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Treatment of wastewater containing linear alkylbenzene sulfonate by bacterial-microalgal biological turntable

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Abstract−Linear alkylbenzene sulfonate (LAS), which is widely used as detergent, is a common toxic pollutant in wastewater. Generally, biodegradation process is applied to remove LAS. However, the efficiency of traditional wastewater treatment cannot meet the growing demand. In this study, an improved biological turntable with a symbiotic system of bacteria and microalgae was primarily used to enhance the biodegradation efficiency of LAS from wastewater. The symbiotic system of bacteria and microalgae was mainly composed of *Scenedesmus dimorphus* and three LAS-degrading bacteria *Plesiomonas* sp. (L3, L7) and *Pseudomonas* sp. (H6). The average removal rate of LAS was up to 94.6%. The LAS concentration of the effluent of the system decreased by 81.7% after the bacterial-microalgae inoculation (the inoculation temperature was 25 °C; microalgae were inoculated at a concentration of 10% only at the start of the system; bacteria were continuously inoculated at 1‰ concentration). After bacterial-microalgae inoculation, the average effluent concentration of COD_{Cr} in the tertiary reaction tank was 24.3 mg/L, the average membrane effluent concentration was 15.8 mg/L, and the average removal rate was 90.5%. Compared with the control group without inoculation, the concentration of COD_{Cr} in the tertiary reaction tank and membrane effluent decreased by 55.7% and 46.4%. The denaturing gradient electrophoresis (DGGE) pattern analysis of the systemic flora showed that there were two dominant species of high LAS degrading bacteria. They were identified to belong to *Plesiomonas* sp. and *Pseudomonas* sp., respectively.

Keywords: Biological Turntable, Bacterial-microalgae, Linear Alkylbenzene Sulfonate, Biodegradation, Molecular Ecology Analysis

INTRODUCTION

Linear alkylbenzene sulfonate (LAS) is the most used anionic surfactant worldwide and is considered as a high-priority pollutant [1-3]. LAS is an important anionic surfactant which is widely used as formulation component of synthetic detergents, cleaning agents, decontamination powder and so on [4,5]. The consumption of LAS in China is over 1 million tons per year [6,7]. Due to the extensive application of LAS, waterbodies are seriously contaminated [8]. Furthermore, LAS can cause large toxic effects on animals and plants because of its long-term accumulation in environment and organisms. LAS's environmental behavior and effects have attracted great concern [9-11]. At present, the main methods of LAS treatment are physical chemistry and biological treatment. Physicochemical treatment has the characteristics of simple equipment, low cost, convenient management and stable treatment effect. Physicochemical treatment is the first choice for inorganic or organic insoluble pollutants and colloidal substances in wastewater [12,13].

Biological treatment is one of the most widely studied methods because of its large scale, simple equipment, low cost and wide application [14,15]. It is a method where microorganisms use surfactants in wastewater as carbon source to degrade LAS. Biological treatment mainly utilizes microbial metabolism to degrade harmful substances in wastewater and convert them into stable and harmless components [16]. Biological treatment is currently recognized as an effective method to treat surfactant wastewater [17]. Eslamia et al. studied LAS biodegradation among different loading rates and fate of LAS in integrated fixed-film activated sludge (IFAS) using synthetic media. The mean removal efficiency of LAS among three LAS loading rates was 92.32%±2.81%, 95.55%±2.74% and 96.22%±2.74%, respectively [18]. Eslamia et al. investigated the performance of IFAS system in treatment of linear alkylbenzene sulfonate (LAS) in synthetic greywater; the best removal efficiency for LAS was obtained as 94.24% [19]. Babaei et al. examined removal of linear alkylbenzene sulfonate and turbidity from greywater by a hybrid multi-layer slow sand filter microfiltration ultrafiltration system; the best removal efficiencies for LAS was 99.97% using the MSSF-MF-UF hybrid system [20]. The main mechanisms of biodegradation are aerobic biodegradation and anaerobic biodegradation [21].

Aerobic biodegradation increases free oxygen molecules in wastewater by aeration and degrades organic matter by the metabolism of aerobic microorganisms and facultative microorganisms. Schulz et al. studied the degradation of LAS by a Delftiaaci-dovorans SPB1 strain isolated from nature. The results showed that although the strain could not directly utilize LAS, it could open the benzene ring,

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which is the most difficult step in the degradation of anionic surfactants [22]. Schleheck et al. isolated an alpha-proteobacterium DS-1 strain from activated sludge of sewage treatment plant, which could transform LAS and linear alkyl diphenyl ether disulfonates into sulfophenyl carboxylates and sulfodiphenyl ether carboxylates at the same time. But the strain can grow normally only if it is fixed to a certain solid support, such as glass filament and polyester wool [23]. The degradation of anionic surfactants by most microorganisms begins with oxidation at the end of alkyl chain and then shortening the long chain by beta-oxidation. However, Yadav et al. studied the degradation of LAS by white rot fungi. The results showed that LAS could be transformed into many sulphonyl carboxylates with different chain lengths, but the degradation ability of the strain was limited. It took six days for 2 mg/L LAS to degrade completely in maltose extract liquid medium [24].

Anaerobic biodegradation is a biological treatment method for degradation of organic matter by facultative bacteria and anaerobic bacteria without free and combined oxygen. Sanz et al. studied the effect of temperature on LAS treatment under anaerobic conditions. The results showed that the optimum degradation temperature was 40 °C [25]. In microalgae degradation, the microalgae enrich organic matter and then migrate along the food chain of organisms or undergo a series of biochemical reactions with the participation of various enzymes to degrade and transform organic pollutants. Relevant studies showed that microalgae had a strong ability to degrade many organic pollutants [26]. Li et al. studied the enrichment and degradation of LAS by Spirulina platensis. The results showed that Spirulina platensis had a good enrichment capacity for LAS. When the concentration of LAS was 0.5, 1 and 2 mg/L, the degradation rates were 87%, 80% and 70.5% within five days, respectively. The degradation of organic pollutants by microalgae was the result of facultative chemical energy and organic nutrition [27]. Eslamia et al. investigated biodegradation and removal of Reactive Red 198 (RR198) dye from aqueous environments using a new bacterial consortium isolated from textile wastewater sludge on laboratory scale via batch study, the removal efficiency of RR198 dye at an initial concentration of 10-25 mg/L was more than 98% [28,29].

In this paper, an improved symbiotic system consisting of bacteria and microalgae was applied to enhance the biodegradation efficiency of LAS from wastewater. Based on our previous study, the bacterial strains isolated from wastewater treatment process and *Scenedesmus dimorphus* were used to form a symbiotic system for biological turntable to enhance the degradation efficiency of LAS in wastewater. The relationship between the performance and bacteria amount, distribution and community structures in the RBC was also investigated by the PCR-DGGE technique.

MATERIALS AND METHODS

1. Source of Strains and Microalgae

The strains L3, L7 and H6 mixed in the experiment were selected from the previous screening of our research group [30]. Three LAS-degrading bacteria L3, L7 and H6 were isolated from activated sludge of Luofang Sewage Treatment Plant and domestic wastewater of Shenzhen University Town (Shenzhen, China) by enrichment culture and plate marking. The microalgae species was the Scenedesmus dimorphus which was cultured in the original wastewater. The influent of the bacteria-microalgae biological turntable was domestic wastewater from Shenzhen University Town. The water quality of the model is shown in Table 1.

2. Experimental Devices and Operating Conditions

A small-scale experimental device was made of fiber reinforced plastics (FRP), and the technological process flow of bacteria and microalgae biological turntable is shown in Fig. 1. The effective volume of a sedimentation tank was 54 L, and its retention time was 1.35 H. The total volume of the microalgae biological turntable was 325 L, the hydraulic retention time (HRT) of the first biological turntable was 2.7 h, the HRT of the second biological turntable

Table 1. Water quality parameters of wastewater

Water quality index	Concentration (mg/L)
COD_{Cr}	256
BOD_5	140
TN	40.4
NH_4^+-N	42.4
TP	4.9
SS	410

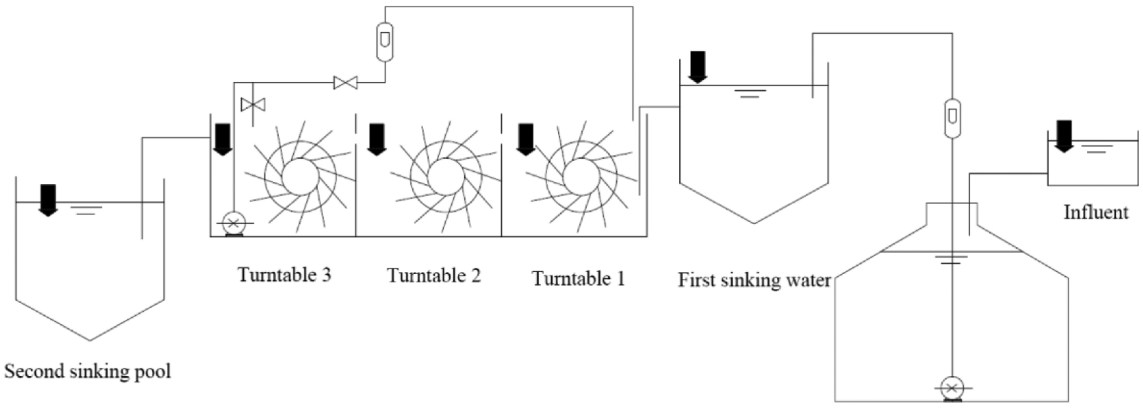

Fig. 1. Schematic diagram of the process flow of the biological turntable process.

was 2.5 h, and the HRT of the third biological turntable was 2.9 H. The size of the main reactor was 150 cm×66 cm×55 cm. A silent aeration pump, six glass rotors and six aeration rods were used to control the intake volume in the three-stage reactor, so as to control the rotational speed and DO of the biological rotating disc. Two 16-watt tricolor tubes were installed at the top of the three-stage reactor. The effective volume of secondary clarifier was 86 L and HRT was 2.14 H. The flow rate of the experimental device was 40 L/h, which was continuous. The inoculated sludge was the activated sludge from Luofang Wastewater Treatment Plant, and the influent was the domestic wastewater of Shenzhen University Town (Shenzhen, China). The experimental devices were as follows: COD Meter (DR/890, HACH, America), PCRAmplifier (PTC-100, MJResearch, America), electrophresis apparatus (Bio-RAD PowerPac basic, Bio-RaD, America), DCode^{TM} Universal Mutation Detection Processs (DCode^{TM}, Bio-RaD, America), electron microscope (BX41, Olympus, Japan).

3. Community Structure Analysis of Bacteria

Biofilm samples and mud-water mixture DNA were extracted according to the instructions of Baitek Soil Genome DNA Rapid Extraction Kit (centrifugal column type). DNA was validated by 1% agarose gel electrophoresis. The bacterial community structure was analyzed by PCR-DGGE technique, and the sequencing results were compared with known sequences in the National Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST). Phylogenetic analysis was carried out by MEGA4.1 software, evolutionary tree was drawn by UPGMA method, and map was digitized by Gel Pro Analyzer 4.0 analysis [31].

4. Degradation Rate Quantification of LAS

Methylene blue reacts with anionic surfactants to form blue ion-pair complexes. These substances that can react with methylene blue are called methylene blue active substances (MBAS). The chromogenic substance can be extracted by chloroform, and the absorbance of chloroform layer can be measured at 650 nm by spectrophotometer. The specific methods were as follows: 100 mL water sample was added into 250 mL separating funnel, phenolphthalein was used as indicator, 4% NaOH was added drop by drop until the solution was peach red, and 3% H_2SO_4 was added until the red disappeared. 25 mL methylene blue solution and 15 mL chloroform were added into the separating funnel. After 30 seconds of

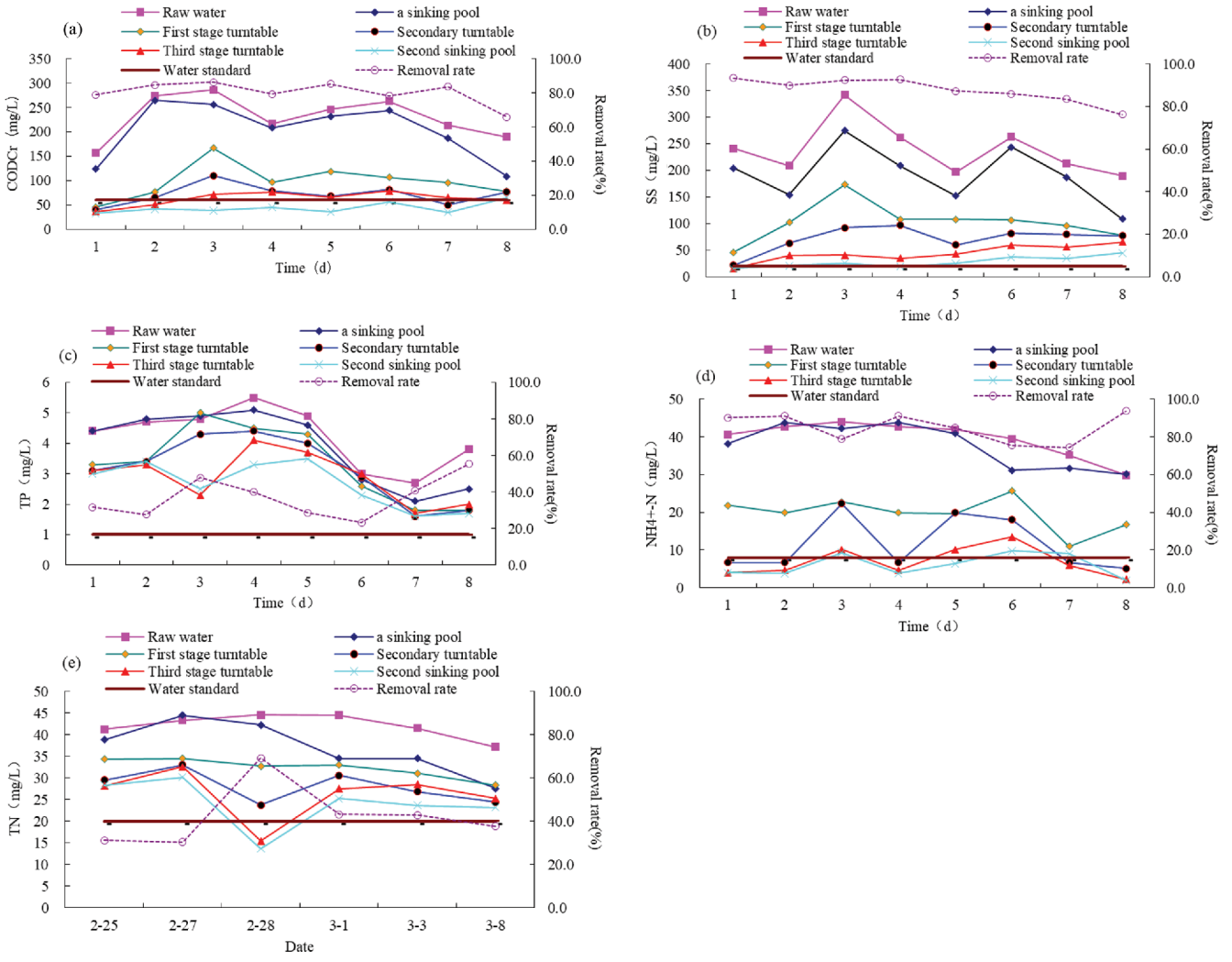

Fig. 2. Removal effect of COD_{Cr} (a), TN (b), SS (c), TP (d), NH_4^+-N (e) in the three-stage biological turntable system.

violent vibration, the separating funnel was rotated slowly to make the solvent layered statically. The chloroform phase was absorbed by degreased cotton and then put into the colorimetric dish. Using pure chloroform as reference, the absorbance of the sample at 650 nm was determined by spectrophotometer. After subtracting the absorbance of the sample from the blank absorbance, the content of LAS could be found on the calibration curve [32,33].

RESULTS AND DISCUSSION

1. Operating Conditions of Three-stage Biological Turntable System without Microalgae and Bacteria

When the influent was 60 L/h, the removal effect of CODcr by the three-stage biological turntable system was shown in Fig. 2(a) without inoculation of bacteria and microalgae. The average influent concentration of COD_{Cr} was about 231 mg/L, and the average effluent concentration was 44 mg/L, and the removal rate was 80%.

The results showed that the removal rates of COD_{Cr} in the three biological turntables were 43%, 13% and 5%, respectively, except for primary and secondary sedimentation tank. The removal of COD_{Cr} was mainly accomplished in the primary biological turntable, which was consistent with some research results [34,35].

The SS removal in three-stage biological turntable system is shown in Fig. 2(b). The system had a minimum removal efficiency of 76% for SS, a maximum of 93%, an average removal rate of 88%, and an average SS concentration of 28 mg/L. The removal effect of TP is shown in Fig. 2(c). The average influent concentration of TP was 4.1 mg/L, the average effluent concentration was 2.4 mg/L, and the average removal rate was 36.9%. The removal effect of ammonia nitrogen is shown in Fig. 2(d). The average influent concentration of ammonia nitrogen was 39.54 mg/L, and the average effluent concentration was 5.88 mg/L, the average removal rate was 85%. Meanwhile, the removal effect of TN was not good, and the average removal rate was only 42.4%. The average influent concentration of TN is 42.1 mg/L, and the average effluent concentration was 24.1 mg/L.

As the design of the three-stage biological turntable was conservative, it could be seen from the results that the removal rates of TN, TP and SS were not up to the emission standards. Therefore, the third biological turntable and the secondary sedimentation tank were removed, the third reaction tank was used as the sedimentation tank. To intercept microalgae in later experiments, hollow fiber membrane was added to filter effluent in three-stage reaction tank.

2. Operating Conditions of Two-stage Biological Turntable with Hollow Fiber Membrane

The removal effect of COD_{Cr} in the two-stage biological turntable system is shown in Fig. 3(a) without adding microalgae and bacteria. The average influent concentration of COD_{Cr} was 208 mg/L, the average effluent concentration of COD_{Cr} was 54.8 mg/L, the average membrane effluent concentration was 29.4 mg/L, and the average removal rate was 85.4%.

The removal effect of LAS in two-stage biological turntable system is shown in Fig. 3(b) without adding microalgae and bacteria. The average influent concentration of LAS was 4.1 mg/L, the average effluent concentration of LAS was 1.2 mg/L, and the average removal rate was 70.1%.

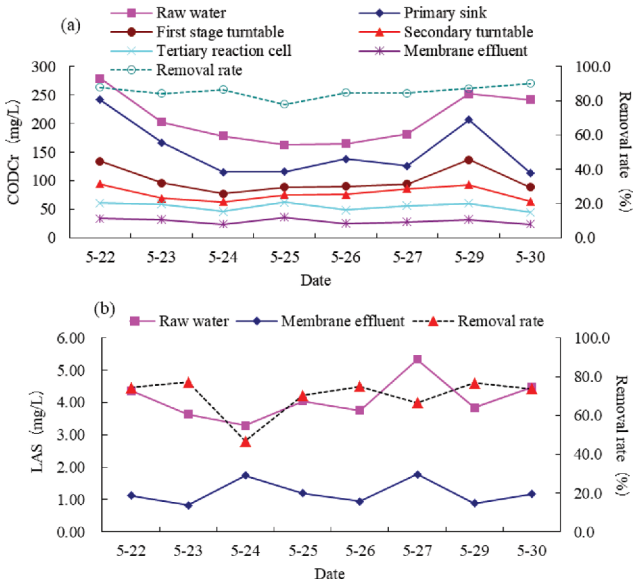

Fig. 3. Removal effect of COD_{Cr} (a) and LAS (b) in two-stage biological turntable system.

3. Running Status of Process when Microalgae were Added

The microalgae were added with a similar method of bacterial inoculation, and the total inoculum was 10% of the total volume of the reactor. The concentration of COD_{Cr} and LAS in the effluent of the reactor was examined after microalgae were added into the system. The removal effect of COD_{Cr} in the two-stage biological turntable system is shown in Fig. 4(a). The average influent concentration of COD_{Cr} was 146.4 mg/L, the average effluent concentration of COD_{Cr} in the tertiary reactor was 39.9 mg/L, the average membrane effluent concentration was 23.9 mg/L, resulting in the average removal rate was 83.6%. Compared with the treatment

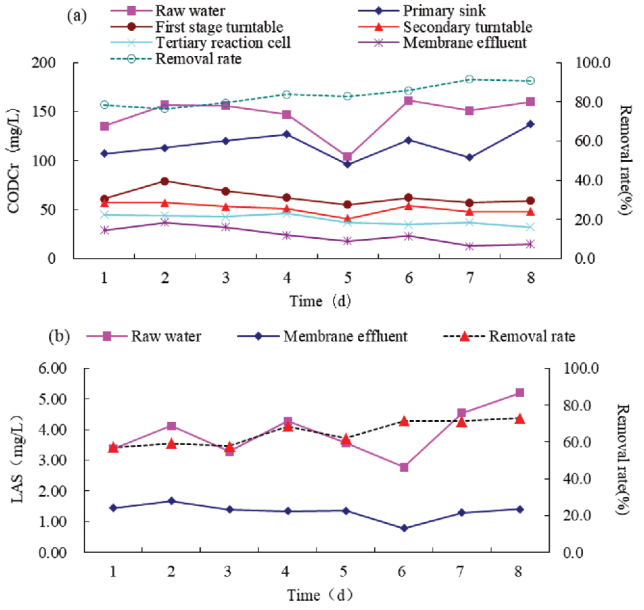

Fig. 4. Removal effect of COD_{Cr} (a) and LAS (b) after microalgae were added into the system.

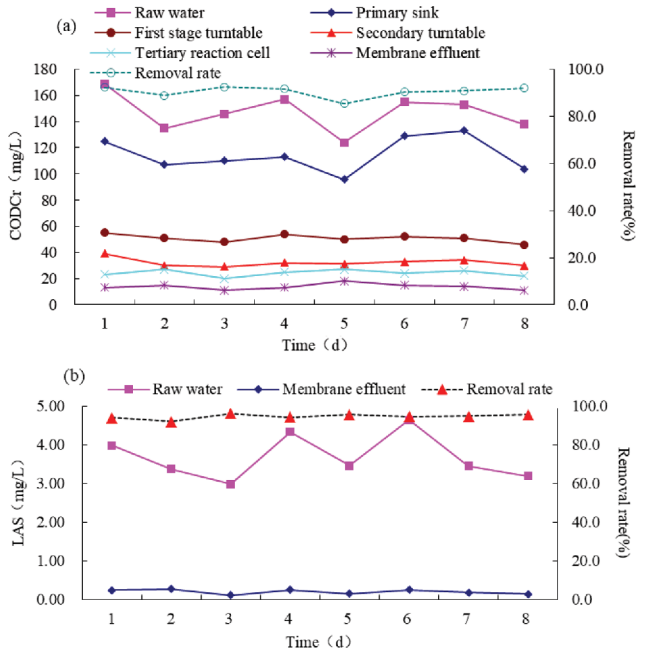

Fig. 5. Removal effect of COD_{Cr} (a) and LAS (b) after microalgae and bacteria were added into the system.

group without adding microalgae, the COD_{Cr} concentration in the effluent of the tertiary reactor decreased by 27.1% and 18.7%, which indicated that microalgae addition could enhance the COD_{Cr} removal capacity of the system. Muoz came to the same conclusion when he used bacterial and algal systems to degrade toxic and harmful organisms. It was also found that 1 kg of microalgae could produce 1.5-1.9 kg of oxygen during photosynthesis [36].

The removal effect of LAS in the two-stage biological turntable system is shown in Fig. 4(b). The average influent concentration of LAS was 3.9 mg/L, the average effluent concentration was 1.34 mg/L, and the average removal rate was 65.0%.

4. Operating Conditions of Two-stage Biological Turntable with Inoculation of Microalgae and Bacteria

The removal effect of COD_{Cr} in two-stage biological turntable system is shown in Fig. 5(a) with microalgae and bacteria addition. The average influent concentration of COD_{Cr} was 174.1 mg/L, the average effluent concentration of COD_{Cr} in the tertiary reactor was 24.3 mg/L, the average membrane effluent concentration was 15.8 mg/L, and the average removal rate was 90.5%. Compared with the treatment group without adding bacteria, the COD_{Cr} concentration in the effluent of the tertiary reactor decreased by 55.7% and 46.4%, which indicated that bacteria addition could enhance the COD_{Cr} removal capacity of the system.

The removal effect of LAS in two-stage biological turntable system is shown in Fig. 5(b) with adding microalgae and bacteria. The average influent concentration of LAS was 3.68 mg/L, the average effluent concentration was 0.20 mg/L, and the average removal rate was 94.6%. The results show that the addition of LAS degrading bacteria could enhance the LAS removal capacity of the system. Dhouib et al. used *Citrobacter* combined with MBR to treat industrial wastewater containing high concentration of anionic surfactants. The volumetric load of the system was 0.6-1.5 g/L·d, the concentration of anionic surfactants in influent was 116-870 mg/L, and the average concentration of anionic surfactants in effluent was 18 mg/L. It could be concluded that the method adding bacteria combined with membrane filtration is effective for the treatment of surfactants [37]. To achieve complete biodegradation of surfactant, it mainly takes three important steps, including terminal oxidation of the alkyl chain by w-oxidation followed with successive β-oxidations forming sulfophenyl carboxylic acids, removal of sulfonate from benzene ring by desulfonation, and aromatic-ring cleavage. But there is no consensus on the sequence of the three mechanisms. The degradation process of LAS alkyl chain is similar to that of common fatty acids. Fatty acids are biodegradable substances, and the catalytic enzymes involved in the degradation process, such as oxygenase and dehydrogenase, are common in algae cells. Therefore, it can be considered that the degradation of alkyl chain in LAS molecule is relatively easy. The biodegradation of benzene ring and sulfonate requires the participation of many enzymes. The process is complex, so it is difficult to degrade. It is concluded that the degradation products of LAS are sulfonylbenzoic acid (such as 2 - (4-sulfonylbenzene) butyric acid), aromatic benzenesulfonic acid (toluene sulfonate), benzene ring, sulfate [38].

5. Molecular Ecology Analysis of Bacterial-microalgae Biological Turntable Process

5-1. Bacterial Population Analysis

The fragment of 16S rDNA V3 region (about 250 bp) was amplified by PCR. The DGGE map was obtained by DGGE electrophoresis and staining as shown in Fig. 6. L0 was mixed bacterial sample, L1 and L2 were primary and secondary rotating disc biofilm samples before inoculation, L3 and L4 were primary and secondary rotating disc biofilm samples after inoculation, L5 and L6 were primary and secondary rotating disc biofilm samples after inoculation. It can be seen from the figure that electrophoretic bands with

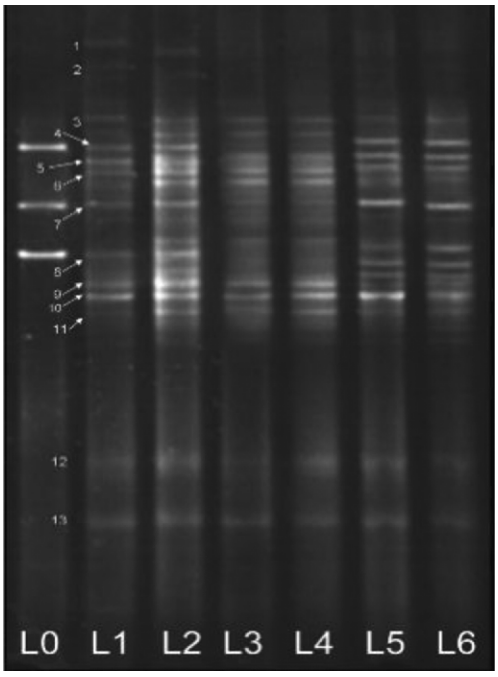

Fig. 6. Comparison of DGGE patterns.

different number and intensity could be obtained by DGGE separation of samples before and after inoculation in each wastewater treatment unit.

Fig. 6 shows that the three strains of high-efficiency LAS-degrading bacteria are present in the pre-inoculation system, corresponding to the No. 4, No. 7, and No. 8 bands in the L1 sample, belonging to the genus *Plesiomonas* sp., *Pseudomonas* sp. and *Plesiomonas* sp., respectively. After the addition of microalgae, the bands 4 and 7 are almost invisible in the L3 and L4 samples. After the addition of LAS degrading bacteria, the bands No. 4, No. 7 and No. 8 in the L5 and L6 lanes are clearly visible, and band No. 10 is also brighter, which is the dominant bacteria in the system after inoculation. The number of total DNA bands in DGGE profiles of bacterial samples decreased after inoculation, which might be due to the competition between some bacteria in bacterial flora, resulting in the reduction or disappearance of some bacteria. Therefore, the addition of LAS efficient degrading bacteria not only enhanced the number of LAS efficient degrading bacteria, but also changed the species of bacteria in the whole system.

5-2. Sequencing Analysis of DGGE Characteristic Bands

The main strips in DGGE electrophoresis were recovered by tapping. After sequencing, the sequencing results were compared with the sequences in the National Center for Biotechnology Information (NCBI), and the results of identifying similar sequences in GenBank are shown in Table 2. The similarity between the obtained sequence and the known sequence in GenBank is more than 90%. Among the 10 bands, 5 and 7 belong to *Pseudomonas* sp., 4 and 8 belong to *Plesiomonas* sp., which could play a major role in the degradation of LAS, 6 belongs to *Nitrospira* sp., which mainly oxidized nitrite to nitrate and played an important role in nitrogen removal in the system.

Table 2. Comparison of the sequence of the DGGE tapping recovery strip with the NCBI database

Band	Most closely related bacteria	Similarity	NCBI
1	*Uncultured cyanobacterium clone*	94%	FJ967896
2	*Haslea salstonica*	96%	AF514854
3	*Spirosoma Lingual*	95%	AY279982
4	*Plesiomonas shigelloides*	98%	GU324301
5	*Pseudomonas* sp.	99%	EU099609
6	*Nitrospira* sp.	99%	Y14640
7	*Pseudomonas aeruginosa*	96%	GU124827
8	*Uncultured Plesiomonas* sp.	96%	GU293171
9	*Uncultured Flectobacillus* sp.	98%	AM941709
10	*Uncultured beta proteobacterium*	97%	FM992016

5-3. Phylogenetic Analysis

Clustawl was used for comparative analysis of target sequence and correlation sequence, and phylogenetic tree was established as shown in Fig. 7 [39]. Fig. 7 shows that the 10 strips could be roughly divided into three categories, band 1 and band 2 could be classified as *Cyanobacteria*, band 3, band 4, band 8, band 5, band 7 and band 9 could be classified as *Gram-negative bacteria*, band 10 and band 6 could be classified as a group; they belonged to *β-proteobacteria*.

5-4. Correlation Analysis between Bacterial Community Characteristics and System Treatment Efficiency

Pearson correlation analysis in DPS software was used to analyze the correlation between bacterial community characteristics and COD_{Cr} and LAS removal efficiency in the system after adding

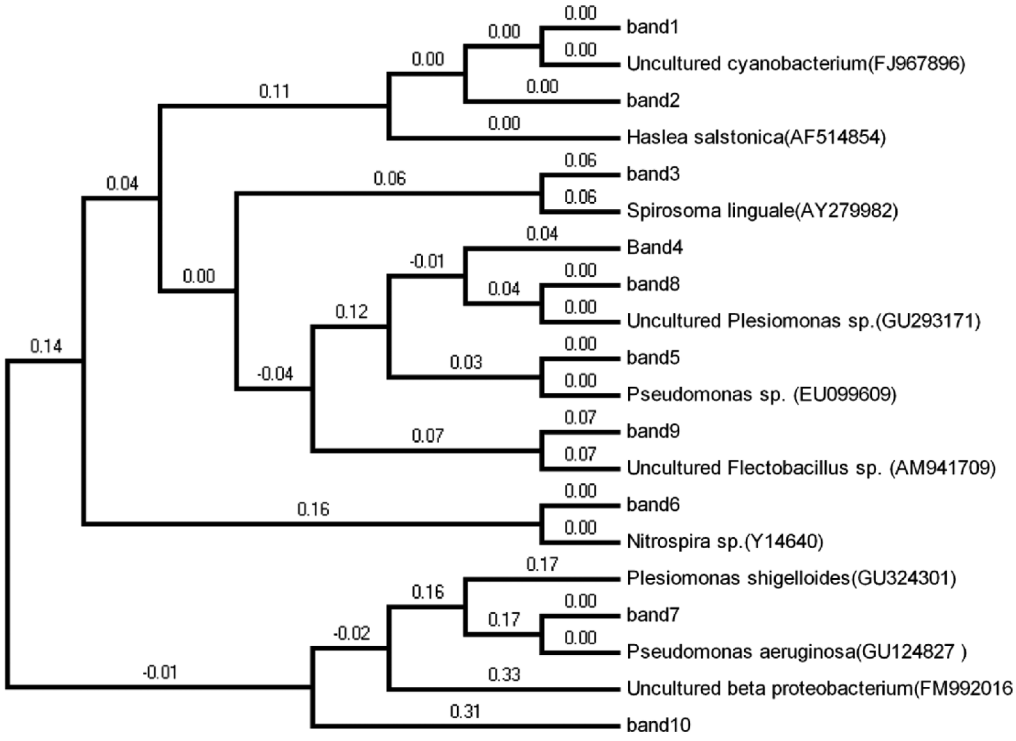

Fig. 7. Construction of phylogenetic tree by recovering 16S rRNA gene from DGGE band.

Table 3. Correlation analysis between bacterial community characteristics and system treatment efficiency

Pearson correlation	Total number of LAS-degrading bacteria	Diversity index	Total number of bacteria	LAS removal rate	COD_{Cr} removal rate
Total number of LAS-degrading bacteria	1				
Diversity index	0.61	1			
Total number of bacteria	0.99**	0.59	1		
LAS removal rate	0.89**	0.41	0.85*	1	
COD_{Cr} removal rate	0.92**	0.47	0.88**	1.00**	1

Note * $p<0.05$, ** $p<0.01$

LAS degradation bacteria (Table 3). It can be seen that the number of LAS degrading bacteria is significantly positively correlated with the total number of bacteria, LAS removal rate and COD_{Cr} removal rate, the Pearson correlation coefficients are 0.99, 0.89 and 0.92, respectively (Table 3). The number of LAS degrading bacteria is not significantly correlated with the diversity index of flora (Pearson correlation R2=0.61). There is no significant positive correlation between diversity index and the total number of bacteria, LAS removal rate and COD_{Cr} removal rate; the Pearson correlation coefficients were 0.59, 0.41 and 0.47, respectively. The structure of the bacteria in the system was stable because the dominant species of LAS-degrading bacteria was basically the same as that of LAS-degrading bacteria in the system. The addition of bacteria only increased the number of the LAS-degrading bacteria in the system but did not change the flora structure. Therefore, the number of LAS-degrading bacteria was more significant than the community structure in the removal of LAS. Wang et al. studied the cultivation of aerobic granular sludge with anaerobic granular sludge in a small-scale SBR reactor. Pearson correlation analysis showed that AOB community size and biodiversity were related to degradation constants, and the impact of community size might be greater than that of biodiversity, which is consistent with our experimental results [40].

CONCLUSION

An improved biological turntable with a symbiotic system of bacteria and microalgae was primarily used to enhance the biodegradation efficiency of LAS from wastewater. The symbiotic system of bacteria and microalgae was mainly composed of *Scenedesmus dimorphus* and three LAS-degrading bacteria L3, L7 and H6. The average effluent concentration of LAS was 0.20 mg/L, and the average removal rate was 94.6%. Compared with the non-inoculation condition, the LAS concentration of the effluent decreased by 81.7%. The DGGE pattern analysis of the systemic flora showed that there were two dominant species of high LAS degrading bacteria. Combined with sequencing analysis, they belonged to *Plesiomonas* sp. and *Pseudomonas* sp., respectively.

ACKNOWLEDGEMENTS

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