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Allelopathy Inhibitory Effects of *Hydrodictyon reticulatum* on *Chlorella pyrenoidosa* under Co-Culture and Liquor-Cultured Conditions

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Abstract: Eutrophication has become increasingly serious in recent years, which severely impairs the aquatic ecosystem. Applying environmentally-friendly methods to effectively control the growth of algae and avoid eutrophication has been proved to be a promising way. Thus, the potential of *Hydrodictyon reticulatum* on eutrophication control was studied in this research. The allelopathy inhibitory effects of *H. reticulatum* on the growth of *Chlorella pyrenoidosa* were investigated under both co-culture and liquor-cultured conditions. The biomass and chlorophyll *a* content of *C. pyrenoidosa* were determined with time during the experimental period. Nitrogen and phosphorus removal capacities of *H. reticulatum* were also examined. Results showed that the growth of *C. pyrenoidosa* was obviously inhibited under both co-culture and liquor-cultured conditions, and the "Hormesis effect" was patently observed. The strength of allelopathy inhibitory effect depended on the relative biomass between *H. reticulatum* and *C. pyrenoidosa*. The allelopathy inhibitory effect of *H. reticulatum* on *C. pyrenoidosa* under co-culture condition was stronger than that under liquor-cultured condition. The decrease speeds of nitrogen and phosphorus concentrations were in direct proportion to the concentration of *H. reticulatum*.

Keywords: *Hydrodictyon reticulatum*; *Chlorella pyrenoidosa*; allelopathy; removal of nitrogen and phosphorus

1. Introduction

With the rapid development of society and economy, human's irrational production ways have resulted in the enrichment of nutrient salts such as nitrogen (N) and phosphorus (P) in water bodies. This leads to global eutrophication becoming increasingly serious in recent years [1–3]. How to effectively control the growth of algae and avoid eutrophication in water bodies have become hot research issues [4–6]. At present, the comprehensive and in-depth studies to control algae growth are mainly physical and chemical methods [2,7–12]. However, the common shortcomings of these two methods are high cost and short effective time. Thus, they are not applicable for prevention and rehabilitation of eutrophication in large-scale water bodies [13–16]. Biological method is one of the most innovative technologies for environmental restoration in recent years, which has become a

hotspot in the field of environment research [17–22]. Studies have shown that many kinds of aquatic plants have the ability to resist and control eutrophication, such as reed, *Ceratophyllum demersum*, *Ruppia* [4,23–25]. Due to the advantages of high efficiency, low cost, and no secondary pollution, the method of algae control by plant allelopathy is of great significance for preventing and repairing water bodies from eutrophication [24,26–28], and maintaining water stability after restoration.

There were more than 20 species of aquatic plants which had been reported to inhibit algal blooms [4,14,29,30], and most of the studies focused on the allelopathy of aquatic on algae growth [19,31–33], less on the removal of nitrogen and phosphorus from water bodies. However, reducing the concentrations of nutrient elements is the fundamental means to prevent algal blooms [1,2]. If a certain aquatic plant has strong abilities to control algae growth and remove nitrogen and phosphorus, it can play an important role in tackling the problem of eutrophication. Both characteristics present in *Hydrodictyon reticulatum* [14,34]; however, few research has been done on its allelopathy inhibitory effects. *H. reticulatum* is one of common and large alga which can live in a wide range of temperatures. It has strong adaptability and fast reproduction speed. During the growth process, it can absorb a large amount of nutrients, including ammonia nitrogen, nitrate nitrogen and inorganic phosphorus. In addition, it is easy to be harvested and removed from water due to its large algae plant [14]. Meanwhile, *Microcystis* was mainly selected in the studies on allelopathy [27,32,35–38], but as another main algal specie inducing blooms [35,39], *Chlorella* was rarely chosen. *Chlorella* grows in freshwater lakes and ponds, and generally begins to grow and multiply when the water temperature is stable and higher than 20 °C. It has very strong abilities of photosynthesis and reproduction in summer, thus often forming blooms.

Therefore, the objective of this research is to study the allelopathy inhibitory effects of *Hydrodictyon reticulatum* on *Chlorella pyrenoidosa*. The effects of *H. reticulatum* on the growth of *C. pyrenoidosa* will be investigated under both co-culture and liquor-cultured conditions. In detail, the biomass and chlorophyll *a* content of *C. pyrenoidosa* will be determined with time during the experimental period. The concentrations of nitrogen and phosphorus in culture medium will also be monitored to examine the removal efficiencies of nitrogen and phosphorus by *H. reticulatum*. The research results of this study will be expected to provide scientific basis for eutrophication prevention and algal bloom control.

2. Materials and Methods

2.1. Experimental Materials

The *Hydrodictyon reticulatum* (FACHB-856) and *Chlorella pyrenoidosa* (FACHB-1222) used in this study were purchased from the Freshwater Algae Species Pool of Wuhan Aquatic Research Institute (Chinese Academy of Sciences, Wuhan, China). BG11 culture medium was used to cultivate these two alga after high-pressure steam sterilization. Table 1 shows the ingredient of BG11 culture medium, and all the reagents were analytical grade and used as-received. Alga were seeded into 500 mL conical flasks containing 500 mL of newly prepared culture medium, and the tops of conical flasks were covered by gauze to avoid dust falling into and contaminating culture. The whole process was carried out on a clean bench (SW-CJ-2FD, Shanghai Boxun, Shanghai, China), then the flasks were moved to a sterilized illumination incubator (PYX-800G-B, Guangdong Keli, Guangzhou, China). The culture temperature was set as 25 ± 2 °C, and the light/dark ratio was 12 h/12 h with illumination intensity at 3000 lx. The flasks were shaken twice a day to reduce accidental error from orientation, and their positions were changed randomly to ensure that the light radiation for each bottle was consistent. When the algae cell density reaching logarithmic growth phase, they were used for experiment.

Table 1. Ingredient of BG11.

Chemical	Dose
$NaNO_3$	1.5 g
$K_2HPO_4 \cdot H_2O$	0.04 g
$MgSO_4 \cdot 7H_2O$	0.075 g
$CaCl_2 \cdot 2H_2O$	0.036 g
Citric acid	0.006 g
Ferric ammonium citrate	0.006 g
EDTA (dinatrium-salt)	0.001 g
Na_2CO_3	0.02 g
A5 + Co Solution	1 mL
Distilled water	919 mL
Composition of the A5 + Co solution	
Distilled Water H_3BO_3	1000 mL
	2.86 g
$MnCl_2 \cdot H_2O$	1.81 g
$ZnSO_4 \cdot 7H_2O$	0.222 g
$CuSO_4 \cdot 5H_2O$	0.079 g
$Na_2MO_4 \cdot 2H_2O$	0.39 g
$Co(NO_3) \cdot 6H_2O$	0.049 g

2.2. Experimental Methods

The biomass of *C. pyrenoidosa* was presented through measuring the spectroscopic absorbance of algae liquid at 680 nm (OD_{680}) [17]. The concentrations of total nitrogen (TN) and total phosphorus (TP) in culture medium were respectively determined by Total Organic Carbon/Total Nitrogen (TOC/TN) analyzer (TNM-1, Shimadzu, Kyoto, Japan) and 5B-6P TP analyzer after filtered through 0.45 μm microporous filter membrane. The chlorophyll *a* content of *C. pyrenoidosa* was measured following the standard method in water and wastewater monitoring analysis [40]. Ten milliliters of algae liquid were filtered through 0.45 μm microporous membrane, and then the membrane was placed in refrigerator to be frozen 8 h. We then transferred the membrane into mortar, added magnesium carbonate powder and 2–3 mL of 90% acetone, and fully grinded to extract chlorophyll *a*. We transferred all the matter to a centrifuge tube and added 90% acetone to 10 mL, then placed the centrifuge tube in refrigerator to be frozen 6 h to continue extracting chlorophyll *a*. After taking out from the refrigerator, the centrifuge tube was centrifuged for 10 min at 4000 r/min, then the spectroscopic absorbance of liquid supernatant was measured at 630, 645, 663, and 750 nm wavelength. The chlorophyll *a* content can be calculated through following equation:

$$\text{Chlorophyll } a \text{ (mg/m}^3\text{)} = (11.64 \times (OD_{663} - OD_{750}) - 2.16 \times (OD_{645} - OD_{750}) + 0.1 \times (OD_{630} - OD_{750})) \times (V_1/V)$$

where V is the volume of algae liquid (L), V_1 is the volume of extracting solution (mL), and OD is the absorbance.

2.3. Experimental Design

2.3.1. Co-Culture Experiment between *Hydrodictyon reticulatum* and *Chlorella pyrenoidosa*

H. reticulatum and *C. pyrenoidosa* were cultured together in 500 mL conical flasks with 500 mL of BG11 culture medium. Four sets of comparative experiments were set up, and the corresponding concentrations of *H. reticulatum* were 1 g/L, 2 g/L, 3 g/L and 4 g/L, respectively. The initial OD_{680} value of the algae suspension of *C. pyrenoidosa* was about 0.1. A blank test and a controlled trial were also set. The initial OD_{680} value of *C. pyrenoidosa* was also about 0.1 in the blank test without *H. reticulatum*, and the concentration of *H. reticulatum* was 4 g/L without adding *C. pyrenoidosa* in the controlled trial. The OD_{680} value of *C. pyrenoidosa* suspension was measured every 24 h, and the

concentrations of TN and TP in the culture medium were measured every 48 h. Two replicates were set for each experiment group, and each parameter was measured twice.

2.3.2. Liquor-Cultured Experiment of *Chlorella pyrenoidosa*

The growing water of *H. reticulatum* was obtained as follows: 1 g/L of *H. reticulatum* in the logarithmic phase was cultivated in a 500 mL conical flask with 500 mL of fresh culture medium. After culturing for 30 days, the culture medium was filtered through regular filter paper first, and then filtered through 0.45 μm microporous filter membrane to obtain growing water of *H. reticulatum*. Four sets of comparative experiments were set up, and 100 mL, 200 mL, 300 mL and 400 mL of growing water were added to 500 mL conical flasks which contained 100 mL of *C. pyrenoidosa* suspension in the logarithmic phase, respectively. The volume of culture medium in each conical flask was adjusted to 500 mL through adding ultrapure water. Thus, different culture media with 20%, 40%, 60% and 80% of growing water were obtained, respectively. A blank test was set with 100 mL of *C. pyrenoidosa* suspension and 400 mL of ultrapure water, without growing water of *H. reticulatum*. The corresponding doses of nutrients of BG11 culture medium were then added to each flask to make sure that the nutrient levels of all experiment groups and the blank test were the same. The initial OD_{680} values of *C. pyrenoidosa* suspension in all conical flasks were measured as about 0.05. The OD_{680} value was measured every 24 h, and the chlorophyll *a* content of *C. pyrenoidosa* was measured every 48 h. Two replicates were set for each experiment group, and all the parameters were measured twice.

The inhibition rate of *H. reticulatum* on *C. pyrenoidosa* can be calculated as [41]:

$$IR_t = (1 - N_t/N_{0,t}) \times 100\%$$

where t is the experiment time, IR_t is the inhibition rate at day t, N_t is the mean value of OD_{680} of *C. pyrenoidosa* suspension in experimental group at day t, and N_0 is the mean value of OD_{680} of *C. pyrenoidosa* suspension in blank test at day t.

3. Results and Discussion

3.1. The Allelopathy Inhibitory Effects of Hydrodictyon reticulatum on Chlorella pyrenoidosa under Co-Culture Condition

Figures 1 and 2 show the growth curves of *C. pyrenoidosa* and the inhibition rates of *H. reticulatum* on *C. pyrenoidosa* under co-culture condition during the experimental period, respectively. The OD_{680} value of the blank test remains stable increase, and reaches 0.383 after culturing for 10 days. It is indicated that *C. pyrenoidosa* grows steadily without the effects from *H. reticulatum*. In the first seven days, 1 g/L and 2 g/L experimental groups have different degrees of growth promotion, and their OD_{680} values increase faster than that of the blank test. The highest promotion rates reach 57.71% and 38.86% at the 5th day, respectively. From the 8th day, *H. reticulatum* begins to inhibit the growth of *C. pyrenoidosa*. The OD_{680} values start to decrease, and the inhibition rates are 16.45% and 44.39% at the 10th day, respectively. As for the 3 g/L group, the OD_{680} value increases to 0.152 in the first three days, but decreases steadily in the next seven days. At the 10th day, the OD_{680} value is 0.112, which is much lower than that of the blank test, and the inhibition rate is 70.76%. The 4 g/L group shows the strongest inhibitory effect on the growth of *C. pyrenoidosa*. The OD_{680} value starts to decrease rapidly on the 3rd day, and lowers to 0.026 at the 10th day, and the final inhibition rate reaches up to 93.21%.

The results showed that, under co-culture condition, *H. reticulatum* at low concentration promoted the growth of *C. pyrenoidosa* in the first few days, but inhibited its growth at high concentration. However, all concentrations of *H. reticulatum* had inhabitation effects on the *C. pyrenoidosa*'s growth in the end. This indicated that the allelopathy inhibitory effects of *H. reticulatum* on *C. pyrenoidosa* were dependent on their relative biomass. For example, 1 g/L and 2 g/L of *H. reticulatum* promoted the growth of *C. pyrenoidosa* in the first seven days, and their OD_{680} values were higher than that of the blank test, but they started to inhibit the *C. pyrenoidosa*'s growth from the 8th day. After seven

days of growth, the relative biomass of *H. reticulatum* exceeded the threshold of growth promotion for *C. pyrenoidosa*, and then inhibited its growth in the rest of culturing period. The concentrations of *C. pyrenoidosa* in 3 g/L and 4 g/L experimental groups increased slowly in the first three days, then continuously decreased to 0.026 at the 10th day. The OD_{680} values were always lower than that of the blank test, indicating the inhibition effect from *H. reticulatum*. This phenomenon may be attributed to the fact that low concentration of allelochemical secreted from *H. reticulatum* can increase the cell membrane permeability of *C. pyrenoidosa*, and thus facilitate *C. pyrenoidosa* to absorb nutrients from culture medium. However, when the concentration of allelochemical exceeds a certain threshold, the cell membrane of *C. pyrenoidosa* would be destroyed, leading to the death of algae cell [18,39,42].

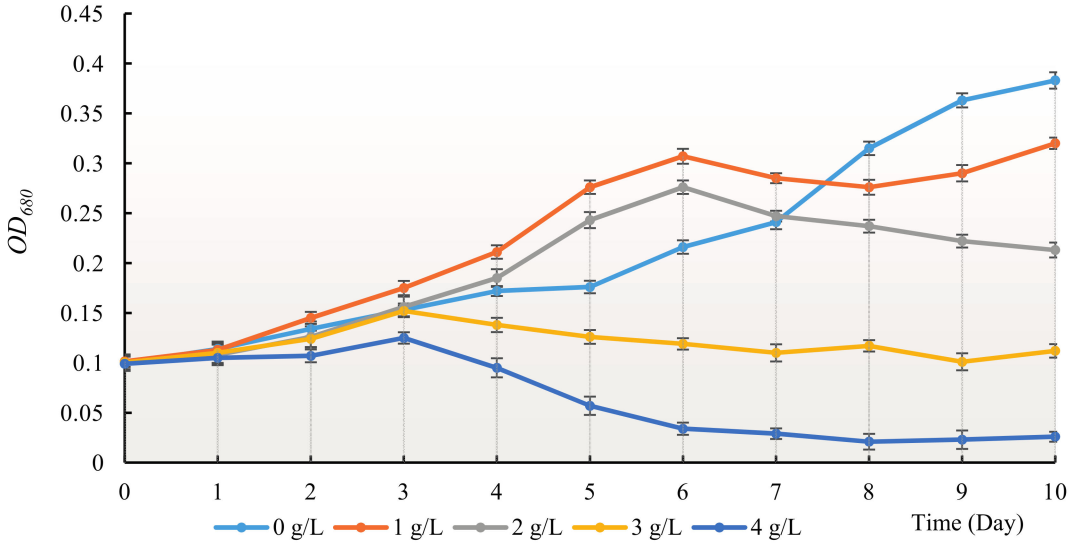


Figure 1. Growth curve of *C. pyrenoidosa* under co-culture condition.

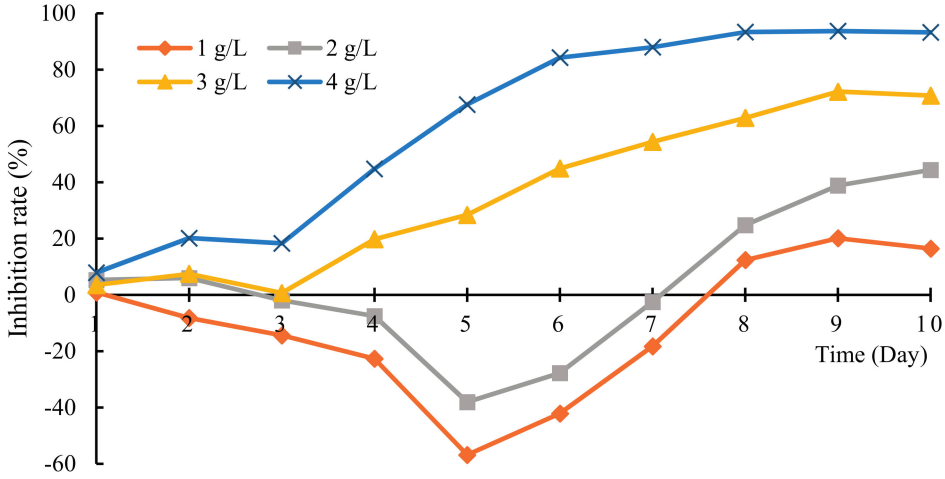


Figure 2. Inhibition rate of *H. reticulatum* on *C. pyrenoidosa* under co-culture condition.

3.2. Effect of H. reticulatum Growing Water on the Growth of C. pyrenoidosa

Figure 3 presents the growth curves of *C. pyrenoidosa* under liquor-cultured condition. *C. pyrenoidosa* in the blank test grow well, and the optical density of algae liquor increases steadily, the OD_{680} value reaching 0.281 after culturing twelve days. As for the four experimental groups, their biomasses of *C. pyrenoidosa* increase in the first two days, and the growth rates were similar. From the

3rd day, *C. pyrenoidosa* in the experimental groups with 20% to 60% of *H. reticulatum* growing water keep increasing, but the growth rate of *C. pyrenoidosa* in the 80% experimental group is significantly lower than that of the other groups. Particularly, the growth rate of *C. pyrenoidosa* in the 20% experimental group rises rapidly and stability in the whole experimental period, and the optical density of its algae liquid remains the largest from the 3rd day

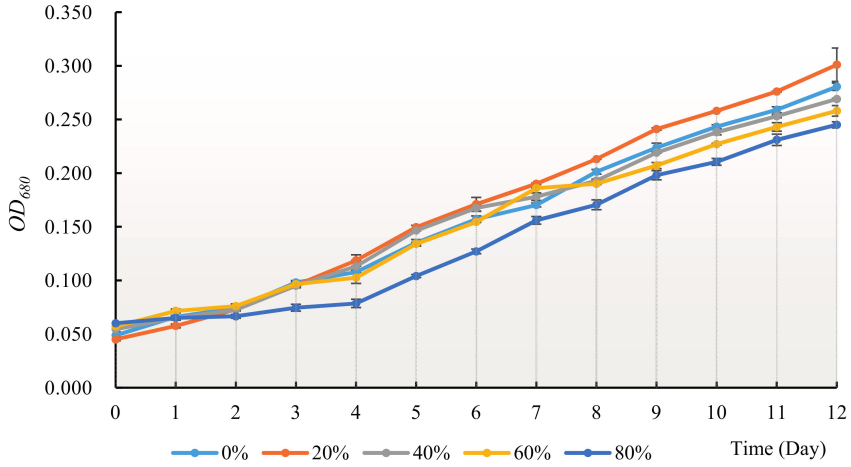


Figure 3. Growth curve of *C. pyrenoidosa* under liquor-cultured condition.

Figure 4 shows the inhibition rates of *C. pyrenoidosa* in different concentrations of *H. reticulatum* growing water. As can be seen, 20% and 40% of concentrations of *H. reticulatum* growing water initially show weak inhibition effect on the growth of *C. pyrenoidosa*. After the 3rd day, the *H. reticulatum* growing water in 20% experimental group has significant and stable promotion effect on the *C. pyrenoidosa*'s growth, and the promotion rate is 7.12% on the 12th day. The growth of *C. pyrenoidosa* is facilitated in the 40% experimental group during the 4th to 7th day, but it is slightly inhibited again from the 8th day. Sixty percent of *H. reticulatum* growing water promotes the *C. pyrenoidosa*'s growth in the first three days, but turns to lightly inhibit its growth from the 4th to 7th day, and the inhibition rate remains stable from the 8th day. Eighty percent of *H. reticulatum* growing water has the most obvious inhibitory effect on the *C. pyrenoidosa*'s growth. The cell concentration of *C. pyrenoidosa* is the lowest during the whole experimental period. The inhibition rate continues to increase rapidly from 1.52% to 27.31% in the first four days, and slowly declines to 11.46% at the 9th day and then remains stable.

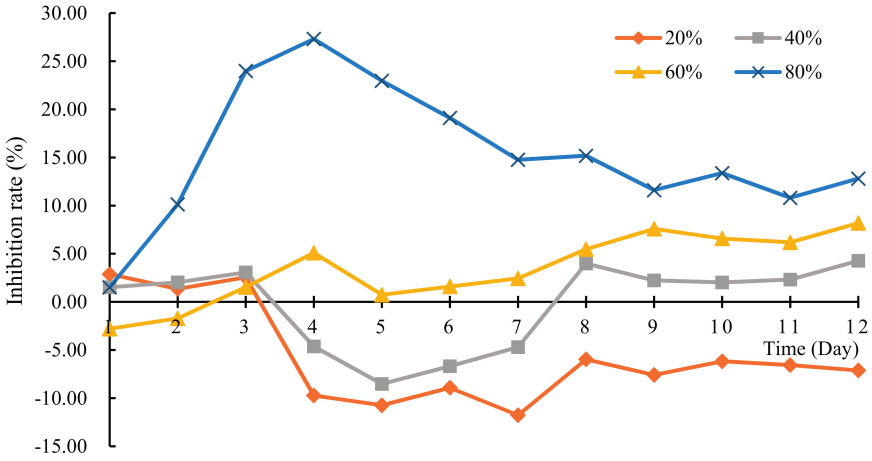


Figure 4. Inhibition rate of *C. pyrenoidosa* under liquor-cultured condition.

The results indicated that the inhibitory effect of *H. reticulatum* growing water on the growth of *C. pyrenoidosa* was different with the concentration of growing water, and the "Hormesis effect" was patently observed [43]. Among the experimental groups, the growth of *C. pyrenoidosa* was slightly promoted at 20% of *H. reticulatum* growing water, but severely inhibited at the 80% experimental group. This proved that *H. reticulatum* growing water contained allelochemical secreted by *H. reticulatum*. The allelochemical would accelerate the growth of *C. pyrenoidosa* if its concentration was lower than a threshold, but would inhibit the growth of *C. pyrenoidosa* if its concentration was higher than the threshold, and then the inhibitory effect would be enhanced with the increase of its concentration.

The chlorophyll *a* content curves with time of *C. pyrenoidosa* under liquor-cultured condition are shown in Figure 5. Chlorophyll *a* is the main photosynthetic pigment in most algae, which can be used to judge the primary productivity of a water body. It is closely related to the growth state and photosynthesis of algae cells, and thus regarded as an important indicator of algae photosynthetic potential in environment monitoring. Compared with the blank test, the chlorophyll *a* content of *C. pyrenoidosa* in the 20% experimental group is higher, which means that the growth of the *C. pyrenoidosa* is promoted by *H. reticulatum* growing water at low concentration. For other experimental groups with higher concentrations of *H. reticulatum* growing water, the chlorophyll *a* contents of *C. pyrenoidosa* decrease after a short period of increasing. That was because the chlorophyll *a* content of *C. pyrenoidosa* was affected by high concentration of *H. reticulatum* growing water, leading to the normal photosynthesis of *C. pyrenoidosa* being inhibited. From the combination of Figures 3 and 5, the chlorophyll *a* content changed with the biomass of *C. pyrenoidosa*, thus their variations were consistent. The decrease of chlorophyll *a* content was due to the decrease of *C. pyrenoidosa* cells. The other reason may be that the allelochemical in *H. reticulatum* growing water inhibited the synthesis of chlorophyll *a* or accelerated the degradation of chlorophyll *a* in *C. pyrenoidosa* cells [14]. This resulted in the decrease of chlorophyll *a* content in *C. pyrenoidosa* cells and thus limited its photosynthetic capacity, which may be another important cause of *C. pyrenoidosa*'s death.

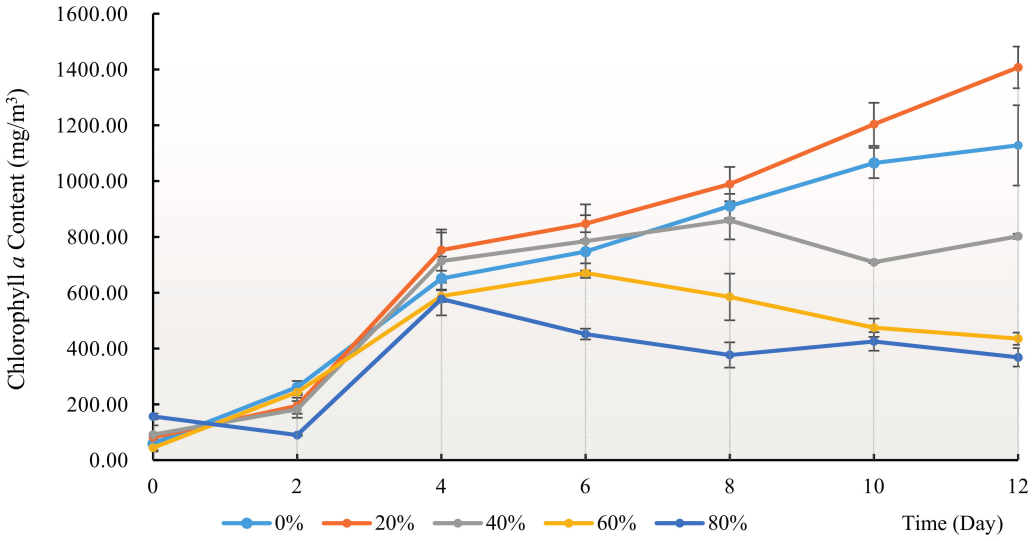
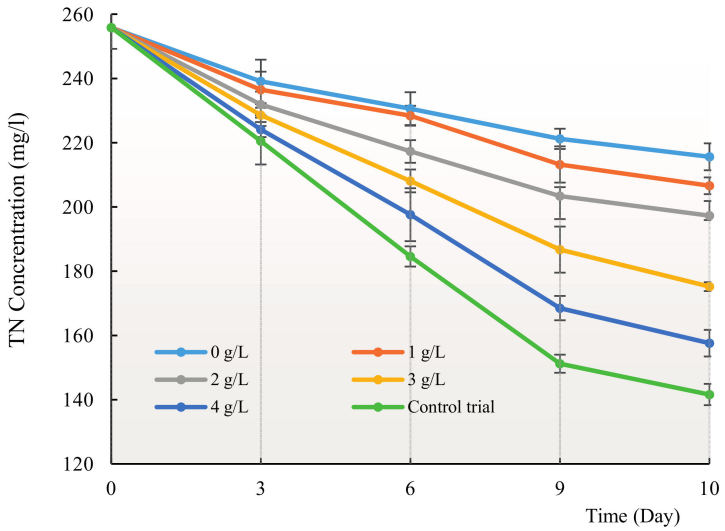


Figure 5. Chlorophyll *a* content of *C. pyrenoidosa* under liquor-cultured condition.

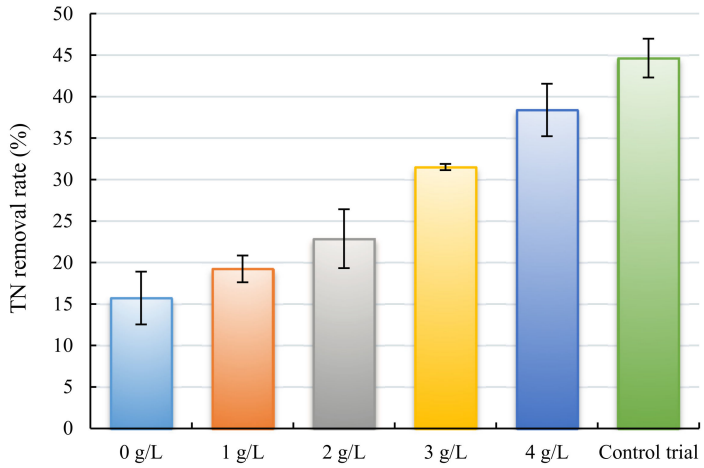
3.3. Removal of Nitrogen and Phosphorus by H. reticulatum under Co-Culture Condition

Figures 6 and 7 summarize the changes of TN and TP concentration under co-culture condition, and the removal rates of TN and TP through *H. reticulatum* under co-culture condition at the 10th day. It can be seen that the concentrations of nitrogen and phosphorus in the culture medium decrease rapidly with time, which indicates that *H. reticulatum* has good abilities to remove nitrogen and phosphorus under co-culture condition. The abilities are enhanced with the increasing concentration

of *H. reticulatum*. The initial concentrations of TN and TP in all groups are about 255.8 mg/L and 5.71 mg/L, respectively. At the end of the experimental period, the concentrations of TN and TP in the 1 g/L experiment group decrease to 206.6 mg/L and 3.28 mg/L, and the removal rates of TN and TP are only 19.23% and 42.56%, respectively. Compared to the 1 g/L experiment group, the removal rates of TN and TP are higher in the other experiment groups. In the 4 g/L experiment group, the concentrations of TN and TP decrease to 157.6 mg/L and 1.13 mg/L, respectively, and the removal rates reach up to 38.4% and 80.2%, respectively. As for the blank test which only contains *C. pyrenoidosa*, the removal abilities of nitrogen and phosphorus are the weakest. The removal rates of TN and TP are even lower than that of the 1 g/L experiment group, only 15.72% and 27.14% at the 10th day, respectively. Conversely, in the control trial with 4 g/L of *H. reticulatum* and without *C. pyrenoidosa*, the removal rates of TN and TP are highest among all groups and up to 44.64% and 86.86%, respectively. According to the obtained results, it has been indicated that the abilities of *H. reticulatum* to remove nitrogen and phosphorus were stronger than that of *C. pyrenoidosa*, which may be an important reason leading to the death of *C. pyrenoidosa*.

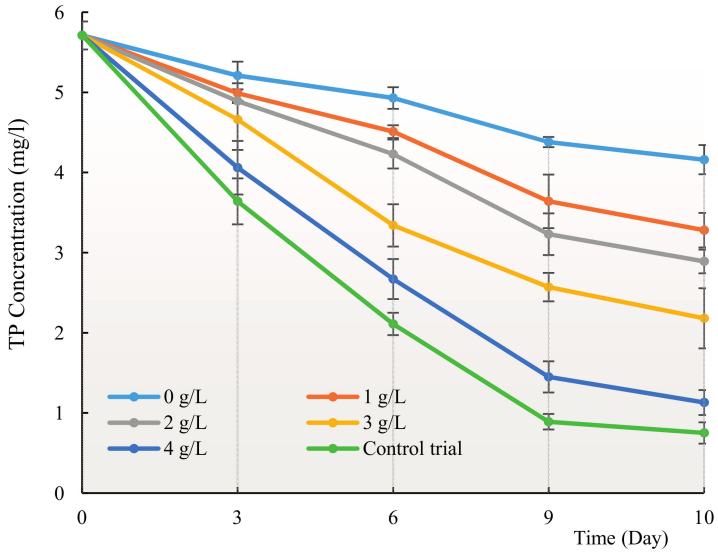


(**a**)



(**b**)

Figure 6. Change curve of TN concentration under co-culture condition and TN removal rate at the 10th day. (**a**) TN concentration; (**b**) TN removal rate.



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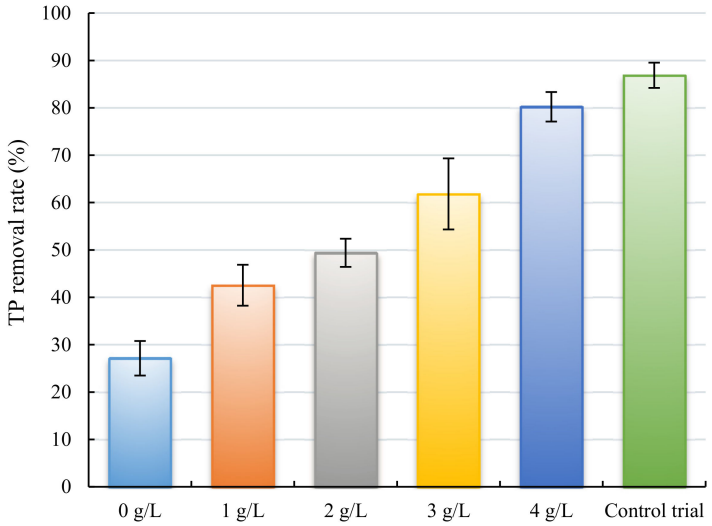

(**b**)

Figure 7. Change curve of TP concentration under co-culture condition and TP removal rate at the 10th day. (**a**) TP concentration; (**b**) TP removal rate.

4. Conclusions

The allelopathy effect of *H. reticulatum* on *C. pyrenoidosa* was studied under both co-culture and liquor-cultured conditions. The obtained results showed that *H. reticulatum* had allelopathy inhibitory effect on the growth of *C. pyrenoidosa*. The extent of allelopathy effect depended on the relative biomass between *H. reticulatum* and *C. pyrenoidosa*, resulting in the observed "Hormesis effect". In the co-cultured condition, *H. reticulatum* at low concentration promoted the growth of *C. pyrenoidosa* in the first few days, but inhibited its growth at high concentration. In the liquor-cultured condition, the growth of *C pyrenoidosa* was facilitated at 20% of *H. reticulatum* growing water, but it was inhibited at 40–80% of *H. reticulatum* growing water. Comparing the death situations of *C. pyrenoidosa* cells under the two conditions, the allelopathy inhibitory effect of *H. reticulatum* on *C. pyrenoidosa* under co-culture condition was stronger than that of under liquor-cultured condition. The concentrations of

TN and TP in culture medium were monitored during the experimental period. Results showed that *H. reticulatum* had strong abilities to remove nitrogen and phosphorus from water body. The removal rates of nitrogen and phosphorus in culture medium increased rapidly with the increase of its biomass. Nevertheless, the specific mechanism of allelopathy in this study is unclear, which can be explored through the extraction and identification of allelochemical in the future research.

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Author Contributions: Xiujuan Chen and Haiyan Fu conceived and designed the experiments; Xiujuan Chen performed the experiments; Xiujuan Chen and Yao Yao analyzed the data; Chunjiang An contributed reagents and materials, Guanhui Cheng and Meiqin Suo offered analysis tools and guides; Xiujuan Chen wrote the paper; Guohe Huang supervised all the authors and helped to revise the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Oliveira, M.; Machado, A. The role of phosphorus on eutrophication: A historical review and future perspectives. *Environ. Technol. Rev.* **2013**, *2*, 117–127. [CrossRef]
2. Schoumans, O.; Chardon, W.; Bechmann, M.; Gascuel-Odoux, C.; Hofman, G.; Kronvang, B.; Rubæk, G.H.; Ulén, B.; Dorioz, J.-M. Mitigation options to reduce phosphorus losses from the agricultural sector and improve surface water quality: A review. *Sci. Total Environ.* **2014**, *468*, 1255–1266. [CrossRef] [PubMed]
3. Zhang, D.; Men, L.; Chen, Q. Ocean Observation with Opto-Microfluidic Devices. *J. Environ. Inform.* **2016**, *28*, 120–125.
4. Hu, H.; Hong, Y. Algal-bloom control by allelopathy of aquatic macrophytes—A review. *Front. Environ. Sci. Eng.* **2008**, *2*, 421–438. [CrossRef]
5. Molot, L.A. The effectiveness of cyanobacteria nitrogen fixation: Review of bench top and pilot scale nitrogen removal studies and implications for nitrogen removal programs. *Environ. Rev.* **2017**, *999*, 1–4. [CrossRef]
6. Graneli, E.; Weberg, M.; Salomon, P.S. Harmful algal blooms of allelopathic microalgal species: The role of eutrophication. *Harmful Algae* **2008**, *8*, 94–102. [CrossRef]
7. An, C.; McBean, E.; Huang, G.; Yao, Y.; Zhang, P.; Chen, X.; Li, Y. Multi-Soil-Layering Systems for Wastewater Treatment in Small and Remote Communities. *J. Environ. Inform.* **2016**, *27*, 27. [CrossRef]
8. Zhang, M.; Fu, H.; Tian, C.; Bin, Y.; Chen, X. A pilot-scale study on continuous self-cleaning microalgae-collection equipment. *J. Environ. Eng.* **2011**, *5*, 2018–2022.
9. Wang, C.; Ao, Y.; Wang, P.; Hou, J.; Qian, J.; Zhang, S. Preparation, characterization, photocatalytic properties of titania hollow sphere doped with cerium. *J. Hazard. Mater.* **2010**, *178*, 517–521. [CrossRef] [PubMed]
10. Wang, C.; Ao, Y.; Wang, P.; Hou, J.; Qian, J. Preparation of cerium and nitrogen co-doped titania hollow spheres with enhanced visible light photocatalytic performance. *Powder Technol.* **2011**, *210*, 203–207. [CrossRef]
11. Wang, P.; Ao, Y.; Wang, C.; Hou, J.; Qian, J. Enhanced photoelectrocatalytic activity for dye degradation by graphene-titania composite film electrodes. *J. Hazard. Mater.* **2012**, *223*, 79–83. [CrossRef] [PubMed]
12. Wang, P.; Ao, Y.; Wang, C.; Hou, J.; Qian, J. A one-pot method for the preparation of graphene–Bi_2MoO_6 hybrid photocatalysts that are responsive to visible-light and have excellent photocatalytic activity in the degradation of organic pollutants. *Carbon* **2012**, *50*, 5256–5264. [CrossRef]
13. Nematian, J. An extended two-stage stochastic programming approach for water resources management under uncertainty. *J. Environ. Inf.* **2016**, *27*, 27. [CrossRef]
14. Zhao, K.; Fu, H.; Chai, T.; Zhang, M.; Liu, Z.; Chen, X.; Hou, M.; Xu, P. Allelopathy of *Hydrodictyon reticulatum* on *Microcystis aeruginosa* and its removal capacity on nitrogen and phosphorus. *Huan Jing Ke Xue* **2011**, *32*, 2267–2272. (In Chinese) [PubMed]
15. Zhao, S.; Huang, G.; Fu, H.; Wang, Y. Enhanced coagulation/flocculation by combining diatomite with synthetic polymers for oily wastewater treatment. *Sep. Sci. Technol.* **2014**, *49*, 999–1007. [CrossRef]

16. Wang, P.; Cao, M.; Ao, Y.; Wang, C.; Hou, J.; Qian, J. Investigation on Ce-doped TiO_2-coated BDD composite electrode with high photoelectrocatalytic activity under visible light irradiation. *Electrochem. Commun.* **2011**, *13*, 1423–1426. [CrossRef]
17. Guo, P.; Liu, Y.; Liu, C. Effects of chitosan, gallic acid, and algicide on the physiological and biochemical properties of Microcystis flos-aquae. *Environ. Sci. Pollut. Res. Int.* **2015**, *22*, 13514–13521. [CrossRef] [PubMed]
18. Huang, H.; Xiao, X.; Lin, F.; Grossart, H.-P.; Nie, Z.; Sun, L.; Xu, C.; Shi, J. Continuous-release beads of natural allelochemicals for the long-term control of cyanobacterial growth: Preparation, release dynamics and inhibitory effects. *Water Res.* **2016**, *95*, 113–123. [CrossRef] [PubMed]
19. Tang, Y.Z.; Gobler, C.J. The green macroalga, Ulva lactuca, inhibits the growth of seven common harmful algal bloom species via allelopathy. *Harmful Algae* **2011**, *10*, 480–488. [CrossRef]
20. Wang, C.; Lu, G.; Peifang, W.; Wu, H.; Qi, P.; Liang, Y. Assessment of environmental pollution of Taihu Lake by combining active biomonitoring and integrated biomarker response. *Environ. Sci. Technol.* **2011**, *45*, 3746–3752. [CrossRef] [PubMed]
21. Wang, C.; Zhang, S.H.; Wang, P.F.; Hou, J.; Zhang, W.J.; Li, W.; Lin, Z.P. The effect of excess Zn on mineral nutrition and antioxidative response in rapeseed seedlings. *Chemosphere* **2009**, *75*, 1468–1476. [CrossRef] [PubMed]
22. Wang, P.; Zhang, S.; Wang, C.; Lu, J. Effects of Pb on the oxidative stress and antioxidant response in a Pb bioaccumulator plant Vallisneria natans. *Ecotoxicol. Environ. Saf.* **2012**, *78*, 28–34. [CrossRef] [PubMed]
23. Dong, J.; Yang, K.; Li, S.; Li, G.; Song, L. Submerged vegetation removal promotes shift of dominant phytoplankton functional groups in a eutrophic lake. *J. Environ. Sci.* **2014**, *26*, 1699–1707. [CrossRef] [PubMed]
24. Gross, E.M.; Hilt, S.; Lombardo, P.; Mulderij, G. Searching for allelopathic effects of submerged macrophytes on phytoplankton—State of the art and open questions. *Hydrobiologia* **2007**, *584*, 77–88. [CrossRef]
25. Hilt, S. Allelopathic inhibition of epiphytes by submerged macrophytes. *Aquat. Bot.* **2006**, *85*, 252–256. [CrossRef]
26. Do Carmo Bittencourt-Oliveira, M.; Chia, M.A.; de Oliveira, H.S.B.; Araújo, M.K.C.; Molica, R.J.R.; Dias, C.T.S. Allelopathic interactions between microcystin-producing and non-microcystin-producing cyanobacteria and green microalgae: Implications for microcystins production. *J. Appl. Phycol.* **2015**, *27*, 275–284. [CrossRef]
27. Meng, P.; Pei, H.; Hu, W.; Liu, Z.; Li, X.; Xu, H. Allelopathic effects of *Ailanthus altissima* extracts on *Microcystis aeruginosa* growth, physiological changes and microcystins release. *Chemosphere* **2015**, *141*, 219–226. [CrossRef] [PubMed]
28. Rice, E.L. *Allelopathy*; Harcourt Brace Jovanovich, Academic Press, INC.: Cambridge, MA, USA, 2012.
29. Wang, C.; Zhang, S.H.; Wang, P.F.; Hou, J.; Li, W.; Zhang, W.J. Metabolic adaptations to ammonia-induced oxidative stress in leaves of the submerged macrophyte Vallisneria natans (Lour.) Hara. *Aquat. Toxicol.* **2008**, *87*, 88–98. [CrossRef] [PubMed]
30. Wang, P.; Yin, Y.; Guo, Y.; Wang, C. Removal of chlorpyrifos from waste water by wheat straw-derived biochar synthesized through oxygen-limited method. *RSC Adv.* **2015**, *5*, 72572–72578. [CrossRef]
31. Graneli, E.; Hansen, P.J. Allelopathy in harmful algae: A mechanism to compete for resources? In *Ecology of Harmful Algae*; Springer: Berlin/Heidelberg, Germany, 2006; pp. 189–201.
32. He, Y.; Zhou, Q.-H.; Liu, B.-Y.; Cheng, L.; Tian, Y.; Zhang, Y.-Y.; Wu, Z.-B. Programmed cell death in the cyanobacterium *Microcystis aeruginosa* induced by allelopathic effect of submerged macrophyte *Myriophyllum spicatum* in co-culture system. *J. Appl. Phycol.* **2016**, *28*, 2805–2814. [CrossRef]
33. Rzymski, P.; Poniedziałek, B.; Kokociński, M.; Jurczak, T.; Lipski, D.; Wiktorowicz, K. Interspecific allelopathy in cyanobacteria: Cylindrospermopsin and *Cylindrospermopsis raciborskii* effect on the growth and metabolism of *Microcystis aeruginosa*. *Harmful Algae* **2014**, *35*, 1–8. [CrossRef]
34. Gao, Y.; Yan, X. Response of *Chara globularis* and *Hydrodictyon reticulatum* to lead pollution: Their survival, bioaccumulation, and defense. *J. Appl. Phycol.* **2012**, *24*, 245–251. [CrossRef]
35. Ma, Z.; Fang, T.; Thring, R.W.; Li, Y.; Yu, H.; Zhou, Q.; Zhao, M. Toxic and non-toxic strains of *Microcystis aeruginosa* induce temperature dependent allelopathy toward growth and photosynthesis of *Chlorella vulgaris*. *Harmful Algae* **2015**, *48*, 21–29. [CrossRef]

36. Ni, L.; Jie, X.; Wang, P.; Li, S.; Wang, G.; Li, Y.; Li, Y.; Acharya, K. Effect of linoleic acid sustained-release microspheres on *Microcystis aeruginosa* antioxidant enzymes activity and microcystins production and release. *Chemosphere* **2015**, *121*, 110–116. [CrossRef] [PubMed]
37. Zhang, S.; Zhang, B.; Xing, K.; Zhang, X.; Tian, X.; Dai, W. Inhibitory effects of golden thread (*Coptis chinensis*) and berberine on *Microcystis aeruginosa*. *Water Sci. Technol.* **2010**, *61*, 763–769. [CrossRef] [PubMed]
38. Zhang, W.; Jeppesen, E.; Wang, M.; Xu, X.; Wang, L. Allelopathic effect boosts *Chrysosporum ovalisporum* dominance in summer at the expense of *Microcystis panniformis* in a shallow coastal water body. *Environ. Sci. Pollut. Res.* **2017**, *24*, 4666–4675. [CrossRef] [PubMed]
39. Qian, H.; Xu, X.; Chen, W.; Jiang, H.; Jin, Y.; Liu, W.; Fu, Z. Allelochemical stress causes oxidative damage and inhibition of photosynthesis in *Chlorella vulgaris*. *Chemosphere* **2009**, *75*, 368–375. [CrossRef] [PubMed]
40. State Environmental Protection Administration. *Water and Wastewater Monitoring and Analysis Methods*, 4th ed.; China Environmental Science Press: Beijing, China, 2002; pp. 244–248.
41. Li, F.; Hu, H.; Men, Y.; Hong, Y.; Guo, M. Effects of allelochemicals on the activity of antioxidant enzymes in *Chlorella vulgaris*. *Environ. Sci.* **2006**, *27*, 89–92.
42. Allen, R.D. Dissection of oxidative stress tolerance using transgenic plants. *Plant Physiol.* **1995**, *107*, 1049–1054. [CrossRef] [PubMed]
43. Qiu, H.; Geng, J.; Ren, H.; Xia, X.; Wang, X.; Yu, Y. Physiological and biochemical responses of *Microcystis aeruginosa* to glyphosate and its Roundup® formulation. *J. Hazard. Mater.* **2013**, *248*, 172–176. [CrossRef] [PubMed]