Comparison of Degradation of Lignin-containing Wastewaters in the Presence of Different Microbial Consortia

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Lignin-containing wastewater treatment by different microbial consortia were studied in this research. The special microbial consortia (J-6 and J-1) obtained from decayed wooden relics were selected. The bacteria of original microbial consortium J-6 mainly included *Shinella*, *Cupriavidus* and *Bosea*. The bacteria of original microbial consortium J-1 mainly included *Serratia* and *Yersinia*. The fungi of J-6 and J-1 were dominated by Saccharomycetales. The performances of two microbial consortia in wastewater treatment were compared, and the changes in community structure were analyzed to study the relationship between microbial consortium structure and degradation efficiency. For the treatment of model Chinese medicine wastewater, the optimal degradation conditions were treatment temperature of 30 °C, initial pH of 7, dissolved oxygen of 2 mg L^{-1}, and treatment time of 96 h. The COD (Chemical Oxygen Demand) removal efficiency reached 95.25 % by J-1. For the treatment of model papermaking wastewater, the optimal degradation conditions were treatment temperature of 30 °C, pH of 5, dissolved oxygen of 3 mg L^{-1}, nitrogen source concentration of 0.1 g L^{-1}, and treatment time of 120 h. The COD removal efficiency reached 86.8 % by J-6. Bacteria played a significant role in the degradation of lignin-containing wastewater, and the bacterial consortium abundance may promote the degradation of organic substances in the wastewater. The dominant strains were different in Chinese medicine wastewater and paper-making wastewater systems. The correlation between microorganisms and the difference in the abundance of bacteria groups may be the reason for the different performances of the two microbial consortia in treating different lignin-containing wastewaters.

Keywords
lignin-containing wastewater, microbial degradation, community diversity and composition

Introduction

Lignin is an organic substance with a content second only to cellulose in nature[1]. Lignin exhibits stable and complex structure, and is difficult to degrade[2]. The effective treatment of lignin-containing wastewater is still a difficult problem[3,4]. Lignin-containing wastewater has been discharged from a considerable number of industries (e.g., traditional Chinese medicine (TCM) businesses, rice milling businesses, and the paper industry)[5,6].

Specifically, TCM businesses wastewater (as well as wastewater from similar medicine production using plants in other countries) and paper-industry wastewater are the most typical. TCM has great potential in treating global diseases such as COVID-19. The pharmaceutical industry represented by TCM will continue to develop in the future. According to data released by the Ministry of Ecology and Environment of China[7], in 2021, the proportion of wastewater treatment facilities in the pharmaceutical industry accounted for 5.4 % of the total industrial wastewater treatment facilities, with a significant increase. As an attractive industry, its impact on the environment has gained urgent attention[8,9]. Furthermore, the paper-industry wastewater primarily originates from the processes of material preparation, pulping, condensation, pulp washing, bleaching, etc. Specifically, the environmental pollution caused by the pulping stage wastewater (also known as papermaking black liquor) is the most serious[10]. According to data from the Ministry of Ecology and Environment of China[7], in 2021, the wastewater from the paper industry accounted for 5.6 % of the total industrial wastewater treatment, reaching 1.69 billion tons. Lignin-containing wastewater has certain similarities in characteristics, and

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the environmental pollution is extremely serious[11]. How to effectively solve the pollution problem caused by lignin-containing wastewater has become the focus of researchers[12,13]. The biological treatment process has been confirmed as a more effective and economic method for the treatment of lignin-containing wastewater[13,14].

At the same time, microorganisms exhibiting high adaptability are required for lasting and effective biological treatment. Therefore, microbial consortia with strong environmental adaptability have been extensively used. For instance, Kong *et al.* used the mixed microbial consortium to degrade the wastewater containing N, N-dimethylformamide, and the removal efficiency was higher than 96 %[15]. Ma *et al.* suggested that the microbial consortium can reduce 90 % chemical oxygen demand under different salinity conditions[16]. The microbial consortium exhibits higher adaptability to different environments than a single microorganism because the structure of the microbial consortium can change with the change in environmental conditions[17,18]. Furthermore, compared with other industrial wastewater, the treatment of lignin-containing wastewater is significantly more difficult since it contains macromolecular lignin. Thus, using special microorganisms to purify lignin-containing wastewater is a promising research direction[19]. The most active microbes relating to lignin biodegradation are fungi[20]. Some research has suggested that white-rot fungi can effectively reduce chemical oxygen demand (COD) and biochemical oxygen demand (BOD) in papermaking wastewater while decolorizing and disinfecting the wastewater[14,19]. Since the environmental adaptability of microorganisms in the wastewater treatment system should be maintained, it is more feasible to use special lignin-degrading microbial consortium to treat lignin-containing wastewater.

In view of the potential advantages of lignin-degrading microbial consortium, our previous study successfully screened four microbial consortia J-1, J-6, J-8, and J-15 with high lignin degradation efficiency from decayed wooden relics[21]. Specifically, the lignin degradation efficiency of J-6 reached 54 % after only 48 h with an initial lignin concentration of 0.5 g L^{-1}. The screened microbial consortia achieved high degradation efficiency in the process of degrading soluble lignin. Thus, it is essential to study the lignin degradation application of these lignin-degrading microbial consortia in lignin-containing wastewater treatment. Moreover, most of the current research on the degradation of lignin is focused on a single microorganism, and few of them study the degradation of a microbial consortium.

In this study, the obtained microbial consortia were selected to treat lignin- containing wastewater. More insight can be gained into the degradation mechanism of lignin-containing wastewater through analysis and comparison of the pollutant's degradation by different microbial consortia. In combination with the characteristics of their respective consortium structure, this study revealed the intrinsic relationship between the microbial consortium structure and the efficiency of wastewater treatment.

Materials and methods

Materials

The original microbial consortia were screened from wooden relics[21]. The bacteria of the original microbial consortium J-6 mainly included *Shinella* (47.38 %), *Cupriavidus* (29.84 %), *Bosea* (7.96 %), *Bacillus* (4.39 %), and *Rhodococcus* (2.72 %). The bacteria of the original microbial consortium J-1 mainly included *Serratia* (95.23 %) and *Yersinia* (2.33 %). The fungi of J-6 and J-1 were dominated by Saccharomycetales. Species with low relative abundance were not listed. The original microbial consortia were preserved at 4 °C. The raw materials of traditional Chinese medicine (*Astragalus membranaceus*, *Fritillaria cirrhosa,* and *Angelica sinensis*) were obtained from a pharmaceutical factory in Qinhuangdao, Hebei Province. The biological reagent used in the experiment was purchased from Sigma Company. Other reagents (analytical grade) were purchased from the Beijing Chemical Factory. Experimental water was prepared using a milli-Q IQ 7000 pure water machine (Merck Millipore, Germany).

Culture media and model wastewater

The enriched medium consisted of $(NH_4)_2SO_4$ 1.0 g L^{-1}, KH_2PO_4 0.5 g L^{-1}, NaCl 1 g L^{-1}, $MgSO_4\cdot 7H_2O$ 0.1 g L^{-1}, $CaCl_2$ 0.05 g L^{-1}, glucose 0.5 g L^{-1}, alkali lignin 0.5 g L^{-1}, and trace elements 1 mL L^{-1}. Trace element solutions included $ZnSO_4\cdot 7H_2O$ 0.1 g L^{-1}, $CoCl_2\cdot 6H_2O$ 0.16 g L^{-1}, $CuSO_4\cdot 5H_2O$ 0.15 g L^{-1}, $MnSO_4\cdot H_2O$ 0.1 g L^{-1}, H_3BO_3 0.02 g L^{-1}, $Na_2MoO_4\cdot 8H_2O$ 0.8 g L^{-1}, and $NiCl_2\cdot 6H_2O$ 0.05 g L^{-1}. Two typical lignin-containing wastewater (traditional Chinese medicine wastewater and papermaking wastewater) were selected for degradation. In order to ensure the repeatability of the experimental results, model wastewater was used in this research. Artificial Chinese medicine wastewater was prepared according to the production process of a pharmaceutical factory in Qinhuangdao, Hebei Province, China. Because the wastewater was varying daily, the concentration of model wastewater was the average concentration of wastewater discharged. The preparation method consisted of boiling the traditional Chinese medicine raw materials for three hours, filtering, and diluting the solution. The average COD of the artificial traditional Chinese medicine wastewater was 1580 mg L^{-1}. The artificial papermaking wastewater was prepared according to reference[22]. The composition was humic acid, lignin, diatomite, microcrystalline cellulose, xylan, glucose, sodium bicarbonate, and potassium dihydrogen phosphate. The COD of the artificial papermaking wastewater was 1030 mg L^{-1}. It should be noted that, considering the fluctuation of wastewater concentration during production, the wastewater treatment effects under different wastewater concentrations were considered in the wastewater treatment and conditions optimization study.

Domestication of the microorganisms

The original microbial consortia were firstly transferred to the enriched medium. Subsequently, the microbial consortia were cultivated at pH 7, 30 °C, and 160 rpm for 72 h. The microbial consortia were then transferred to the mixed culture medium of model wastewater (inoculation amount of 10 %), and enrichment medium for multiple rounds of culture, and the proportion (volume proportion) of model wastewater in the culture medium tended to increase from 10 % to 100 %. The culture conditions remained unchanged. Thus, the microbial consortia could adapt to the environment of TCM wastewater and papermaking wastewater, respectively. Following their domestication, the microbial consortia were transferred to the simulated TCM wastewater and papermaking wastewater, respectively. Three cycles of incubation in the wastewater were performed followed by the wastewater treatment experiments. The domestication process of activated sludge was the same as that of microbial consortia domestication.

Wastewater treatment and conditions optimization

In this experiment, the model wastewater was prepared for wastewater treatment experiments. The free microbial consortium was inoculated into the wastewater for degradation. The volume of activated sludge introduced in the contrast experiment and the microbial consortium suspension was the same. The effects of treatment time, temperature, pH, dissolved oxygen, wastewater concentration, and nitrogen source supplement on the wastewater treatment were investigated according to Table 1. The optimal degradation conditions were obtained. The control experiments were set in the same operating mode without adding microorganisms. The aforementioned wastewater experiments were performed in the wastewater treatment unit reported previously[18]. The effective volume of the wastewater treatment unit was 8 L (20 cm × 20 cm × 20 cm), and 200 mesh nets were covered in the experiment to prevent other sundries from falling into it. The heating device (at the bottom of the reactor) controlled the temperature, and the dissolved oxygen was controlled through aeration device. The pH value of wastewater was adjusted using sulfuric acid and sodium hydroxide. The equipment was cleaned and disinfected before each round of use.

Analysis methods

The ammonia nitrogen (AN) concentration of the wastewater was measured by Nessler's reagent method[23]. The measurement method of SS was filter paper filtration[13]. The chromaticity of wastewater was detected using an accurate colorimeter (LH-SD500, Luheng Co. China). Dissolved oxygen was determined using a dissolved oxygen meter (HQ30D, HACH, USA).

The mixed solution samples were taken from the wastewater treatment equipment. The sampling time was the logarithmic growth period of microorganisms. The microorganisms were then collected by low-speed centrifugation. The microorganism samples were washed 1-3 times with sterile water, and stored at –80 °C. Microbial community genomic DNA was extracted from the samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-Tek, USA). The extracted DNA was preserved at –20 °C for test[24]. The primer pair 515F (5'-GTGCCAGCMGCCGCGG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') was used to amplify bacterial 16S rRNA genes in the community. The primer pair ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') was used to amplify fungal ITS genes. The purified amplicons were sequenced on MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, USA).

Fungal and bacterial biomasses were measured by the respiratory inhibition method[25]. Agar slices were used for microscopic examination of microbial consortia, and samples were placed in a microscope (CX23, Olympus, Japan) for microscopic examination.

The lignin concentration was measured by measuring the absorbance at 280 nm according to the reported method[26]. COD (mg L^{-1}) was measured on HACH equipment (HACH Co.), which consisted of HACH, DR/4000U spectrophotometer and COD heating reactor, using the standard HACH testing kits. The removal efficiency of COD and other parameters can be calculated as follows:

$$\text{Removal efficiency}(\%) = \frac{\text{initial COD} - \text{COD after treatment}}{\text{initial COD}} \cdot 100 \quad (1)$$

Table 1 – *Experimental design for wastewater treatment conditions*

Experimental sequence	Experimental parameters	
1	Temperature*: 30 °C pH*: 7 Dissolved oxygen*: 2.0 mg L^{-1} Wastewater concentration*: 100 % Nitrogen source supplement*: 0 g L^{-1} Inoculation amount*: 10 %	Treatment time: 0 h, 24 h, 48 h, 72 h, 96 h, 120 h and 144 h
2	pH*: 7 Dissolved oxygen*: 2.0 mg L^{-1} Wastewater concentration*: 100 % Nitrogen source supplement*: 0 g L^{-1} Inoculation amount*: 10 %	Temperature: 20 °C, 25 °C, 30 °C, 35 °C and 40 °C; Treatment time: 0-144 h
3	Temperature*: the optimal treatment temperature obtained from experimental sequence 1 Dissolved oxygen*: 2.0 mg L^{-1} Wastewater concentration*: 100 % Nitrogen source supplement*: 0 g L^{-1} Treatment time*: optimal treatment time obtained from experimental sequence 1 and 2 Inoculation amount*: 10 %	pH: 5, 6, 7, 8 and 9
4	Temperature*: optimal treatment temperature obtained from experimental sequence 1 pH*: optimal pH obtained from experimental sequence 3 Wastewater concentration*: 100 % Nitrogen source supplement*: 0 g L^{-1} Treatment time*: optimal treatment time obtained from experimental sequence 1 and 2 Inoculation amount*: 10 %	Dissolved oxygen: 1.0 mg L^{-1}, 2.0 mg L^{-1}, 3.0 mg L^{-1}, 4.0 mg L^{-1} and 5.0 mg L^{-1}
5	Temperature*: optimal treatment temperature obtained from experimental sequence 1 pH: optimal pH obtained from experimental sequence 3 Dissolved oxygen*: optimal DO value obtained from experimental sequence 4 Nitrogen source supplement*: 0 g L^{-1} Treatment time*: optimal treatment time obtained from experimental sequence 1 and 2 Inoculation amount*: 10 %	Wastewater concentration: 50 %, 100 %, 150 %, 200 %, and 250 %
6	Temperature*: optimal treatment temperature obtained from experimental sequence 1 pH*: optimal pH obtained from experimental sequence 3 Dissolved oxygen*: optimal DO value obtained from experimental sequence 4 Wastewater concentration*: Papermaking wastewater 100 % Treatment time*: optimal treatment time obtained from experimental sequence 1 and 2 Inoculation amount*: 10 %	Nitrogen source supplement: 0 g L^{-1}, 0.025 g L^{-1}, 0.05 g L^{-1}, 0.1 g L^{-1}, and 0.15 g L^{-1}

*invariant parameters

Statistical analysis

All the mentioned experiments were repeated three times, and the average data were reported. One-way ANOVA was used to detect any significant differences in the results (Tukey's test, $p<0.05$).

Results and discussion

Conditions optimization and analysis of wastewater degradation effect

Firstly, to determine the effectiveness of the screened microbial consortia, comparative experiments of different lignin-degrading microbial consortia and activated sludge were designed. The treatment conditions were as follows: temperature of 30 °C, inoculation amount of 10 % (v/v), pH of 7, and dissolved oxygen of 2.0 mg L^{-1}. The treatment time was 48 h. The experimental results are shown in Table 2.

The experimental results indicated that the COD removal efficiency of the obtained lignin-degrading microbial consortia was higher than that of activated sludge under current treatment conditions, thus confirming the superiority of the selected microorganisms in lignin-containing wastewater treatment. In addition, the screened microbial consortia had different performance in the treatment of different wastewater. The COD degradation effect of the two microbial consortia on TCM wastewater was better than that on papermaking wastewater. A considerable amount of research[6,14] has identified the difficulty of biodegradation of papermaking wastewater. Moreover, the domestication is beneficial for the microorganisms to adapt to the wastewater environment, thus increasing the wastewater degradation efficiency. Both the screened microbial consortia and activated sludge achieved better degradation efficiency after domestication, consistent with other research[27]. Furthermore, in-depth research was conducted on the optimized use of the screened microbial consortia and the factor for the degradation of lignin-containing wastewater.

Effect of treatment time on degradation

Existing research has suggested that, during the degradation of lignin, the degradation efficiencies of the obtained lignin-degrading microbial consortia on the 1st and 2nd days were often higher, and then reached a plateau on the 6th day[21]. Accordingly, in this experiment, the degradation time gradient was set to 24 h, 48 h, 72 h, 96 h, 120 h, and 144 h. The two microbial consortia were compared. Fig. 1 presents the results.

When treating TCM wastewater, the degradation efficiency tended to increase over time, and the COD removal efficiency of was stable at 96 h. The COD removal efficiency of papermaking wastewater continuously increased over time, and the COD removal efficiency was stable at 120 h. Metabolic inhibition occurred with the extension of the culture time, thus affecting the removal rate of COD[28]. Lignin is difficult to degrade, and high lignin degradation efficiency by fungal degradation always requires more than 15 days[29]. Compared with fungal degradation, the microbial consortia degraded faster.

3.1.2 Effect of temperature on COD removal

Fig. 2 presents the treatment of wastewater at different temperatures. The result showed that the

Table 2 – *Degradation of lignin-containing wastewater by different microorganisms*

Traditional Chinese medicine wastewater treatment			
Undomesticated microorganisms	Average removal efficiency of COD (%)	Domesticated microorganisms	Average removal efficiency of COD (%)
J-6	43.7±3.17	J-6	57.39±5.49
J-1	32.15±3.90	J-1	69.09±4.98
Activated sludge	27.09±1.66	Activated sludge	44.13±3.76
Blank test	0		
Papermaking wastewater treatment			
Undomesticated microorganisms	Average removal efficiency of COD (%)	Domesticated microorganisms	Average removal efficiency of COD (%)
J-6	31.94±4.27	J-6	45.65±1.04
J-1	20.13±1.89	J-1	31.45±3.28
Activated sludge	14.17±1.98	Activated sludge	23.77±2.13
Blank test	0		

In the blank experiment, no microorganisms were added.

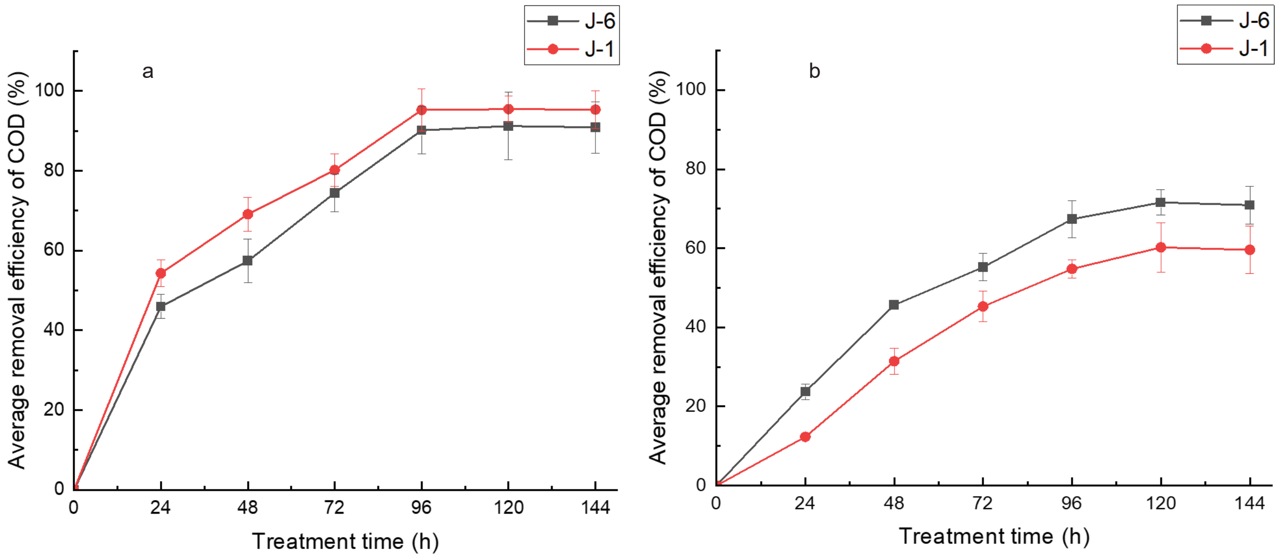

Fig. 1 – *Effect of treatment time on lignin-containing wastewater treatment (a: traditional Chinese medicine wastewater; b: papermaking wastewater)*

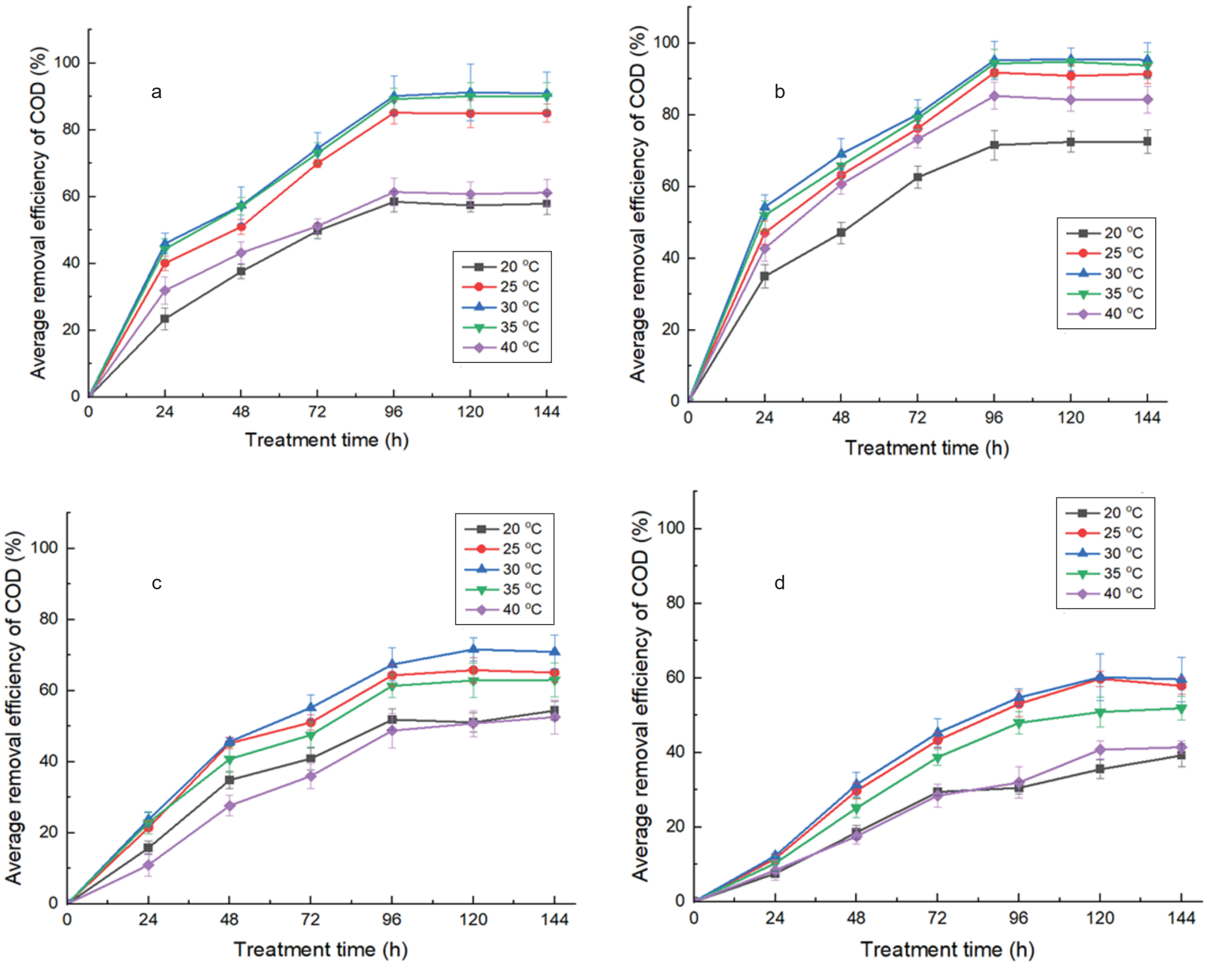

Fig. 2 – *Effect of temperature on lignin-containing wastewater treatment (a: traditional Chinese medicine wastewater treatment by J-6; b: traditional Chinese medicine wastewater treatment by J-1; c: papermaking wastewater treatment by J-6; d: papermaking wastewater treatment by J-1)*

optimal treatment temperature of the two microbial consortia was 30 °C. According to reference[30], the optimal growth temperature of most microorganisms was 25-35 °C, and excessive high or low temperatures had an effect on the activity of microbial anabolic enzymes. However, the result of the statistical analysis suggests that temperatures do not significantly affect the removal efficiency of COD in the two types of wastewaters, consistent with the effect of temperature on the degradation of lignin by the microbial consortia in our existing study[21]. In addition, according to the temperature factor experiment, it can be verified again that the best treatment times of the two types of wastewater were 96 h and 120 h, respectively. Therefore, in the subsequent treatment, the treatment time of traditional Chinese medicine wastewater was 96 h, and that of papermaking wastewater was 120 h.

Effect of pH on COD removal

The metabolism activities of microorganisms are closely correlated with the environmental pH value, such that the effect of pH should be considered in the process of wastewater treatment and lignin degradation. Over-acidic or alkaline environment will reduce or even inactivate the activity of some microbial enzymes[31]. As presented in Fig. 3a-b, the pH value affected the treatment process of papermaking wastewater more significantly than it did the TCM wastewater. In the treatment of TCM wastewater, COD degradation efficiency was the highest under neutral and weak acid conditions (pH = 6, 7). Under acidic conditions (pH = 4, 5), the removal efficiency of COD by J-6 was higher than that by J-1. Under neutral and weak alkaline conditions (pH = 7, 8), the treatment effect of J-1 was better. The differences in treatment effects between the two microbial consortia under different pH conditions may be correlated with their different consortium structure. In the treatment of papermaking wastewater, COD removal efficiency was higher under acidic conditions (pH = 4, 5). The lignin content in papermaking wastewater was high. Some existing research has suggested that the activity of lignin-degrading enzyme is higher under acidic conditions[32], such that the acidic conditions are more applicable to the treatment of papermaking wastewater. However, the original papermaking wastewater is always alkaline; therefore, the pH of the wastewater should be adjusted during microbial treatment.

Effect of dissolved oxygen on COD removal

In the process of lignin degradation, microorganisms have a certain demand for oxygen. For instance, in the shaking flask experiment of microbial lignin degradation, it is often necessary to adjust the shaking speed[33]. The previous experiments showed that the optimal dissolved oxygen concentration of the microbial consortia was 2.0-4.0 mg L^{-1}. In general, the dissolved oxygen concentration of aerobic biological wastewater treatment is controlled in the range from 1.0 mg L^{-1} to 5.0 mg L^{-1} [34]. Fig. 3c-d present the effect of dissolved oxygen on wastewater degradation. The results indicated that the COD removal efficiency of the two types of organic wastewater initially increased and then decreased with higher dissolved oxygen concentration. However, the result of the statistical analysis indicated that the effect of dissolved oxygen on the COD removal efficiency was not significant, thus suggesting that the microbial consortia J-6 and J-1 had high adaptability to the changes in dissolved oxygen environment, consistent with the conclusions of our previous study[21]. For the treatment of TCM wastewater, the COD removal efficiency reached 95.25 % by J-1 after 96 h at the dissolved oxygen concentration of 2.0 mg L^{-1}. For the treatment of papermaking wastewater, the removal efficiency of COD reached 75.6 % by J-6 after 120 h at the dissolved oxygen concentration of 3.0 mg L^{-1}. Microorganisms grow slowly under low dissolved oxygen. However, this will result in waste energy, and cause cell oxidation and death due to relative lack of nutrition if the concentration of dissolved oxygen is too high[35].

Effect of wastewater concentration on COD removal

Wastewater concentration has been found as a vital factor for the COD removal efficiency of wastewater[36]. The concentration of wastewater is easy to fluctuate under different production conditions. Fig. 3e-f illustrate the effects of wastewater concentration on COD removal efficiency. For the treatment of TCM wastewater in the range of 50 %-250 % of wastewater concentration, the removal efficiency of COD still achieved 89.7 % after 96 h by J-1 at the wastewater concentration of 200 %. For the treatment of papermaking wastewater, with the wastewater concentration of 200 %, the removal efficiency of COD decreased to 54.3 % through the treatment of J-6, and that reached 44.8 % through the treatment of J-1. High wastewater concentration will lead to the increase in microbial osmotic pressure, thus affecting their normal life activities and the degradation of organic matter[37]. The result of the one-way ANOVA of Tukey test indicated that the concentration of papermaking wastewater significantly affected the degradation efficiency of J-6 and J-1, whereas the concentration of TCM wastewater did not significantly affect the degradation efficiency of J-6 and J-1. The treatment efficiency of papermaking wastewater was insufficient, and the process should be optimized.

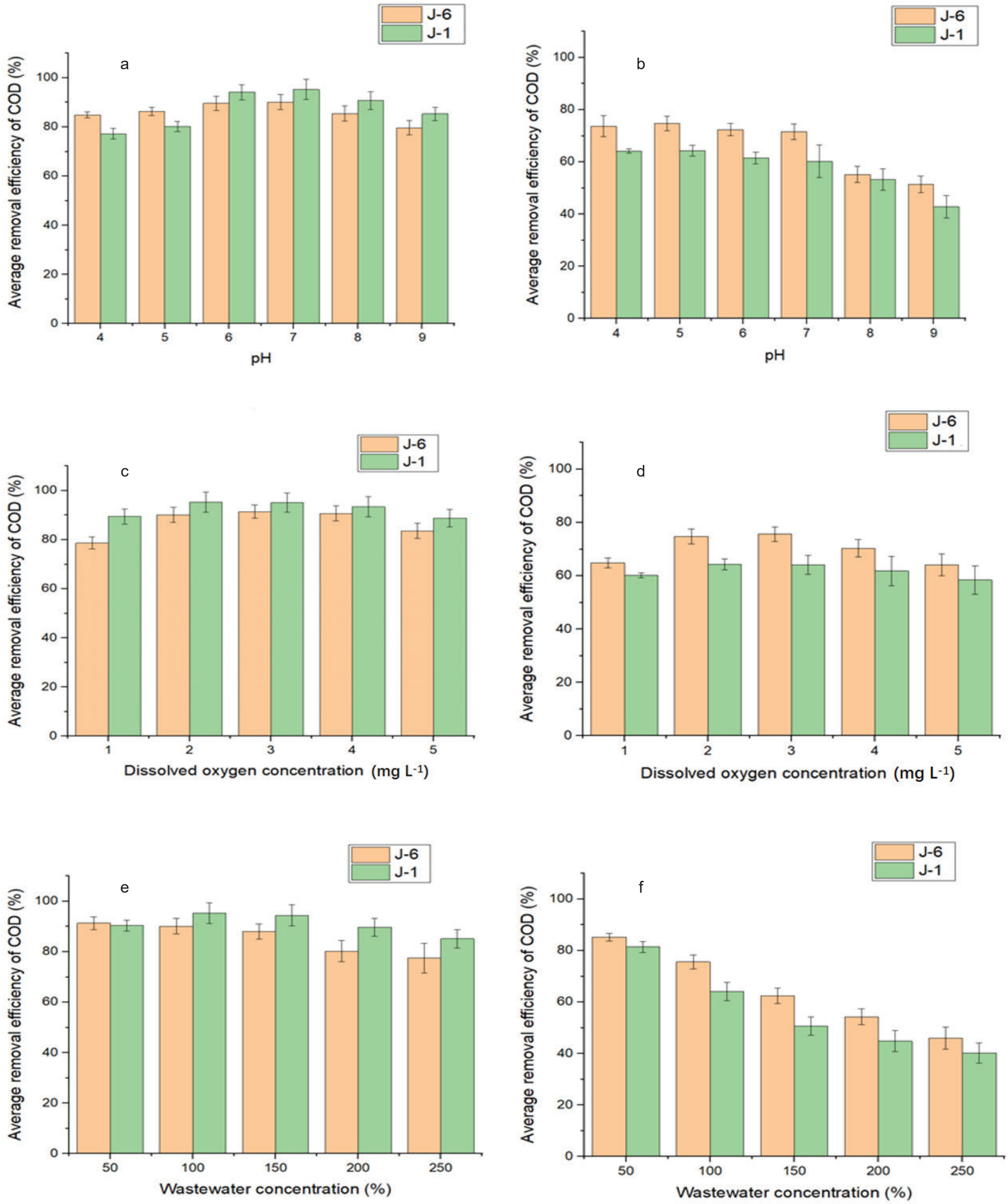

Fig. 3 – *Effect of different factors on the treatment of lignin-containing wastewater (a: effect of pH on traditional Chinese medicine wastewater treatment; b: effect of pH on papermaking wastewater treatment; c: effect of DO on traditional Chinese medicine wastewater treatment; d: effect of DO on papermaking wastewater treatment; e: effect of wastewater concentration on traditional Chinese medicine wastewater treatment; f: effect of wastewater concentration on papermaking wastewater treatment)*

Effect of nitrogen source addition on papermaking wastewater treatment

Nitrogen sources were always added when biological treatment of papermaking wastewater was performed because it is nitrogen-deficient[38]. The effect of nitrogen source addition on the removal of COD in wastewater was studied. The results are presented in Fig. 4. The results indicated that, at the concentration of nitrogen source of 0.1 g L^{-1}, the COD removal efficiency of microbiota J-6 in the treatment of papermaking wastewater was 86.8 %; the treatment effect increased significantly, and the ammonia nitrogen concentration of effluent was stable and lower than the discharge standard value (15 mg L^{-1}). The effluent ammonia nitrogen concentration reached 20.8 mg L^{-1} at the supplemental concentration of ammonium sulfate of 0.15 g L^{-1}, thus exceeding the discharge standard. Accordingly, the supplemental ammonium sulfate concentration should be controlled. The nitrogen source can be used effectively by microorganisms. Nitrogen is a vital element of microorganisms. The addition of nitrogen source can effectively facilitate the growth and reproduction of microorganisms in the microbial consortium and increase the treatment effect[39].

In addition, the effect of inoculation amount on wastewater degradation was explored. The results indicated that in the range of 2 %–15 % inoculation amount, the effect of inoculation amount of microbiota on COD degradation of wastewater was relatively small. The inoculation amount had a slight effect on the wastewater treatment process, consistent with other research[27].

In summary, the optimal treatment conditions of the two types of wastewaters were presented as follows. For the treatment of TCM wastewater, the optimal degradation conditions included the treatment temperature of 30 °C, inoculation amount of 10 % (v/v), initial pH of 7, DO of 2 mg L^{-1}, as well as treatment time of 96 h. The COD removal efficiency was 95.25 % by J-1. Through the test of other water quality parameters, the degradation efficiency of lignin reached 95 %, and decolorization efficiency of 96.8 % under the optimal conditions. Moreover, the microbial consortium played a certain role in the adsorption and sedimentation of the suspended solids, and the removal efficiency of SS by J-1 was 91.72 %. For the treatment of papermaking wastewater, the optimal degradation conditions included treatment temperature of 30 °C, inoculation amount of 10 % (v/v), pH of 5, DO of 3 mg L^{-1}, nitrogen source concentration of 0.1 g L^{-1}, and treatment time of 120 h. The COD removal efficiency was 86.8 % by J-6. Through the test of other water quality parameters, the degradation efficiency of lignin reached 87.4 %, and achieved decolorization efficiency of 90.3 % under the optimal conditions. In addition, the removal efficiency of SS by J-6 was 89.84 %. The degradation of real wastewater was also tested, and the results showed that the average degradation efficiency of COD in traditional Chinese medicine wastewater reached 92 %, and the average degradation efficiency of COD in papermaking wastewater reached 80 %. The research results were compared with other references. Chen *et al.*, used membrane bioreactor (MBR) method to treat TCM wastewater, and almost all the COD was removed through the physical and biological process[13]. Liang *et al.* used a composite method to treat pulp and paper wastewater, and the results showed that the physical, anaerobic, aerobic, and chemical steps accounted for 41.6 %, 40.0 %, 11.9 %, and 6.5

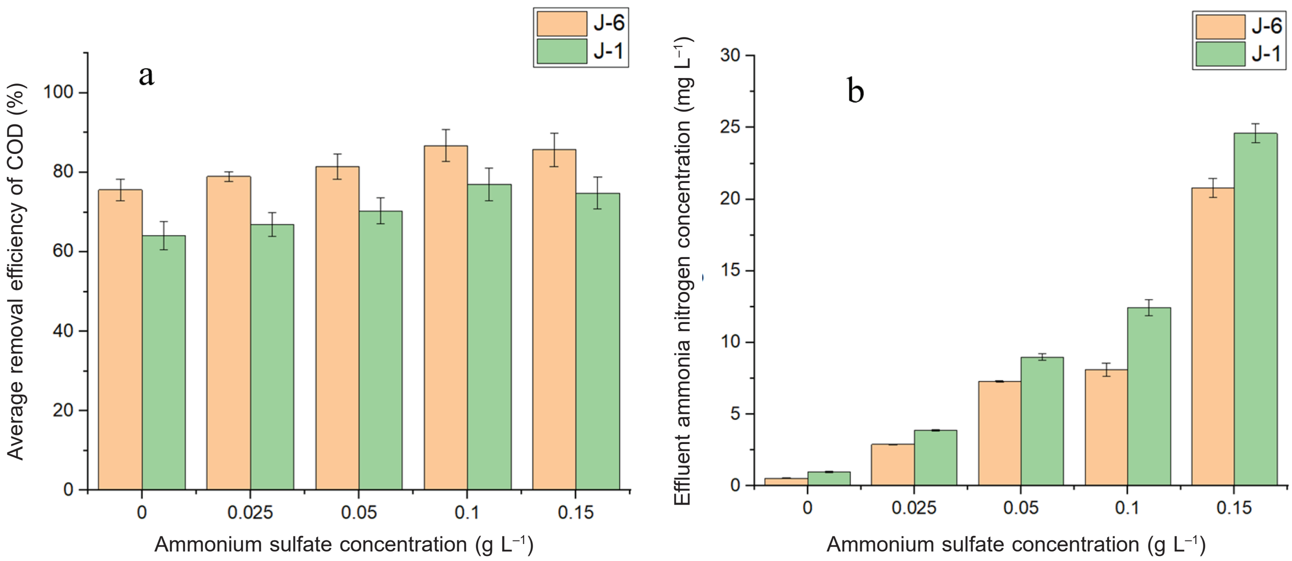

Fig. 4 – *Effect of nitrogen source addition on papermaking wastewater treatment (a: average removal efficiency of COD under different nitrogen addition; b: effluent ammonia nitrogen concentration under different nitrogen addition)*

% of COD removal, and physical and anaerobic treatments contributed significantly to COD degradation[14]. The aerobic biological method in this study can have good treatment effects on the two types of wastewaters. It is found that the lignin-degrading microbial consortia have strong application prospects.

Consortium structure and diversity analysis

Existing research has suggested that the two microbial consortia are capable of effectively remove COD from wastewater in different wastewater degradation systems. The treatment efficiency of the two microbial consortia was different. J-6 was more efficient in treating papermaking wastewater. J-1 was more efficient in treating TCM wastewater, but the efficiency difference between J-6 and J-1 in treating TCM wastewater was minor. To reveal the mechanism of microbial degradation under different wastewater conditions, high-throughput sequencing analysis of microbial consortia in different wastewater was performed. In different wastewater degradation systems, the consortium structures of microbial consortia will change to adapt to different wastewater environments, and enhance the treatment effect of wastewater[40].

Analysis of consortium diversity and composition

The abundance index Chao and OTUs (Table 3) indicated that the bacterial consortium abundance in TCM wastewater group was higher than that in the papermaking wastewater group. The possible reason for this result is that the high toxicity of papermaking wastewater has a certain effect in inhibiting the activity of microorganisms during the microbial consortium domestication. However, there was little difference between the TCM wastewater group and the papermaking wastewater group in the consortium diversity index faith_ pd, Simpson and Shannon indexes of different wastewater treatment groups. Furthermore, the bacterial abundance and diversity of J-6 were higher than were those of J-1, especially in the papermaking wastewater system. Compared with previous research results, J-6 had significantly higher degradation efficiency in the papermaking wastewater system. Thus, the bacterial consortium abundance may promote degradation of organic substances in the wastewater.

The consortium structural changes of the two microbial consortia in different lignin-containing wastewater were also investigated to analyze the dominant strains in different wastewater degradation systems. Fig. 5 presents the comparison results of bacterial consortium composition. The bacterial composition in J-6 in the papermaking wastewater treatment system, primarily comprised *Sphingomonas* (47.8 %), *Rhodoplanes* (29.9 %), *Lactobacillus* (2.54 %), *Pseudomonas* (1.94 %), *Serratia* (1.71 %), *Ralstonia* (1.37 %), unspecified_ Clostridiales (1.37 %), unspecified_ Alcaligenaceae (1.34 %), etc. Moreover, the bacterial composition in J-6 in the TCM wastewater treatment system changed to *Rhodoplanes* (70.9 %), *Pseudomonas* (2.56 %), *Serratia* (2.46 %), unspecified_ Clostridiales (21.0 %), *Lactobacillus* (1.76 %), *Ralstonia* (1.61 %), unspecified_ Alcaligenaceae (1.60 %), *Bacteroides* (0.84 %), etc. The result indicated that some microbial species in the two wastewater systems were the same, whereas the dominant strains were different. *Sphingomonas* and *Rhodoplanes* became the dominant strains in the treatment of papermaking wastewater, and *Rhodoplanes* was the dominant strain in the treatment of TCM wastewater. *Rhodoplanes* accounted for a large proportion in the two lignin-degradation systems. *Rhodoplanes* is a common bacterium in lignin degradation systems or lignocellulosic biomass degradation systems, which has been reported in numerous studies[41,42]. This strain has high adaptability to this type of environment, and it is very reasonable for them to exist in large quantities in the treatment system of lignin-containing wastewater. *Sphingomonas* is a microbial type existing in the environment that can degrade aromatic pollutants. It has a significant advantage in degrading monocyclic aromatic hydrocarbons, polycyclic aromatic hydrocarbons, and chlorinated aromatic compounds[43]. As we know, the partial degradation of lignin, bleaching and bleaching processes in the papermaking process produce certain toxic aromatic compounds. As a result, the proportion of *Sphingomonas* may increase in the degradation system of papermaking wastewater. *Sphingomonas* is also a very common microbial type in organic wastewater systems and environmental remediation systems[44].

Table 3 – *Bacterial community diversity in different samples*

Sample	observed_otus	faith_pd	Chao	Simpson	Shannon
Papermaking wastewater J-6	574	44.83625655	575.721519	0.684778507	3.101135517
Traditional Chinese medicine wastewater J-6	645	46.06183038	646.7662338	0.505831166	2.989028359
Papermaking wastewater J-1	275	31.96264288	275.5806452	0.666234765	2.221483538
Traditional Chinese medicine wastewater J-1	356	34.9931803	356.6707317	0.533884532	1.911123386

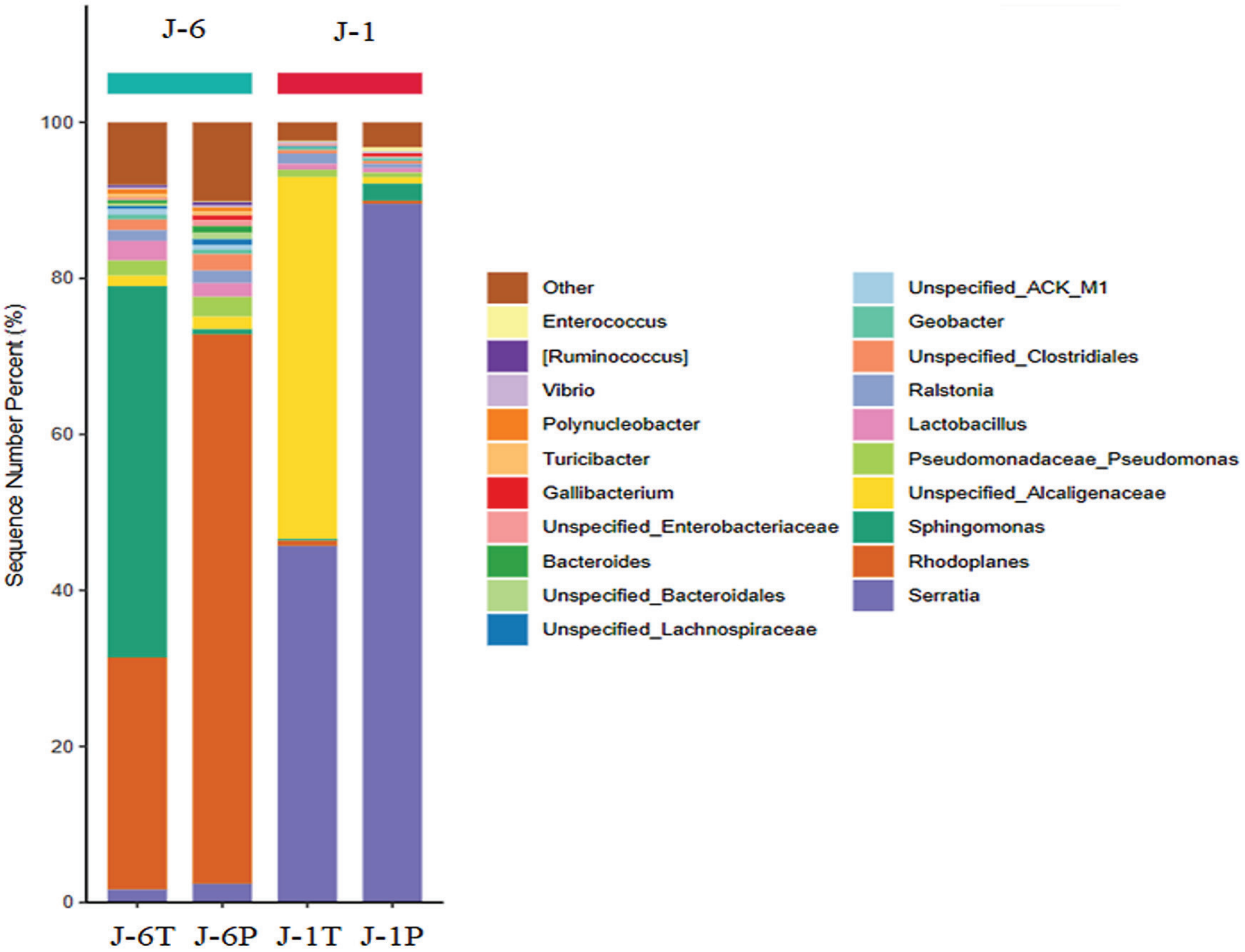

Fig. 5 – *Bacterial community composition of different wastewater treatment systems (T: traditional Chinese medicine wastewater; P: papermaking wastewater)*

The bacterial composition of J-1 in the papermaking wastewater treatment system largely comprised unspecified_ Alcaligenaceae (46.5 %), *Serratia* (45.7 %), *Ralstonia* (1.33 %), *Pseudomonas* (0.93 %), *Lactobacillus* (0.76 %), *Rhodoplanes* (0.68 %), unspecified_ Clostridiales (0.42 %), *Geobacter* (0.37 %), etc. Moreover, the bacterial composition of J-1 in the TCM wastewater treatment system changed to *Serratia* (89.7 %), *Sphingomonas* (2.20 %), unspecified_ Alcaligenaceae (0.82 %), *Pseudomonas* (0.60 %), *Lactobacillus* (0.58 %), *Ralstonia* (0.55 %), etc. The result indicated that some microbial species in the two wastewater systems were also the same, whereas the microbial consortium composition in the papermaking wastewater group was inconsistent with that in the TCM wastewater group. The species with the highest abundance was different. It was *Serratia* in the degradation system of TCM wastewater, whereas Alcaligenaceae and *Serratia* were the dominant strains in the degradation system of papermaking wastewater. Alcaligenaceae and *Serratia* accounted for a high proportion in the original J-1 microbial consortium. The mentioned microorganisms have also been reported in other existing research. Moraes *et al.*, screened the microbial consortium with lignin-degrading function from plant soil samples, and found that the Alcaligenaceae content in the microbial consortium increased significantly compared with the original soil, which verified the adaptability of Alcaligenaceae in such environment[45]. The *Serratia* bacteria isolated from sewage-polluted soil can degrade and treat pulp-making wastewater and paper-mill wastewater, significantly reduce the lignin concentration, and remove the toxicity of papermaking wastewater[46]. Accordingly, *Serratia* can also become a dominant bacterium in the degradation of papermaking wastewater.

In addition, the result of the genome sequencing analysis indicated that the fungal consortium composition of the microbiota of different systems was almost consistent with that of the original microbial consortium. Our previous research and other studies have suggested that fungi also have great significance in the lignin-degradation system[21,47]. However, the biomass of fungi was lower than that

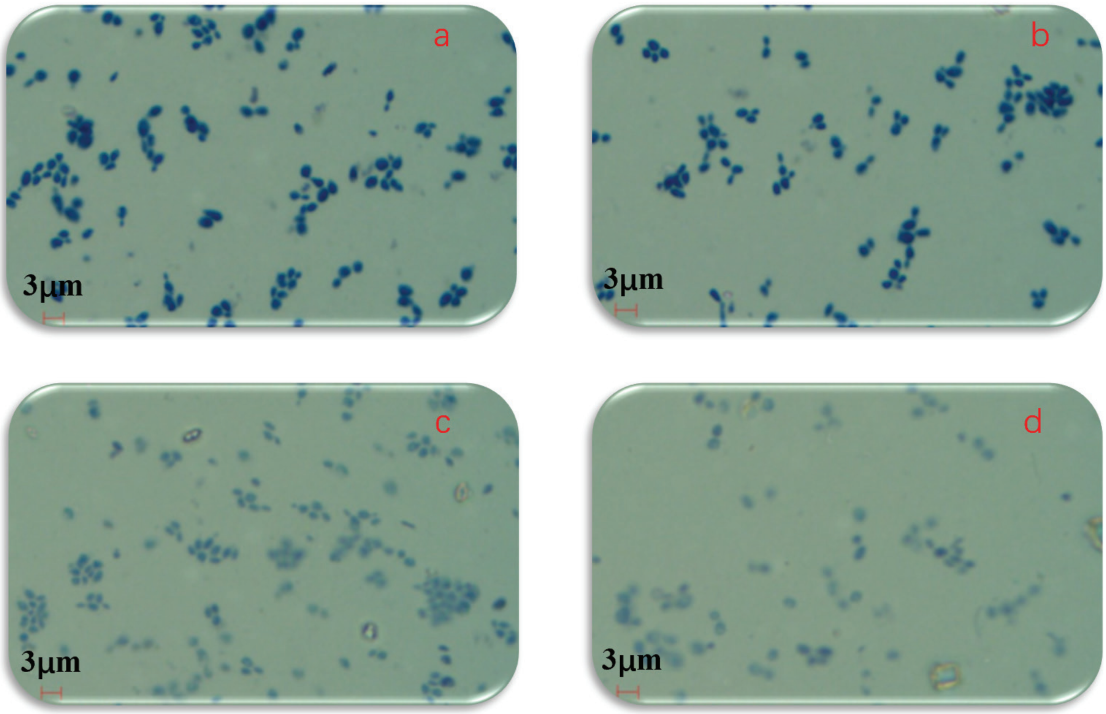

Fig. 6 – *Microscopic examination of lignin degradation microbial consortia in different wastewater treatment systems (a: traditional Chinese medicine wastewater treatment by J-1; b: papermaking wastewater treatment by J-1; c: traditional Chinese medicine wastewater treatment by J-6; d: papermaking wastewater treatment by J-6)*

of the original microbial consortium compared with the changes in the biomass of fungi and bacteria. In addition, microscopic examination (Fig. 6) showed that J-1 and J-6 contained oval and spherical single cells, and no large fungal hyphae were present. Some microorganisms agglomerated to form aggregates, which could also adsorb pollutants in water. It is therefore speculated that bacteria played a significant role in the degradation of lignin-containing wastewater.

It is noteworthy that, although the treatment equipment was cleaned and disinfected before the respective rounds of use, the two types of wastewaters and treatment equipment was not well sterilized. As a result, the effect of external microorganisms on the system cannot be excluded. However, the results indicated that the existing microorganisms in the original microbial consortium accounted for a large proportion in the two wastewater treatment systems, thus confirming the environmental adaptability of the used microbial consortium and its feasibility in the real wastewater treatment.

Microorganism correlation analysis

The correlation between different species in the sample or in the sample group was examined based on the relative abundance among species through the analysis of multiple samples of the two wastewater treatment systems[47]. Subsequently, the species interaction network was constructed using the visualization software showing the interrelationship among species (Fig. 7). The above analysis is capable of searching for mutually antagonistic or synergistic species, and finding the information regarding microbial consortium synergy or mutual inhibition in the environmental samples to examine the microorganisms that work together or inhibit each other in the degradation system of lignin-containing wastewater. The circle in the figure represents a species, and the size represents its relative abundance. The different colors represent different species phyla classifications. The lines between the circles indicate the significant correlation between the two species ($P<0.05$). The red line represents the positive correlation, and blue represents the negative correlation. The thick line suggests the high absolute value of the correlation coefficient. The results indicated that there were many positive correlations among microorganisms in the lignin-containing wastewater treatment systems, and the negative correlations were concentrated under different phyla. The *Serratia* bacteria with the highest abundance were not significantly correlated with the species with higher abundance (e.g., *Rhodoplanes*, *Sphingomonas,* and *Pseudomonas*). The above strains can play their respective roles in the wastewater treatment system to achieve the wastewater degradation effect. However, *Serratia* was negatively correlated with *Geobacter* and *Lactoba-*

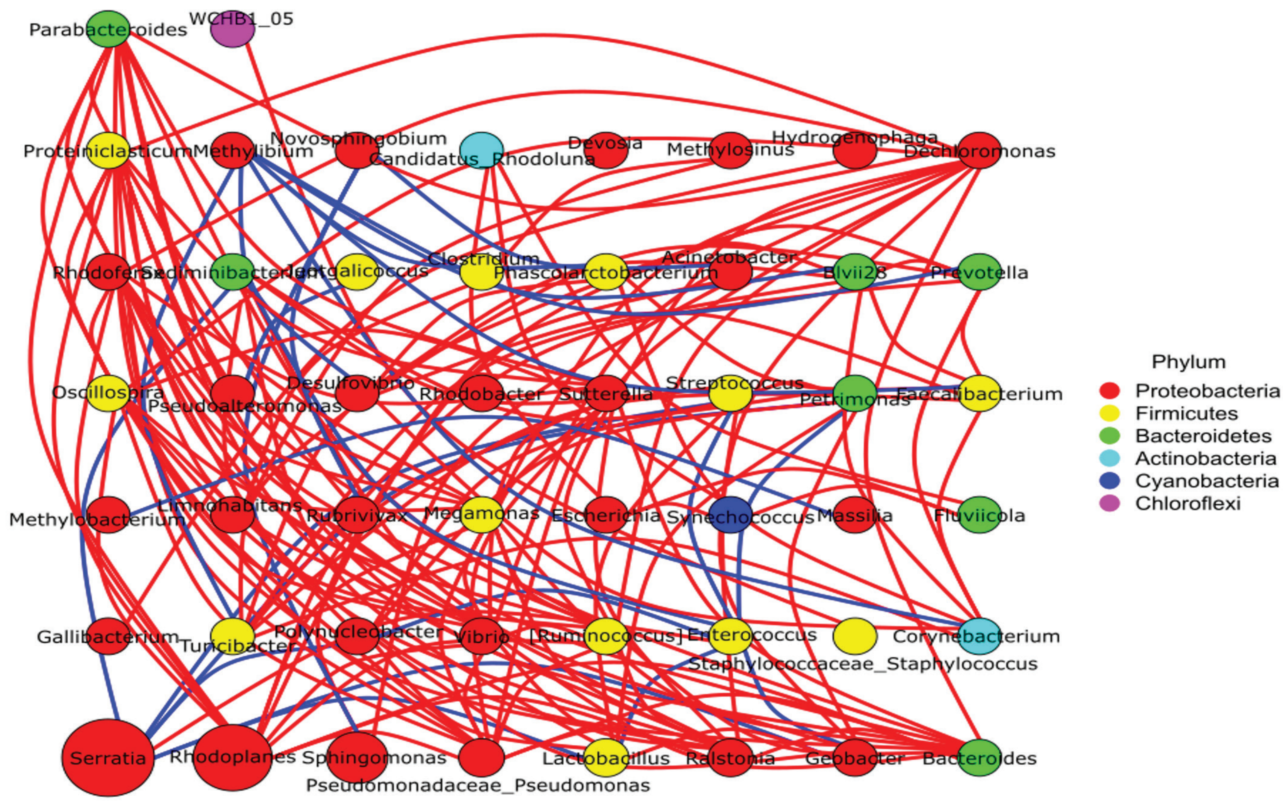

Fig. 7 – *Correlation between different species in the lignin-containing wastewater treatment systems*

cillus with certain abundance in the consortium. *Serratia* coexisted with *Geobacter* and *Lactobacillus* in the consortium J-1 in the treatment of papermaking wastewater, such that the degradation effect of J-1 on papermaking wastewater may be affected. Nevertheless, no negative correlations were identified between the species with high abundance in J-6, which may explain the reason why J-6 has a better effect on degrading papermaking wastewater compared to J-1.

Conclusion

In this research, the obtained microbial consortia with high lignin degradation efficiency and strong environment adaptability were selected to treat two kinds of lignin-containing wastewater. For the treatment of Chinese medicine wastewater, the COD removal efficiency reached 95.25 % with J-1 under the optimal degradation conditions. For the treatment of papermaking wastewater, the COD removal efficiency reached 86.8 % with J-6 under the optimal degradation conditions. The performances of two microbial consortia in wastewater treatment were compared, and the changes in community structure analyzed to study the relationship between microbial consortium structure and degradation efficiency. It was speculated that bacteria played a significant role in the degradation of lignin-containing wastewater, and the bacterial consortium abundance may promote the degradation of organic substances in the wastewater. The correlation between microorganisms and the difference in the abundance of bacteria groups may be the reason for the different performances of the two microbial consortia in treating different kinds of lignin-containing wastewater.

In future research, the treatment process of lignin-containing wastewater can be further optimized. The combined treatment of microbial consortium and other physical and chemical methods can be used to improve treatment efficiency.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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