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RESEARCH ARTICLE

Effects of multiple environmental factors on the growth and extracellular organic matter production of *Microcystis aeruginosa*: a central composite design response surface model

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Abstract
In this study, statistically designed experiments using response surface methodology were conducted on *Microcystis aeruginosa*. A central composite design response surface model was established to investigate the multiple effects of various physical and chemical factors (total nitrogen, total phosphorus, temperature, and light intensity) on algal density and extracellular organic matter. The results of the experiments reveal that nitrate and phosphate had significant interactive effects on algal density, both iron and light intensity had synergic effects on the production of microcystins (MC-LR) and extracellular polysaccharides (EPS), and light intensity and nitrite had clear interactive effects on EPS release. Results did not show significant interactive effects on extracellular dissolved organic carbon (DOC) production. The contribution of extracellular dissolved organic matter of *Microcystis aeruginosa* during the logarithmic phase was further identified using a three-dimensional excitation emission matrix (3-DEEM). This study contributes to our theoretical knowledge of the prediction and analysis of *M. aeruginosa* growth and extracellular organic matter production.

Keywords Algal growth · Extracellular polysaccharides · Nitrogen · Phosphorus · Light intensity · Excitation emission matrix

Introduction

Microcystis aeruginosa is one of the most harmful freshwater bloom-forming cyanobacteria (Black et al. 2011; Yang et al. 2013). In addition to the growth of algal biomass, the dissolved organic matter released from *M. aeruginosa* considerably contributes to water degradation and interferes with the drinking water treatment processes (Henderson et al. 2008). Microcystins and extracellular polysaccharides (EPS) are two significant types of extracellular organic matter (EOM) that influence the buoyancy regulation, anti-predator defense behavior, and colony formation of *M. aeruginosa* (Brookes and Ganf 2001; Guo and Xie 2006; Li et al. 2013; Ma et al. 2015). These factors may contribute to the dominance of bloom-forming *M. aeruginosa* in freshwater lakes. Thus, controlling EOM, as well as the algal density of *M. aeruginosa*, will allow the effective prevention and control of harmful algal blooms.

Algal growth and extracellular organic matter release into water bodies are complex processes characterized by a large number of interior physical, chemical, and biological mechanisms, and impacted by a series of exterior environmental factors. Light intensity, temperature, nitrogen, phosphorus, and iron have been identified as the primary environmental factors affecting the growth of *M. aeruginosa* and EOM production in laboratory batch or continuous cultures (Fujii et al. 2015; Graham et al. 2004; Li et al. 2013; Vézie et al. 2002). However, previous studies have only explored single or coupled effects of these factors on EOM production. For example, Zhang et al. (2016) investigated the effects of phosphate loading on the generation of *M. aeruginosa* EOM and its derived disinfection by-products. Dotson and Westerhoff (2009) studied the effects of nitrogen concentration on algal-releasing dissolved organic matter. Biermann et al. (2014) evaluated the combined effects of light intensity and temperature on organic matter production during a phytoplankton bloom. Yang et al. (2012) studied the combined effects of temperature, light intensity, and nitrogen concentration on the growth and EPS content of

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M. aeruginosa in a batch culture. Li et al. (2009) evaluated the effect of iron content on the growth and microcystin production of *M. aeruginosa*. However, the interactive effects of multiple environmental factors have been ignored by previous studies. Although these factors exist individually, they occur simultaneously; thus, knowledge of their interactive effects on the growth of *M. aeruginosa* and EOM production will provide further understanding of the formation of harmful algal blooms in natural freshwater bodies. For this reason, we investigate the synergic effects of all influential factors to determine the major factors that affect algal growth and EOM production.

Statistically based experimental design has proved to be more efficient than individual evaluation of the different factors (Liu et al. 2003; Soumya et al. 2016; Wen and Chen 2001). As a unique type of statistically based experimental design, central composite design (CCD) is one of the most commonly used response surface methodologies (RSM). Jiang et al. (2008) used CCD to study the effects of environmental factors on the growth and microcystin production of *M. aeruginosa*. Wang et al. (2016) presented a new approach to quantify the nutrient threshold of harmful algal blooms using CCD. Nevertheless, no previous studies have attempted to apply CCD to investigate the simultaneous interaction effects of these environmental factors on the production of extracellular organic matter. The aim of this study is to evaluate the interactive influences of various environmental factors on the algal growth and extracellular organic matter release from *M. aeruginosa* using a second-order polynomial regression model established by CCD. Furthermore, differences between the major compositions of extracellular dissolved organic matter of *M. aeruginosa* during the logarithmic phase were identified using fluorescence excitation–emission matrix (EEM) spectroscopy.

Materials and methods

Cyanobacteria cultivation

The cyanobacteria *M. aeruginosa* (M.A., FACHB-912), which was previously isolated from algal blooms in Taihu Lake, China, was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences (FACHB, Wuhan City, China), and cultured in a BG11 medium (Rippka et al. 1979). Cultures were grown in 1000-mL Erlenmeyer flasks (250 mL medium in each) under controlled conditions with a light intensity of 4500 lx (12-h light/12-h dark cycle), a temperature of 28 ± 2 °C, and a relative humidity of 70–90%. During the experimental period, the cultures were homogenized using magnetic stirrers.

Determination of algal density and EOM production

Algal density analysis

Algal cell density of *M. aeruginosa* was performed using a Sedgwick–Rafter counting chamber under a microscopic magnification of ×200–400. Each sample was measured at least three times, with a maximum deviation of approximately 20% (Li et al. 2015).

DOC analysis

Dissolved organic carbon (DOC) can be regarded as the most comprehensive measurement used to quantify the EOM content of an aquatic system (Leenheer and Croué 2003). DOC concentrations of the *M. aeruginosa*-EOM were analyzed using a total organic carbon analyzer (TOC-L CPH, Shimadzu, Japan) as the difference between total carbon and inorganic carbon. The relative deviation of DOC analysis was less than 2%, and each sample was analyzed in triplicate.

MC-LR analysis

MC-LR is the most toxic and frequently occurring isoform of microcystin (Máthé et al. 2007). A 30 mL cultural broth was collected and centrifugated at 3000 rpm for 20 min, and the supernatant was collected for analysis using a MC-LR enzyme-linked immunosorbent assay kit (GRBio, China) following the instructions supplied by the manufacturer. All treatments were analyzed in triplicate.

EPS analysis

In this study, EPS represents bond EPS (b-EPS) and soluble EPS (s-EPS). The EPS content was calculated as the sum of the concentration of b-EPS and s-EPS. The concentration of the two forms of EPS was analyzed using the method of Xu et al. (2013b). Each sample was measured three times.

Fluorescence measurement

Fluorescence EEM spectra were obtained using a fluorescence spectrophotometer (Hitachi, Tokyo, Japan; light source xenon lamp; voltage = 650 V; excitation 200–400 nm; emission 250–600 nm; slit width 10.0 nm; scanning interval 1.0 nm; scan speed 1200 m/s). Data were expressed as isoline maps (SigmaPlot, Systat Software, San Jose, CA, USA).

Table 1 Central composition design of variables (in coded levels) with algal density, DOC, MC-LR, and EPS content as the responses

Run	Variables					Response			
	T	PPFD	TN	TP	Fe	Cell density (10^6 cells/mL)	DOC (mg/L)	MC-LR (μg/L)	EPS (mg/L)
1	−2	0	0	0	0	0.45	3.68	2.10	1.83
2	−1	−1	1	1	1	1.35	4.60	2.58	1.69
3	−1	−1	1	1	−1	1.16	4.35	2.08	1.81
4	−1	−1	1	−1	1	1.05	4.39	3.77	2.01
5	−1	1	1	1	−1	2.43	5.71	3.86	2.17
6	−1	1	1	−1	1	0.85	4.41	5.87	2.17
7	−1	−1	−1	−1	1	1.02	4.29	1.88	2.37
8	−1	−1	−1	−1	−1	0.80	4.61	1.50	2.41
9	−1	1	1	1	1	2.18	5.44	5.58	2.52
10	−1	−1	1	−1	−1	1.38	4.52	2.18	2.61
11	−1	1	1	−1	−1	1.91	5.19	2.42	2.71
12	−1	−1	−1	1	−1	0.93	4.76	1.59	2.83
13	−1	−1	−1	1	1	0.87	4.29	1.28	2.84
14	−1	1	−1	−1	−1	1.66	5.48	2.17	2.90
15	−1	1	−1	1	−1	1.46	4.93	1.26	3.15
16	−1	1	−1	1	1	1.46	4.94	3.86	3.31
17	−1	1	−1	−1	1	2.24	6.18	2.45	3.84
18	0	0	0	0	0	2.13	4.92	2.61	1.81
19	0	0	0	0	0	2.12	4.90	5.47	1.99
20	0	0	2	0	0	2.53	4.91	6.54	2.31
21	0	0	0	0	0	2.85	5.54	4.44	2.33
22	0	0	0	0	0	2.14	4.81	4.11	2.36
23	0	0	0	0	−2	2.13	4.78	3.47	2.42
24	0	0	0	−2	0	0.95	3.67	4.05	2.45
25	0	0	0	2	0	2.46	5.11	4.77	2.45
26	0	−2	0	0	0	2.11	4.88	2.84	2.59
27	0	0	0	0	0	2.55	5.21	2.56	2.59
28	0	0	0	0	0	2.79	5.45	3.44	2.72
29	0	0	0	0	0	2.47	5.25	4.02	2.82
30	0	2	0	0	0	3.22	5.76	3.70	2.87
31	0	0	0	0	0	2.14	4.8	4.84	3.09
32	0	0	0	0	2	0.92	3.83	8.67	3.25
33	0	0	−2	0	0	1.63	4.87	3.64	4.08
34	1	−1	1	1	1	2.94	5.36	4.85	2.33
35	1	−1	1	−1	1	3.47	5.56	4.18	2.40
36	1	1	1	−1	−1	3.18	5.57	4.26	2.47
37	1	−1	−1	1	1	2.66	5.80	3.55	2.49
38	1	−1	1	−1	−1	2.38	4.81	5.46	2.56
39	1	1	1	1	−1	3.56	5.78	3.73	2.59
40	1	−1	1	1	−1	3.96	6.01	4.28	2.64
41	1	1	1	1	1	4.92	6.82	6.82	2.77
42	1	1	1	−1	1	3.44	5.78	6.65	2.96
43	1	−1	−1	1	−1	2.30	5.39	2.39	3.09
44	1	−1	−1	−1	1	2.66	5.46	3.83	3.19
45	1	1	−1	−1	−1	2.58	5.65	2.72	3.68
46	1	−1	−1	−1	−1	2.42	5.16	3.33	3.72
47	1	1	−1	1	−1	2.51	5.63	2.85	4.10
48	1	1	−1	1	1	3.35	6.36	5.69	4.20
49	1	1	−1	−1	1	2.87	5.79	4.64	4.35
50	2	0	0	0	0	3.75	6.21	6.04	2.94

T temperature, *PPFD* photosynthetic photon flux density, *TP* total phosphorus, *TN* total nitrogen, *DOC* dissolved organic carbon, *MC-LR* microcystin-LR, *EPS* extracellular polysaccharide

Experimental design

Central composite design (CCD), which includes factorial design and regression analysis, helps in understanding interactions among factors at varying levels and selecting optimum conditions for the design response. This method has been widely used for the optimization of various processes in biotechnology. In this study, we set algal growth and EOM production as the design responses. Five major environmental factors, TN ($NaNO_3$ as the N source), TP (K_2HPO_4 as the P

source), Fe (ferric ammonium citrate as the Fe source), T (water surface temperature), and L (photosynthetic photon flux density (PPFD)) were studied at five levels (− 2, − 1, 0, 1, 2) using CCD. Except TN, TP, and Fe, the concentrations of other nutrients were set according to BG-11 medium. In addition, the interactive effects of these factors on algal growth and EOM production were investigated. As shown in Table 1, a fractional factorial design composed of 8 central points, 10 axial points, and 32 factorial points (a total of 50 different combination runs) was derived from the standard CCD quadratic model. The initial algal concentration was set as 50,000 cells/mL, and the experimental period of each run in our study was set to 7 days to ensure that the logarithmic phase was reached according to the pre-experiment. Sampling was performed at the end of the experiment. The true value of each variable is given in Table 2. The variable i in the CCD was coded as follows:

$$x_i = \frac{X_i - X_i}{\Delta X} i = 1, 2, 3\text{K K}, k \quad (1)$$

where x_i is the coded level, X_i is the real value of an independent variable, X_{ci} is the real value of an independent variable at the central point, and ΔX_i is the step change of variable i.

The cell density and EOM production (including DOC, MC-LR, and EPS) of *M. aeruginosa* can be written as the following second-polynomial model:

$$Y = \beta_0 + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_{ii}^2 + \Sigma\beta_{ij} x_i x_j \quad (2)$$

where Y is the predicted response (i.e., cell concentration, DOC content, MC-LR content, EPS content), β is the coefficient of the equation, and x_i and x_j are the coded levels of the variables i and j.

The correlation coefficients of the above second-polynomial model were obtained by non-linear regression analysis of the responses using Design Expert (DX) 8.0.6 (Stat-Ease, Inc., Minneapolis, MN, USA). The estimates of the coefficients with confidence levels above 95% ($p < 0.05$) were accepted in the final models. The F test was used to evaluate the significance of the models.

Table 2 Real values of independent variables in central composition design

Level	T (°C)	PPFD (μmol/m^2/s)	TN (mg/L)	TP (mg/L)	Fe (mg/L)
+ 2	36	100	5.00	1.00	1.25
+ 1	32	80	3.78	0.75	1.00
0	28	60	2.55	0.51	0.75
− 1	24	40	1.33	0.26	0.50
− 2	20	20	0.10	0.01	0.25

Results and discussion

Development of CCD regression model

The experimental design and responses are listed in Table 1, and runs 18, 19, 21, 22, 27, 28, 29, and 31 at the center point design were used to determine the experimental error for algal density (1.09×10^5 cell/mL) and production of DOC (0.10 mg/L), MC-LR (0.36 μg/L), and EPS (0.15 mg/L). The estimated coefficients and statistical significance of parameters in second-order polynomial CCD regression models of algal density and EOM (including DOC, MC-LR, and EPS) production are shown in Table 3. To describe the responses of the most significant variables, a reduced model that only included the estimates with a significance level higher than 95% (i.e., $p < 0.05$) were generated and are listed in Table 4. The regression models were significant, as indicated by relatively high values of R^2 obtained for these models. The F test value listed in Table 4 also verified the goodness and rationality of the regression models. Figure 1 presents the predicted values versus actual values for the four responses. The predicted values obtained were very close to the experimental values, indicating that the CCD regression models listed in Table 4 were successful in capturing the correlations between the environmental factors and the four responses.

Main effects of environmental factors on *M. aeruginosa* responses

The main (first order) effects of variables on the four responses and their significance can be interpreted from Table 3. In case of algal density, all the effects were significant (p value < 0.05) except for the coefficient for Fe (p value > 0.05), and all the significant first-order coefficients of variables had a positive effect on algal density. In the case of DOC content, three environmental factors (temperature, PPFD, and TN) had a significant positive influence on the DOC production of *M. aeruginosa*. As for the release of MC-LR, the variables of temperature, PPFD, TN, and Fe showed a clear promoting effect. Temperature and PPFD had a positive effect, while TN had an adverse effect on EPS production. Compared to the main effects, the second-order coefficients had a comparatively less significant or even non-existent effect on *M. aeruginosa* responses. Earlier studies only focused on the main effects of the environmental factors on these responses (Otten et al. 2016; Wu et al. 2015; Xu et al. 2013b), and while this is not the only way, factors impact the responses; the interactive effects of environmental factors on the responses have the same significance.

Table 3 Regression coefficients of variables in the central composite design in response to cell density, DOC, MC-LR, and EPS content

Variables	Model of algal density		Model of DOC		Model of MC-LR		Model of EPS	
	Estimate	*p* value	Estimate	*p* value	Estimate	*p* value	Estimate	*p* value
Intercept	2.350	*< 0.0001*	5.010	*0.0007*	4.130	*< 0.0001*	2.460	*< 0.0001*
A-T	0.830	*< 0.0001*	0.450	*< 0.0001*	0.820	*< 0.0001*	0.260	*< 0.0001*
B-L	0.290	*0.0003*	0.300	*0.0003*	0.450	*0.005*	0.240	*0.0002*
C-N	0.250	*0.001*	− 0.008	*0.9106*	0.730	*< 0.0001*	− 0.440	*< 0.0001*
D-P	0.180	*0.0158*	0.160	*0.0431*	0.010	0.9464	− 0.045	0.4216
E-Fe	0.007	0.9189	0.000	0.9984	0.800	*< 0.0001*	0.042	0.4623
AB	− 0.062	0.4299	− 0.082	0.3242	− 0.160	0.3379	0.017	0.7925
AC	0.140	0.0735	0.041	0.6192	− 0.034	0.8352	− 0.067	0.2935
AD	0.070	0.3742	0.110	0.2054	− 0.024	0.8832	− 0.014	0.823
AE	0.130	0.1092	0.120	0.143	0.030	0.8581	− 0.010	0.8716
BC	0.010	0.8943	− 0.003	0.9685	0.110	0.5097	− 0.130	*0.0402*
BD	0.067	0.3973	− 0.006	0.9397	0.190	0.2629	0.040	0.5235
BE	0.041	0.6035	0.051	0.5359	0.470	*0.0071*	0.150	*0.0251*
CD	0.170	*0.0329*	0.140	0.108	− 0.031	0.8515	− 0.030	0.6377
CE	− 0.070	0.3751	− 0.034	0.6827	0.083	0.6152	− 0.044	0.4894
DE	0.004	0.9587	0.006	0.9414	0.092	0.5798	− 0.015	0.8134
A^2	− 0.009	0.9076	0.078	0.3512	− 0.210	0.2195	− 0.009	0.8829
B^2	0.130	0.1003	0.170	*0.0463*	− 0.400	*0.0195*	0.077	0.2276
C^2	− 0.014	0.8624	0.064	0.4405	0.050	0.7643	0.190	*0.0042*
D^2	− 0.110	0.1764	− 0.060	0.471	− 0.120	0.4646	0.008	0.8992
E^2	− 0.150	0.0592	− 0.082	0.3291	0.290	0.0823	0.100	0.1059

p values lower than 0.05 were all shown in italics

Interactive effects of environmental factors on *M. aeruginosa* responses

Effect of interactive variables TN-TP on algal density

Response surface plots were constructed to determine changes in algal density due to the interactive variables TP-TN according to the equations in Table 4. As shown in Fig. 2, the response 3D plots and contour plots of algal density with varying TN-TP reflected the synergic growth-promoting effects of TN and TP on algal growth, with a PPFD of 60 μmol/m^2/s at 28 °C, which represents proper conditions for the growth of *M. aeruginosa* (Jingtian 2010). The single increase of TN or TP can only bring limited or no enhancement of algal density, while the joint increase of TN and TP can raise algal density to a large extent. The interactive effects of TN and TP on the cell density of *M. aeruginosa* found in this study might be due to the absorption efficiency of nitrate influenced by the availability of phosphate ions, which has an important role in cellular energetics as part of ATP (adenosine triphosphate), and which influences the activity of many enzymes required for cell metabolism, including the nitrate reduction process (Hu et al. 2000).

2D contour plots of the 3D surface help to identify the major interactions that take place between environmental

Table 4 Reduced models for algal density, DOC, MC-LR, and EPS content as the functions of independent variables[a]

Responses	Model	R^2	*F* value
Algal density (10^6 cells/mL)	$2.35 + 0.83[\text{Tem.}] + 0.29[\text{Light}] + 0.25[NaNO_3] + 0.18[K_2HPO_4] + 0.17[NaNO_3][K_2HPO_4]$	0.87	10.05
DOC (mg/L)	$5.01 + 0.45[\text{Tem.}] + 0.30[\text{Light}] + 0.16[K_2HPO_4] + 0.17[\text{Light}]^2$	0.72	3.71
MC-LR (μg/L)	$4.13 + 0.82[\text{Tem.}] + 0.45[\text{Light}] + 0.73[NaNO_3] + 0.8[Fe^{3+}] + 0.47[\text{Light}][Fe^{3+}] - 0.4[\text{Light}]^2$	0.80	5.93
EPS (mg/L)	$2.46 + 0.26[\text{Tem.}] + 0.24[\text{Light}] - 0.44[NaNO_3] - 0.13[\text{Light}][NaNO_3] + 0.15[\text{Light}][Fe^{3+}] + 0.19[NaNO_3]^2$	0.82	6.48

[a] Only coefficients (Table 3) in second-order polynomial [Eq. (2)] with $p < 0.05$ were included

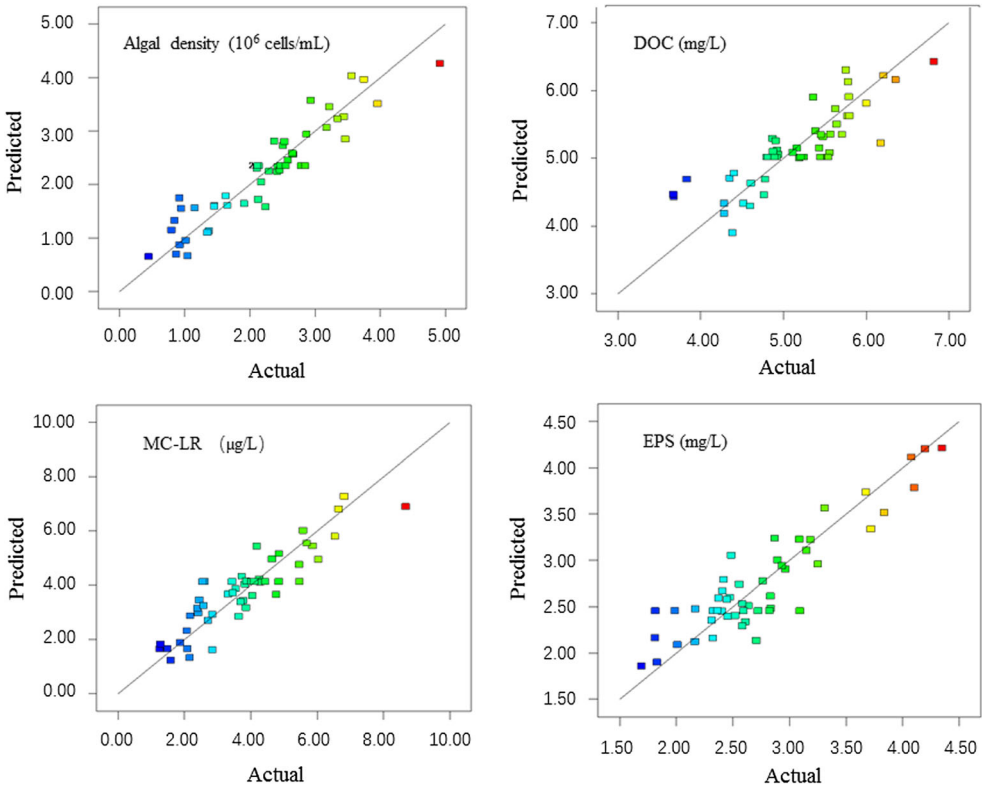

Fig. 1 Relationship between the observed and predicted responses for algal density, DOC, MC-LR, and EPS content

factors. The 2D contour graph, as shown in Fig. 2, exhibited a clear optimal boundary; the optimum TN/TP ratio was calculated using the model of algal density as approximately 12:1 under the proper PPFD and temperature conditions. The growth of *M. aeruginosa* might be restricted upon divergence from the optimal TN/TP ratio when the concentrations of N or P are individually increased or decreased. The optimum TN/TP ratio is widely considered as an important quantitative basis in determining the limiting factor of a eutrophic waterbody (Hauss et al. 2012; Vrede et al. 2010; Zhu et al. 2015). Redfield (1958) first suggested that the optimum N/P mass ratio for phytoplankton growth was approximately 16:1, with seasonal variations, which is higher than the value in this study. Smith (1983) reported that when the N/P mass ratio falls below approximately 29:1, blue-green algal blooms occur, and blue-green algae is rare when the N/P ratio exceeds this value. The N/P mass ratios from previous literature were used to predict species dominance; this study predicted that rapid growth of *M. aeruginosa* will occur at the optimum N/P mass ratio of 12:1.

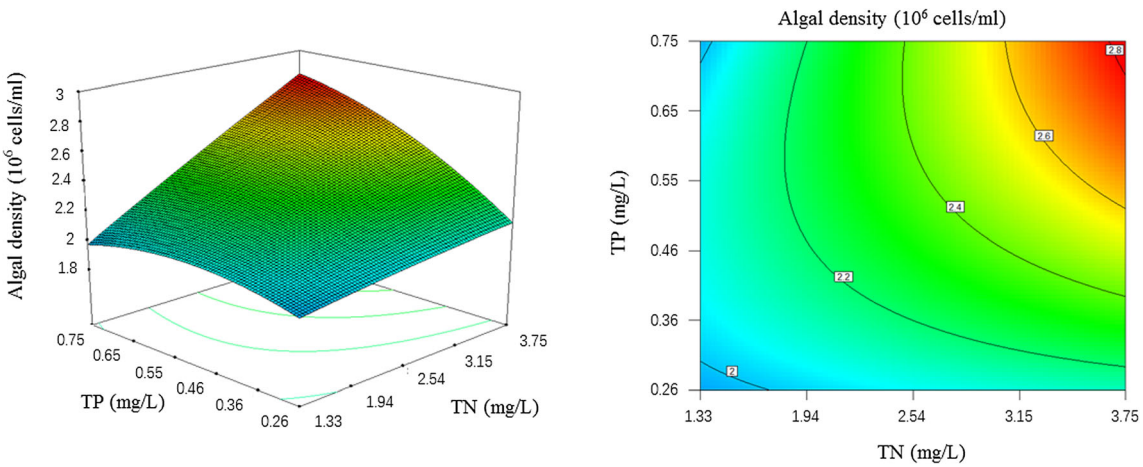

Fig. 2 3D surface plots of algal density as a function of TP and TN and corresponding contour graph (Fe, temperature, and PPFD were maintained at the central point)

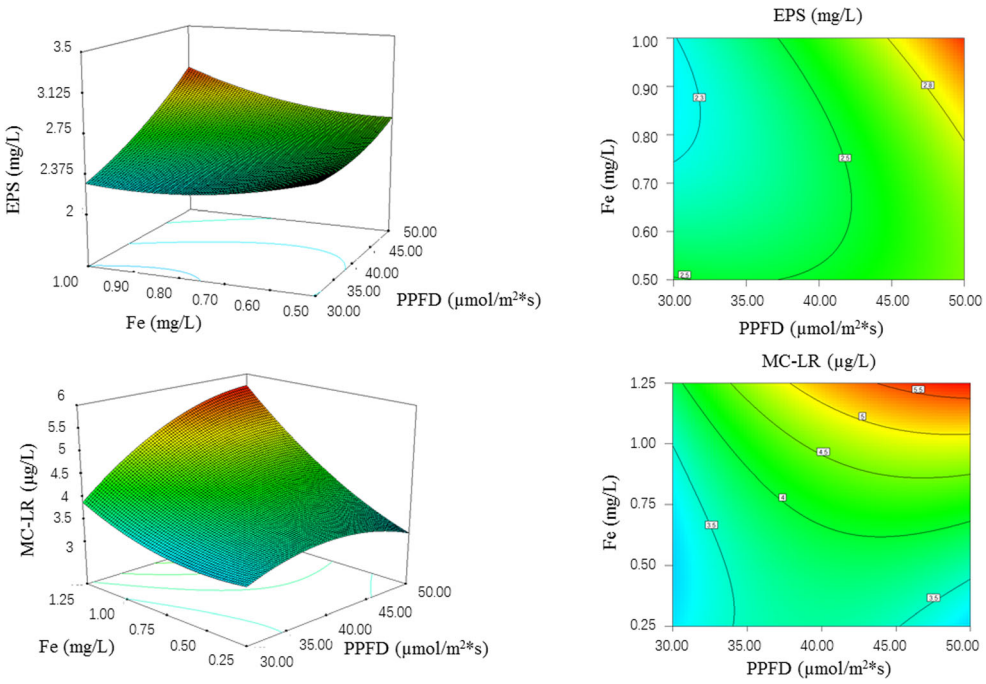

Fig. 3 3D surface plots of MC-LR and EPS as a function of Fe and PPFD and corresponding contour graphs (TP, TN, and temperature were maintained at the central point)

Effect of interactive variables PPFD-Fe on MC-LR and EPS production

The response 3D plots and contour plots of the effects of interactive variables PPFD-Fe on MC-LR and EPS production are presented in Fig. 3. As shown in this figure, the MC-LR and EPS content was stimulated well when the Fe and PPDF were set at high levels and other factors were set to central levels. It is possible that Fe^{3+} in the medium was reduced to Fe^{2+} by light before it was transported into algal cells and PPFD positively affected the Fe uptake rate. The light-induced reduction species Fe^{2+} from Fe^{3+} in the medium enhance the ability of the algae to process, which strengthens the bioactivity of iron and improves the secretion of EPS and MC-LR (Finden et al. 1984).

For MC-LR concentration, increasing the Fe concentration from 0.5 to 1 mg/L facilitated an increase in MC-LR production from 3.30 to 3.89 μg/L. As PPFD increased from 30 to 40 μmol/m^2/s, MC-LR content increased to a peak level of 3.63 μg/L and then showed a slight decrease as PPFD increased from approximately 40 to 50 μmol/m^2/s. MC-LR content was found to have a maximum value in the presence of a varying amount of PPFD; results in this study show that this maximum value was approximately 40 μmol/m^2/s, which is generally in accordance with previous findings (Utkilen and Gjølme 1992). This is probably because the PPDF has a positive effect on MC-LR production up to the point where the maximum content is reached, and higher levels of PPFD inhibit MC-LR production (Wiedner et al. 2003).

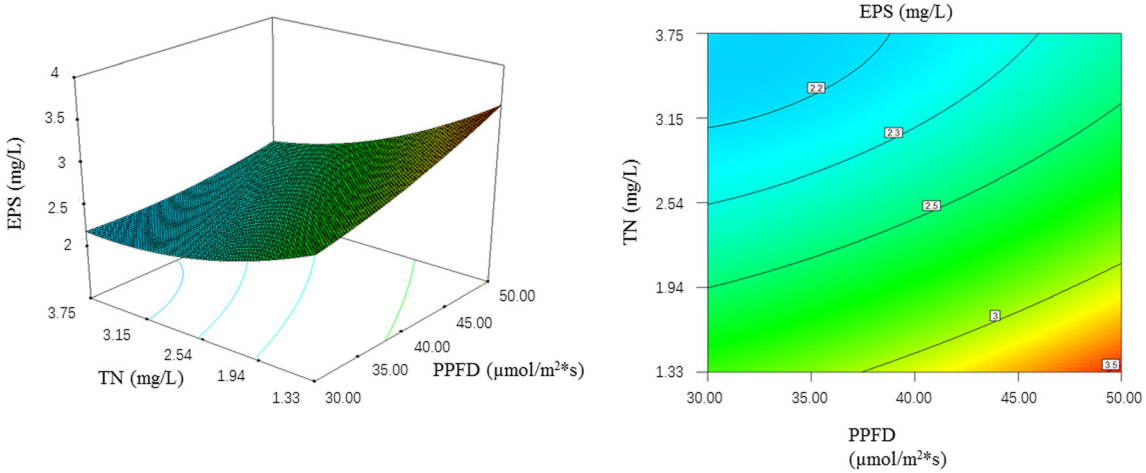

Fig. 4 3D surface plots of EPS as a function of TN and PPFD and corresponding contour graph (TP, Fe, and temperature were maintained at the central point)

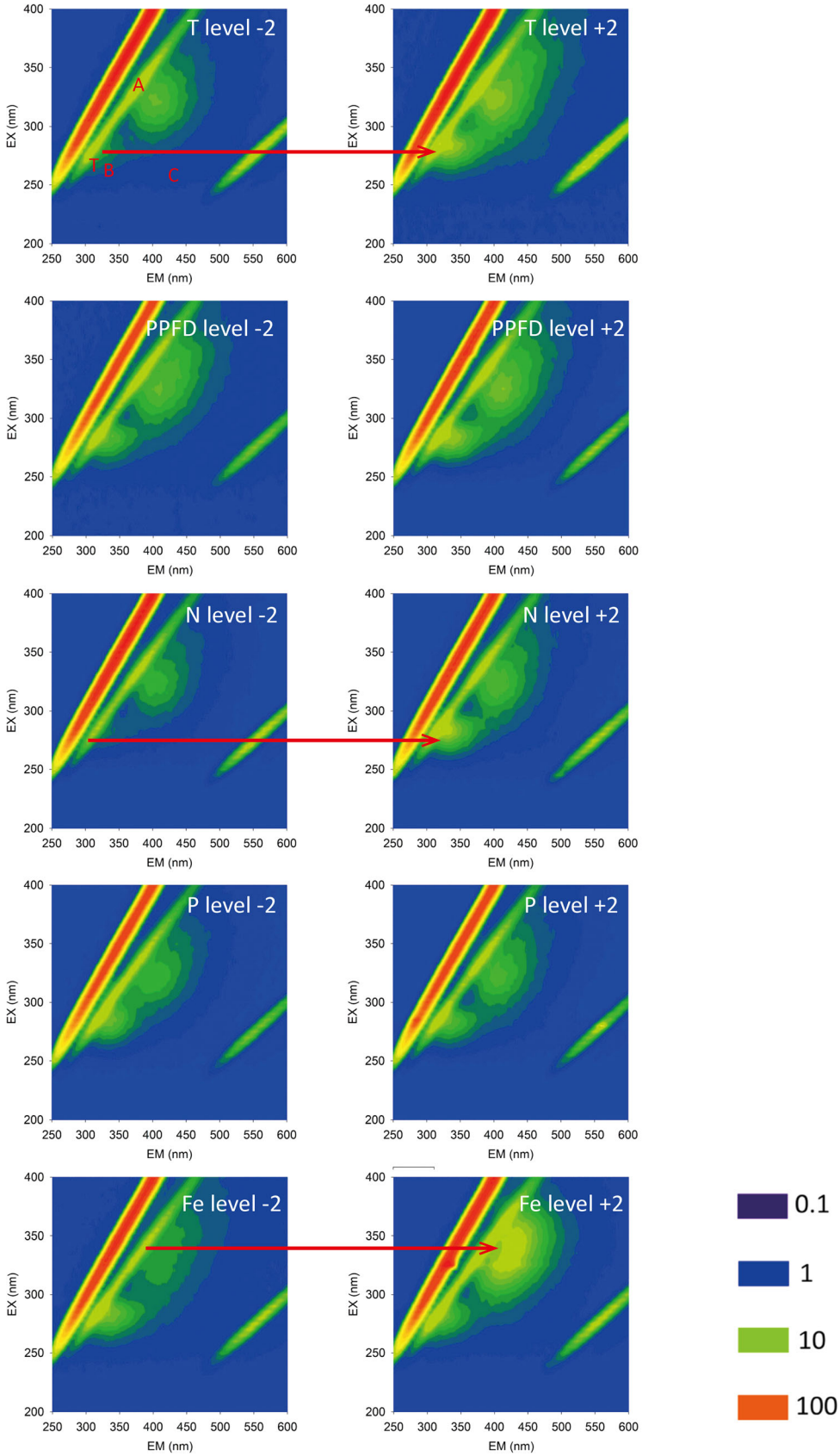

Fig. 5 Excitation/emission matrix fluorescence spectra of EOM samples of *M. aeruginosa* under 10 different axial CCD design environmental conditions (logarithmic phase)

Effect of interactive variables PPFD-TN on EPS production

There is still no consensus among previous studies on how nitrogen affects EPS production. EPS levels are species specific in cyanobacterium and change with the cultural conditions (Philippis et al. 2001; Philippis and Vincenzini 1998). Results in this study indicate that nitrogen deficiency stimulates EPS synthesis in *M. aeruginosa*. Nitrogen limitation can increase the EPS production of various algal species (Magaletti et al. 2004; Moreno et al. 1998). This increase in EPS production in algae under nutrient stress is believed to serve as a sink for the excess fixed carbon generated under an unbalanced carbon and nitrogen metabolism (Otero and Vincenzini 2004). High PPFD and low nitrogen limitation can stimulate EPS production in *M. aeruginosa*, and there was significant interaction between PPFD and nitrogen concentration in this study, as shown in Fig. 4. High PPFD promotes photosynthesis, thereby increasing the EPS yield. At very high PPFD levels, microcystic cell division is inhibited, causing further excess of intracellular fixed carbon and a large increase in EPS content (Li et al. 2013).

Fluorescence characterization of EOM

To investigate changes in EOM composition, ten trails of CCD axial points were measured using fluorescence spectroscopy. Figure 5 presents fluorescence EEM contours for the EOM released by *M. aeruginosa* during the logarithmic phase. Three fluorescence maxima were observed in the ten samples: one humic-like fluorescence peak at Ex/Em values of 330–350 nm/420–480 nm (peak A) and two protein-like fluorescence peaks: one tryptophan-like at Ex/Em values of 270–280 nm/300–320 nm (peak T) and one tyrosine-like at Ex/Em values of 270–280 nm/320–350 nm (peak B). There was no fluorescent substance in the culture (Xu et al. 2013a). According to Fig. 5, changes of environmental factors only affected the content of species without modifying their chemical components. The concentration of protein-like substances increased with increasing nitrogen concentration and temperature (Fig. 5), while the changes of PPFD and phosphorus had little or no impact on the composition or concentration of EOM in this study. Iron concentration had a positive impact on the release of humic-like substances; an excess concentration may lead to the death or autolyzing of algal cells, restrain the growth of *M. aeruginosa*, and accelerate deterioration of the aqueous environment, thus raising the content of humic-like substances (Lv et al. 2006). Humic-like substances were considered as the main precursors of wastewater disinfection byproducts (DBPs), such as trihalomethanes (THMs) and haloacetic acid (HAA), which severely affect water quality (Wei et al. 2011).

Conclusion

In this study, CCD response surface design was applied to study the interactive effects of selected environmental factors (TN, TP, Fe, PPFD, and temperature) on algal growth and EOM release. This study contributes to our theoretical knowledge of the prediction and analysis of *M. aeruginosa* growth and EOM production in the following aspects:

1. CCD response surface models of algal density, DOC, MC-LR, and EPS were established by fitting the experimental data. These models effectively captured the correlation between the environmental factors and the responses and can be used to help alert environmental managers to the eutrophication status of selected water bodies.

2. Results indicated that TN and TP had positive interactive effects on algal density, both iron and PPFD had synergic effects on MC-LR and EPS production, and PPFD and TN had clear interactive effects on EPS release. The optimum TN/TP mass ratio for the growth of *M. aeruginosa* was 12:1, derived from the second polynomial regression model of algal density.

3. The EOM composition of *Microcystis aeruginosa* during the logarithmic phase was determined using fluorescence analysis. The results showed that the EOM consisted primarily of humic-like substances and protein-like substances. Changes of environmental factors can influence the content of the substances without modifying their composition. Elevated levels of temperature and TN boosted levels of protein-like substances, and an increase of iron concentration promoted the content of humic-like substances, which were considered as DBP precursors.

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References

Biermann A, Engel A, Riebesell U (2014) Changes in organic matter cycling in a plankton community exposed to warming under different light intensities. J Plankton Res 36:658–671

Black K, Yilmaz M, Phlips EJ (2011) Growth and toxin production by Microcystis Aeruginosa PCC 7806 (Kutzing) Lemmerman at elevated salt concentrations. J Environ Prot 2:669–674

Brookes JD, Ganf GG (2001) Variations in the buoyancy response of *Microcystis aeruginosa* to nitrogen, phosphorus and light. J Plankton Res 23(1313):1399–1411

Dotson A, Westerhoff P, Krasner SW (2009) Nitrogen enriched dissolved organic matter (DOM) isolates and their affinity to form emerging disinfection by-products Water Sci Technol J Int Assoc Water Pollut Res 60:135–143

Finden DAS, Tipping E, Jaworski GHM, Reynolds CS (1984) Light-induced reduction of natural iron(III) oxide and its relevance to phytoplankton. Nature 309:783–784

Fujii M, Dang TC, Rose AL, Omura T, Waite TD (2015) Effect of light on iron uptake by the freshwater cyanobacterium *Microcystis aeruginosa*. Environmental Science & Technology 45:1391–1398

Graham JL, Jones JR, Jones SB, Downing JA, Clevenger TE (2004) Environmental factors influencing microcystin distribution and concentration in the Midwestern United States. Water Res 38:4395–4404

Guo N, Xie P (2006) Development of tolerance against toxic Microcystis aeruginosa in three cladocerans and the ecological implications. Environ Pollut 143:513–518

Hauss H, Franz JMS, Sommer U (2012) Changes in N:P stoichiometry influence taxonomic composition and nutritional quality of phytoplankton in the Peruvian upwelling. J Sea Res 73:74–85

Henderson RK, Baker A, Parsons SA, Jefferson B (2008) Characterisation of algogenic organic matter extracted from cyanobacteria, green algae and diatoms. Water Res 42:3435–3445

Hu Q, Westerhoff P, Vermaas W (2000) Removal of nitrate from groundwater by cyanobacteria: quantitative assessment of factors influencing nitrate uptake. Appl Environ Microbiol 66:133–139

Jiang Y, Ji B, Wong RNS, Wong MH (2008) Statistical study on the effects of environmental factors on the growth and microcystins production of bloom-forming cyanobacterium—Microcystis aeruginosa. Harmful Algae 7:127–136

Jingtian Z (2010) Optimization of growth conditions of *Microcystis aeruginosa* using response surface methodology Beijing University of Chemical Technology

Leenheer JA, Croué JP (2003) Characterizing aquatic dissolved organic matter. Environmental Science & Technology 37:18A

Li H, Murphy T, Guo J, Parr T, Nalewajko C (2009) Iron-stimulated growth and microcystin production of Microcystis novacekii UAM 250. Limnol- Ecol Manag Inland Waters 39:255–259

Li M, Zhu W, Gao L, Lu L (2013) Changes in extracellular polysaccharide content and morphology of Microcystis aeruginosa at different specific growth rates. J Appl Phycol 25:1023–1030

Li J, Wang Z, Cao X, Wang Z, Zheng Z (2015) Effect of orthophosphate and bioavailability of dissolved organic phosphorous compounds to typically harmful cyanobacterium Microcystis aeruginosa. Mar Pollut Bull 92:52–58

Liu C, Liu Y, Liao W, Wen Z, Chen S (2003) Application of statistically-based experimental designs for the optimization of nisin production from whey. Biotechnol Lett 25:877–882

Lv X, Zhang X, Kang R, Hu H, Cong W, Tan T (2006) Effects of Fe3 + on growth and photosynthesis of *Microcystis aeruginosa*. J Beijing Univ Chem Techenol 33:27–30

Ma J, Qin B, Paerl HW, Brookes JD, Wu P, Zhou J, Deng J, Guo J, Li Z (2015) Green algal over cyanobacterial dominance promoted with nitrogen and phosphorus additions in a mesocosm study at Lake Taihu, China. Environ Sci Pollut Res 22:5041–5049

Magaletti E, Urbani R, Sist P, Ferrari CR, Cicero AM (2004) Abundance and chemical characterization of extracellular carbohydrates released by the marine diatom Cylindrotheca fusiformis under N- and P-limitation. Eur J Phycol 39:133–142

Máthé C, M-Hamvas M, Vasas G, Surányi G, Bácsi I, Beyer D, Tóth S, Tímár M, Borbély G (2007) Microcystin-LR, a cyanobacterial toxin, induces growth inhibition and histological alterations in common reed (Phragmites australis) plants regenerated from embryogenic calli. New Phytol 176:824–835

Moreno J, Vargas MA, Olivares H, Rivas JN, Guerrero MG (1998) Exopolysaccharide production by the cyanobacterium Anabaena sp. ATCC 33047 in batch and continuous culture. J Biotechnol 60:175–182

Otero A, Vincenzini M (2004) Nosto (cyanophyceae) goes nude: extracellular polysaccharides serve as a sink for reducing power under unbalanced C/N metabolism. J Phycol 40:74–81

Otten TG, Xu H, Qin B, Zhu G, Paerl HW (2016) Spatiotemporal patterns and ecophysiology of toxigenic microcystis blooms in Lake Taihu, China: implications for water quality management. Environ Sci Technol 46:3480–3488

Philippis RD, Vincenzini M (1998) Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol Rev 22:151–175

Philippis RD, Sili C, Paperi R, Vincenzini M (2001) Exopolysaccharide-producing cyanobacteria and their possible exploitation: a review. J Appl Phycol 13:293–299

Redfield AC (1958) The biological control of chemical factors in the environment. Sci Prog 11:205–221

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Microbiology 111(1):1–61

Smith VH (1983) Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science 221:669–671

Soumya PS, Lakshmi MSK, Nambisan P (2016) Application of response surface methodology for the optimization of laccase production from Pleurotus ostreatus by solid state fermentation on pineapple leaf substrate. J Sci Ind Res 75:306–314

Utkilen H, Gjølme N (1992) Toxin production by *Microcystis aeruginosa* as a function of light in continuous cultures and its ecological significance. Appl Environ Microbiol 58:1321

Vézie C, Rapala J, Vaitomaa J, Seitsonen J, Sivonen K (2002) Effect of nitrogen and phosphorus on growth of toxic and nontoxic Microcystis strains and on intracellular microcystin concentrations. Microb Ecol 43:443–454

Vrede T, Ballantyne A, Mille-Lindblom C, Algesten G, Gudasz C, Lindahl S, Brunberg AK (2010) Effects of N : P loading ratios on phytoplankton community composition, primary production and N fixation in a eutrophic lake. Freshw Biol 54:331–344

Wang C, Wang Z, Wang P, Zhang S (2016) Multiple effects of environmental factors on algal growth and nutrient thresholds for harmful algal blooms: application of response surface methodology. Environ Model Assess 21:247–259

Wei YY, Liu Y, Zhang Y, Dai RH, Liu X, Wu JJ, Zhang Q (2011) Influence of soluble microbial products (SMP) on wastewater disinfection byproducts: trihalomethanes and haloacetic acid species from the chlorination of SMP. Environ Sci Pollut Res 18:46–50

Wen ZY, Chen F (2001) Application of statistically-based experimental designs for the optimization of eicosapentaenoic acid production by the diatom Nitzschia laevis. Biotechnol Bioeng 75:159–169

Wiedner C, Visser PM, Fastner J, Metcalf JS, Codd GA, Mur LR (2003) Effects of light on the microcystin content of Microcystis strain PCC 7806. Appl Environ Microbiol 69:1475–1481

Wu X, Yan Y, Wang P, Ni L, Gao J, Dai R (2015) Effect of urea on growth and microcystins production of Microcystis aeruginosa. Bioresour Technol 181:72–77

Xu H, Cai H, Yu G, Jiang H (2013a) Insights into extracellular polymeric substances of cyanobacterium Microcystis aeruginosa using fractionation procedure and parallel factor analysis. Water Res 47:2005–2014

Xu H, Yu G, Jiang H (2013b) Investigation on extracellular polymeric substances from mucilaginous cyanobacterial blooms in eutrophic freshwater lakes. Chemosphere 93:75–81

Yang Z, Geng L, Wang W, Zhang J (2012) Combined effects of temperature, light intensity, and nitrogen concentration on the growth and polysaccharide content of Microcystis aeruginosa in batch culture. Biochem Syst Ecol 41:130–135

Yang C, Zhang AW, Ren BM, Song AL, Li AT, JZA B (2013) Whole-genome sequence of Microcystis aeruginosa TAIHU98, a nontoxic bloom-forming strain isolated from Taihu Lake, China. Genome Announce 1:650–658

Zhang N, Xu B, Qi F (2016) Effect of phosphate loading on the generation of extracellular organic matters of Microcystis Aeruginosa and its derived disinfection by-products. Water Air Soil Pollut 227:264

Zhu W, Sun Q, Chen F, Li M (2015) Cellular N:P ratio of Microcystis as an indicator of nutrient limitation—implications and applications. Environ Earth Sci 74:4023–4030