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RESEARCH ARTICLE

Characterization of extracellular polymeric substance (EPS) fractions produced by *Microcystis aeruginosa* under the stress of linoleic acid sustained-release microspheres

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Abstract This paper focuses on the characterization of extracellular polymeric substances (EPS), which are composed of soluble EPS (SL-EPS), loosely bound EPS (LB-EPS), and tightly bound EPS (TB-EPS) produced by *Microcystis aeruginosa* under the stress of linoleic acid (LA) and LA sustained-release microspheres. Three-dimensional excitation-emission matrix (3D-EEM) fluorescence spectroscopy and Fourier transform infrared (FTIR) spectrometry were used to characterize three forms of EPS while the content of polysaccharide and protein was tested, respectively. The results showed that the highest inhibitor rate (IR) occurred when *M. aeruginosa* were exposed to LA sustained-release microspheres of 0.3 g L^{-1}. The 3D-EEM contour demonstrated that tryptophan and protein-like substances were detected in all three EPS fractions, whereas humic acid-like substance was only distributed in SL-EPS, and aromatic proteins merely existed in LB-EPS and TB-EPS. The infrared spectrum showed that functional groups in three EPS fractions had no obvious change in all experimental groups. Polysaccharide (1120–1270 cm^{-1}, C–O–C and C–O stretching vibration) and protein (1384–1670 cm^{-1}, C–N and N–H stretching) were detected in three forms of EPS.

Keywords Algal inhibition · LA sustained-release microspheres · Extracellular polymer substance

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Introduction

Due to climatic change and nutrient enrichment, algal blooms spread globally and threaten the sustainability of freshwater ecosystems (Paerl and Paul 2012). Once the amount of cyanobacteria substantially increased, the water quality and human health would suffer colossal menace. Algal blooms will bring about not only the undesirable taste and odor but also the release of toxin and specific organics (Dixon et al. 2011; Fang et al. 2010). Over the past 20 years, there has been significant interest in the growth inhibition of cyanobacteria via allelochemicals released by aquatic macrophytes (Gross 2003; Sun et al. 1989; Zhang et al. 2007). Among all allelochemicals, unsaturated fatty acids have been studied most and some of them have been confirmed to significantly inhibit the growth of *Microcystis aeruginosa* (*M. aeruginosa*) (Gallardo-Williams et al. 2002; Tønnesen and Karlsen 2002). In our previous study, it was found that incorporation of linoleic acid (LA) into alginate-chitosan had a considerable algal control effect, which could release LA sustainably into distilled water up to 150 days; furthermore, these LA sustained-release microspheres could inhibit *M. aeruginosa* to the nongrowth state (Ni et al. 2015a). However, the changes of algal secretions (extracellular polymeric substances (EPS)) are unknown and this is vital to explore the algal inhibition mechanisms.

EPS are the products of cellular lysis and hydrolysis of macromolecules with a high molecular weight, and they usually secreted by microorganisms (Wingender et al. 2012). The

EPS typically are composed of polysaccharides, proteins, glycoproteins, glycolipids, and nucleic acids. Amphiphilic compounds not only provide nutrient source for periphytic organisms, but also serve as a protective barrier against toxic compounds, such as nonessential trace metals (Cogan and Keener 2004; Neu and Lawrence 2009). The cyanobacteria EPS are reported to be associated with aggregation formation and colony development, which can further protect cyanobacteria from unfavorable environment to survive (Pereira et al. 2009). Based on the binding force with cells, the microbial EPS are usually fractionated into soluble EPS (SL-EPS) (soluble macromolecules, colloids, and slimes) and bound EPS (sheaths, capsular polymers, condensed gels, loosely bound polymers, and attached organic materials) (Laspidou and Rittmann 2002; Qu et al. 2012). The SL-EPS are dissolved into solution while the bound EPS are closely bound with cells. The bound EPS inherently exhibit a dynamic double-layered structure and can be further divided into loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) for better understanding (Sheng et al. 2010; Xu et al. 2010). Many functional groups are found in EPS, such as carboxyl, phosphoric, sulfhydryl, phenolic, and hydroxyl groups, which can bind with not only heavy metals but also organic pollutants (Joshi and Juwarkar 2009; Liu et al. 2001). Chiou et al. (2010) investigated the attached EPS of three algae species (*Chlorella vulgaris*, *Chodatella* sp., *Microcystis* sp.) by Fourier transform infrared (FTIR) spectra and discovered that they had similar functional groups, while the major constituents of them are protein-like and polysaccharide-like substances. The tryptophan-like substances in the LB-EPS and TB-EPS were positively correlated with *Microcystis* growth, whereas in the SL-EPS, the tryptophan-like as well as humic-like substances were associated with the growth of *M. aeruginosa* (Xu et al. 2013). Previous studies often focused on the EPS secreted by algae under normal growth conditions; however, the investigation of *M. aeruginosa* EPS fractions under effective algaecides is limited, which was necessary for further study of algal inhibiting mechanism.

The aims and objectives of this study are (1) to compare the algal inhibition effect of pure LA and LA sustained-release microspheres, (2) to measure the polysaccharide and protein content of EPS fractions, and (3) to characterize EPS secreted by *M. aeruginosa* under the stress of pure LA and LA sustained-release microspheres.

Materials and methods

Algal cultivation

M. aeruginosa FACHB-905, purchased from Freshwater Algae Culture Collection of the Institute of Hydrobiology (China), was cultured in the laboratory at Hohai University with sterilized BG-11 medium. The algae were grown in 250-mL flasks with 50 mL sterilized culture medium (BG-11) at 25 °C under 40–60 μmol photons m^{-2} s^{-1} (14-h light/10-h dark). The algae were cultivated in batch cultures (transferred and inoculated once every 5–6 days) to ensure that the algae were all at the exponential growth phase throughout the experiment.

LA and LA sustained-release microspheres

The LA (purity ≥99%) was purchased from Aladdin Industrial Corporation. The optimal preparation of LA sustained-release microsphere was based on our previous study (Ni et al. 2015a). The LA was emulsified and a latent solvent (ethylis oleas) was added into sodium alginate solution. The mixture liquor was dripped into an aqueous acid solution containing chitosan and calcium chloride to form microspheres. After cooling to room temperature, the cross-linking agent glutaraldehyde was dosed at a mixing speed of 500 rpm on a magnetic stirrer (S25-2, Changzhou, China). The microspheres were rinsed several times with distilled water and petroleum ether after filter pumping to get wet LA sustained-release microspheres. Finally, the wet microspheres were air-dried. The LA sustained-release microspheres are shown in Fig. 1. From Fig. 1, the wet LA anti-algal sustained-release granules were shapely white spheres approximately 3 mm in diameter and the dry granules were 1 mm in diameter.

Algal inhibition test of LA and LA sustained-release microspheres

Algal (*M. aeruginosa*) inhibition tests were in accordance with the National Standard of China (GB/T 21805-2008). *M. aeruginosa* was inoculated into a culture medium in a 500-mL flask using 2×10^7 cells·mL^{-1} as the initial algal density. The experimental groups included a control group, a blank microsphere group (without LA in the microspheres), three pure LA groups with diverse concentration (0.1, 0.2, and 0.4 mg L^{-1}), and three LA microsphere groups with diverse concentration (0.1, 0.3, 0.5 g L^{-1}). There were three replicates for each group. All flasks were cultivated at 25 °C under 40–60 μmol photons m^{-2} s^{-1} (14-h light/10-h dark) conditions. Algal density was estimated according to a conversion of optical density every 3 days to obtain the optimal inhibition dose. The inhibition ratio (IR) was calculated by the following equation (Xu et al. 2007):

$$\textit{Inhibition Ratio}\ (\mathrm{IR}) = \left(1\text{-}\frac{N}{N_0}\right) \times 100\%$$

where N is the algal density (cell·mL^{-1}) of the treatment group and N_0 is the algal density (cell·mL^{-1}) of control group.

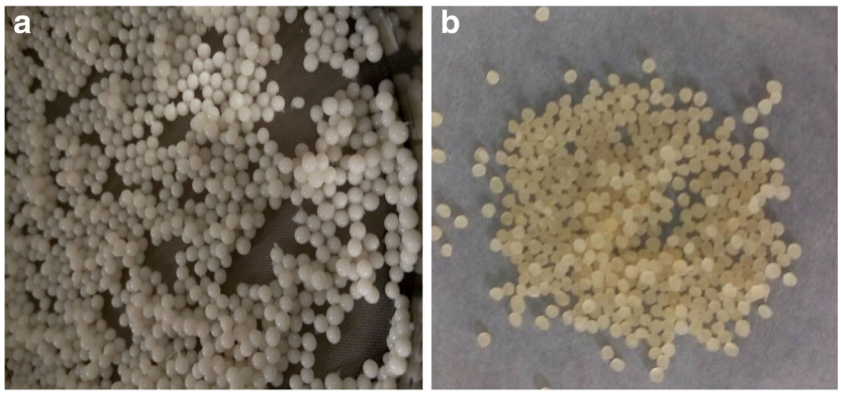

Fig. 1 Images of **a** wet LA microspheres and **b** dry LA microspheres

Extraction of EPS

The centrifugation method (Xu et al. 2013) was used in the extraction of EPS to obtain three EPS fractions. Firstly, culture samples were centrifuged at 2500*g* for 15 min at room temperature, and then, the supernatant was collected as SL-EPS in a 50-mL centrifuge tube. Secondly, the harvested algal samples were suspended with 0.05% NaCl solution and centrifuged at 5000*g* for 15 min, and the second supernatant was collected carefully for measurement of LB-EPS. Thirdly, the remaining harvested algal samples were resuspended with 0.05% NaCl solution and heated in thermostatic water bath at 60 °C for 30 min. The heated samples were centrifuged at 15,000*g* for 20 min, and the supernatant was collected as TB-EPS. Finally, all the collected EPS fractions were filtered by 0.45-μm PTFE membranes (Xingya Purification Materials Co., Shanghai, China) for test.

Quantification of polysaccharide and protein in EPS fractions

Polysaccharides and proteins are the main components of EPS, and the two compounds influence the concentration of EPS fractions. The phenol-sulfuric acid method was used to quantify polysaccharides with glucose as a standard, and the sample absorbance was measured at 485 nm (Dubois et al. 1956). The method of Bradford (1976) was usually applied for protein determination in EPS, with bovine serum albumin (BSA) (Shanghai Huixing Biotechnology Co. Ltd., China) as a standard, and the sample absorbance was measured at 595 nm.

Characterization of EPS

At day 12, the polysaccharide and protein both changed a lot. In order to study the composition and characteristic functional group of three EPS fractions secreted by *M. aeruginosa* (day 12) under different conditions, 24 samples of EPS were characterized by three-dimensional fluorescence excitation-emission matrix (3D-EEM) technology and FTIR analysis. 3D-EEM is a sensitive and selective method that only requires very small samples and will not destroy the structure of the samples. This technique can be used to elucidate the functional group variety and element compositions in EPS or microbial aggregates (Chabaliná et al. 2013). Twenty-four samples of EPS were assayed by the Hitachi F-7000 fluorescence spectrometer (Hitachi High Technologies, Tokyo, Japan) in scan mode with a 700-V xenon lamp at room temperature (25 ± 1 °C). Emission spectra were scanned from 250 to 550 nm with 5-nm increments, and excitation spectra were scanned from 200 to 450 nm with 5-nm increments. The spectra were recorded at a scan rate of 12,000 nm/min. The Ex slit bandwidth was 5 nm, and the Em slit bandwidth was 10 nm. FTIR spectrophotometer (Nicolet iS5, Thermo Fisher, USA) was used to identify the functional groups of EPS that were extracted from algae under different conditions. Samples were firstly freeze-dried, then mixed with potassium bromide (KBr) to form pellets at a mass ratio of 1:100 (2 mg EPS in 200 mg KBr), and scanned at a wavelength range of 4000–400 cm^{-1} to obtain the FTIR spectrum simultaneously. Baseline shifts were used to correct the baseline to reduce systematic errors.

Statistical analysis

Statistical analysis was performed using SPSS for Windows Version 17.0 (SPSS, Chicago, IL, USA). All data were first log transformed so that the normality requirement for ANOVA was met, and then analyzed using one-way ANOVA followed by a test for significance at the $p = 0.05$ level.

Results and discussion

Inhibition effect of LA sustained-release microspheres on *M. aeruginosa*

The algal density and inhibition ratio (IR) in different concentration groups are shown in Fig. 2. The algal density of all groups increased generally when exposed to pure LA

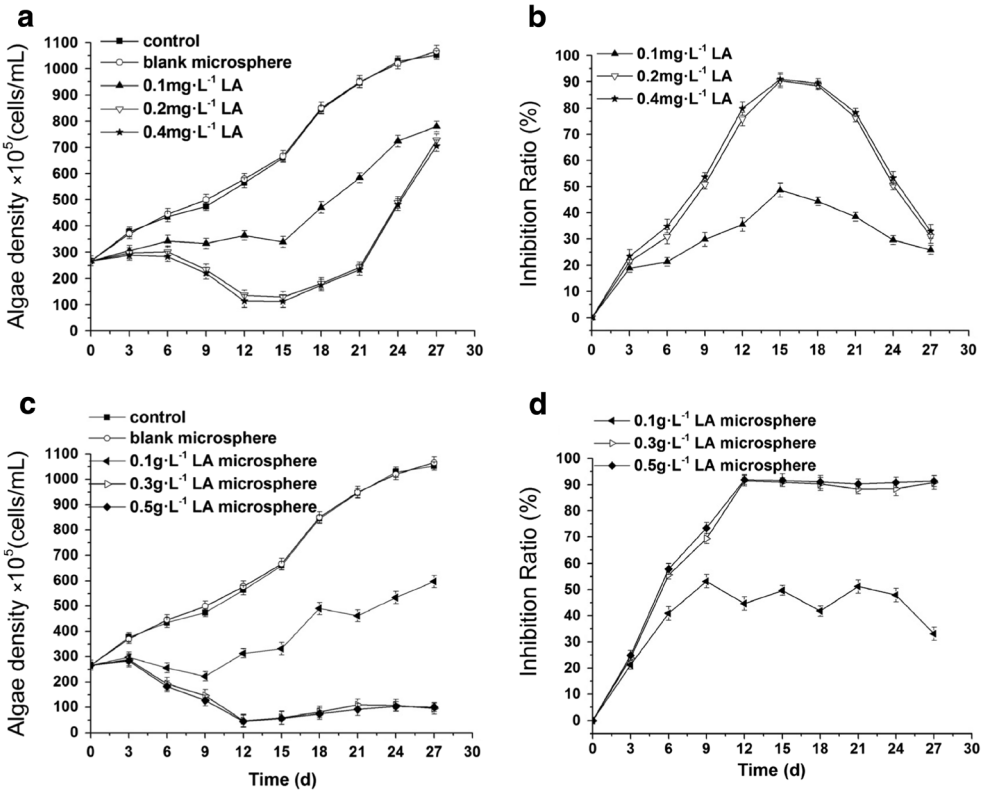

Fig. 2 Influence of different concentrations of pure LA and LA sustained-release microspheres on algae density (**a**, **c**), inhibition ratio (**b**, **d**) of *M. aeruginosa*

(Fig. 2a). The IRs increased first and then decreased (Fig. 2b). In lower-concentration group of pure LA (0.1 mg L^{-1}), the algal density was multiplied at a lower growth rate than control group. The highest IR was up to 48.6% on day 15. In higher-concentration group (0.2 and 0.4 mg L^{-1} LA), the growth tendency of algae was similar. The inhibition rate (Fig. 2b) presented the increasing trend and reached to highest value (90.3%) on day 15 as well. After 15 days, the algal density raised again in all pure LA groups, which implied that the algae recovered to grow (Fig. 2a). When exposed to 0.1 g L^{-1} LA microspheres, the growth curve of the algae was resemble with the control group especially after day 9. In high-concentration groups of LA microspheres (0.3 and 0.5 g L^{-1}), the algal density decreased after day 3 and was stable at 100 × 10^5 cells/mL latterly (Fig. 2c). The IRs exhibited a similar trend in two high-concentration groups: IR stayed around 95% after 12 days, which indicated that the LA microspheres had a strong and lasting algal inhibiting effect (Fig. 2d). The algal growth in the blank microsphere group was similar to the control group, which suggested that the blank microspheres had litter impact on algal growth. When using pure LA exclusively, the algal growth was inhibited first and then began to recover after a period of cultivation (day 15) as shown in Fig. 2a, b. This can be attributed to the degradation or transformation of the effective anti-algal components (Hong and Hu 2009). However, LA microspheres could continually release LA to replenish the effective anti-algal components and inhibit *M. aeruginosa* to a nongrowth state (Fig. 2c, d). Comparison of the results between 0.3 and 0.5 g L^{-1} LA microsphere groups revealed that these two dosages yielded stronger inhibitory effects on algal growth and the IRs both reached nearly 95% (Fig. 2c). It demonstrated a threshold effect (Chang et al. 2012) in these two groups, which declared little effect of the concentration augment (>0.3 g L^{-1}) on IR. This result was also consistent with our previous study (Ni et al. 2015b). Therefore, from an economic point of view, 0.3 g L^{-1} LA sustained-release microspheres could be the optimal dose with good anti-algal effect.

Contents of polysaccharide and protein in EPS fractions

Concentrations and variations of polysaccharide and protein in each EPS fraction are shown in Figs. 3 and 4. Contents of proteins in all groups were found to be higher than polysaccharides. The results showed that the polysaccharide concentration changed from 7.7 to 50.4 μg/10^7 cells and the protein concentration changed from 31.8 to 66.4 μg/10^7 cells during the whole test. Figure 3a–h shows that polysaccharide concentration in EPS was higher when exposed to the LA sustained-release microsphere group than that in pure LA groups. Samples in the control group contained the lowest content of polysaccharides in EPS. Above all, it could be summarized that polysaccharides in EPS acted as a defender when the algae suffered from the algal inhibitor (LA and LA microspheres) (Stadnik and Freitas 2014). The distribution of

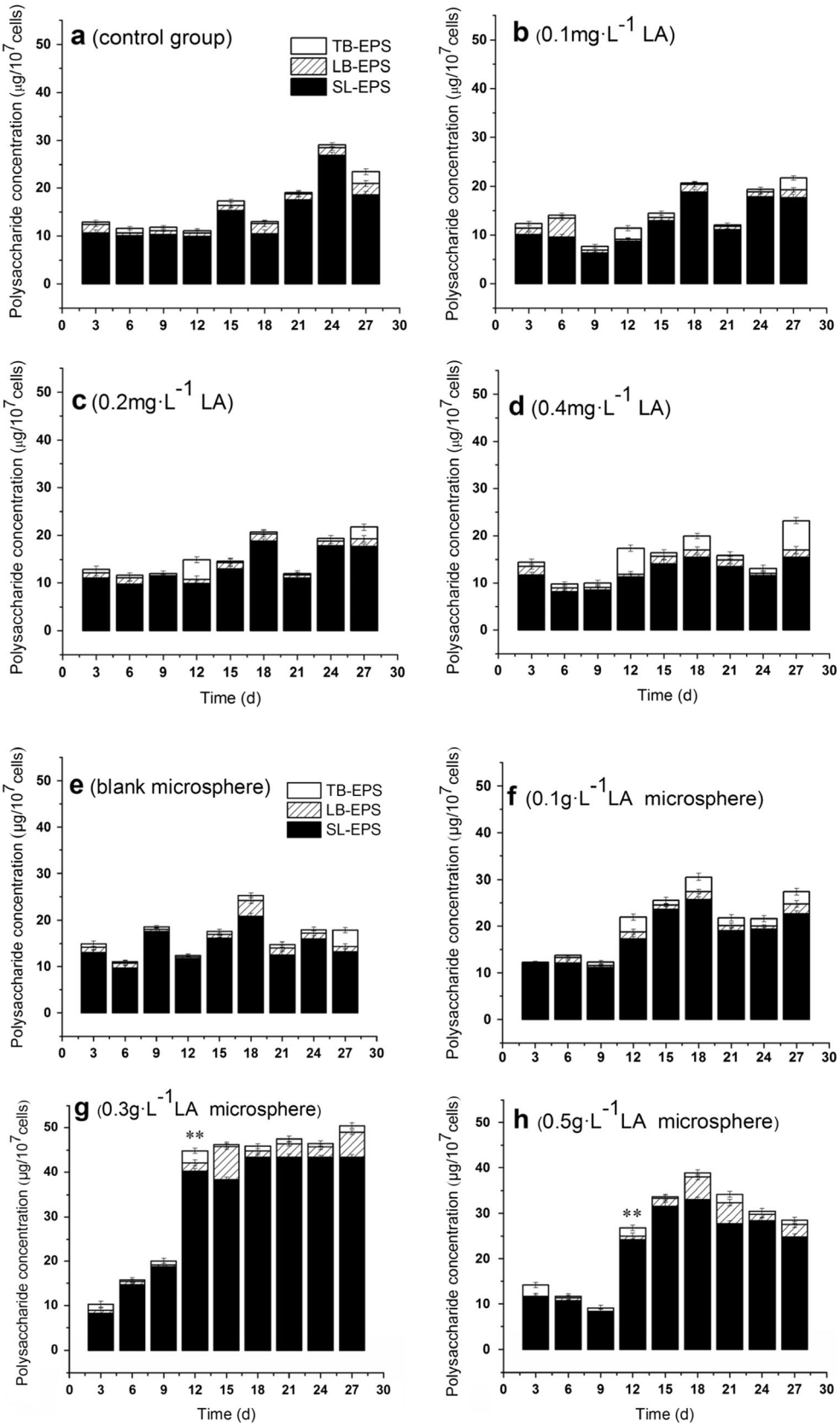

Fig. 3 Variations of polysaccharides in EPS excreted by *M. aeruginosa* exposed to different concentrations of LA and LA sustained-release microspheres. **a** Control group, **b** 0.1 mg L^{-1} LA, **c** 0.2 mg L^{-1} LA, **d** 0.4 mg L^{-1} LA, **e** blank microsphere, **f** 0.1 g L^{-1} LA microsphere, **g** 0.3 g L^{-1} LA microsphere, and **h** 0.5 g L^{-1} LA microsphere

polysaccharides in three EPS fractions was different. There was a large amount of polysaccharides in SL-EPS but only a few of them in LB-EPS and TB-EPS. The results clearly suggested that most polysaccharides were scattered in the

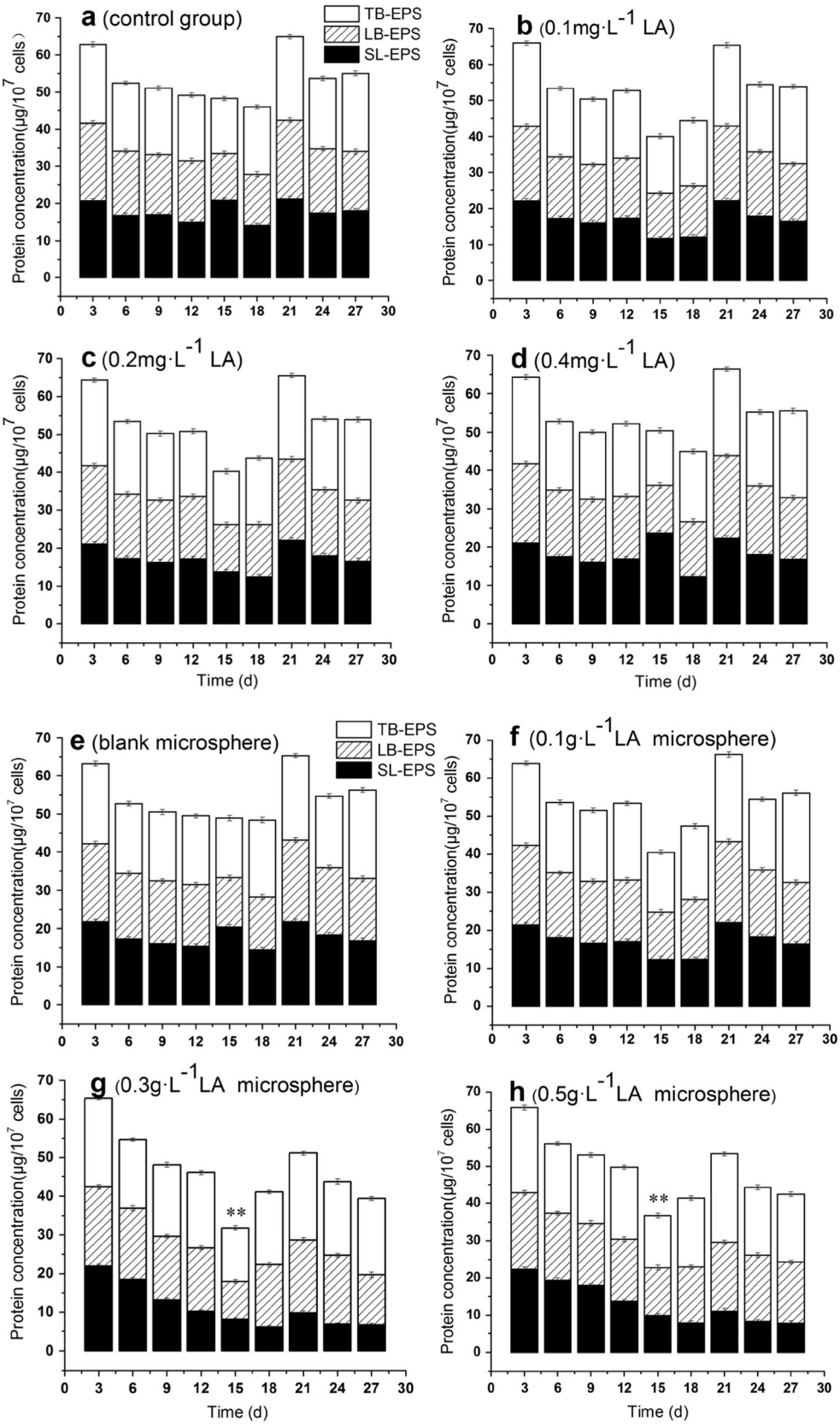

Fig. 4 Variations of proteins in EPS excreted by *M. aeruginosa* exposed to different concentrations of LA and LA sustained-release microspheres. **a** Control group, **b** 0.1 mg L^{-1} LA, **c** 0.2 mg L^{-1} LA, **d** 0.4 mg L^{-1} LA, **e** blank microsphere, **f** 0.1 g L^{-1} LA microsphere, **g** 0.3 g L^{-1} LA microsphere, and **h** 0.5 g L^{-1} LA microsphere

outermost layer of extracellular polymer providing first line of defense from external environmental pressure (De Philippis and Vincenzini 1998). From Fig. 3a–d, no significant change of polysaccharide concentration was found between the

control group and the pure LA groups ($p > 0.05$). However, in high-concentration LA microsphere groups (0.3 and 0.5 g L^{-1}), polysaccharide concentration increased significantly in SL-EPS especially after day 12 (Fig. 3g, h) compared to other groups ($p < 0.01$). The total polysaccharide concentration (in 0.3 g L^{-1} LA microsphere group) reached up to 45 μg/10^7 cells, which was more than twice amount in pure LA groups and control group.

Figure 4 shows the changes of protein contents in each EPS fraction and the change trend of protein was contrary to the polysaccharides. In the control group and pure LA groups, protein was evenly distributed in three EPS fractions and total protein contents had a slight decline near day 15 (Fig. 4a–d). However, in high-concentration microsphere groups (0.3 and 0.5 g L^{-1}), the concentration of protein had a substantial decline in SL-EPS ($p < 0.01$) while had little change in LB-EPS and TB-EPS on day 15 (Fig. 4g, h). At the same time, the total amount of protein was 31.6 μg/10^7 cells in 0.3 g L^{-1} LA microsphere group, which was lower than the blank microsphere group (50.3 μg/10^7 cells).

Based on Figs. 2, 3, and 4, it could be concluded that great changes have taken place in *M. aeruginosa* after 12 days. The algal density decreased to the lowest level no matter exposure to pure LA (≥0.2 mg L^{-1}) or LA microspheres (≥0.3 g L^{-1}), and the IR could both reached to the highest (95%), while the inhibition effect of LA microspheres on *M. aeruginosa* was more long-term than pure LA. At the same time, the polysaccharide concentration in EPS increased a lot and the protein concentration decreased conversely. These variations indicated that the *M. aeruginosa* and its secretions were affected by LA to some extent. The inhibited algae would secrete more polysaccharides in EPS. Therefore, the polysaccharides in EPS play a more important role in protecting algae from the external environment pressure than protein (Xiao and Zheng 2016). The actual mechanism of this effect deserved further study.

Fluorescence EEM

In this study, 24 fluorescence EEM spectra of EPS produced by *M. aeruginosa* were collected on day 12. The EEM contours of three EPS are depicted in Figs. 5, 6, and 7. According to Fig. 5, it was found that only one peak (peak A) was present in SL-EPS fraction in control and pure LA groups. Here, peak A (275/320–335 nm) belongs to a protein-like substance containing tryptophan (Ismaili et al. 1998, Reynolds and Ahmad 1997). The similar peak means that pure LA almost has little effect on protein in SL-EPS. So it could be concluded that pure LA was almost exhausted completely after day 12, which was corresponded with the earlier results of the declining algal IR after day 12. Another peak (345/430 nm, peak C) which attributed to humic-like substances (Chen et al. 2003; Mounier et al. 1999) was determined in SL-EPS. They probably originated from the decomposition of dead cells or macromolecular organics such as proteins (Parlanti et al. 2000). Unlike fluorescence spectra of SL-EPS, another peak (peak B) was detected in LB-EPS when exposed to pure LA and LA sustained-release microspheres (Fig. 6). Peak B (230–235/305–330 nm) was identified as aromatic proteins including tyrosine (aromatic proteins 220–250/280–380 nm) (Ahmad and Reynolds 1999). At the same time, the second peak was located at 280/330 nm (peak A) in LB-EPS in all groups. It also belonged to tryptophan substances and protein-like substances (Li et al. 2012). However, there were some differences in LA substance-release groups that the intensities of peak A and peak B in LB-EPS were both stronger than those in the control and pure LA groups. It is concluded that the LA sustained-release microspheres may increase the aromatic proteins and tryptophan substances in LB-EPS. In TB-EPS fractions, peak A (275–280/325–345 nm) and peak B (225/305–345 nm) showed up in each group (Fig. 7), which implied the existence of tryptophan, protein-like substances, and aromatic proteins. The results of 3D-EEM contour demonstrated that tryptophan and protein-like substances were detected in three EPS fractions, whereas humic acid-like substance was only distributed in SL-EPS. Aromatic proteins merely exist in LB-EPS and TB-EPS. The main compositions in bound EPS fractions were similar. It was also proved that the environmental pressure exerted more influence on SL-EPS than bound EPS.

FTIR spectroscopy

FTIR spectra of SL-EPS, LB-EPS, and TB-EPS excreted by *M. aeruginosa* are shown in Fig. 8. The spectra of SL-EPS were similar under the effect of pure LA and LA sustained-release microspheres (Fig. 8a, d). Some stronger bands were detected, which were assigned to the stretching vibration of O–H at 3430–3470 cm^{-1}, the C=O stretching vibration of carboxylic group at 1790 cm^{-1}, the C=O and C–N (amide I) stretching vibrations in protein at 1648–1669 cm^{-1}, the C–H bending in methyl and C–N stretching (amide III) at 1384 cm^{-1}, and the C–O–C stretching vibrations at 1120–1159 cm^{-1} (Chen et al. 2013; Guibaud et al. 2005; Zhu et al. 2012). The visible weak bands at 2851–2927 cm^{-1} were attributed to C–H stretching vibrations of aliphatic CH_2 group (Hung and Liu 2006). In addition, the bands below 900 cm^{-1} could be assigned to the fingerprint region phosphate or sulfur functional groups (You et al. 2015) and the unsaturated bonds in the sample (Guibaud et al. 2005). This suggested that functional groups in SL-EPS changed little under the effect of pure LA and LA sustained-release microspheres. Figure 8b, e shows that LB-EPS excreted by naturally growing algae only had one band at 1174 cm^{-1}, which belonged to the C–O–C stretching vibration of polysaccharides (Chen et al. 2013), while the bands at 1384, 1657, 1790, 2925, and 3445 cm^{-1}

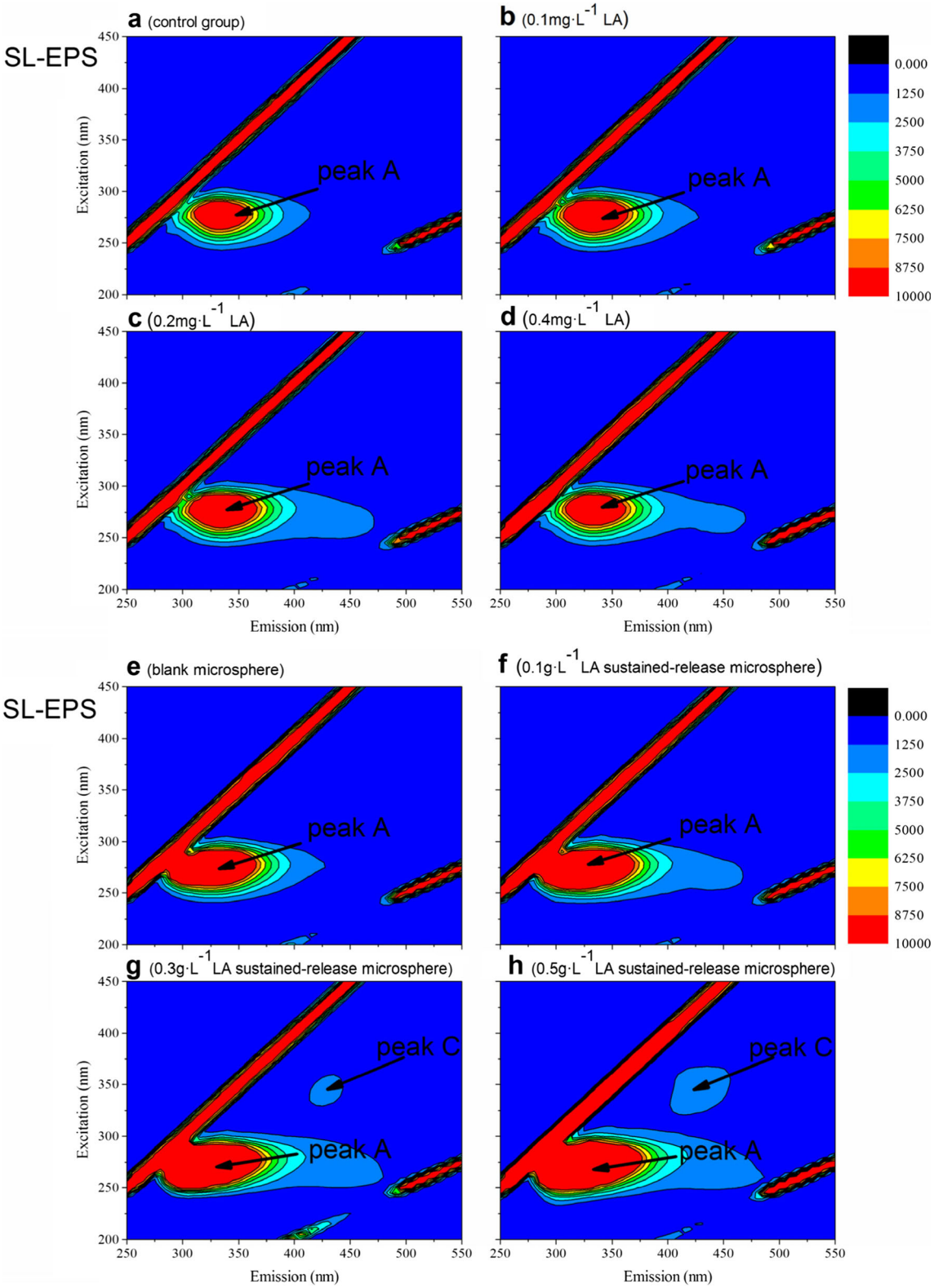

Fig. 5 Typical EEM contours of SL-EPS fractions excreted by *M. aeruginosa* exposed to different concentrations of LA and LA sustained-release microspheres at the 12th day. **a** Control group, **b** 0.1 mg L^{-1} LA, **c** 0.2 mg L^{-1} LA, **d** 0.4 mg L^{-1} LA, **e** blank microsphere, **f** 0.1 g L^{-1} LA sustained-release microsphere, **g** 0.3 g L^{-1} LA sustained-release microsphere, and **h** 0.5 g L^{-1} LA sustained-release microsphere

were all strengthened when exposed to pure LA and LA sustained-release microspheres, which suggested that the content of polysaccharides and protein in LB-EPS increased. These results corresponded with the fluorescence EEM and

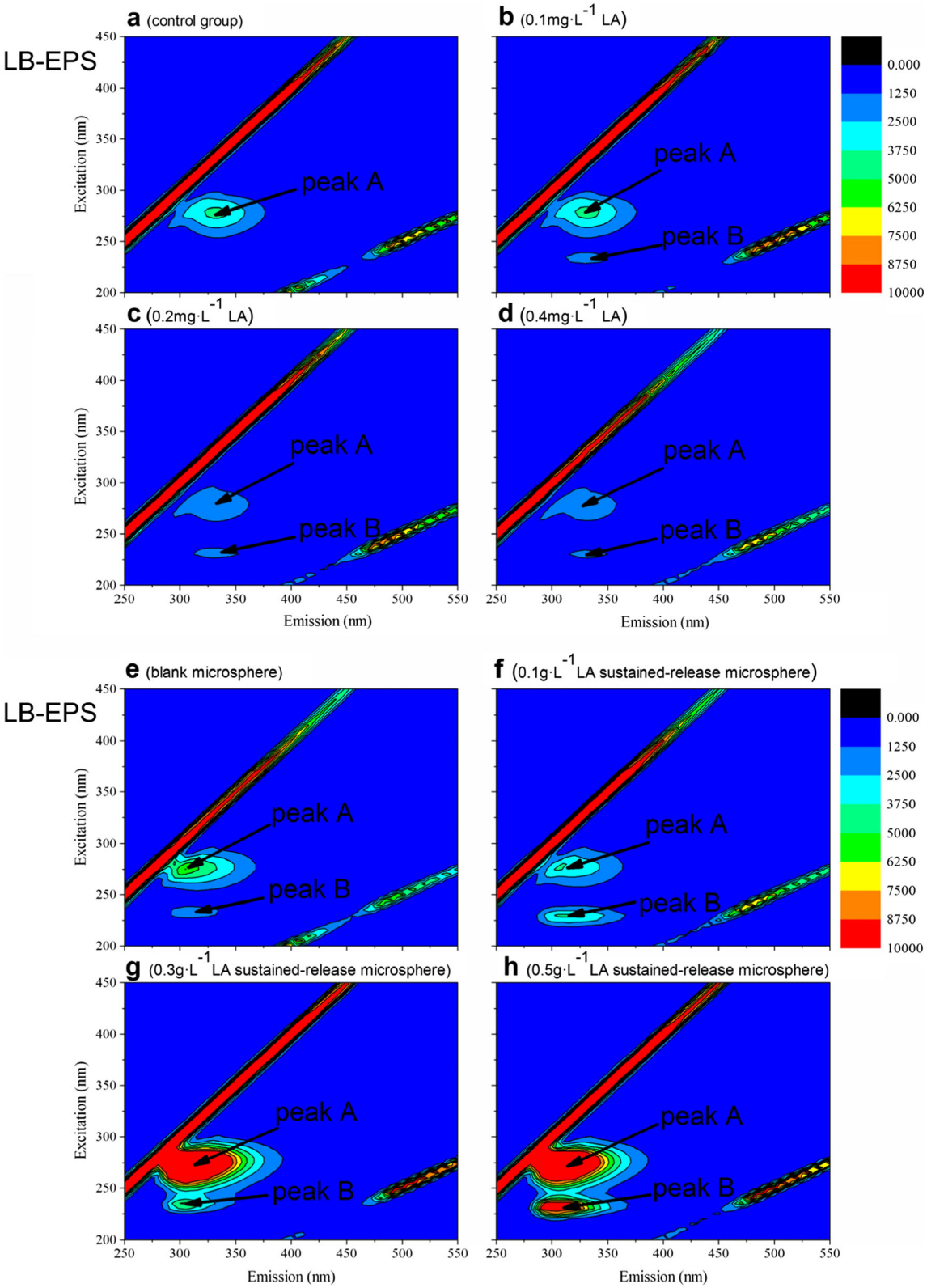

Fig. 6 Typical EEM contours of LB-EPS fractions excreted by *M. aeruginosa* exposed to different concentrations of LA and LA sustained-release microspheres at the 12th day. **a** Control group, **b** 0.1 mg L^{-1} LA, **c** 0.2 mg L^{-1} LA, **d** 0.4 mg L^{-1} LA, **e** blank microsphere, **f** 0.1 g L^{-1} LA sustained-release microsphere, **g** 0.3 g L^{-1} LA sustained-release microsphere, and **h** 0.5 g L^{-1} LA sustained-release microsphere

LB-EPS component analysis. In addition, there were slight band shifts especially in SL-EPS from 1159 to 1120 cm^{-1}, from 1648 to 1629 cm^{-1}, and from 2851 to 2927 cm^{-1}, which meant that LA influenced more on SL-EPS than bound EPS.

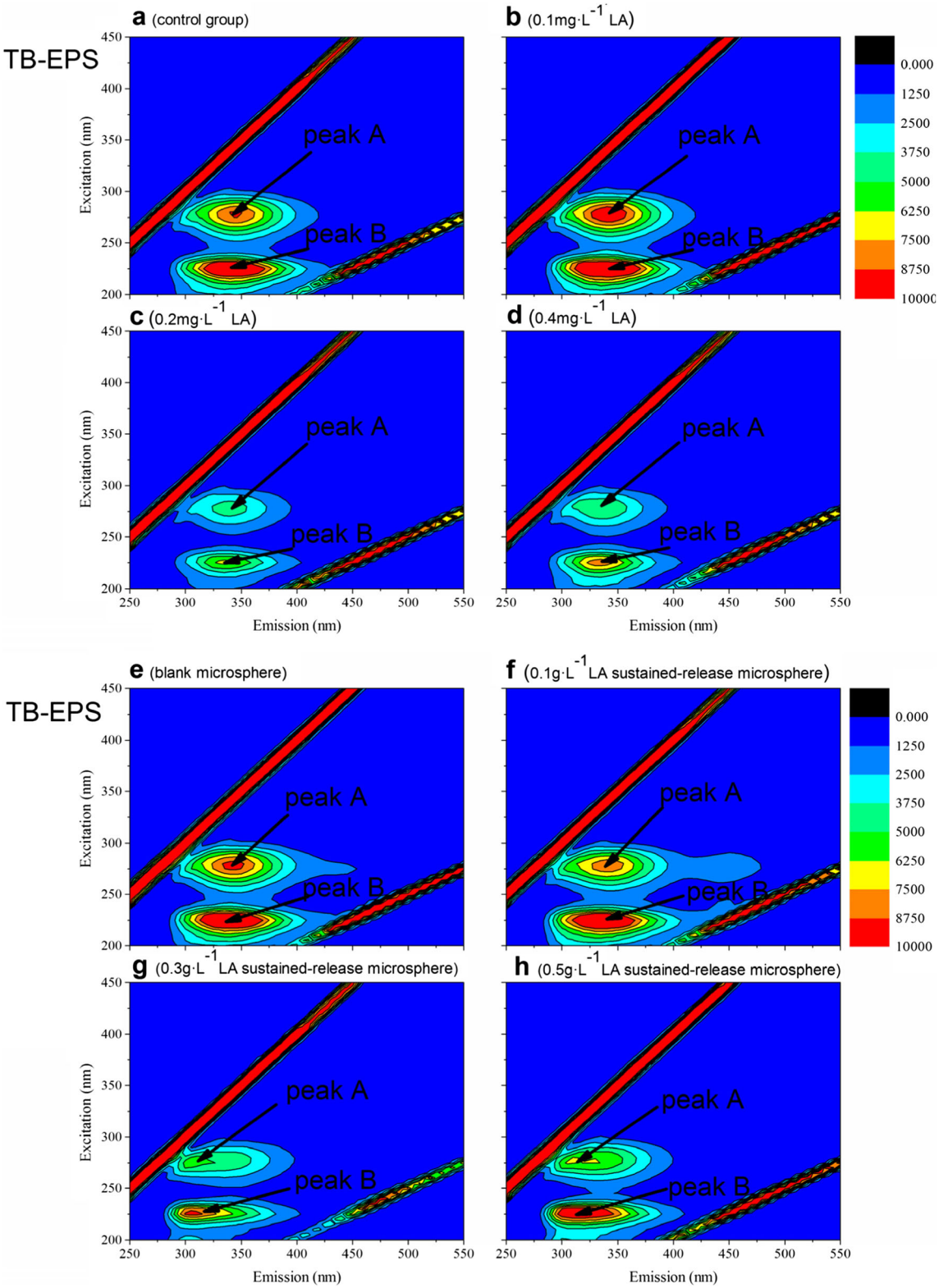

Fig. 7 Typical EEM contours of TB-EPS fractions excreted by *M. aeruginosa* exposed to different concentrations of LA and LA sustained-release microspheres at the 12th day. **a** Control group, **b** 0.1 mg L^{-1} LA, **c** 0.2 mg L^{-1} LA, **d** 0.4 mg L^{-1} LA, **e** blank microsphere, **f** 0.1 g L^{-1} LA sustained-release microsphere, **g** 0.3 g L^{-1} LA sustained-release microsphere, and **h** 0.5 g L^{-1} LA sustained-release microsphere

While in TB-EPS (Fig. 8c, g), under the effect of LA sustained-release microspheres, the band at 1790 cm^{-1} (C=O stretching vibration) disappeared owing to the absence of carboxylic acids and increase of protein. It meant that there were

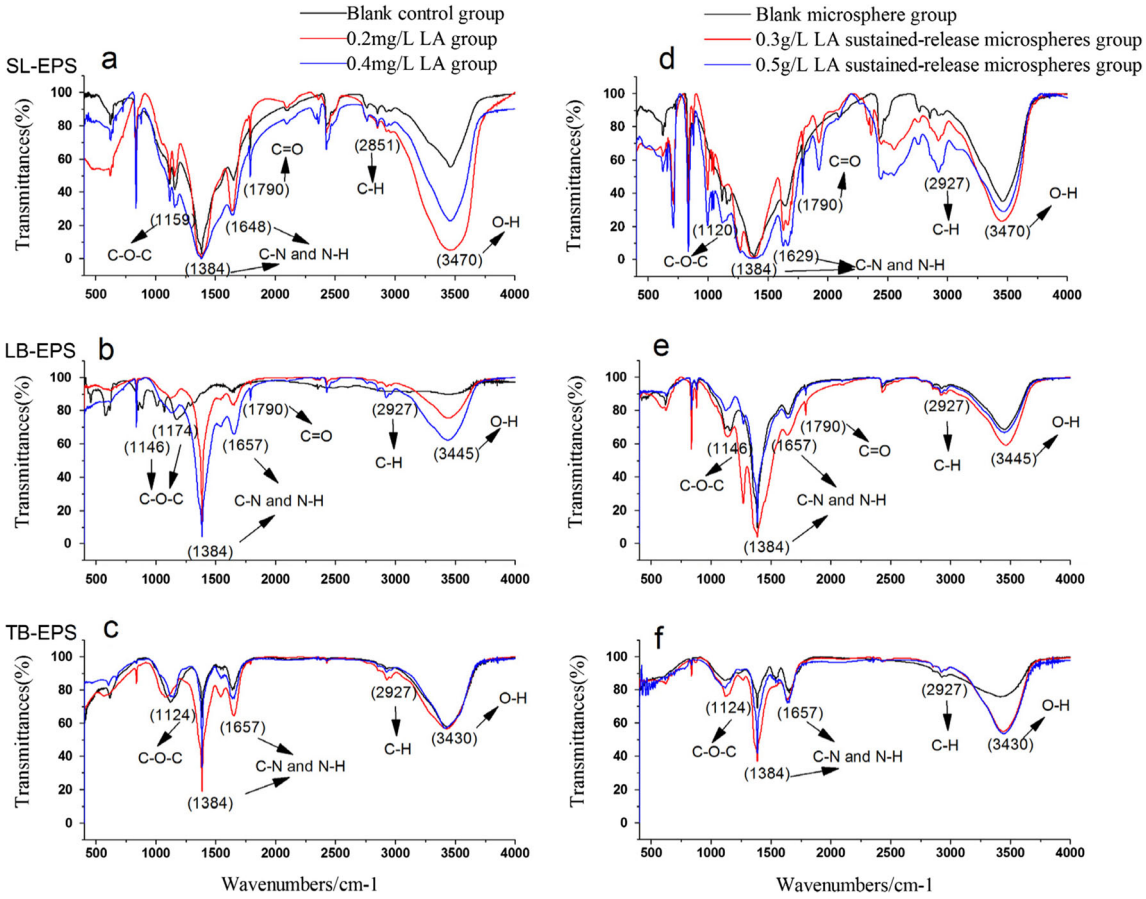

Fig. 8 FTIR spectrum of EPS excreted by *M. aeruginosa* exposed to different concentrations of LA and LA sustained-release microspheres at the 12th day. **a**, **d** SL-EPS. **b**, **e** LB-EPS. **c**, **f** TB-EPS

more proteins in TB-EPS than in SL-EPS and LB-EPS, which conformed to the protein change trend and was similar to the previous study by Cai and Singh (1999).

Conclusion

It was demonstrated that both pure LA and LA sustained-release microspheres have inhibitory effect on the growth of *M. aeruginosa*, while the LA sustained-release microspheres (95%) were superior and long-lasting to pure LA (90.3%). The optimal dose with good anti-algal effect of LA sustained-released microspheres was 0.3 g L^{-1}. The LA sustained-released microsphere can inhibit the algae to nongrowth state. At the same time, the algae will produce a lot of EPS during the inhibition process. The 3D-EEM contour demonstrated that tryptophan and protein-like substances were detected in three EPS fractions, whereas humic acid-like substance was only distributed in SL-EPS, and aromatic proteins merely existed in LB-EPS and TB-EPS. Polysaccharides were mostly found in SL-EPS while only a very small number of polysaccharides existed in LB-EPS and TB-EPS. It is suggested that polysaccharides are the protective layer of algae when the algae suffer from the external pressure. Contrary to polysaccharide content, protein in SL-EPS displayed an obvious decline. The FTIR spectra results suggested that protein (1648–1669 cm^{-1}) and polysaccharide-like substances (1120–1159 cm^{-1}) were the major components in three forms of EPS. The results of this study indicated that algae would lose activities under the stress of LA sustained-release microspheres.

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