Growth and phosphate uptake of microalgae under different phosphate conditions

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Abstract: Microalgae could utilize the nutrients in the environment and therefore are widely used in wastewater treatment. The variety and complexity of phosphates in the water column and different types of wastewater can affect the growth of microalgae, which further affects the treatment efficiency of phosphorus-containing wastewater. In this study, phosphate uptake and microalgal growth were investigated under different phosphate conditions. The results showed that the increase of phosphate concentration could promote the growth of microalgae. Phosphate species affected the phosphate uptake and transformation process. Orthophosphate and polyphosphate were first adsorbed on the surface of microalgae and then transported inside the cell. Polyphosphates produce desorption in the early stages of uptake. The adsorbed polyphosphates were released back into the culture medium and were taken up and utilized again in the later stages of incubation. Polyphosphates were taken up and utilized by microalgae for a longer period of time than orthophosphates. It was also found that orthophosphate was more readily utilized by microalgae compared to polyphosphate. An increase in phosphate concentration promotes the growth of microalgae.

1. Introduction

Microalgae are important target organisms for biotechnology development and are widely used as biofuel feedstocks, food supplements, animal feed, pesticide applications and wastewater treatment. The advantages of microalgae for wastewater treatment are enormous. Microalgae can be successfully integrated with existing wastewater systems; low energy requirements, low cost and high nutrient removal efficiency. Biomass synthesized by microalgae can be used for biofuels [1], fish feed [2] and industrial pigments [3]; certain microalgae have a high tolerance for high nutrient wastewater compared to conventional wastewater treatment techniques [4].

Microalgae cultures are usually categorized into autotrophic, heterotrophic and mixed cultures. Autotrophic culture is a growth mode in which Chlorella utilizes inorganic carbon sources such as HCO_3^- and CO_2 for photosynthesis [5]. Hybrid culture can reduce the dependence of microalgae on a single energy source, and its advantages as a renewable feedstock for biofuels and bioproducts have been widely investigated, showing high photosynthetic efficiency, mitigation of photoinhibition, mitigation of photo-oxidative damage, and tolerance of stress conditions [6, 7].

Currently, phosphorus comes mainly from the mining of phosphate minerals and will be depleted within 100-200 years[8]. The biogeochemical cycling of natural phosphorus is balanced, with phosphorus cycling between the lithosphere and the hydrosphere: bedrock gradually weathering to form soils, which are then transported to rivers and oceans by wind/water erosion, and eventually sinking to the seafloor along with the remains of aquatic organisms to form sedimentary rocks, which eventually rise to the surface due to tectonic uplift [9]. With the development of human technology, mankind is breaking the natural phosphorus cycle, and about 90% of phosphorus ore is used for food production: 82% for fertilizers, 5% for animal feed, and 2-3% for food additives[10]. Increased release of phosphorus-containing wastes into rivers and oceans, resulting in catastrophic problems represented by the phenomenon of eutrophication [11]. Wastewater phosphorus recovery is an effective solution to the dual problems of phosphorus mineral scarcity and water eutrophication. Phosphorus in phosphorus-containing wastewater takes various forms and is mainly influenced by the wastewater treatment process, especially phosphorus removal, and the conditions of the wastewater sludge treatment process. Researchers have developed numerous physical, chemical, and biological techniques to remove and recover phosphorus, mainly chemical precipitation, adsorption, crystallization, ion exchange, biological methods, and combinations of these methods [12]. It is now shown that microalgae cells utilize nutrients (nitrogen, phosphorus, COD and antibiotics, etc.) in the water column during their growth process, which can improve the quality of wastewater discharges, and therefore microalgae have a clear advantage in the treatment of phosphorus-containing wastewater. Microalgae are widely used in wastewater treatment due to their ability to utilize nutrients in the environment. Microalgae cells utilize nutrients in the water column during growth to improve the quality of

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*Corresponding author: cw1041@stu.ouc.edu.cn wastewater discharge, and the longer the cultivation time, the greater the uptake of nutrients by microalgae [13-15].

The uptake of inorganic phosphorus by microalgae is considered to be direct, and phosphorus uptake is mainly accomplished by multiple phosphate transporter proteins in microalgal cell membranes. Phosphate transport was mainly influenced by the form and abundance of the transporter proteins: the rate of transporter proteins was low but the affinity was high in phosphorus-deficient conditions, and the rate of transporter proteins was high but the affinity was low in phosphorus-enriched conditions. P-related metabolism in all microalgal cells begins when phosphate was transported into the cell interior.

Phosphorus in water bodies occurs in a variety of forms, and its quantitative assessment is the key to accurate water ecological restoration. In wastewater and surface water, phosphorus exists in the form of various phosphates, which are mainly divided into inorganic phosphates (e.g., orthophosphates, polyphosphates, metaphosphates, and pyrophosphates) and organic phosphates (e.g., sodium β-glycerophosphate, sodium glucose-6-phosphate, and adenosine triphosphate), and in particular, yellow phosphorus. Since inorganic phosphates are preferentially taken up by Chlorella, there is a need to study the process of uptake of different species of phosphates by Chlorella.

2. Materials and methods

2.1. Chlorella culture

After obtaining Chlorella, the initial expanded culture of Chlorella was carried out to ensure the subsequent experiments. Firstly, f/2 seawater medium was prepared for the growth of Chlorella as shown in Tables 1, Tables 2 and Tables 3. Chlorella was inoculated into the medium on an ultra-clean bench. Finally, it was incubated in a constant temperature light incubator. The conical flasks were shaken three times a day to ensure the uniform suspension and distribution of Chlorella in the culture medium. The optical density of Chlorella was monitored during the incubation process, and after reaching the logarithmic growth period, the culture was re-expanded or experimented.

Table 1 Formula of f/2 medium

substance	concentration (/L)
$NaNO_3$	75 mg
$NaH_2PO_4 \cdot 2H_2O$	5.72 mg
trace element	1 mg/L
vitamins	1 mg/L

Table 2 Biotin formulations for f/2 medium

substance	concentration (/L)
Vitamin B1	0.10 g
Vitamin B12	0.50 mg
biotin	0.50 mg

Table 3 Trace element formulations for f/2 medium

substance	concentration (/L)
$CuSO_4$	6.40 mg
$MnCl_2 \cdot 4H_2O$	178.00 mg
$ZnSO_4 \cdot 7H_2O$	28.30 mg
$Na_2MoO_4 \cdot H_2O$	7.30 mg
$Na_2EDTA \cdot 2H_2O$	4.82 g
$CoCl_2 \cdot 6H_2O$	12.00 mg
$FeC_6H_5O_7 \cdot 5H_2O$	3.90 g

2.2. Microalgae growth

The conical flask was shaken three times a day at regular intervals to reduce the walling and settling of the microalgae for uniform distribution. The homogeneous algal solution was aspirated with a sterilized lance tip and the absorbance OD_{685} of the samples was measured at 685 nm on a UV-visible spectrophotometer.

2.2. Phosphorus concentration of substrate

The extracellular total phosphorus concentration (WP) of Chlorella vulgaris was determined to analyze the phosphorus uptake. After shaking, 10 mL of algal solution was centrifuged at 4500 r/min for 10 min, and the supernatant was taken and filtered with a 0.45 μm filter head, and the external total phosphorus (WP) was digested and analyzed by phosphomolybdate blue spectrophotometry.

2.3. Phosphorus concentration of adsorption

Appropriate amount of the algal solution was taken, the pH was adjusted to 2~3, desorbed for 30 min, and filtered with a 0.45 μm filter tip, and the phosphorus adsorbed on the surface of the algal cells (AP) was determined by phosphomolybdate blue spectrophotometric method by subtracting the phosphate content in the culture medium.

3. Results and discussion

3.1. Absorption of orthophosphate

Fig. 1(a) shows the changes in total phosphorus concentration in the medium under orthophosphate conditions. Orthophosphate concentration decreased rapidly in the pre-culture period and stabilized at 50 min, with the removal rates reaching 79.29%, 58.23%, 36.72%, respectively. Fig.1(b) shows the concentration of phosphate adsorbed on the cell surface. As the concentration of phosphorus in the medium decreased, the concentration of phosphate adsorbed on the cell membrane surface increased. It indicated that the process of orthophosphate utilization by microalgae is divided into two steps. Firstly, orthophosphate was adsorbed to the surface of the cell membrane, and then phosphate on the surface of the cell membrane was transported to the inside of the cell for further metabolism.

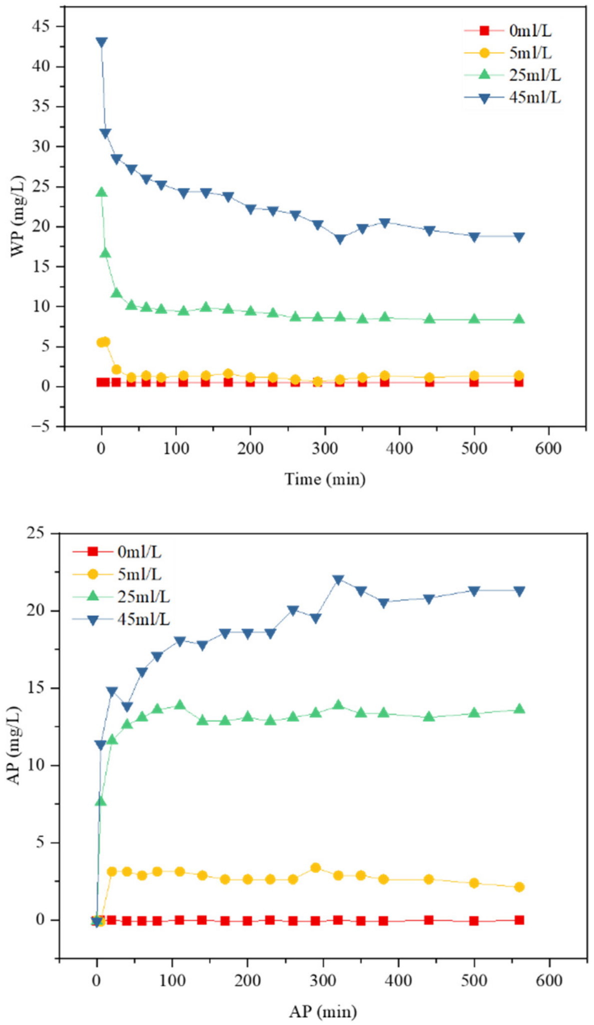

Fig. 1 the changes in total phosphorus concentration in the medium and cell surface under orthophosphate conditions (a: orthophosphate; b: polyphosphate).

3.2. Absorption of polyphosphate

Fig. 2(a) shows the changes in total phosphorus concentration in the medium under polyphosphate conditions. During the first 20 minutes of incubation, the concentration of polyphosphate in the medium decreased rapidly and the removal rates reached 67.93%, 64.65% and 69.10%, respectively. After 20 min, the phosphorus concentrations in the medium started to increase. It was only at the late stage of incubation that a stable decreasing trend was observed. Fig. 2(b) shows the variation of surface adsorbed phosphate concentration under polyphosphate conditions. The surface adsorbed phosphorus concentration firstly reached the maximum at 20 min, with the cocentrations of 0.148 mg/L, 4.890 mg/L and 8.681 mg/L, respectively. Combined with the decreasing phosphate concentration in the medium in Fig. 2(a), it could be known that the phosphate in the medium was transferred to the surface of the cell membrane. This was attributed to the adsorption of polyphosphate by the microalgae. At the end of the incubation period, the concentration of surface-adsorbed phosphorus decreased while the concentration of phosphorus in the medium remained stable, indicating that phosphate on the surface of the microalgae began to be translocated to the interior of the cell.

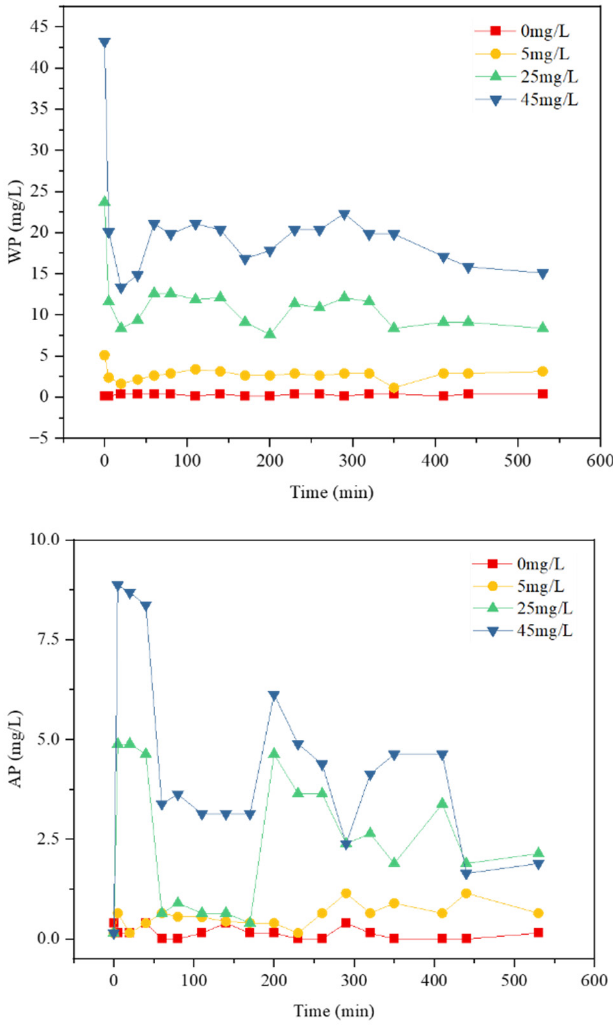

Fig. 2 the changes in total phosphorus concentration in the medium and cell surface under polyphosphate conditions (a: orthophosphate; b: polyphosphate).

3.3. Microalgae growth

Fig.3 shows the growth of microalgae under different phosphate conditions. Fig. 3(a) shows the change in algal density under orthophosphate conditions. The addition of orthophosphate could promote the increase of microalgae algal density. The density of microalgae increased rapidly within 2 hours of incubation, and the growth rate decreased at the later stage. 45 mg/L condition reached the maximum at the later stage of incubation, and the optical density value was 0.355. This indicates that the increase of orthophosphate concentration promotes the growth of microalgae. Figure 3(b) shows the change in algal density under polyphosphate conditions. The change in microalgae density under polyphosphate conditions was similar to that under orthophosphate conditions. Increasing polyphosphate concentration can also increase microalgae density. Notably, the algal density under the polyphosphate condition was less than that of the orthophosphate condition in the later stages. This indicated that orthophosphate was more suitable for microalgae growth. In addition, the maximum algal density under polyphosphate conditions was obtained at a concentration of 25 mg/L, with an algal density value of 0.277.

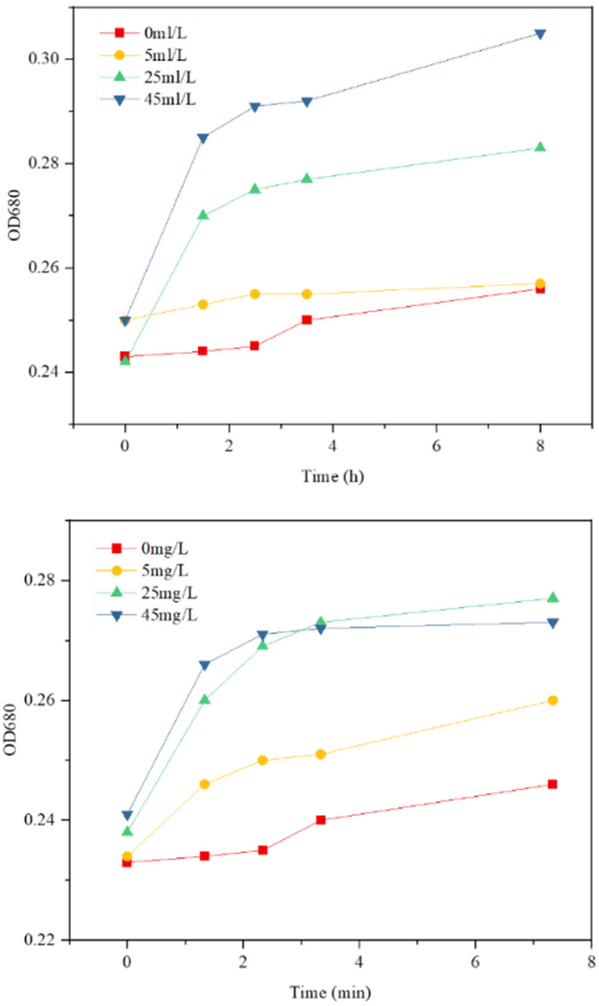

Fig.3 the growth of microalgae under different phosphate conditions (a: orthophosphate; b: polyphosphate).

4. Conclusion

Under different phosphate conditions, microalgae will show different uptake and transformation characteristics. With the addition of orthophosphate, phosphate was adsorbed on the surface of microalgae cells. After a short stay, it entered into the cell interior via transporter proteins on the surface of the algal cells to supply cell growth. Unlike orthophosphate, The phosphorus concentration in the medium decreased first and would increase temporarily after 20 min. This might be due to the polyphosphate being re-released into the medium. Finally, it showed a steady decrease at the late stage of incubation. Phosphate was an important factor affecting the growth of microalgae. Phosphate concentration affects the growth of microalgae. As the orthophosphate concentration increased, the density of microalgae increased.

References

1. Chu, F.F., Chu, P.N., Cai, P.J., Li, W.W., Lam, P.K.S., Zeng, R.J. 2013. Phosphorus plays an important role in enhancing biodiesel productivity of Chlorella vulgaris under nitrogen deficiency. Bioresour. Technol., **134**, 341-346. (2013)
2. Yaakob, M.A., Mohamed, R., Al-Gheethi, A., Gokare, R.A., Ambati, R.R. 2021. Influence of Nitrogen and Phosphorus on Microalgal Growth, Biomass, Lipid, and Fatty Acid Production: An Overview. Cells, **10**(2), 393. (2021)
3. You, X., Yang, L., Zhou, X., Zhang, Y. 2022. Sustainability and carbon neutrality trends for microalgae-based wastewater treatment: A review. Environmental Research, **209**, 112860. (2022)
4. Nagarajan, D., Lee, D.-J., Chen, C.-Y., Chang, J.-S. 2020. Resource recovery from wastewaters using microalgae-based approaches: A circular bioeconomy perspective. Bioresour. Technol., **302**, 122817. (2020)
5. Chiu, S.Y., Kao, C.Y., Chen, T.Y., Chang, Y.B., Kuo, C.M., Lin, C.S. 2015. Cultivation of microalgal Chlorella for biomass and lipid production using wastewater as nutrient resource. Bioresour. Technol., **184**, 179-189. (2015)
6. Lu, Q., Chen, P., Addy, M., Zhang, R.C., Deng, X.Y., Ma, Y.W., Cheng, Y.L., Hussain, F., Chen, C., Liu, Y.H., Ruan, R. 2018. Carbon-dependent alleviation of ammonia toxicity for algae cultivation and associated mechanisms exploration. Bioresour. Technol., **249**, 99-107. (2018)
7. Chojnacka, K., Noworyta, A. 2004. Evaluation of Spirulina sp growth in photoautotrophic, heterotrophic and mixotrophic cultures. Enzyme and Microbial Technology, **34**(5), 461-465. (2004)
8. Cordell, D., Drangert, J.-O., White, S. 2009. The story of phosphorus: Global food security and food for thought. Global Environmental Change, **19**(2), 292-305. (2009)
9. Filippelli, G.M. 2011. Phosphate rock formation and marine phosphorus geochemistry: The deep time perspective. Chemosphere, **84**(6), 759-766. (2011)
10. Cordell, D., White, S. 2014. Life's Bottleneck: Sustaining the World's Phosphorus for a Food Secure Future. Annual Review of Environment and Resources, **39**(1), 161-188. (2014)
11. Daneshgar, S., Callegari, A., Capodaglio, A.G., Vaccari, D. 2018. The Potential Phosphorus Crisis: Resource Conservation and Possible Escape Technologies: A Review. Resources, **7**(2), 37. (2018)
12. Chai, W.S., Tan, W.G., Munawaroh, H.S.H., Gupta, V.K., Ho, S.H., Show, P.L. 2021. Multifaceted roles of microalgae in the application of wastewater biotreatment: A review. Environmental Pollution, **269**, 116236. (2021)

13. Abbas Azeez, R. 2010. A Study on The Effect Of Temperature on The Treatment of Industrial Wastewater Using Chlorella Vulgaris Alga. Engineering and Technology Journal, **28**(4), 785-792. (2010)
14. Rasoul-Amini, S., Montazeri-Najafabady, N., Shaker, S., Safari, A., Kazemi, A., Mousavi, P., Mobasher, M.A., Ghasemi, Y. 2014. Removal of nitrogen and phosphorus from wastewater using microalgae free cells in bath culture system. Biocatalysis and Agricultural Biotechnology, **3**(2), 126-131. (2014)
15. Emparan, Q., Harun, R., Danquah, M.K. 2019. Role of phycoremediation for nutrient removal from wastewaters: a review. Applied Ecology and Environmental Research, **17**(1), 889-915. (2019)