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A promising application of chitosan quaternary ammonium salt to removal of *Microcystis aeruginosa* cells from drinking water

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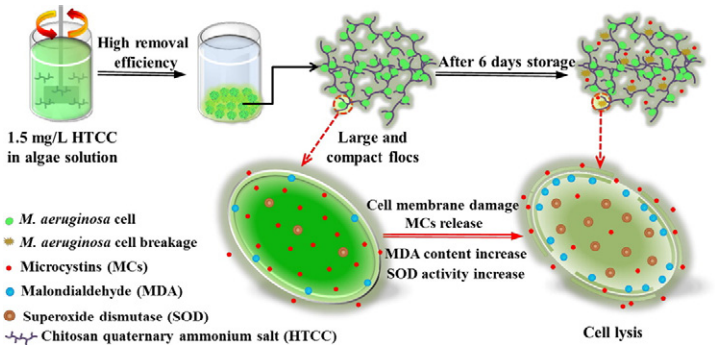
HIGHLIGHTS

- HTCC is first used to coagulate *Microcystis aeruginosa* cells.
- HTCC shows better coagulation property than other coagulants.
- The flocs formed by HTCC have large size and compact structure.
- HTCC can inhibit the growth of *Microcystis aeruginosa* while the flocs can restrain the cells lysis.

GRAPHICAL ABSTRACT



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ABSTRACT

This work was aimed toward studying the new application of chitosan quaternary ammonium salt (HTCC), a water-soluble chitosan derivative, on removal of *Microcystis aeruginosa* (*M. aeruginosa*) cells during HTCC coagulation and floc storage. Results showed that all cells were removed without damage under optimum coagulation conditions: HTCC dosage 1.5 mg/L, rapid mixing for 0.5 min at 5.04 *g* and slow mixing for 30 min at 0.20 *g*. The high removal efficiency was due to the large size and compact structure of flocs formed by HTCC, which readily settled. During floc storage, HTCC could induce production of reactive oxygen species (ROS), which would accelerate *M. aeruginosa* cell lysis. But the flocs, into which the cells aggregated, could protect cells from cellular oxidative damage caused by ROS, thus keeping the cells intact for a longer time.

1. Introduction

Eutrophication, caused by excessive nutrient and pollution input, has become a serious issue (Reichwaldt and Ghadouani, 2012). It is known that eutrophication always accompanies algal blooms, which can result in serious problems in aquatic systems (de Vicente et al.,

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Table 1
Response of cell removal efficiency to changes in individual coagulation conditions. (Optimal conditions shown in bold.)

System	HTCC (mg/L)	Rapid mix		Slow mix		Removal efficiency (%)
		Speed (g)	Time (min)	Speed (g)	Time (min)	
1	0.1	1.26	0.5	0.02	10	0.0
2	0.1	2.24	1	0.09	15	0.0
3	0.1	3.50	1.5	0.20	20	0.0
4	0.1	5.04	2	0.36	25	0.0
5	0.1	6.85	2.5	0.56	30	0.0
6	0.5	1.26	1	0.20	25	24.5
7	0.5	2.24	1.5	0.36	30	6.2
8	0.5	3.50	2	0.56	10	40.4
9	0.5	5.04	2.5	0.02	15	88.2
10	0.5	6.85	0.5	0.09	20	13.4
11	1.5	1.26	1.5	0.56	15	99.0
12	1.5	2.24	2	0.02	20	99.0
13	1.5	3.50	2.5	0.09	25	99.0
14	**1.5**	**5.04**	**0.5**	**0.20**	**30**	**100.0**
15	1.5	6.85	1	0.36	10	98.0
16	3	1.26	2	0.09	30	84.2
17	3	2.24	2.5	0.20	10	83.8
18	3	3.50	0.5	0.36	15	84.3
19	3	5.04	1	0.56	20	85.6
20	3	6.85	1.5	0.02	25	74.5
21	4.5	1.26	2.5	0.36	20	71.7
22	4.5	2.24	0.5	0.56	25	50.0
23	4.5	3.50	1	0.56	30	66.3
24	4.5	5.04	1.5	0.09	10	66.7
25	4.5	6.85	2	0.20	15	76.5

2010; Haywood et al., 2012). Among these algae, the most notorious is *Microcystis aeruginosa* (*M. aeruginosa*). It is one of the typical toxin-producing cyanobacteria that can impart taste, odors and a wide range of toxins. Microcystins (MCs), which are hepatotoxins (Kemal et al., 2009; Chen et al., 2011; Adamovsky et al., 2015), are the most harmful toxins produced by *M. aeruginosa*, and are dangerous to animal and human health (Shi et al., 2015; Li and Pan, 2015). In recent years, more and more research has focused on the regulation and treatment of *M. aeruginosa* and MCs (Fujii et al., 2014).

As coagulation is the key step in the drinking water treatment processes, many conventional coagulants such as $FeCl_3$, $AlCl_3$, poly aluminium chloride (PAC) and poly aluminium ferric chloride (PAFC) are reported to remove *M. aeruginosa* with intact cells (Li et al., 2015; Sun et al., 2012; Sun et al., 2013; Song et al., 2015). But to remove algal cells effectively, high dosages of coagulant must be added, which increases the treatment costs and simultaneously forms large volumes of sludge that need to be disposed of. Furthermore, the sludge produced by the inorganic metallic coagulants (*i.e.*, aluminium and iron salts) are toxic to the environment (Fast et al., 2014), and the flocs formed by inorganic metallic coagulants are hard to settle due to their low density (Wu et al., 2011). Considering the above issues, application of organic polymers as coagulants may be a more feasible and sustainable alternative. Chitosan, the second most abundant natural polymer (Huang et al., 2015; Shchipunov et al., 2009; Dekamin et al., 2013), used to be considered as a 'new generation' of coagulant. However, its water-insolubility restricts its application in drinking water treatment processes (Dao et al., 2015). Therefore, some water-soluble chitosan derivatives have been synthesized and used to remove *M. aeruginosa* cells. Recently, a chitosan quaternary ammonium salt (HTCC) has been synthesized as a novel chitosan derivative, which can easily dissolve in water. However, most research into HTCC was focused on its antimicrobial activity (Jia et al., 2001; Yang et al., 2012; Wan et al., 2013). In our laboratory, Zhu et al. first studied the antialgal activity of HTCC; results showed that 1.2 mg/L HTCC could effectively inhibit the growth of *M. aeruginosa* and simultaneously reduce the release of MCs (Zhu et al., 2016). Until now, no research has been done into the application of HTCC in drinking water treatment, especially as a coagulant used for *M. aeruginosa* removal.

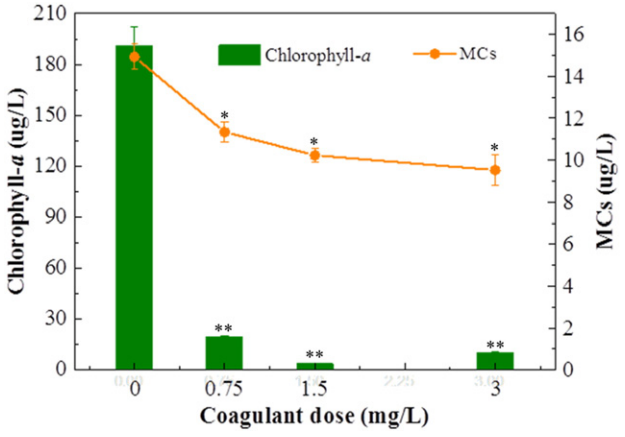

Fig. 1. The changes of chlorophyll-*a* and MCs concentrations in the coagulation supernatants at different coagulation doses with a standardised stirring condition: rapid mix at 5.04 *g* for 0.5 min, slow mix at 0.20 *g* for 30 min.

Cell lysis during floc storage processes also needs to be studied, as this can help us avoid high levels of toxic MCs being released back into the supernatant. Lysis of *M. aeruginosa* cells could be affected by many factors. For example, *M. aeruginosa* grows well in alkaline environments, while it tends to lyse in acidic environments (Shapiro, 1997). On the other hand, coagulant types and doses could affect cell lysis during floc storage. In research by Xu et al., the lysis of *M. aeruginosa* in $AlCl_3$ and $FeCl_3$ sludges was more severe than that in PAFC sludges, which was because Al and excessive Fe were toxic to cells; however, in PAFC sludge, low levels of Al showed little toxic effect on *M. aeruginosa* growth and moderate amounts of Fe were beneficial to growth (Xu et al., 2016). So it is also necessary to study the influence factors on lysis and the mechanisms of lysis of *M. aeruginosa* cells during storage of flocs generated with HTCC coagulant.

Hence, the purposes of this study were to: (1) find the optimum coagulation conditions of HTCC for the effective removal of cyanobacterial cells; (2) assess the effects of HTCC dose and coagulation stirring on *M. aeruginosa* cells during coagulation processes; (3) study the fate of *M. aeruginosa* cells during floc storage and the lysis mechanism.

2. Materials and methods

2.1. Materials

2.1.1. Cyanobacterial culture

M. aeruginosa FACHB-905 (purchased from Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China)) was used as the model alga in this study. This strain was grown in BG11 medium with a light/dark cycle (12 h/12 h) and constant temperature (25 °C) under 2000 lux. The cultures were harvested at the late exponential phase of growth and had a final cell yield up to 10^7 cells/mL (generally 10 days after inoculation). In this phase, cells have higher metabolic and enzymatic activity, more consistent size, and keep most MCs within the actively growing cells.

2.1.2. Water

In the experimental system, the water was collected from the Queshan Reservoir (a drinking water source, Jinan, Shandong province) and filtered through 0.45 μm fiber membranes (Xinya Co. Ltd., Shanghai, China) to remove any natural algae. The raw water quality parameters were: temperature 16.7 °C, pH 8.4, color 8 PCU, turbidity 4.2 NTU, COD_{Mn} 14.4 mg/L, DO 8.84 mg/L, NH_3-N 0.19 mg/L, TN 2.2 mg/L, TP 0.03 mg/L, alkalinity 133.9 mg/L. The raw water quality parameters were measured following the Chinese state standard testing methods (State Environmental Protection Administration, 2002).