organic matter, waste volume, and odors and to recover biogas[1]. However, nutrient levels (especially ammonium) are not reduced during anaerobic digestion because the microorganisms employed are generally incapable of sufficient autotrophic metabolism of inorganic nitrogen[2]. The discharge of digested but otherwise untreated piggery wastewater results in excessive nutrient transport and induces aquatic eutrophication, groundwater contamination, and soil degradation[3]. For these reasons, many studies have focused on effective secondary treatments to remove nutrients from digested piggery wastewater (DPW) prior to disposal.

Most of the nitrogen retained in DPW after anaerobic digestion is in the form of ammonium nitrogen, which is readily available for use by algae in photosynthesis[4-5]. Previous studies have demonstrated that many genera of microalgae, such as *Chlorella*, *Scenedesmus* and *Neochloris*, are capable of nutrient removal from digested livestock effluent via uptake into cells[6-7]. For example, ammonium at an initial concentration between 81 and 178 mg/L was completely removed within 21 days from digested and diluted dairy manure and then used as a nutrient supplement for the cultivation of *Chlorella sp.*[8]. *Scenedesmus sp.* removes ammonium at rates of 5.20～6.46 mg/(L·d) when seeded at a concentration range of 0.5～1.5 g/L in anaerobically digested livestock waste effluent[9]. *Neochloris oleoabundans* assimilates 90％～95％ of the initial nitrate and ammonium in effluent after six days[10].

The reported NH_4—N removal efficiencies vary by algae species, initial nutrient concentration, and environmental conditions. A comparison of the abilities of four green microalgae (*Hydrodictyaceae reticulatum Lag.*, *Scenedesmus obliquus*, *Oedogonium sp.*, and *Chlorella pyrenoidosa*) and three blue-green algae (*Anabaena flos-aquae*, *Oscillatoria amoena Gom.*, and *Spirulina platensis*) to remove nutrients from diluted DPW suggests that *Oedogonium sp.* is very effective at removing nutrients, especially NH_4—N, which is present at an initial concentration of approximately 55 mg/L[11]. The use of microalgae for wastewater treatment offers other advantages, such as low cost, the possibility of recycling nutrients assimilated into algal biomass as fertilizer, and the discharge of oxygenated effluent into water bodies[3].

Although an *Oedogonium sp.*-based secondary wastewater treatment process provides great potential for NH_4—N removal from DPW, the main mechanisms involved require further investigation to optimize the design of the treatment system utilizing this alga. The stable isotope ^{15}N has been traditionally used to evaluate ammonium transformation pathways in ecosystems, such as wetlands, in situ mesocosms, and laboratory microcosms[12-13]. Thus, in this study, we used the ^{15}N mass balance approach to investigate the main mechanisms by which *Oedogonium sp.* removes ammonium from DPW. We describe here the relationship between algal growth and ammonium removal, the influence of bacteria initially present in DPW upon ammonium removal, and the dominant ammonium removal pathway.

1 Materials and methods

1.1 Algae strain

Algal strains of *Oedogonium sp.* were obtained from the Freshwater Microalgae Culture Collection of the Institute of Hydrobiology (FACHB-Collection), Chinese Academy of Sciences, China. Cells were grown in culture media[14] at 25±2 ℃ in an incubator with a 12-h photoperiod. Cells in the exponential growth phase were filtered and then used in the experiments.

1.2 Wastewater

The DPW was collected from a piggery in Changsha, China; it contained 401 mg/L chemical oxygen demand (COD), 50.36 mg/L total phosphorus (TP), 525.4 mg/L total nitrogen (TN), and 508 mg/L ammonia nitrogen (NH_4—N). Pretreatment in an autoclave reduced the COD, TP, TN, and NH_4—N to 369, 17.88, 169.7, and 110 mg/L, respectively. Before being used as culture media, the raw and autoclaved DPW samples were diluted with distilled water at factors of 9.2 and 2, respectively, to reduce the toxic influence of the ammonia on algal growth and obtain initial ammonia concentrations of 55 mg/L.

1.3 Experimental operation

The experiment was carried out in an incubator set at 25±2 ℃ with a 12-h photoperiod. *Oedogonium sp.* cells were cultivated in 2 L flasks with a total working volume of 1 L. Cultures were continuously agitated by atmospheric air bubbling (0.5 L/min). To investigate the fate of ammonium in the *Oedogonium sp.* microcosm and the effect of initial bacteria in wastewater on ammonium removal, 0.67 mg ^{15}N (as ammonium sulfate) was added with the diluted raw (DRW) or diluted autoclaved (DAW) DPW. To eliminate isotopic effects, the DRW or DAW treatments without the addition of ^{15}N were also arranged in triplicate. The initial inoculum density of *Oedogonium sp.* was in the range of 300 mg/L dry weight for all treatments. DRW or DAW without *Oedogonium sp.* was prepared as the control in triplicate.

1.4 Analytical procedures

During the 9 days incubation period, 50 mL samples were taken for assay on days 0, 1, 3, 5, 7, and 9, and water evaporation losses were supplemented with distilled water before sample collection. NH_4—N, pH, and dissolved oxygen (DO) analyses were performed following standard methods[15]. The cells were collected by centrifugation at 4 000 rpm for 10 min and then transferred into a 10 mL tube for algal Chl-a content (mg/L) analysis[16]. The specific growth rate, μ (day^{-1}), was determined on the basis of the Chl-a content[16]. At the end of the experiment, a mass spectrometer (Thermo Scientific MAT 253, USA) was used to measure the ^{15}N composition of TN in water and dried cells after filtration. As algal uptake, stripping volatilization, and microbial removal are the three main N removal processes in the *Oedogonium sp.* microcosm, the ^{15}N mass balance analysis was expressed using equations modified from Zhang et al.[12].

$$M_s = M_i - (M_w + M_m + M_a) \tag{1}$$

$$M_m = M_c - M_a \tag{2}$$

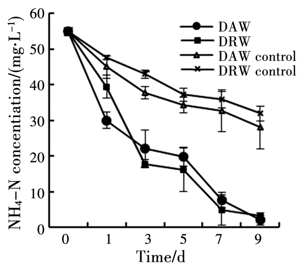
where M_i is the initial ^{15}N load in wastewater, M_w is the final ^{15}N load in DRW wastewater, M_s is the estimated ^{15}N loss by stripping volatilization in DRW, M_c is the ^{15}N uptake by the cells (algal and bacterial) in DRW, M_a is the ^{15}N uptake by the algae in DAW, and M_m is the estimated microbial N removal.

The regression and path analyses relating NH_4—N removal in DAW and the independent variables were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

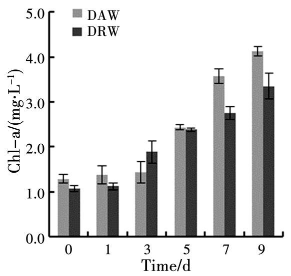
2 Results and discussion

2.1 Algal growth and ammonium removal efficiencies

During the incubation period, NH_4—N concentrations decreased from the initial 55 mg/L to 3.2 mg/L (DRW) and 2.1 mg/L (DAW), and the NH_4—N concentrations in the DRW and DAW controls decreased to 28.1 and 32.0 mg/L on day 9, respectively (Fig. 1(a)). Meanwhile, the Chl-a content increased from 1.07 to 3.35 mg/L (DRW) and 1.29 to 4.13 mg/L (DAW) (Fig. 1(b)). Data related to the growth rate of *Oedogonium sp.* and the ammonium removal rate are summarized in Table 1. Most of the NH_4—N was removed during the first five days, accounting for 64.1%～70.4% of NH_4—N removal in both treatments. By day 9, 96.2% (DAW) and 94.1% (DRW) of NH_4—N was removed. After an initial adjustment to the new growth conditions, after day 3 *Oedogonium sp.* grew rapidly and at a steady, specific growth rate.



(a) Changes in NH4-N concentration of DRW and DAW over time



(b) Changes in Chl-a concent of DRW and DAW over time

Fig. 1 Changes in NH_4—N concentration and Chl-a content of DRW and DAW over time

Table 1 Specific growth rates of *Oedogonium sp.* and ammonium removal rates during the incubation period

Time/days	Specific growth rate of *Oedogonium sp.* /d^{-1}		NH_4—N removal rate/%			
	DAW	DRW	DAW	DRW	DAW control	DRW control
1	0.07	−0.14	45.5	28.3	17.9	13.4
3	0.04	0.13	59.7	67.5	31.1	34.8
5	0.13	0.12	64.1	70.4	37.7	32.2
7	0.15	0.11	86.1	91.0	40.4	34.8
9	0.13	0.11	96.2	94.1	48.9	41.8

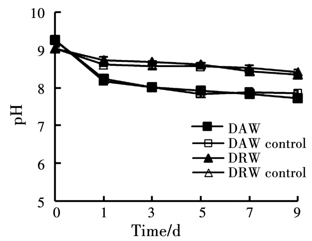
Nitrogen often exists as ammonium in anaerobically digested wastewater[17]; thus, we focused our investigation of microalgal nitrogen removal on ammonium. Algal growth and ammonium removal rates were higher for DAW than for DRW, which could be due to the relatively higher initial concentration of P in DAW than in DRW, while both treatments had similar initial NH_4—N levels. In the case of *Nannochloropsis sp.* cultivated in nutrient media, a lower N:P supply ratio favors biomass productivity and nutrient removal[18]. It is worth noting that 41.8%～48.9% of the ammonium removed was observed on day 9 in the DRW and DAW controls without algal uptake.

2.2 Changes in pH and DO concentration

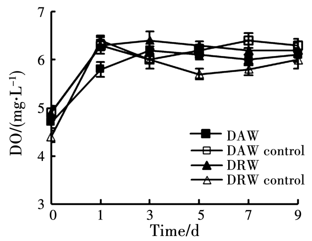
From an initial pH value of 9.0, the pH of the DRW and DRW controls decreased to 8.3 and 8.4 on day 9, respectively. The pH of DAW decreased from 9.3 to 8.0 on day 3 and reached a pH of 7.7 on day 9. The DAW control followed a similar trend, reaching a pH of 7.8 on day 9 (Fig. 2(a)). These data demonstrate that during ammonium removal, the pH of the wastewater was maintained within 7.7～9.3 and mostly > 8.0. Such high values (> 8.0) favor ammonia stripping due to the shifting equilibrium from NH_4 to ammonia (NH_3)[19]. Therefore, the extent of ammonia removal with time can be explained by NH_3 volatilization.

The DO concentrations in DRW and DAW increased from 4.9 and 4.7 mg/L to 6.3 and 5.8 mg/L on day 1, respectively, and then remained between 6.0 and 6.4 mg/L for both treatments. The DO concentration of the DAW control increased to 6.3 mg/L on day 1 and then remained around 6.2 mg/L, while that of the DRW control ranged from 5.7～6.4 mg/L after day 1 (Fig. 2(b)). Molecular oxygen is normally released during photosynthesis when treating wastewater with microalgae[20]. However, DO saturation (6.2

mg/L) can be maintained by appropriate aeration, which is favorable for algal cell growth and algal ammonia uptake[9]. The net algal growth rate without aeration is enhanced 1. 7-fold by aeration. Moreover, the ammonium removal rate in algal cultures with aeration is approximately three times that of algal cultures without aeration[9]. At the same time, aeration contributes to ammonium removal by stripping ammonia out of wastewater directly; this process is highly favored at high pH[19]. Therefore, the NH_4—N removal rate in this experiment was probably due to gas loss by NH_3 volatilization under conditions of high pH conditions and the stripping of ammonia by aeration in the controls as well as in DAW and DRW. These results are consistent with the aforementioned observation that 41. 8%~48. 9% of ammonium was removed in the controls without algal uptake.



(a) Changes in pH for DRW and DAW over time



(b) Changes in DO concentrations for DRW and DAW over time

Fig. 2 Changes in pH and DO concentrations for DRW and DAW over time

2. 3 ^{15}N mass balance analysis

The ^{15}N mass balance in *Oedogonium sp.* cultures at the end of the incubation period is shown in Fig. 3. We assumed that the influence of the other microorganisms on ammonium removal was negligible compared to the initial bacteria in DRW and the added algae. Based on the value of ^{15}N uptake by *Oedogonium sp.* in DAW and DRW with isotope and the equations (1) and (2), the estimated quantities of ^{15}N in the final wastewater, algae, and bacteria were 0. 12, 0. 27, and 0. 06 mg, respectively, accounting for 17. 31%, 40. 97%, and 9. 13% of the initial 0. 67 mg ^{15}N in the DRW *Oedogonium sp.* culture. The residual ^{15}N ratio in the final wastewater at day 9 was higher than that of NH_4—N in the final DRW without isotope, indicating that ^{14}N is preferentially incorporated relative to ^{15}N. This has been observed previously in studies on nitrogen isotopic fractionation associated with N uptake and assimilation by microorganisms[21-22]. Mass balance analysis showed that 0. 22 mg ^{15}N disappeared from the *Oedogonium sp.* culture, indicating that 32. 59% of the added ^{15}N was lost through gas. Ammonia stripping could be considered an important pathway for NH_4—N removal from DRW; the results of the ^{15}N tracer analysis showed that NH_4—N removal from DRW could be mainly attributed to NH_4—N incorporation into *Oedogonium sp.* and gaseous loss.

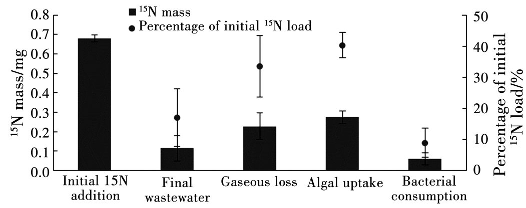


Fig. 3 ^{15}N mass balance in Oedogonium sp. culture

2. 4 Ammonium removal regression and path analyses

The Shapiro-Wilk test was used to analyze the normality of NH_4—N removal in DAW as the dependent variable. The resulting Shapiro-Wilk statistic is 0. 950 (p value of 0. 736 > 0. 05), which means that the dependent variable (Y) is distributed normally, that is, Y is a normal variable for regression analysis. The regression equation to define the relationship between Y and the main influencing factors or independent variables, namely, DO (X_1), pH (X_2), and Chl-a content (X_3), was established by stepwise regression using quadratic polynomials expressed as follows:

$$Y = -0.022 - 0.012X_2^2 + 0.027X_3^2 + 0.032X_1X_2 \quad (3)$$

The correlation coefficient resulting from the equation is close to 1 ($R=0.994$) with a small standard deviation ($S=0.045$), indicating the high accuracy of fit of the equation.

The correlation values between different independent variables and NH_4—N removal are shown in Table 2. The results suggest that the degree of influence of the three independent variables on NH_4—N removal is ranked as follows: Chl-a content > DO > pH. However, it is often difficult to clearly show which variable plays the major decisive or restrictive role on the dependent variable based on subdividing the correlation coefficient between different variables. Path analysis can check whether X_i has a significant effect on Y, and most importantly, it determines whether X_i directly or indirectly affects the dependent variable through other independent variables.

Table 2 Correlation coefficients between different variables

R_{ij} or R_{iy}	DO (X_1)	pH (X_2)	Chl-a content (X_3)	NH_4-N removal rate (Y)
DO(X_1)	1	−0.562	0.200	0.386
pH(X_2)		1	−0.922	−0.966
Chl-a content(X_3)			1	0.968
NH4-N removal rate(Y)				1

The path analysis yields the path coefficient between three independent variables and NH_4—N removal (Table 3). Among the three independent variables, Chl-a content has the greatest direct effect on Y (P_{3y}) followed by pH, and DO has the smallest direct effect. An analysis of various indirect path coefficients demonstrated that, despite the large value of P_{2y}, the indirect effect of pH on Y through DO and Chl-a content produced a large negative value ($P_{21y}=-1.192$ and $P_{23y}=-1.956$), and the correlation coefficient R_{2y} between pH and Y is highly negative (−0.966). The Chl-a content had the greatest indirect effect on Y through DO, yielding an indirect path coefficient P_{31y} of 0.542, while DO had the second greatest indirect effect on Y through Chl-a content, yielding an indirect path coefficient P_{13y} of 0.207. Therefore, Chl-a content plays an important role in NH_4—N removal. To improve NH_4—N removal, Chl-a content and DO must increase while the pH is maintained in a weakly alkaline range.

Table 3 Path coefficients between different variables

X_i	P_{iy}	X_j	P_{ijy}
DO (X_1)	1.035	pH (X_2)	−0.582
		Chl-a content (X_3)	0.207
pH (X_2)	2.121	DO (X_1)	−1.192
		Chl-a content (X_3)	−1.956
Chl-a content (X_3)	2.717	DO (X_1)	0.542
		pH (X_2)	−2.506

3 Conclusion

In conclusion, 96.2% ammonium removal and 0.04~0.15 specific growth rate of *Oedogonium sp.* were observed in DAW during the incubation period, while 94.1% ammonium removal and −0.14~0.13 specific growth rate of *Oedogonium sp.* were observed in DRW. High pH and aeration provided a significant benefit to ammonium removal by NH_3 volatilization and the stripping effect during the incubation period. ^{15}N isotope mass balance analysis indicates that bacteria initially present in DPW have little effect on ammonium removal in *Oedogonium sp.* cultures in DRW, as this occurs primarily through algal uptake, ammonia volatilization, and stripping processes. The statistical analysis between three independent variables and NH_4—N removal indicates that increased levels of *Oedogonium sp.* and DO under weakly alkaline conditions can improve the efficiency of NH_4—N removal. Using the ammonium-based isotope tracing method, we hypothesize that *Oedogonium sp.* is effective for treating DPW with high ammonium loading under adapted DO and pH values. Thus, culture conditions should be evaluated to improve the efficiency of nutrient treatment when microalgae are used for the secondary agricultural wastewater treatment process. Further investigation of whether *Oedogonium sp.* has the capacity to simultaneously remove other pollutants, such as phosphorus and heavy metals, will be useful in assessing the practical potential of this organism as a biological agent for secondary treatment of DPW. In addition, as it affects the growth of algae, the influence of light time on ammonium removal will be further studied.

Acknowledgements

This work was nancially supported by the Research Project of Education Department of Hunan Province (No. 19C0026), the Natural Science Foundation of Hunan Province (2017JJ5061). We gratefully acknowledge anonymous reviewers for their constructive comments and suggestions.

References:

[1] CHENG J, LIU B. Swine wastewater treatment in anaerobic digesters with floating medium [J]. Transactions of the ASAE, 2002, 45(3): 799-805.

[2] NOIKE T, GOO I S, MATSUMOTO H, et al. Development of a new type of anaerobic digestion process equipped with the function of nitrogen removal [J]. Water Science & Technology, 2004, 49(5/6): 173-179.

[3] ASLAN S, KAPDAN I K. Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae [J]. Ecological Engineering, 2006, 28(1): 64-70.

[4] KIM M K, PARK J W, PARK C S, et al. Enhanced production of Scenedesmus spp. (green microalgae) using a new medium containing fermented swine wastewater [J]. Bioresource Technology, 2007, 98(11): 2220-2228.

[5] KUMAR M, MIAO Z, WYATT S. Influence of nutrient loads, feeding frequency and inoculum source on growth of Chlorella vulgaris in digested piggery effluent culture medium [J]. Bioresource Technology, 2010, 101: 6012-6018.

[6] MICHELON W, SILVA M L B D, MEZZARI M P, et al. Effects of nitrogen and phosphorus on biochemical composition of microalgae polyculture harvested from phycoremediation of piggery wastewater digestate [J]. Applied Biochemistry and Biotechnology, 2015, 178: 1407-1419.

[7] SUN Z L, SUN L Q, CHEN G Z. Microalgal cultivation and nutrient removal from digested piggery wastewater in a thin-film flat plate photobioreactor [J]. Applied Biochemistry and Biotechnology, 2019, 187(4): 1488-1501.

[8] WANG L, LI Y, CHEN P, et al. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella sp.* [J]. Bioresource Technology, 2010, 101: 2623-2628.

[9] PARK J, JIN H F, LIM B R, et al. Ammonia removal from anaerobic digestion effluent of livestock waste using green alga *Scenedesmus sp.* [J] Bioresource Technology, 2010, 101: 8649-8657.

[10] LEVINE R B, COSTANZA-ROBINSON M S, SPATAFORA G A. Neochloris oleoabundans grown on anaerobically digested dairy manure for concomitant nutrient removal and biodiesel feedstock production [J]. Biomass and Bioenergy, 2011, 35(1): 40-49.

[11] WANG H P, HU Z Q, XIAO B, et al. Ammonium nitrogen removal in batch cultures treating digested piggery wastewater with microalgae *Oedogonium sp.* [J]. Water Science and Technology, 2013, 68(2): 269-275.

[12] ZHANG S N, LIU F, XIAO R L, et al. Nitrogen removal in Myriophyllum aquaticum wetland microcosms for swine wastewater treatment: 15N-labelled nitrogen mass balance analysis [J]. Journal of the Science of Food and Agriculture, 2017, 97(2): 505-511.

[13] HARRISON M D, GROFFMAN P M, MAYER P M, et al. Nitrate removal in two relict oxbow urban wetlands: A ^{15}N mass-balance approach [J].

Biogeochemistry, 2012, 111(1/2/3): 647-660.

[14] FACHB-Collection. Fresh-water algae collection[EB/OL], http://algae.ihb.ac.cn/Dictionary.aspx. 2019.

[15] APHA. Standard methods for the examination of water and wastewater [S]. American Public Health Association/ American Water Works Association/ Water Environment Federation, Washington, DC, USA, 1998.

[16] WANG H P, LIU F, LUO P, et al. Allelopathic effects of *Myriophyllum aquaticum* on two cyanobacteria of *Anabaena flos-aquae* and *Microcystis aeruginosa* [J]. Bulletin of Environmental Contamination and Toxicology, 2017, 98(4): 556-561.

[17] ULUDAG-DEMIRER S, DEMIRER G N, FREAR C, et al. Anaerobic digestion of dairy manure with enhanced ammonia removal [J]. Journal of Environmental Management, 2008, 86(1): 193-200.

[18] MAYERS J J, FLYNN K J, SHIELDS R J. Influence of the N∶P supply ratio on biomass productivity and time-resolved changes in elemental and bulk biochemical composition of *Nannochloropsis sp.* [J] Bioresource Technology, 2014, 169: 588-595.

[19] DAMMAK M, HADRICH B, MILADI R, et al. Effects of nutritional conditions on growth and biochemical composition of *Tetraselmis sp.* [J/OL]. Lipids in Health and Disease, 2017, 16, 41, https://doi.org/10.1186/s12944-016-0378-1.

[20] MARQUEZ F J, SASAKI K, NISHIO N, et al. Inhibitory effect of oxygen accumulation on the growth of *Spirulina platensis* [J]. Biotechnology Letters, 1995, 17(2): 225-228.

[21] WASER N A D, HARRISON P J, NIELSEN B, et al. Nitrogen isotope fractionation during the uptake and assimilation of nitrate, nitrite, ammonium, and urea by a marine diatom [J]. Limnology and Oceanography, 1998, 43(2): 215-224.

[22] PENNOCK J R, VELINSKY D J, LUDLAM J M, et al. Isotopic fractionation of ammonium and nitrate during uptake by *Skeletonema costatum*: Implications for δ15N dynamics under bloom conditions [J]. Limnology and Oceanography, 1996, 41(3): 451-459.

（编辑　胡英奎）