

Improvement on tolerance of *Spirodela polyrrhiza* to ammonia at presence of nitrate

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Abstract: The response of *Spirodela polyrrhiza* to ammonia with the existence of different concentrations of nitrate was investigated in this study. Four series media with different concentrations of ammonia and nitrate were set up in experiments. The relative growth rate and the frond area of *S. polyrrhiza* in different conditions were studied. The results showed that the relative growth rate and the frond area of *S. polyrrhiza* decreased with the increasing concentration of ammonia. The maximum concentration of ammonia that *S. polyrrhiza* can bear is about $\rho(\text{NH}_4^+-\text{N})$ of $400 \text{ mg} \cdot \text{L}^{-1}$ without nitrate. But *S. polyrrhiza* can bear the concentration of ammonia up to $\rho(\text{NH}_4^+-\text{N})$ of $500 \text{ mg} \cdot \text{L}^{-1}$ in the presence of nitrate. The relative growth rate of *S. polyrrhiza* at high ammonia level [$\rho(\text{NH}_4^+-\text{N}) > 100 \text{ mg} \cdot \text{L}^{-1}$] with nitrate was clearly higher than ones without nitrate. The area of fronds with nitrate was larger than ones without nitrate at higher ammonia level [$\rho(\text{NH}_4^+-\text{N}) > 300 \text{ mg} \cdot \text{L}^{-1}$]. It demonstrated that the presence of nitrate could enhance the tolerance of *S. polyrrhiza* to ammonia.

Key words: ammonia; nitrate; *Spirodela polyrrhiza*; wastewater treatment

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硝酸盐氮对紫背浮萍氨耐受能力的影响

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摘要: 为了分析硝酸盐氮对紫背浮萍氨耐受能力的影响, 该文通过在 4 个系列氨氮培养液中加入不同水平硝酸盐氮, 研究了不同情况下紫背浮萍相对生长速率、叶面积等的变化。研究表明, 在没有硝酸盐氮的情况下, 紫背浮萍的相对增长速率和叶状体大小随氨浓度增加而逐渐降低, 能够耐受的最大氨质量浓度约为 400 mg/L [$\rho(\text{NH}_4^+-\text{N})$]。在硝酸盐存在情况下, 紫背浮萍生长则可耐受 500 mg/L [$\rho(\text{NH}_4^+-\text{N})$], 并且相同氨氮质量浓度下, 硝酸盐存在使紫背浮萍相对增长速率和叶状体面积均增加。从该研究结果来看, 水中硝酸盐氮的存在可提高紫背浮萍对氨的耐受性。

关键词: 氨; 硝酸盐氮; 紫背浮萍; 废水处理

Duckweed is a free-floating aquatic macrophyte that can reproduce by vegetative budding of new frond and grow rapidly^[1]. Due to high protein content in their biomass, duckweed can accumulate lot of nutrients by rapid multiplication^[2, 3]. Therefore duckweeds have an important potential use in wastewater treatment, especially the species

with rich nitrogen and phosphorus. More and more duckweed-based systems are being used for wastewater treatment and resource recovery from wastewater throughout world^[4-7]. The concentration of ammonia in wastewater is usually high and varies greatly after the organic matter was degraded. Ammonia is one of nitrogen resource which

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duckweed can take up. However, it is also a factor to inhibit the growth of duckweed because it has toxic effect on plant cell above a certain level^[8]. Many investigators studied the response of duckweed to ammonia in water^[9-11], but the results were not always in line with each other because the conditions of experiment were not completely same. Many factors, such as pH, temperature, medium composition, affect the tolerance of duckweed to ammonia^[12]. Among these factors, the effect of nitrate in medium was few studied. In fact, most of ammonia in wastewater is nitrified to nitrate under the aerated treatment. Furthermore, nitrate is also a nitrogen resource which duckweed can employ^[8]. The existence of nitrate in wastewater possibly affects the response of duckweed to ammonia. The purpose of this study was to clarify the tolerance of *S. polyrrhiza* by increasing level of ammonia and the effect of nitrate on the tolerance.

1 Materials and methods

1.1 Collection and culture of duckweeds

S. polyrrhiza is a common specie of duckweed in China. It has larger area of leaf and more root than other species of duckweed^[13]. It is also one of species used in wastewater treatment systems. *S. polyrrhiza* used in this study were collected from a wetland nearby Dianchi Lake (located in Kunming City, Yunnan Province, China). They are acclimatized to the effluent water [pH 6-8, COD: 10-50 mg·L⁻¹, ρ(NH₄⁺-N)=5-10 mg·L⁻¹, ρ(N)=40 mg·L⁻¹] of a biological treatment process treating domestic wastewater and got a rapid multiplication in laboratory condition before use for study.

The experiments were conducted in laboratory condition with fluorescent lamps of light intensity of 2 000-3 000 lx (16 hours light, 8 hours dark). The temperature was 26-30 °C. The growth media of experiment was based on the artificial media suitable to culture of duckweed^[14], nitrogen resource of the media was modified by experimental requirement.

The cultivation of *S. polyrrhiza* was performed in 250 mL plastic breaker with 200 mL medium each one. Twenty healthy fronds of *S. polyrrhiza* were put in the plastic container at the beginning of each experiment. Each experiment had two replicates.

1.2 Experimental set up

The composition of nitrogen in the culture medium was adjusted by replacing NH₄NO₃ with NH₄Cl and

NaNO₃. Four series media with different levels of ammonia and nitrate were set up (Table 1). Each medium was replaced once every 5 days to avoid the nutrient exhausted and the growth of alga. The pH of each medium was always kept about 7 (6.5-7.5) in all experiments by adding 1 mol/L HCl or KOH every day. Loss of water by evaporation was compensated by addition of de-mineralised water. Each experiment was duplicated and the cultivation period was 24 days. The fronds were counted and the average area of fronds was measured every 5 days. The fresh mass was determined at last. Then the fronds were collected to measure chlorophyll content and activities of glutamine synthetase, respectively.

Tab. 1 Four series media with different levels of ammonia and nitrate

	ammonia					nitrate		mg·L ⁻¹
ρ(NH ₄ ⁺ -N)	25	50	100	200	300	400	500	
ρ(NO ₃ ⁻ -N)	0	0	0	0	0	0	0	
ρ(NH ₄ ⁺ -N)	25	50	100	300	500			
ρ(NO ₃ ⁻ -N)	10	10	10	10	10			
ρ(NH ₄ ⁺ -N)	25	50	100	300	500			
ρ(NO ₃ ⁻ -N)	20	20	20	20	20			
ρ(NH ₄ ⁺ -N)	500	500	500					
ρ(NO ₃ ⁻ -N)	30	40	50					

1.3 Analytical methods

The fresh mass of duckweeds was determined after the water on surface of frond being absorbed by filter paper. The growth of duckweeds was evaluated on the basis of relative growth rate (RGR)^[15] given by following equation:

RGR=(ln m₂-ln m₁)/t,

Where, m₁ and m₂ are fresh mass at beginning and at the end of experiment. RGR above zero means the fronds can tolerate the condition of experiment and could produce new biomass, nearly zero means the fronds only could tolerate but not grow, below zero means the fronds are damaged and die gradually.

The chlorophyll content was determined according Li's method^[16]. The activity of glutamine synthetase was determined according Wang's method^[17]. Each couple of data from two replicates was taken average value.

2 Results and discussion

The results showed that the relative growth rate (RGR) and the frond area of *S. polyrrhiza* decreased with the increasing concentration of ammonia (Fig. 1). The maximum concentration of ammonia that *S. polyrrhiza* can bear is about ρ(NH₄⁺-N) of 400 mg·L⁻¹ without ni-

trate. At low ammonia level [$\rho(\text{NH}_4^+ - \text{N}) < 50 \text{ mg} \cdot \text{L}^{-1}$] the RGRs and average area of fronds were not affected by the existence of nitrate. But at high ammonia level [$\rho(\text{NH}_4^+ - \text{N}) > 100 \text{ mg} \cdot \text{L}^{-1}$], the RGRs of *S. polyrrhiza* with nitrate was higher than ones without nitrate. The average area of fronds with nitrate was larger than ones without nitrate at higher ammonia level [$\rho(\text{NH}_4^+ - \text{N}) > 300 \text{ mg} \cdot \text{L}^{-1}$]. And *S. polyrrhiza* can bear ammonia up to $\rho(\text{NH}_4^+ - \text{N})$ of $500 \text{ mg} \cdot \text{L}^{-1}$ in the presence of nitrate. Moreover, the relative growth rate and area of *S. polyrrhiza* increased by increasing concentration of nitrate nitrogen (Fig.2). Clearly, the presence of nitrate could enhance the tolerance of *S. polyrrhiza* to ammonia.

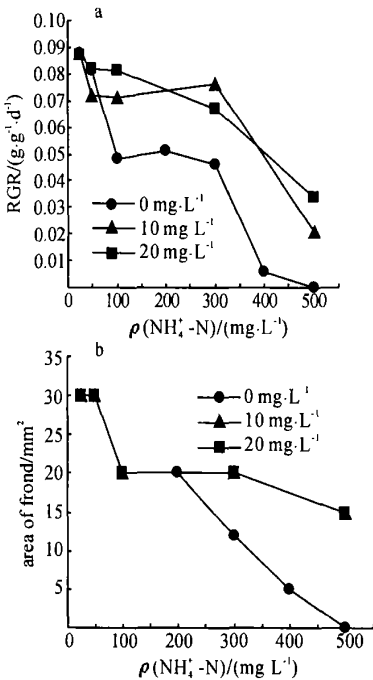


Fig. 1 The effect of ammonia on *S. polyrrhiza* with different concentrations nitrate nitrogen (pH=7)

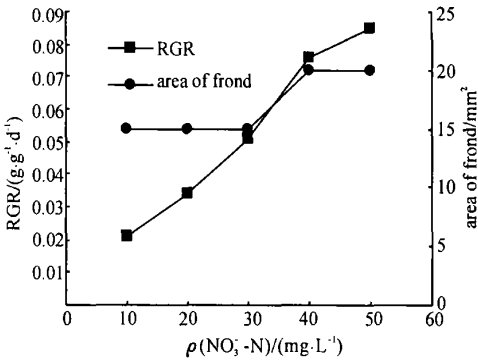


Fig. 2 The effect of nitrate on the growth rate of *S. polyrrhiza* under $\rho(\text{NH}_4^+ - \text{N})$ of $500 \text{ mg} \cdot \text{L}^{-1}$ (pH=7)

$\text{mg} \cdot \text{L}^{-1}$) also inhibited the synthesis of chlorophyll of *S. polyrrhiza*, the chlorophyll content decreased with increasing concentration of ammonia without nitrate (Fig. 3a). When adding nitrate from $\rho(\text{NO}_3^- - \text{N})$ of 10 to $20 \text{ mg} \cdot \text{L}^{-1}$, chlorophyll content increased compared to ones without nitrate at same ammonia concentration. Moreover the chlorophyll content in the presence of nitrate had almost no change with increasing ammonia concentration. The activity of glutamine synthetase of *S. polyrrhiza* increased with increasing concentration of ammonia (Fig. 3b). It had higher values in medium without nitrate than ones with nitrate.

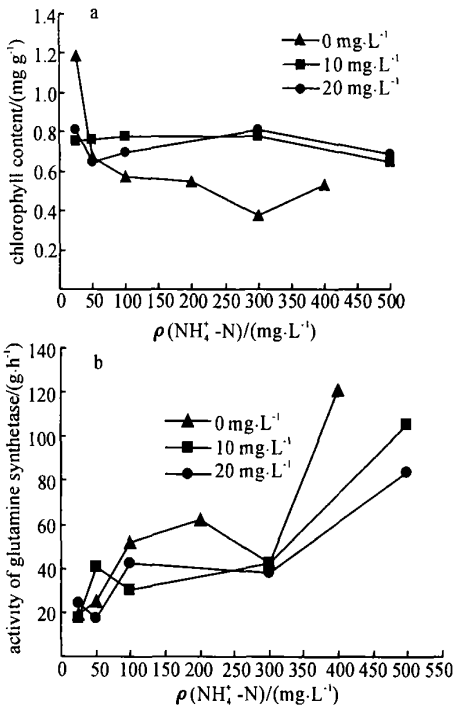


Fig. 3 The chlorophyll content and the activity of glutamine synthetase of *S. polyrrhiza* under different concentrations of ammonia and nitrate nitrogen

3 Discussion and conclusions

Ammonia exists in aqueous solution in two principal forms the ionised form (NH_4^+) and un-ionised form (NH_3). NH_3 is lipid-soluble and therefore easily enters into plant cell through membrane, and disturb cell metabolism^[18]. High NH_4^+ level can cause strong depolarization of membrane. Many investigations suggested that the inhibition of ammonia to duckweed growth was a combined effect of both NH_3 and NH_4^+ ^[19]. In certain extent, the plant can abate the toxicity by some mechanisms^[20].

In our study, although the relative growth rate of

S. polyrrhiza was obviously inhibited when ammonia nitrogen above $50 \text{ mg} \cdot \text{L}^{-1}$, it still could keep to grow until ammonia nitrogen concentration above $300 \text{ mg} \cdot \text{L}^{-1}$. At the same time, the area of frond became smaller and smaller and the activity of glutamine synthetase increased. By glutamine synthetase, the plant cell generally transforms overmuch NH_3 into glutamine to avoid its toxic effect. In addition, the structure of cell may possibly be changed to stop the entering of NH_4^+ so that the area of frond became small. Further research for the mechanisms is needed.

Except for the factors of duckweed itself, the presence of nitrate in medium seems to abate the toxic effect of ammonia. Nitrate is one of nitrogen resource that plant can employ, it can be taken up easily by cell. The use of nitrate generally stimulates the synthesizing of chlorophyll and the vegetable growth of plant. But nitrate needs to be deoxidized into NH_3 firstly before entering the nitrogen metabolism in cell. This step need consume energy. So the plant prefers to ammonia on nitrate^[8]. From the results of experiments, *S. polyrrhiza* employed mainly the ammonia at low level and nitrate did not affect the growth. But increases of chlorophyll content and area of frond with presence of nitrate at high ammonia level suggested that overfull ammonia caused the toxic effect and the cell started to employ the nitrate more. At the same time the NO_3^- possibly competed with NH_4^+ at membrane of cell and abated the absorbing of NH_4^+ .

Ammonia in wastewater can be transformed to nitrate after aerobic biological treatment. Our experimental results suggest that the presence of nitrate can enhance the tolerance of *S. polyrrhiza* to ammonia. So it is helpful to the growth of duckweed if wastewater is treated by aerobic biological process firstly when choosing duckweed-base system for wastewater treatment.

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