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COMPARISON OF PACKING MEDIA ON STRATIFIED EPS AND THEIR ROLE IN THE BIOFILM OF TRICKLING BIOFILTER

The research focused on such packing media as ceramsite, polypropylene balls, and elastic fillers, and analyzed the main characteristics of extracellular polymeric substances (EPS) in their filter biofilms. The EPS were categorized as soluble EPS (S-EPS) and bound EPS (B-EPS). The component characteristics of stratified EPS were investigated via UV-Vis spectroscopy and a three-dimensional excitation-emission matrix (3D-EEM). The results showed that the EPS content of ceramsite biofilm was 245.2 mg/g VSS, which was higher than those of elastic filler material and polypropylene ball by 1.26 and 1.51 times, respectively. The protein (PN) and polysaccharide (PS) ratio of EPS in the ceramsite filter material was highest in S-EPS and B-EPS, indicating that the EPS have a stable structure. More than 67.58% of EPS formed by ceramsite was tightly bound EPS (TB-EPS), which was beneficial to maintaining the stability of biofilms. The levels of proteins (PN) and humic substances formed by ceramsite contained in the TB-EPS were higher than those of elastic filter material and polypropylene balls. 3D-EEM fluorescence spectra revealed that TB-EPS formed by a ceramsite contained high concentrations of tryptophan, tyrosine, and humic substances. The dehydrogenase activity of biofilm formed by ceramsite filler was higher than that formed by elastic fillers and polypropylene balls.

1. INTRODUCTION

Biological trickling filters (BTPFs) contain microorganisms used to purify wastewater that adhere to the filter material to form a biofilm [1]. Microbial biofilms play an important role in pollutant removal and reactor operation [2]. Effective media play a key role in the development of microbial community/biofilm of trickling filters [3]. Ceramsite, polypropylene balls, and elastic fillers are durable, insoluble, and resistant to spalling. They have

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high specific surface areas; their voids plug and limit the passage of liquid and air [4]. However, studies on the biofilm characteristics of different filter media of BTPFs are lacking. Thus, biofilm characteristics must be studied in-depth and comprehensively [5]. The major components of biofilm are microorganisms and extracellular polymeric substances (EPS) [6]. EPS are generally categorized as soluble EPS (S-EPS) and bound EPS (B-EPS) [7]. B-EPS are further classified into loosely bound EPS (LB-EPS) in the outer layer and tightly bound EPS (TB-EPS) in the inner layer; stratified EPS have different physical and chemical characteristics [8]. EPS play an essential role in the mechanical stability, surface adhesion, and formation of biofilms [9]. EPS can affect the performance of wastewater treatment by changing biofilm characteristics [10]. Characterizing the compositions and variations in EPS can help in understanding the mechanism of pollutant removal and explaining the potential drivers behind biofilm functions [6]. In addition, the spatial structure and composition of EPS have important effects on the structure and stability of biofilms and determine the size of mass transfer resistance of the matrix in biofilms. Therefore, the research on the composition and distribution of EPS organic matter is important [11]. However, limited studies compared the effects of different packing media on stratified EPS and their role in biofilms of trickling biofilters.

In this research, the effects of three packing media (ceramsite, polypropylene balls, and elastic fillers) on stratified EPS have been investigated to find the relationships between packing media and EPS and analyze the main characteristics of EPS of different packing media of filter biofilms and the organic composition characteristics of biofilms. The EPS were categorized as S-EPS and B-EPS, and UV-Vis spectroscopy and 3D-EEM were used to analyze the components of stratified EPS. The main purpose was to investigate the effects of different filter media on the EPS characteristics of biofilters and their role in biofilm formation.

2. MATERIALS AND METHODS

Experimental setup and operation. The experimental device consisted of a 40 dm^3 water tank, a water pump, a uniform water distributor, and three filter columns. The height of the filter column was 1200 mm, and the inner diameter was 150 mm (Fig. 1).

Activated sludge and synthetic wastewater. The simulated wastewater was composed of $C_6H_{12}O_6$ (carbon source, 490 mg/dm^3), NH_4Cl (nitrogen source, 50 mg/dm^3), K_2HPO_4, and KH_2PO_4 (phosphorus source, 10 mg/dm^3). The activated sludge was collected as the reflux sludge from a secondary sedimentation tank in Wangtang Wastewater Treatment Plant (Hefei, China). The activated sludge (40 dm^3) was cultivated in a water tank fed with $C_6H_{12}O_6$ (490 mg/dm^3), NH_4Cl (50 mg/dm^3), K_2HPO_4, and KH_2PO_4 (10 mg/dm^3) at 20±4 °C and aerated air 6 h every day, with daily replacement of nutrient medium for one week [5].

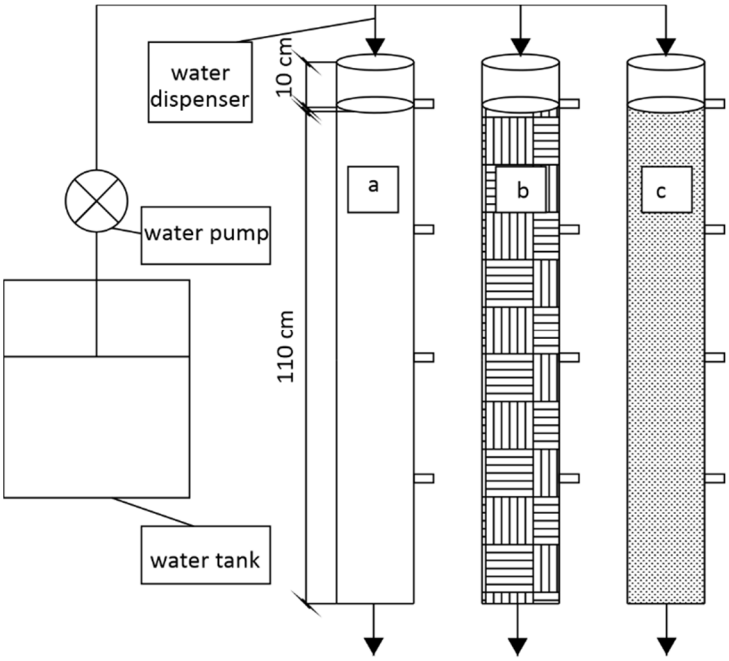

Fig. 1. Experimental setup of a biological trickling filter:
a – ceramsite, b – elastic fillers, c – polypropylene ball

Ceramsite, elastic fillers, and polypropylene balls were selected as experimental packing media (Table 1). Each filter column was filled with packing media (110 cm) and inoculated with activated sludge (10 dm^3, 0.5 g/dm^3) twice a day to ensure full contact of the sludge and filter material. After 3 days, the columns were fed with simulated domestic wastewater. A peristaltic pump (Millipore) operated in continuous mode was used to pump the simulated domestic wastewater to the columns at a flow rate of 5 dm^3/h. BTPFs run four cycles a day, six hours per cycle, run for 4 h and still for 2 h.

Table 1

Physical properties of fillers applied in the study

Filler	Effective particle size [mm]	Density [g/cm^3]	Specific surface area [m^2/m^3]	Volume weight [g/cm^3]	Porosity [%]	Water absorption [%]
Ceramsite	25	1.1	398	0.9	53	32
Elastic filler	150	0.003	762	0.002	41	21
Polypropylene balls	25	0.11	406	0.08	32	17

Biofilm and EPS extraction. The filter material was removed from the reactors, and its surface was washed with high-purity water. Then the filter material was added with high-purity water and centrifuged at 3000 rpm for 5 min, after which the suspension was collected [12]. Biofilm samples were collected and stored at 4 °C.

S-EPS, LB-EPS, and TB-EPS polymers were extracted during the extraction of the extracellular polymer of the biofilms [13]. A total of 10 cm^3 biofilm suspension was centrifuged at 4 °C and 2000 g for 15 min, and the supernatant was filtered through a 0.45 μm filter. The filtrate was collected to obtain a soluble extracellular polymer, and 10 cm^3 of high-purity water was added to the bottom of the centrifuge tube. Then, centrifugation was performed at 4 °C and 5000 g for 15 min, the supernatant was filtered through a 0.45 μm filter, and the filtrate was collected to obtain the LB-EPS. Then, 10 cm^3 of high-purity water and 10 cm^3 of 2% ethylenediaminetetraacetic acid were added to the sediment at the bottom of the tube, which was afterward placed in a constant temperature shaker, and reacted at 4 °C and 200 rpm for 3 h. Next, the mixture was centrifuged at 4 °C and 10 000 g for 15 min. The supernatant was filtered with a 0.45 μm filter, and the filtrate was collected to obtain TB-EPS.

Biofilm and EPS analysis. The biomass was characterized by suspended solids (SS) using the gravimetric method [1]. The biofilm activity was characterized by dehydrogenase activity and measured by the triphenyltetrazolium chloride (TTC) colorimetric method [14].

Extracted EPS samples were stored at 4 °C. The EPS were analyzed by the method described by Yu et al. [15]. The protein (PN) content was determined by the modified Lowry method, using bovine serum albumin (A116563 – 5 g, Aladdin, Shanghai Jingchun Biochemical Technology Co., Ltd., Shanghai, China) as the standard substance. The polysaccharide (PS) content was measured by the anthrone method, with glucose as the standard substance. A UV-Vis spectrophotometer (Shimadzu UV-3600, Japan) was used with scanning wavelengths ranging from 200 nm to 500 nm to determine S-EPS, LB-EPS, and TB-EPS.

3D-EEM analysis. The EEM spectra were gathered at the scanning emission (Em) range of 210–750 nm at 5 nm increments by varying the excitation (Ex) wavelength from 200 nm to 600 nm. The slit width was 5 nm for all samples.

Statistical analysis. SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. All the results of assays were expressed as mean±standard deviation. Analysis of variance was used to test the significance of results, and $p < 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1. EPS CONTENT AND COMPOSITION

Figure 2 shows the EPS content of the BTPF reactor under different packing conditions. The EPS showed significant differences among the fillers. The content of total EPS (T-EPS) in the ceramsite filler reached up to 245.2 mg/g VSS, which was larger than those on polypropylene balls and elastic fillers by 1.51 and 1.26 times, respectively. The PN content of EPS on ceramsite filler was 180 mg/g VSS, which was higher than those on polypropylene balls and elastic fillers, especially polypropylene ball fillers. The polysaccharide (PS) of ceramsite filler was 56.2 mg/g VSS, which was higher than that of the others. The PN/PS value of ceramsite filler was the largest, which means that the EPS exhibited better secretion and structural stability than the other two fillers. The higher contents of EPS matrix PN than those of PS was consistent with the results of previous studies [16]. PN/PS ratio may help maintain the structure and stability of the EPS matrix. Thus, ceramsite which formed a stable structure of EPS is a better filler than elastic fillers and polypropylene ball.

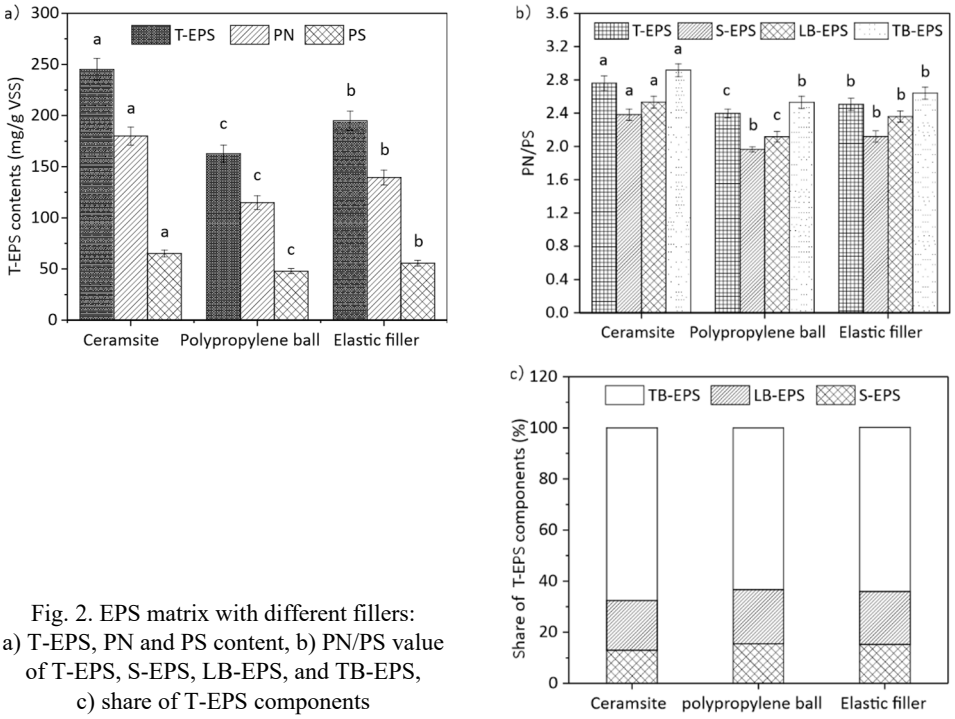

Fig. 2. EPS matrix with different fillers: a) T-EPS, PN and PS content, b) PN/PS value of T-EPS, S-EPS, LB-EPS, and TB-EPS, c) share of T-EPS components

The PN/PS value of the ceramsite filler was higher than those of polypropylene ball and elastic fillers in S-EPS, LB-EPS, and TB-EPS. The higher the value of PN/PS, the

greater content of EPS and the more stable the structure is [17]. Especially in TB-EPS, the ceramsite filler achieved the largest PN/PS value, which implies that ceramsite filler EPS has better secretion and structural stability than polypropylene balls and elastic fillers. The TB-EPS of ceramsite filler accounted for 67.58% of the T-EPS (Fig. 2b), which was higher than that of elastic filler and polypropylene balls. TB-EPS was a part of the binding to cellular secretion, and the high proportion is conducive to maintaining the stability of the biofilm structure [18]. Compared with the other two fillers, the ceramsite filler has a rough surface, mesoporous structure, and rich void, which are more conducive to the growth of biofilms [19].

3.2. ANALYSIS AND CHARACTERIZATION OF EPS

Figure 3 shows the observed UV-Vis spectra. All EPS of ceramsite, elastic filler, and polypropylene spheres exhibited two absorption bands at 210–220 and 250–285 nm; these absorption bands are attributed to amide bonds in PNs, carboxyl groups or esters in EPS, and EPS aromatic and polyaromatic compounds [20].

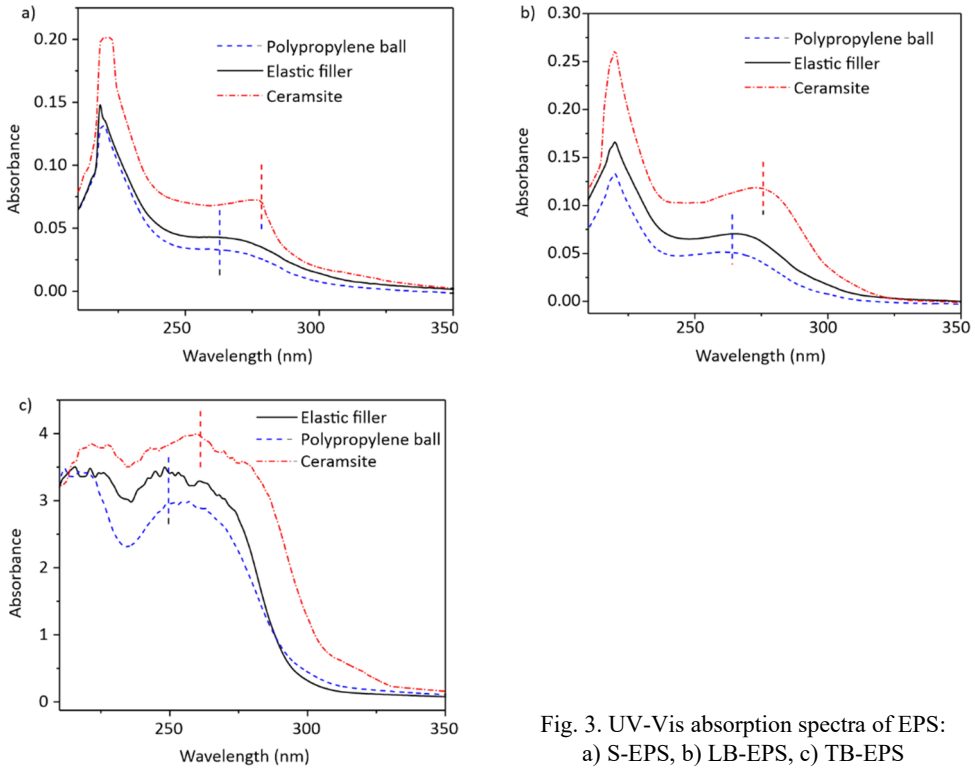

Fig. 3. UV-Vis absorption spectra of EPS: a) S-EPS, b) LB-EPS, c) TB-EPS

The two peaks of ceramsite filler in S-EPS, which were due to the amide bond in the PN and PN aromatic compounds and polyaromatic compounds, were higher than those of elastic fillers and polypropylene balls; the UV absorption peak of ceramsite filler at 265 nm shifted compared with those of the elastic fillers and polypropylene balls due to PN tyrosine, tryptophan, and aromatic hydrocarbons containing conjugated planar ring systems, indicating that the level of proteinaceous substances in the ceramsite filler was higher than of those in elastic fillers and polypropylene balls [10]. Compared with S-EPS, the two absorption peaks of LB-EPS were enhanced, and the peak increase of ceramsite filler was mainly due to the increase in tryptophan, tyrosine, and phenylalanine-related amino acids or PNs and humic substances [17]. Compared with those of S-EPS and LB-EPS, the intensity of the absorption band of TB-EPS, which was composed of multiple absorption peaks, was significantly enhanced, and the absorption bands at 220–285 nm constantly fluctuated, showing that TB-EPS was rich in PN tyrosine, tryptophan, and aromatic hydrocarbon substances, EPS aromatic and polyaromatic compounds, and EPS carboxyl or ester substances [5]. The ceramsite filler exhibited the largest absorption band, which moved to a longer wavelength region compared with those of elastic fillers and polypropylene balls, mainly because of the remarkably enhanced levels of PN substances, such as tryptophan, tyrosine, phenylalanine-related amino acids, and humus substances [17].

Figure 4 shows the 3D fluorescence of S-EPS, LB-EPS, and TB-EPS under different filler biofilms. S-EPS fluorescence was mainly due to peak A of tyrosine aromatic PN-like substance in the elastic filler and polypropylene balls. Meanwhile, ceramsite fillers displayed peaks A and B due to tyrosine aromatic PN-like and tryptophan PN-like substances, respectively, and the enhanced fluorescence intensity indicates that the level of tyrosine PN-like substance was higher than that in the other two fillers and added tryptophan PN-like substance in S-EPS [20]. The LB-EPS fluorescence showed peaks A, B, and D, which were mainly due to tryptophan, tyrosine PN-like substance, and humic acids in polypropylene balls, respectively. The elastic fillers exhibited peaks A, B, D, and F, whereas ceramsite fillers manifested peaks A, B, D, and E due to tryptophan, tyrosine PN-like substance, and humic acids. The peak intensity of ceramsite filler was higher than that of the other two fillers, indicating that the former contained more PN and humic substances [21]. Six peaks (peaks A, B, C, D, E, and F) of TB-EPS were observed for polypropylene spheres, elastic fillers, and ceramsite fillers, but the intensity and range of ceramsite fillers were significantly larger than those of the other fillers, mainly because of the increase in tryptophan, tyrosine PN-like, and humic substances [20]. In addition, compared with S-EPS and LB-EPS, TB-EPS showed the largest number of fluorescence peaks; the increase in peak C was due to the fluorescence of proteinaceous substances and related to the aromatic ring amino acid structure in EPS [22]. The fluorescence intensity also increased, indicating that TB-EPS contained the most abundant substances and was the

main component of EPS.EPS of Ceramsite filler showed the highest intensity of fluorescence peak due to the highest levels of PN and humic substances [23].

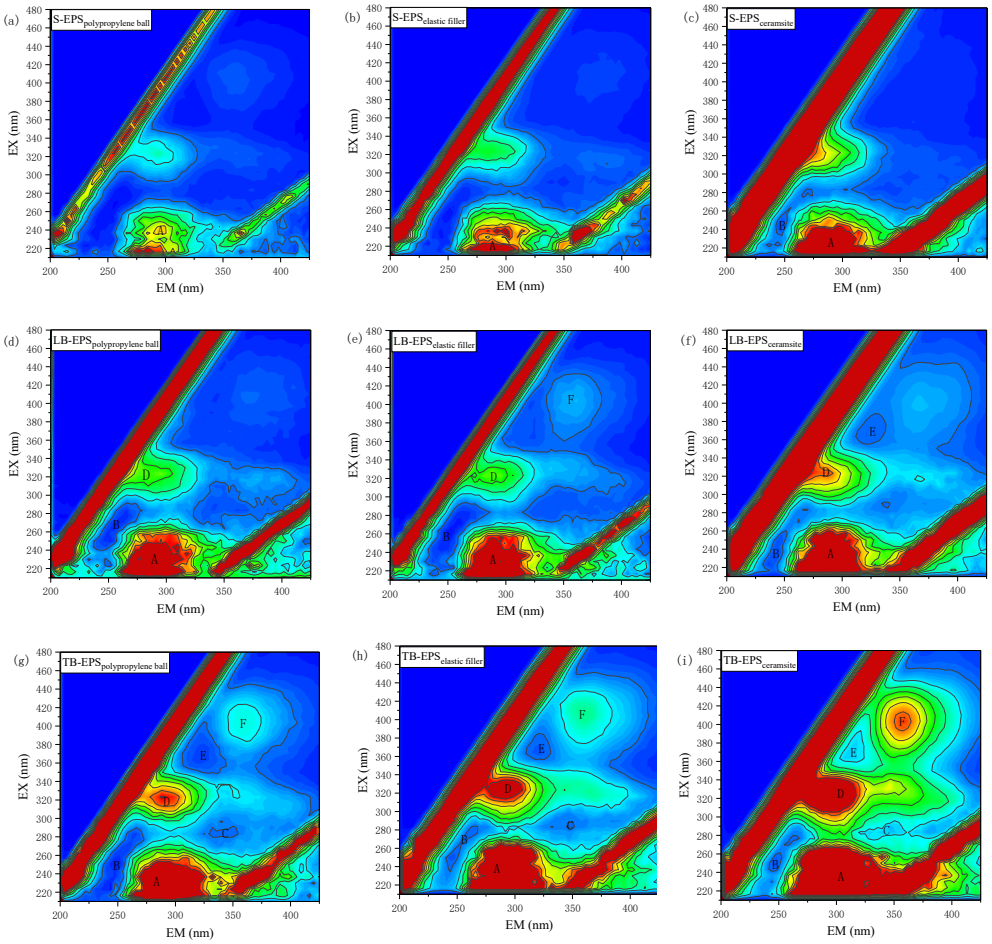

Fig. 4. Three-dimensional fluorescence of EPS; S-EPS: a) polypropylene balls, b) elastic filler, c) ceramsite; LB-EPS: d) polypropylene balls, e) elastic filler, f) ceramsite; TB-EPS: g) polypropylene balls, h) elastic filler, i) ceramsite under different fillers

In S-EPS taken from elastic fillers and polypropylene balls, one tyrosine-aromatic PN-like substance was found. The EPS of ceramsite filler were not only rich in tyrosine--aromatic PN-like substances but also tryptophan-like substances. The LB-EPS generated with polypropylene balls contained tryptophan, tyrosine PN-like, and marine humic substances. The LB-EPS formed with elastic and ceramsite fillers contained tryptophan, tyrosine PN-like, and two humic acid substances, but ceramsite filler substances were

more abundant. In TB-EPS formed with elastic fillers, polypropylene balls, and ceramsite fillers tryptophan, tyrosine PN-like, and three humic substances were found, but ceramsite fillers contained the most substances due to the highest fluorescence intensity.

3.3. BIOFILM BIOMASS AND BIOMASS ACTIVITY

Figure 5 shows the contents of biofilm suspended solids (SS) and dehydrogenase activities of biofilm formed at various fillers. Ceramsite filler, polypropylene ball, and elastic filler biofilm suspended solids (SS) contents were 31.689, 18.129, and 21.342 mg/g, respectively. The content of biofilm suspended solids (SS) of ceramsite filler was 1.5 times that of polypropylene balls and 1.48 times that of elastic fillers. Ceramsite filler, polypropylene ball, and elastic filler biofilm dehydrogenase activities were 44.589, 21.654, and 27.375 mg TPF/(g SS·h), respectively. The contents of biofilm suspended solids (SS) of ceramsite filler were 2.06 and 1.63 times that of polypropylene ball and elastic filler, respectively. The biofilm suspended solids (SS) content and dehydrogenase activity of polypropylene balls and elastic fillers were inconspicuous but the biofilm suspended solids (SS) content and dehydrogenase activity of ceramsite fillers were higher than those of the other two fillers. These results imply that the growth and activity of ceramsite filler biofilm are superior to those of the other two fillers.

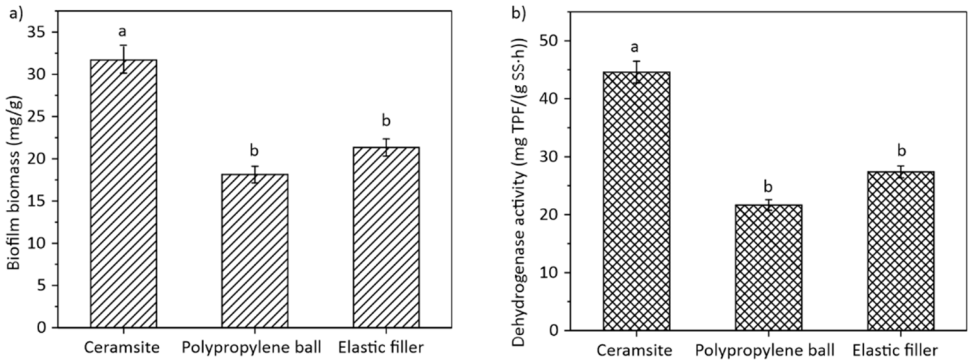

Fig. 5. Biofilm biomass (a), and dehydrogenase activity (b) of biomass formed at various fillers

Analysis of different filler media showed a greater effect on the growth and activity of biofilms [24]. The carrier interface roughness has a great effect on the adhesion of bacterial cells, and the key reason for effective BOD and COD removal is the rapid development of microbial films [3]. Thus, these removals have an important effect on the growth and activity of biofilms [24]. In addition, the pores inside the ceramsite filler can provide sufficient space for the attachment of microorganisms whereas the roughness of surface filler can enhance microorganism attachment [4]. Studies have shown that the presence or absence of pores inside the fillers has a great influence on the number of attached microorganisms, and the pores in the fillers cannot only accommodate

individual microorganisms but also provide space for the diffusion and exchange of substances between the microbial cells and the matrix, which increases the growth of biofilm and activity [25]. The ceramsite filler has a rough surface, is a light, porous material, and has a strong adsorption force. Thus, the quantity and activity of the biofilm formed with ceramsite filler are higher than those of elastic filler and polypropylene ball.

4. CONCLUSION

The content of EPS in the biofilm formed with ceramsite filler was 245.2 mg/g VSS, which was 1.26 and 1.51 times higher than those of elastic filter material and polypropylene balls, respectively. The PN/PS value of the ceramsite filter biofilm was higher than that of polypropylene ball and elastic filler material in S-EPS, LB-EPS, and TB--EPS, indicating that EPS have a more stable structure, especially TB-EPS. The PN/PS value of biofilm formed with ceramsite filter media was the largest, and the TB-EPS of the biofilm ceramsite filter material accounted for 67.58% of the T-EPS, which was higher than those of the biofilm of elastic filter material and polypropylene balls. Thus, ceramsite filter media are beneficial to maintain biofilm stability.

The UV-Vis spectrum showed that the content of three components (S-EPS, LB-EPS, and TB- EPS) of ceramsite filter material EPS, especially the PN and humic substances contained in TB-EPS, were higher than those in the elastic filter material and polypropylene balls. The 3D-EEM fluorescence spectra revealed that the content of S-EPS, LB--EPS, and TB-EPS in ceramsite filter material was higher than those of elastic filler and polypropylene balls, especially TB-EPS. The EPS of biofilm from ceramsite filler contains high amounts of tryptophan, tyrosine, PNs, and humic substances.

Dehydrogenase activity of the biofilm formed by ceramsite is higher than that of elastic filler and polypropylene ball.

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