Pol. J. Environ. Stud. Vol. 31, No. 2 (2022), 1355-1362
DOI: *10.15244/pjoes/141816* ONLINE PUBLICATION DATE: 2022-01-18

Original Research

Effects of Different Conditions on the Growth of *Microcystis aeruginosa*

Meichen Wang[1,2], Weihua Peng[2,3], Herong Gui[2]*, Jun Li[1,2], Hao Yu[2]

[1]School of Resources and Environmental Engineering, Hefei University of Technology, Hefei, China
[2]National Engineering Research Center of Coal Mine Water Hazard Controlling (Suzhou University), Suzhou, China
[3]School of Resources and Civil Engineering, Suzhou University, Suzhou, China

Received: 25 May 2021
Accepted: 30 August 2021

Abstract

Microcystis aeruginosa is a common microalgae in nature. It is also the dominant algae species to form freshwater blooms. Water bloom will do harm to water environment, so this paper studies the growth law of *Microcystis aeruginosa*. Single factor experiment and orthogonal experiment were carried out by changing the influencing factors such as photoperiod, initial dosage and initial pH. The results showed that within a certain range, the larger the photoperiod, the larger the initial dosage, the higher the initial pH, the better the growth of *Microcystis aeruginosa* and the greater the increase of pH value. This study is to deepen the understanding of the growth law of *Microcystis aeruginosa*. It provides a scientific basis for controlling the growth of *Microcystis aeruginosa*.

Keywords: initial dosage, initial pH, *Microcystis aeruginosa*, microalgae growth, photoperiod

Introduction

Water eutrophication is becoming increasingly serious, which has become a global water pollution problem. Eutrophication of water will lead to algae blooms and red tides, among which Microcystis aeruginosa is the dominant algae species for freshwater blooms. To study the factors affecting the growth of *Microcystis aeruginosa* and find out the influence of each factor on the growth of microalgae. It is of great significance for controlling microalgae growth and reducing water eutrophication.

In recent years, many scholars have conducted in-depth research on the effects of different conditions on the growth of microalgae. Yuan et al. [1] analyzed the biofilm structure formed by microalgae under different illumination conditions and found that the interaction between cells has an impact on the biofilm structure. Kong et al. [2] took *Chlorella* as the research object. It was found that the higher CO_2 concentration in a certain range, the greater the maximum specific growth rate of microalgae, and the better the removal effect of nitrogen and phosphorus in wastewater. This study provides a scientific basis for the simultaneous realization of biomass growth, CO_2 fixation and nutrient removal. Swati et al. [3] used *Chlorella* to treat comprehensive wastewater under different photoperiod conditions. It is found that when the photoperiod is 24:0, the cost is the lowest and the growth rate of organisms is the highest. Christos et al. [4] studied the response of microalgae biomass yield, composition and growth rate to light conditions. It is considered that there are obvious differences among light-treated microalgae with

e-mail: guiherong@163.com

different colors. The research of Li et al. [5] shows that light quality and culture temperature have interactive effects on the protein and carbohydrate content of microalgae; Gao et al. [6] confirmed that microalgae are beneficial to lipid accumulation in microalgae when the carbon-nitrogen ratio is 110-130; Kristian et al. [7] think that the distribution of fatty acids in microalgae will change under different temperature, light and nutrition conditions.

Up to now, a large number of studies mainly focus on the influence of a single experiment, and there are few comprehensive studies on the factors affecting the growth of microalgae. *Microcystis aeruginosa* has good adaptability to the environment, which can be used as an indicator organism of environmental toxicology and an important indicator of environmental quality monitoring and evaluation [8]. In this paper, *Microcystis aeruginosa* was taken as the research object, and the light period, initial dosage, and initial pH were comprehensively studied. To find out the influence of different factors on the growth of *Microcystis aeruginosa*, and provide scientific basis for controlling the growth of *Microcystis aeruginosa* and restraining the outbreak of water bloom.

Materials and Methods

Microalgae Sources

Microcystis aeruginosa used in the experiment was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences, and cultured in a constant temperature incubator with BG11 medium.

Experimental Design

1) Single-factor experiment: only one factor is changed, and other factors remain unchanged, and the influence of this factor on the experimental results is observed.

a) Photoperiod: centrifuge the algae solution of Microcystis aeruginosa, discard the supernatant and keep the algae, clean the algae three times with sterile ultrapure water and transfer it to the experimental solution (initial pH = 7). Adjust the initial OD_{680} of each experimental group to 0.15, and set the illumination period of each experimental group to be 16:8, 14:10, 12:12, 10:14, 8:16 respectively.
b) Initial dosage: The inoculation procedure is the same as above. In the experimental group, the initial OD_{680} was 0.05, 0.13, 0.21, 0.27, 0.32. The temperature was 25ºC. The illumination was 3500 Lx. The photoperiod was 12:12.
c) Initial pH: The inoculation procedure is the same as above. The initial pH of the experimental group was adjusted to 5, 6, 7, 8 and 9 respectively. The temperature was 25ºC. The illumination was 3500 Lx. The photoperiod was 12:12.

Table 1. Three factors and three levels orthogonal experimental table.

	Photoperiod (A)	Initial Dosage (B)	Initial pH (C)
1	12:12	0.22	7
2	12:12	0.27	8
3	12:12	0.32	9
4	10:14	0.22	8
5	10:14	0.27	9
6	10:14	0.32	7
7	8:16	0.22	9
8	8:16	0.27	7
9	8:16	0.32	8

2) Orthogonal experiment: It is a scientific method to design an experimental scheme by using the orthogonal table, and can effectively analyze experimental results and put forward optimal process conditions [9].

On the premise of achieving the experimental purpose, in order to shorten the experimental period and reduce the experimental cost, orthogonal experiment was proposed to study the influencing factors of heavy metal removal by microalgae, and the results of single factor experiment were tested. The orthogonal test in this study is a three-factor and three-level test. These three factors are photoperiod (A), initial dose (B) and initial pH (C). According to the results of single factor test, the level of each factor in orthogonal test was determined. The orthogonal experiment table is designed by SPSS software, as shown in Table 1.

All the above operations were carried out in sterile environment, and all experimental groups were set in parallel. Sample and test each experimental group at a fixed time point every day.

Index Determination

1) OD_{680}

Using a visible spectrophotometer at 680 nm wavelength, taking ultrapure water as a reference, the absorbance of fresh algae solution was measured, which is the optical density (OD) of algae cells.

2) Chlorophyll

Taking 20 mL algae solution, centrifuge to remove supernatant, and collect algae. Washing with ultrapure water twice, freezing and thawing in a refrigerator at -20ºC for more than 6 times, adding 20 mL 90% acetone, and extracting in the dark for more than 24 hours. After centrifugation, the supernatant was collected, and the absorbance of the supernatant at 630 nm, 647nm, 664 nm and 750 nm was measured by visible spectrophotometer with 90% acetone as reference. And calculate chlorophyll concentration (mg/L) according to the following formula:

$$C_{chla} = [11.85\times(A_{664nm}-A_{750nm})-1.54\times(A_{647nm}-A_{750nm})$$

$$-0.08\times(A_{630nm}-A_{750nm})]\times\frac{V_2}{V_1}$$

In which V_1 is the volume of algae solution to be measured; V_2 is the volume of acetone added.

3) pH

Take appropriate amount of algae solution to be tested at fixed time every day. Centrifuge and stand, pour out the supernatant slowly, and record the supernatant by PHS-3E.

Results and Discussion

The Influence of Photoperiod

Different photoperiods have different effects on the growth of *Microcystis aeruginosa*, and the effects of photoperiod on OD_{680} and chlorophyll during the growth of *Microcystis aeruginosa* are shown in Fig. 1. It can be seen from the figure that under different photoperiods, both OD_{680} and chlorophyll increase with the increase of culture time. OD_{680} and chlorophyll are directly proportional to photoperiod, and the larger the photoperiod, the greater the increase of OD_{680} and chlorophyll.

In Fig. 1b), it can be seen from the slope of the broken line that the increase of chlorophyll presents two different laws. Chlorophyll in the experimental group with a longer illumination time (≥12h) increased greatly in the early stage of the experiment, but decreased in the later stage of the experiment. However, the experimental group with a shorter illumination time (<12 h) had a lower growth rate in the early stage of the experiment, but a higher growth rate in the later stage of the experiment. For the experimental group with a long illumination time, algae can quickly absorb nutrients in the environment for chlorophyll synthesis in the early stage of the experiment [10]. In the later stage of the experiment, the rate of chlorophyll synthesis decreased due to the limitation of nutrients. For the experimental group with short illumination time, the chlorophyll synthesis rate of algae is low due to the limitation of illumination time in the early stage of the experiment. However, in the later stage of the experiment, the chlorophyll synthesis rate increased rapidly. On the one hand, microalgae adapt to shorter illumination time, on the other hand, microalgae accumulate enough nutrients in the early growth process [11].

As shown in Fig. 2, it can be seen that the pH value of the experimental group with different photoperiods gradually increases with the increase of culture time. And the greater the photoperiod, the greater the pH value increases, which has a stable trend in the later stage of the experiment, which is consistent with the results of Han et al. [12]. In a certain range, the longer the illumination time, the better the growth of microalgae. The stronger photosynthesis, the consumption of a large amount of CO_2 destroys the circulation of inorganic carbon in water environment and increases the pH value [13]. In the later stage of the experiment, due to the limited photosynthesis of microalgae, the pH growth rate decreased and gradually stabilized [14].

Influence of Initial Dosage

Different initial dosage will have different effects on the growth of *Microcystis aeruginosa*. The effects of initial dosage on OD_{680} and chlorophyll during the growth of *Microcystis aeruginosa* are shown in Fig. 3. It can be seen from the figure that the curves of culture time, OD_{680} and chlorophyll are different under different initial dose conditions, but both have the same trend and increase with the increase of culture time.

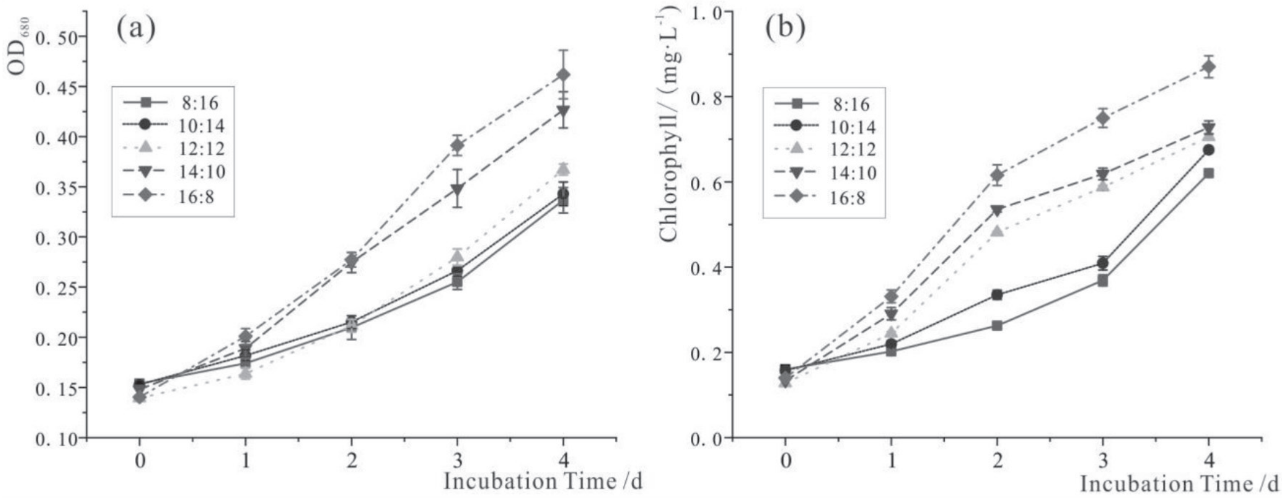

Fig. 1. Growth of *Microcystis aeruginosa* under different light and dark ratios a) OD_{680}; b) Chlorophyll.

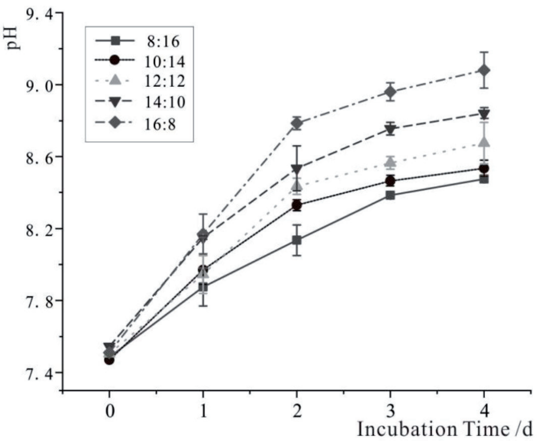

Fig. 2. Changes in pH of water treated with *Microcystis aeruginosa* under different light and dark ratios.

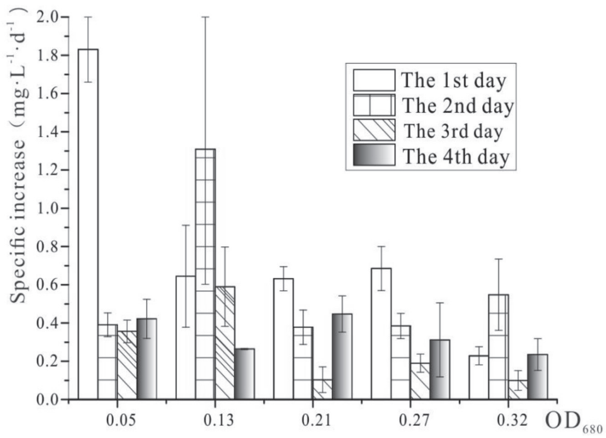

Fig. 4. The increment of chlorophyll ratio of *Microcystis aeruginosa* under different initial dosage conditions.

It can be seen from Fig. 3 that the more the initial dosage of *Microcystis aeruginosa*, the more the increase of OD_{680} and chlorophyll. Because chlorophyll is proportional to the number of microalgae to a certain extent. The experimental group with more initial dosage has more microalgae proliferation and growth, which can produce more chlorophyll.

The specific increment is the ratio of increment to initial increment, which can reflect the increment of unit original microalgae under different conditions. It can be seen from Fig. 4 that the specific increase of chlorophyll is inversely proportional to the initial dosage of microalgae, that is, the more the initial dosage of microalgae, the less the specific increase of chlorophyll. The analysis showed that microalgae appeared a competitive relationship to a certain extent in the experimental group with more initial dosage, which inhibited the synthesis and accumulation of chlorophyll [15].

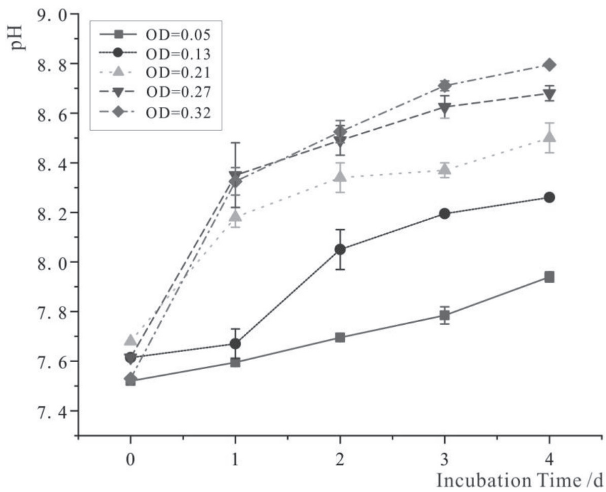

Fig. 5. Changes of pH of water treated with *Microcystis aeruginosa* under different initial dosing conditions.

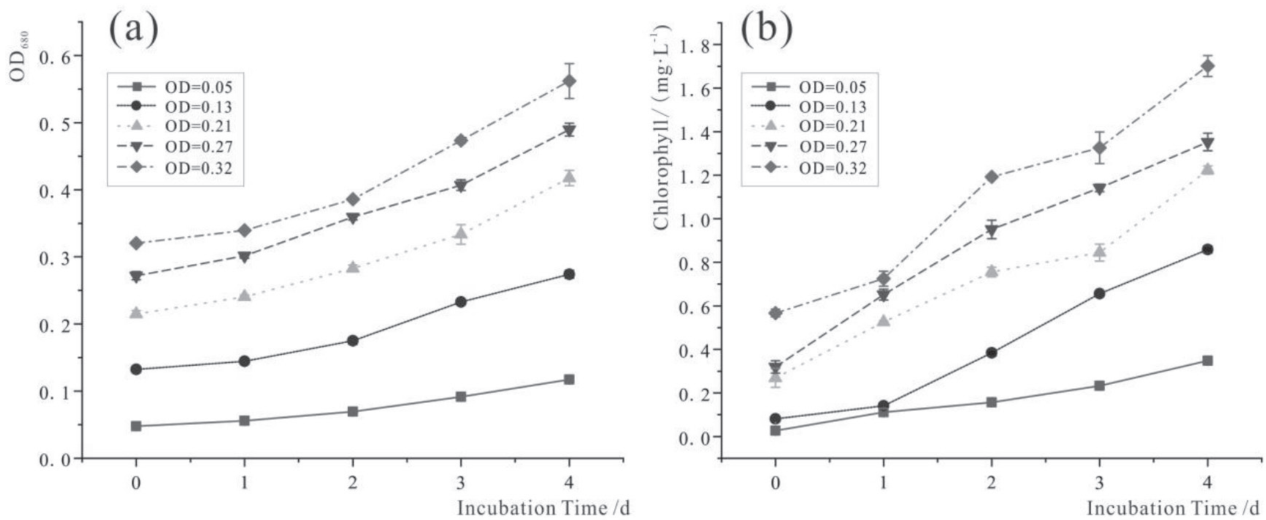

Fig. 3. Growth of *Microcystis aeruginosa* under different initial dosages a) OD_{680}; b) Chlorophyll.

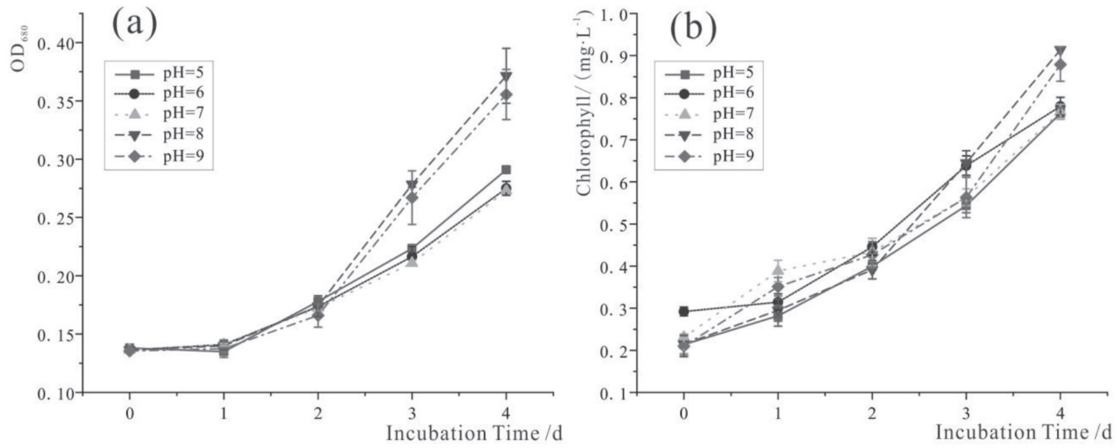

Fig. 6. Growth of *Microcystis aeruginosa* under different initial pH conditions a) OD_{680}; b) Chlorophyll.

The relationship between water pH and treatment time under different initial dosages is shown in Fig. 5. The pH increased with the increase of treatment time, but the experimental groups with different initial dosages showed different growth rules. The experimental group with more initial dosage (OD_{680} = 0.21, 0.27, 0.32) increased rapidly in the early stage of the experiment but decreased in the later stage of the experiment. In addition, the pH of experimental groups with OD_{680} = 0.27 and 0.32 tended to be saturated and stable gradually. The pH value of the experimental group with low initial dosage (OD_{680} = 0.05) always maintained a uniform growth rate, while the experimental group with OD_{680} = 0.13 showed a short-term rapid increase in the middle of the experiment.

Effect of Initial pH

Different initial pH will have different effects on the growth of *Microcystis aeruginosa*. The effects of initial pH on OD_{680} and chlorophyll during the growth of *Microcystis aeruginosa* are shown in Fig. 6. It can be seen from the figure that under different pH conditions, both OD_{680} and chlorophyll increase with the increase of culture time, and the increase of OD_{680} and chlorophyll is related to the initial pH value. The growth of *Microcystis aeruginosa* in alkaline initial environment was larger than that in neutral and acidic initial environment.

It can be seen from Fig. 6 that OD_{680} of experimental groups with different initial pH increased slowly in the early stage of the experiment. In the later stage of the experiment, all experimental groups increased, but the growth rate of alkaline experimental group was obviously higher than that of neutral and acidic experimental groups. When *Microcystis aeruginosa* enters a new environment with different pH values, it takes some time for algae cells to adapt, so the growth rate in the early stage of the experiment is slow. When microalgae adapt to the new environment, the OD_{680} of all experimental groups increases rapidly.

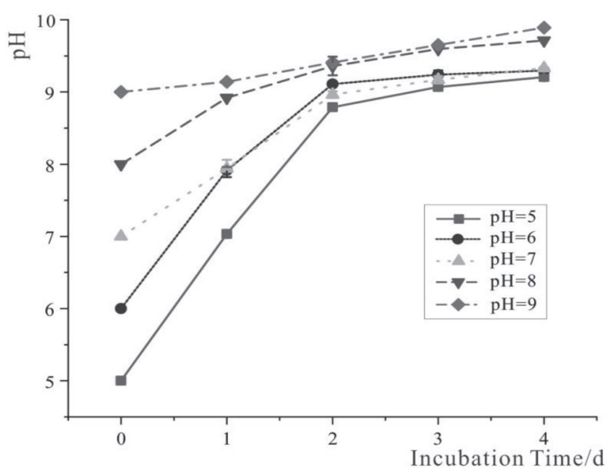

Fig. 7. Changes of pH of water treated with *Microcystis aeruginosa* under different initial pH conditions.

Table 2. The result of orthogonal experiment.

	Factor			Index	
Number	A	B	C	ΔOD_{680}	ΔC_{Chla}
1	1	1	1	0.133	0.614
2	1	2	2	0.156	0.696
3	1	3	3	0.188	0.899
4	2	1	2	0.114	0.439
5	2	2	3	0.148	0.692
6	2	3	1	0.157	0.739
7	3	1	3	0.103	0.391
8	3	2	1	0.120	0.541
9	3	3	2	0.143	0.791

Table 3. Range analysis of growth index in orthogonal experiment.

Index		A	B	C
ΔOD_{680}	K_1	0.476	0.350	0.409
	K_2	0.419	0.424	0.413
	K_3	0.366	0.487	0.439
	k_1	0.159	0.117	0.136
	k_2	0.140	0.141	0.138
	k_3	0.122	0.162	0.146
	R	0.110	0.138	0.030
Primary and secondary order		B>A>C		
ΔC_{Chla}	K_1	2.210	1.445	1.894
	K_2	1.870	1.929	1.926
	K_3	1.723	2.429	1.982
	k_1	0.737	0.482	0.631
	k_2	0.623	0.643	0.642
	k_3	0.574	0.810	0.661
	R	0.486	0.984	0.088
Primary and secondary order		B>A>C		

The growth rate of alkaline experimental group (pH = 8,9) is obviously higher than that of neutral and acidic experimental groups (pH = 5,6,7), so *Microcystis aeruginosa* may be more suitable for alkaline environment [16]. *Microcystis aeruginosa* can grow in a wide pH range but can obtain larger biomass in an alkaline environment, which is consistent with the research results of Khalil et al. [17].

As shown in Fig. 7, it can be seen that the pH values of the experimental groups with different initial pH values gradually increase with the increase of treatment time, and gradually tend to be stable. This is consistent with the previous research results. In the later stage of the experiment, the experimental groups with different initial pH values tended to be stable near pH = 9.5, which reflected the algae solution's good adjustment ability to pH. In the experimental group with acidic and neutral initial environment, the pH increased rapidly in the early stage of the experiment, and kept rising after rising to 8.8 the next day, but the growth rate decreased rapidly and gradually stabilized. In the experimental group in alkaline initial environment, the pH value always kept rising slowly and finally stabilized.

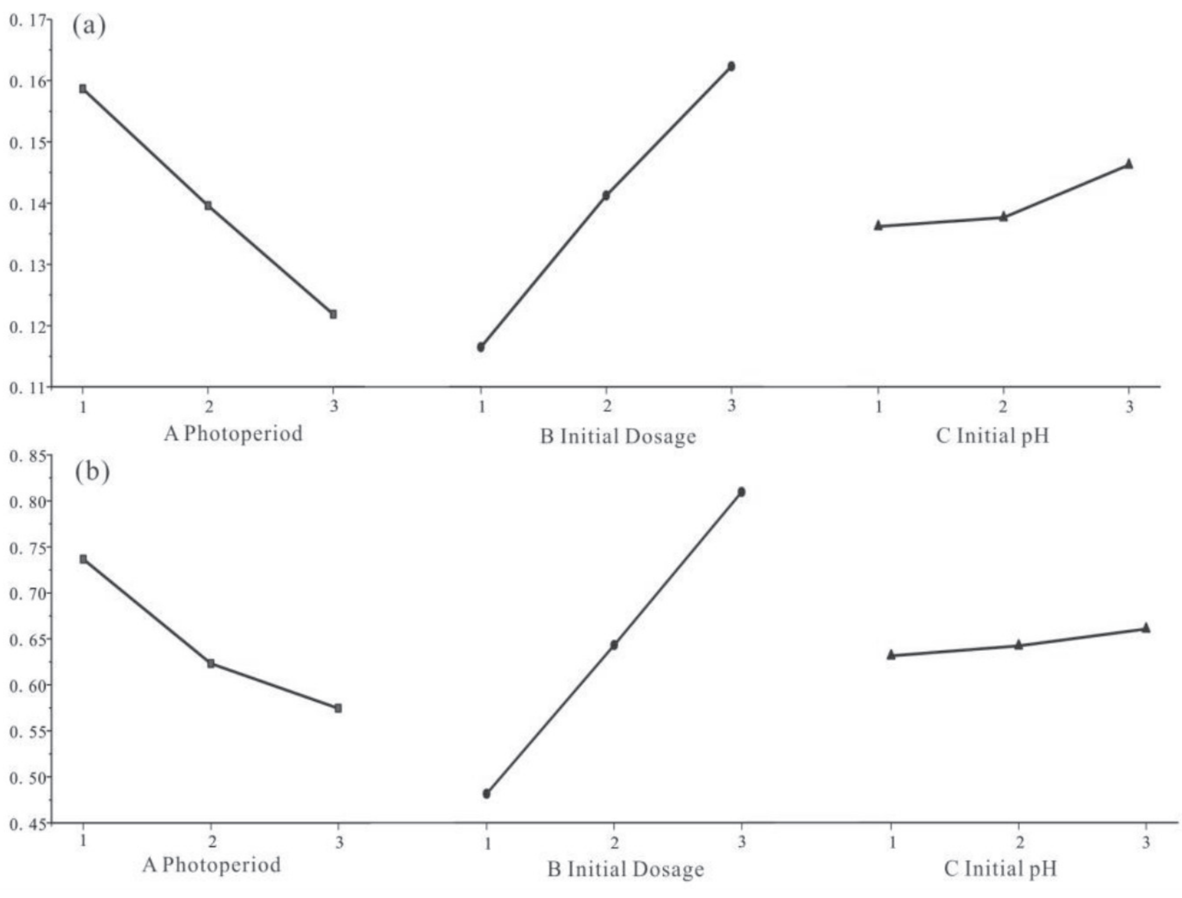

Fig. 8. Horizontal trend chart of growth index factors in orthogonal experiment a) OD_{680}; b) Chlorophyll.

The Influence of Multi-Factor Orthogonality

Many domestic and foreign scholars have carried out a lot of research by orthogonal experiments [18-20]. Through orthogonal experiments, Wang et al. [20] found that soluble inorganic nitrogen is the key factor affecting the growth, accumulation and transformation of As (III) of Microcystis aeruginosa. According to the selected key environmental factors, it is helpful to guide the practical application of algae bioremediation of As polluted water. According to the results of single-factor experiment, the orthogonal experiment was designed by SPSS software, as shown in Table 1.

The results of orthogonal experiments are shown in Table 2. In order to eliminate the influence of different initial dosages on the experimental results, ΔOD_{680} and ΔC_{Chla} were used to analyze the orthogonal experimental results. According to the data in Table 2, the orthogonal conditions and growth indexes (ΔOD_{680}, ΔC_{Chla}) were analyzed. This study mainly uses range analysis, and the analysis results are shown in Table 3.

The magnitude of the range can reflect the importance of each factor to the index, and the greater the range, the more important the factor is [21]. It can be seen from Table 3 that $R_B>R_A>R_C$, so the primary and secondary order of the three factors affecting microalgae growth is: initial dosage >photoperiod >initial pH. The analysis shows that the group with more initial dosage is still within the environmental tolerance in a short time, and there is no obvious competition and inhibition phenomenon. Therefore, the higher the initial dosage, the better the growth of microalgae.

Fig. 8 is taken from experimental factors and levels and k_1, k_2 and k_3. The effects of various factors and levels on ΔOD_{680} and ΔC_{Chla} [22]. It can be seen from the figure that ΔOD_{680} and ΔC_{Chla} have the same change trend under the influence of three factors. The shorter the photoperiod, the less the increase of OD_{680} and chlorophyll. The more the initial dosage, the greater the increase of OD_{680} and chlorophyll. The larger the initial pH value, the greater the increase of OD_{680} and chlorophyll. The orthogonal experiment showed that the effects of photoperiod, initial dosage, and initial pH on the increase of OD_{680} and chlorophyll were consistent with those obtained by single factor experiment.

From the point of view of *Microcystis aeruginosa* growth, the greater the increase of OD_{680} and chlorophyll, the better *Microcystis aeruginosa* growth. Therefore, the optimal combination of OD_{680} and chlorophyll is $A_1B_3C_3$, that is, the photoperiod is 16:8, the initial OD_{680} is 0.32, and the initial pH is 9.

Conclusions

1) In a certain range, the greater the illumination period and the longer the illumination time, the greater the speed and increase of pH, which is beneficial to the growth of *Microcystis aeruginosa*, that is, the greater the OD_{680} and chlorophyll value of *Microcystis aeruginosa*.

2) In a certain range, the more the initial dosage, the better the growth of *Microcystis aeruginosa*, but there may be a certain competitive relationship between *Microcystis aeruginosa*.

3) In a certain range, the growth of *Microcystis aeruginosa* becomes better with the increase of initial pH value, and the pH increases with the increase of culture time and is stable at pH = 10.

4) The order of factors affecting *Microcystis aeruginosa* growth is initial dosage >photoperiod >initial pH, aiming at increasing microalgae growth. The optimal experimental conditions are as follows: photoperiod ratio is 16:8, initial OD_{680} is 0.32, and initial pH is 9.

Acknowledgments

We acknowledge supports from National Natural Science Foundation of China (41773100), Funding projects for research activities of academic and technological leaders of Anhui Province (2020D239) and Anhui Provincial Natural Science Foundation of China (2008085MD122).

Conflict of Interest

The authors declare no conflict of interest.

References

1. HAO Y., YI W., ZL B. Analyzing Microalgal Biofilm Structures Formed Under Different Light Conditions by Evaluating Cell–Cell Interactions. Journal of Colloid and Interface Science. **583**, 563, **2020**.
2. WENEN K., JIA K., JIAO M. Chlorella vulgaris cultivation in simulated wastewater for the biomass production, nutrients removal and CO_2 fixation simultaneously. Journal of Environmental Management. **284**, 112070, **2021**.
3. RANI S., OJHA C. Chlorella sorokiniana for integrated wastewater treatment, biomass accumulation and value-added product estimation under varying photoperiod regimes: A comparative study. Journal of Water Process Engineering. **39**, 101889, **2021**.
4. LATSOS C., HOUCKE J.V., BLOMMAERT L. Effect of light quality and quantity on productivity and phycoerythrin concentration in the cryptophyte Rhodomonas sp. Journal of Applied Phycology. **1**, 13, **2021**.
5. LI X., MANUEL J., SLAVENS S. Interactive effects of light quality and culturing temperature on algal cell size, biomass doubling time, protein content, and carbohydrate content. Applied Microbiology and Biotechnology. **105**, 587, **2021**.

6. GAO B., WANG F., HUANG L. Biomass, lipid accumulation kinetics, and the transcriptome of heterotrophic oleaginous microalga Tetradesmus bernardii under different carbon and nitrogen sources. Biotechnology for Biofuels. **14** (1), 1, **2021**.
7. SPILLING K., SEPPÄLÄ J., SCHWENK D. Variation in the fatty acid profiles of two cold water diatoms grown under different temperature, light, and nutrient regimes. Journal of Applied Phycology. **1**, 9, **2021**.
8. BARINOVA S., TAVASSI M., GLASSMAN H. Algal indication of pollution in the Lower Jordan River, Israel. Applied Ecology and Environmental Research. **8** (1), 19, **2010**.
9. CHOUDHURY S.R., CHAKRABORTY R. Intensified wheat husk conversion employing energy-efficient hybrid electromagnetic radiations for production of fermentable sugar: process optimization and life cycle assessment. Environmental Science and Pollution Research. **1**, 13, **2021**.
10. CSAB C., TC A. Single-phase and two-phase cultivations using different light regimes to improve production of valuable substances in the anoxygenic photosynthetic bacterium Rhodopseudomonas faecalis PA2. Bioresource Technology. 124855, **2021**.
11. EN-JUNG H., WATERS B.M. Alkaline stress and iron deficiency regulate iron uptake and riboflavin synthesis gene expression differently in root and leaf tissue: implications for iron deficiency chlorosis. Journal of Experimental Botany. **67** (19), 5671, **2016**.
12. HAN F., HUANG J., LI Y. Enhanced lipid productivity of Chlorella pyrenoidosa through the culture strategy of semi-continuous cultivation with nitrogen limitation and pH control by CO_2. Bioresource Technology. **136** (0), 418, **2013**.
13. TORRES-FRANCO A.F., ZULUAGA M., HERNÁNDEZ-ROLDÁN D. Assessment of the performance of an anoxic-aerobic microalgal-bacterial system treating digestate. Chemosphere. **270**, 129437, **2021**.
14. HUANG W., JIN Q., YIN L. Responses of CO_2-concentrating mechanisms and photosynthetic characteristics in aquatic plant Ottelia alismoides following cadmium stress under low CO_2. Ecotoxicology and Environmental Safety. **202**, 110955, **2020**.
15. ZHI G., TONG Y.W. The interactions between Chlorella vulgaris and algal symbiotic bacteria under photoautotrophic and photoheterotrophic conditions. Journal of Applied Phycology. **26** (3), 1483, **2014**.
16. NOURI H., ROUSHANDEH J.M., HALLAJISANI A. The effects of glucose, nitrate, and pH on cultivation of Chlorella sp. Microalgae. Global Journal of Environmental Science and Management. **7** (1), 103, **2021**.
17. KHALIL Z.I., ASKER M.M.S., EL-SAYED S. Effect of pH on growth and biochemical responses of Dunaliella bardawil and Chlorella ellipsoidea. World Journal of Microbiology and Biotechnology. **26** (7), 1225, **2010**.
18. YD A., MJ A., ST B. Impact of rice straw biochar addition on the sorption and leaching of phenylurea herbicides in saturated sand column. Science of The Total Environment. **769**, 144536, **2021**.
19. GU W., ZHAO Y., LI Q. Plant-microorganism combined remediation of polychlorinated naphthalenes contaminated soils based on molecular directed transformation and Taguchi experimental design-assisted dynamics simulation. Journal of Hazardous Materials. 122753, **2020**.
20. WANG Z., LUO Z., YAN C. Impacts of environmental factors on arsenate biotransformation and release in, Microcystis aeruginosa, using the Taguchi experimental design approach. Water Research. **118**, 167, **2017**.
21. MOTA T.R., DMD OLIVEIRA, SIMISTER R. Design of experiments driven optimization of alkaline pretreatment and saccharification for sugarcane bagasse. Bioresource Technology. **321**, 124499, **2021**.
22. WANG Z., LUO Z., YAN C. Biokinetics of arsenate accumulation and release in Microcystis aeruginosa regulated by common environmental factors: Practical implications for enhanced bioremediation. Journal of Cleaner Production. **199**, 112, **2018**.