SUPPORTING INFORMATION FOR

Flocculation of *Microcystis aeruginosa* using modified larch tannin

Li Wang, Wenyan Liang, Jian Yu, Zhixia Liang, Lingling Ruan, Yuanchun Zhang*

College of Environmental Science and Engineering, Beijing Forestry University, Beijing 100083, China.

This Supporting Information contains:

- The method of Tannin purification and SEM/EDS analysis;
- Figure S1. The monomer structures of the tannin (TN), raw product without modification;
- Figure S2. The monomer structures of A-TN, modified by *Mannich* reaction;
- Figure S3. The monomer structure of Q-TN, modified by *Mannich* reaction and then quaternization reaction;
- Figure S4. Photograph and SEM (×1000) of tannins before and after modification;
- Table S1. The element analysis of tannins before and after modification using SEM/EDS；
- Figure S5. Flocculation kinetics curves in a series of Q-TN dosage;
- Figure S6. The variations in zeta potential with cell density and dosage;
- Figure S7. SEM images of tannin-algae flocs;
- Figure S8. The change of residual phenols with culture time in supernatant;
- Figure S9. The change of algae cell density with culture time；
- Figure S10. 3DEEMs for algae samples with EOM before centrifugation (a) and after centrifugation (b);
- Figure S11. 3DEEMs of algae samples from different culture times.

Number of Pages: 12

Number of Tables: 1

Number of Figures: 11

Materials and methods

Tannin purification

One gram of tannin was dissolved in 30 mL distilled water, and any insoluble substances, such as phlobaphen and cellulose, were removed by 0.45-μm filtration. The filtrate was poured into a separating funnel, and 90 mL petroleum ether (100%) was added to remove the flavonoid-like organics. After mixing vigorously for 3 min and allowing to stand for 30 min, the mixture was separated into two layers, from which the lower aqueous layer of approximately 20 mL was dropped into a beaker. Then 180 mL ethanol (100%) was added to the beaker and the mixture was centrifuged at 4000 rpm for 10 min. The supernatant was collected and condensed by rotary evaporation. Finally, the concentrated solution was freeze-dried and purified powders were stored in 4 ºC.

SEM/EDS analysis

Scanning electron microscopy (SEM) (S-570, Hitachi, Japan) was used to observe the morphology of the tannin flocs after the flocculation. The flocs were collected by centrifugation (4000 rpm, 3 min), then placed in a phosphate buffer solution (pH = 7) and fixed with 2.5% glutaldehyde at 4 ºC overnight. Next day, the samples were washed using a phosphate buffer solution, dehydrated with successively different concentrations of ethanol (50%, 70%, 85% 95%, 100%), and dried by critical point drying (HCP-2, Hitachi, Japan). Dried samples were mounted on copper stubs and sputter coated with gold-palladium (IB-5, Hitachi, Japan). The specimens were observed and photographed at 10 kV.

For the power of TN, A-TN, and Q-TN, their morphology was observed by scanning electron microscope with energy dispersive spectrometer (SEM/EDS, S-3400N, Hitachi, Japan). The element content of tannins before and after modification was measured using EDS analysis and at least 10 sampling spots were selected to calculate the average results.

Results and Discussion

Monomer structure of tannin

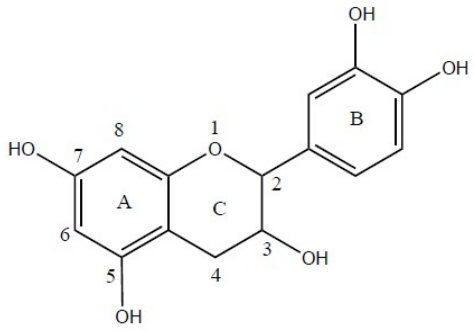


Figure S1. The monomer structure of the tannins (TN), raw product without modification.

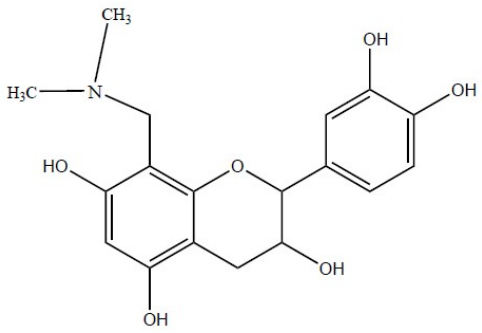


Figure S2. The monomer structure of A-TN, modified by *Mannich* reaction.

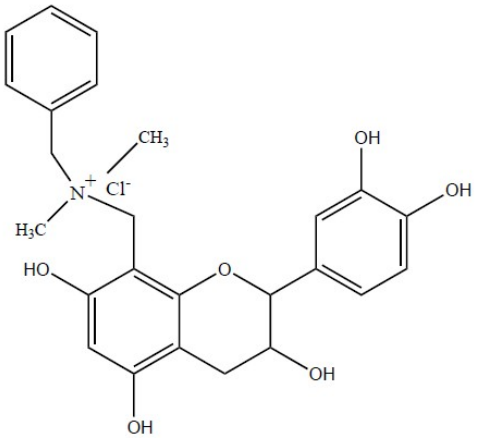


Figure S3. The monomer structure of Q-TN, modified by *Mannich* reaction and then quaternization reaction.

Efficiency of the modification reaction

From Table S1, the atom percentage of nitrogen increased from 1.75% and to 5.92%, and chlorine increased only from 0.19% to 1.11%. Based on the mechanism of *Mannich* reaction and quaternization reaction (Figure S2 and S3), the final percentage of N and Cl for Q-TN should be the same, after subtracting the initial ones of TN. The results indicated that the efficiency of quaternization reaction was less than that of *Mannich* reaction. Therefore, the total efficiency was limited by the quaternization reaction. Because TN was the mixture of polyphenols and there were some impurities in them, it was hard to calculate the reaction efficiency precisely. According to the equation (1), the final efficiency of Q-TN was about 22%.

$$\text{Reaction efficiency (\%)}=\frac{\text{atom percentage of Cl}}{\text{atom percentage of N}}=\frac{1.11\%-0.19\%}{5.92\%-1.75\%}=22\% \qquad (1)$$

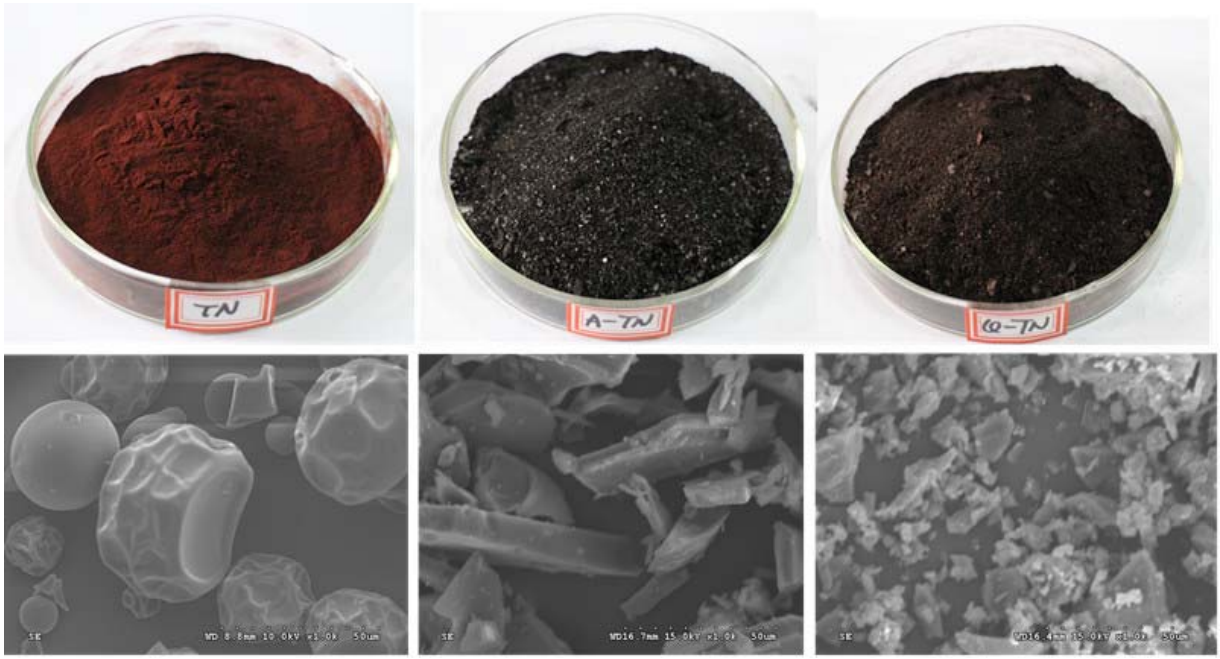


Figure S4. Photograph of tannins before and after modification by SEM, ×1000.

Table S1. The element analysis of tannins before and after modification using SEM/EDS

Element	TN		A-TN		Q-TN	
	W%	A%	W%	A%	W%	A%
Carbon (C)	71.21	77.17	67.73	73.24	69.93	76.25
Nitrogen (N)	2.81	1.75	8.55	7.93	6.33	5.92
Oxygen (O)	25.13	19.93	22.69	18.42	20.11	16.46
Chlorine (Cl)	0.45	0.19	0.36	0.13	3.01	1.11

Note: W% is the percentage of mass. A% is the percentage of atom numbers. The data are the average results of 10 sampling spots.

The kinetics of Q-TN flocculation processes

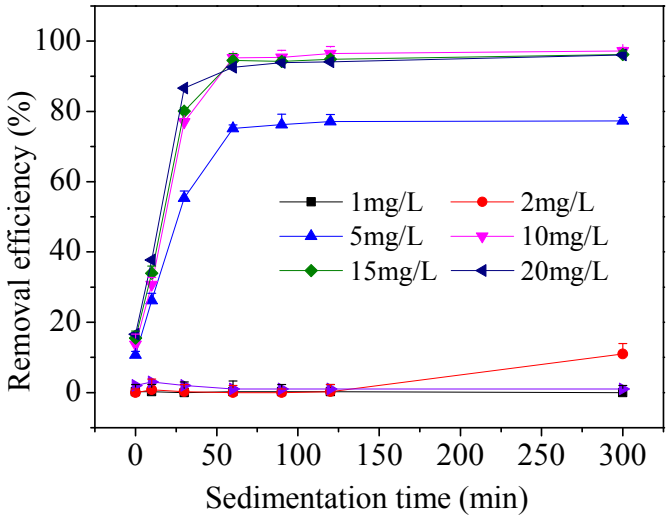


Figure S5. Flocculation kinetics for different dosage of Q-TN. (initial algae cell density was 1×10^9 cells/L; initial pH=7.0 before flocculation)

Zeta potential of algae flocs

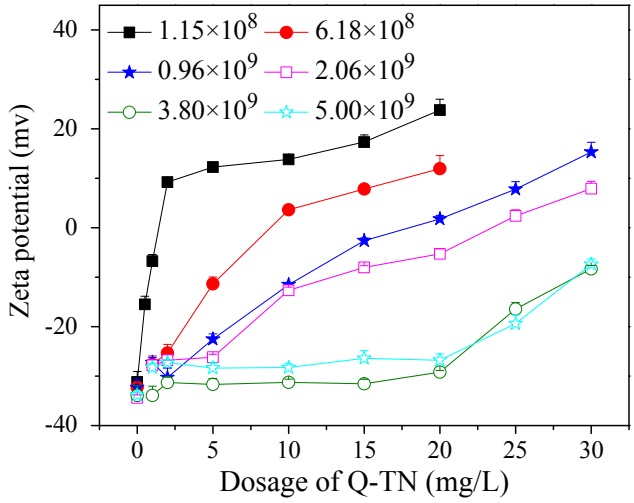
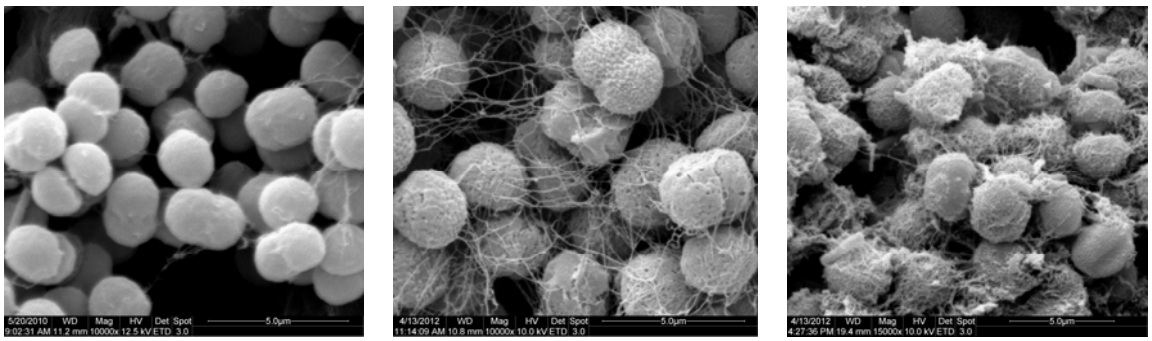


Figure S6. The variations in zeta potential during the flocculation processes. (The dosage of Q-TN was from 0.05 to 30 mg/L; initial algae densities from 1×10^8 cells/L to 5×10^9 cells/L; initial pH=7.0 before flocculation)

SEM of algae cells

During the flocculation of MA cells by A-TN and Q-TN, the formation of tannin-algae flocs was visible to the naked eye during the slow agitation procedure. The flocs were fibrous and stuck to the stirrer or formed large entangled masses resembling cobwebs. The tannin-algae flocs produced by Q-TN appeared to be formed more rapidly and grew to a larger size than those produced by A-TN. As can be seen in Fig. S7, the algae cells were wrapped by the modified tannin flocculants. Moreover, the size and density of the algae flocs aggregated by Q-TN was much greater than for A-TN, which was consistent with observation by the naked eye.



(a) Control sample (×10000) (b) A-TN-algae flocs (×10000) (c) Q-TN-algae flocs (×15000)

Figure S7. SEM images of tannin-algae flocs (b- pH 6.0, 15 mg/L A-TN; c- pH 6.0, 5 mg/L Q-TN; algae cells were cultured after 35 days with ~1×10^9/L density).

The release of polyphenols

After flocculation, the whole water sample including algal flocs at the bottom was cultured in the incubator (30 ºC, 28 μmol/m^2•s, 14:10 light:dark). To prevent the algal flocs from resuspending from the sediment, there wasn't any stirring during the 5-day culture. The polyphenol concentration and the cell density of the supernatant were detected at certain intervals.

As shown in Figure S8, when the dosage of Q-TN exceeded 5 mg/L, the release of polyphenols from flocculants into the supernatant was obvious. During 5-day culture, the concentration of polyphenols in the surpernatant was increased slightly for the samples after Q-TN flocculation. Whereas, it rised a lot for the control samples, which suggested that the algae cells would excrete the polyphenol-like substances during growth. Therefore, the small increment of polyphenols for Q-TN samples resulted from the algae growth, not from the tannin flocculants. Although there were some polyphenols in the supernatant, the remaining cells could still grow during 5-day culture (shown in Figure S9). Due to the lack of the nutrients, the algae cells grew slower than that of the control samples. However, the modified tannins didn't show the obvious inhibition effects to the algae growth, compared with the traditional flocculant of $Al_2(SO_4)_3$,. It indicated that the residual polyphenols in the supernatant wouldn't affect the cell growth. Therefore, the modified tannins were safe to the aquatic organisms.

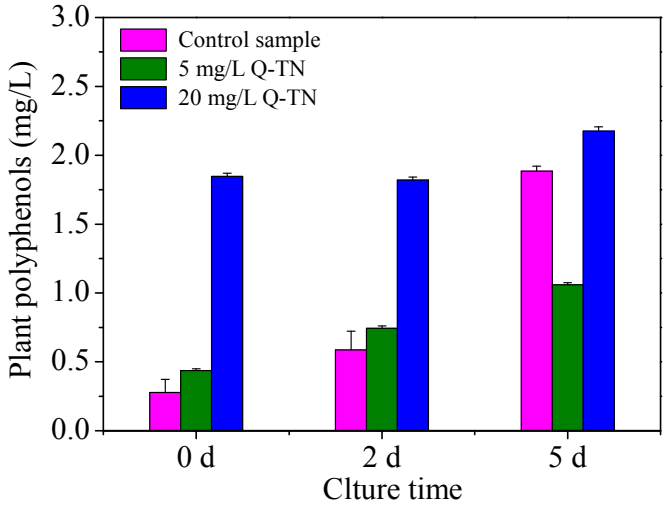


Figure S8. The concentration of polyphenols in the supernatant during culture

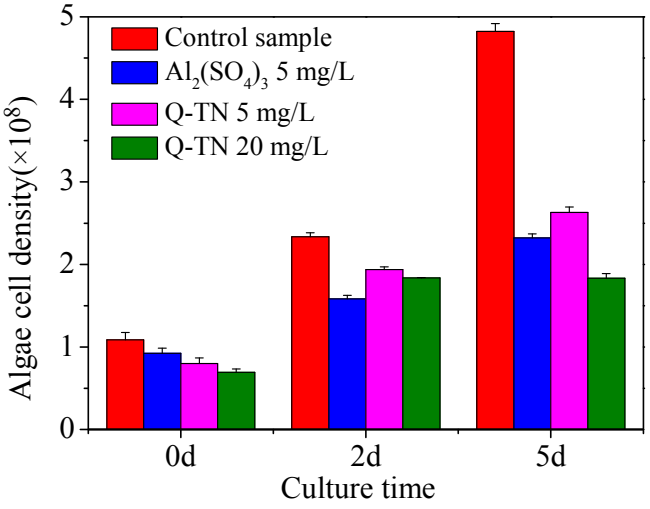


Figure S9. The cell density of MA cells in the supernatant after flocculation processes

3DEEMs of algae samples

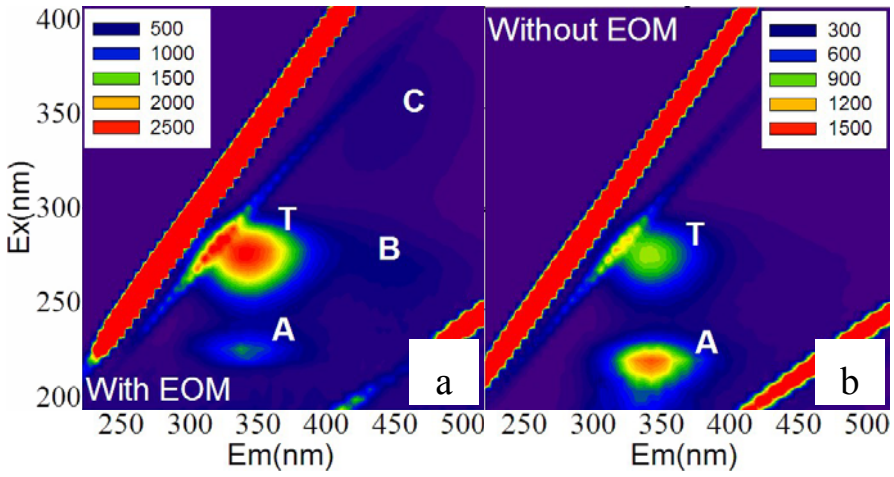


Figure S10. Three-dimensional excitation–emission matrix spectra for algae samples (initial algae density was ~2×10^9 cells/L) with EOM before centrifugation (a) and after centrifugation (b). Ex means the excitation wavelengths. Em means the emission wavelengths. The digital number in the white box is the emission intensity of fluorescent light.

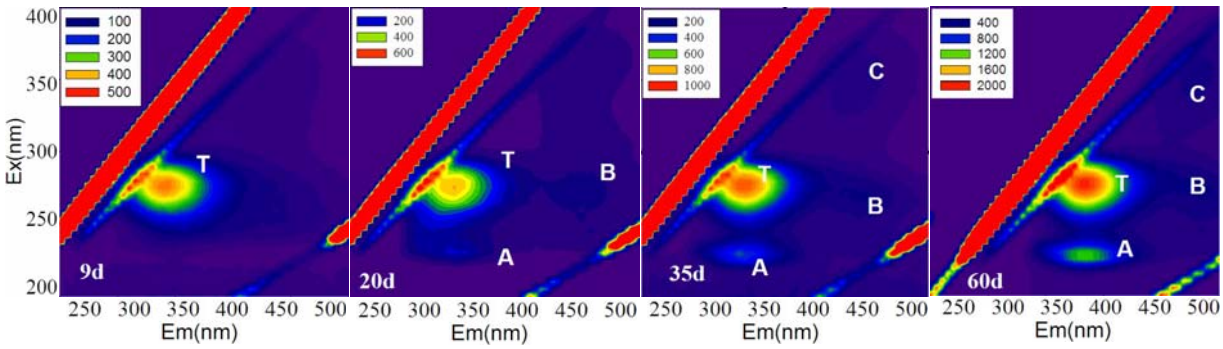


Figure S11. Three-dimensional excitation–emission matrix spectra of algae samples from different culture times. (initial algae density was ~1×10^9 cells/L, Day 9, 20, 35, and 60 were referred to early exponential phase, middle was exponential phase, early stationary phase, and late stationary phase, respectively.)