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Research Article

Immobilized Microbial Catalytic Oxidation Preparation and Application of Biopolymeric Ferric Sulfate

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A novel inorganic polymer flocculant, biopolymeric ferric sulfate (BPFS), was prepared by immobilization technology of microorganisms and by oxidation of ferrous sulfate using domestic *Thiobacillus ferrooxidans* (*T. f*) under acidic condition. *T. f* was isolated on the agarose single-plate medium, which exhibited an unusual trait on the utilization of low concentration of the nitrogen source and phosphorus as the nutrient substance. Under the optimal conditions, the microorganism could grow and reproduce normally and maintain the strong catalytic oxidation activity to Fe^{2+}. The immobilization of *T. f* on the polyurethane as the support matrix was investigated. Cycling batch operation was applied to the preparation of 40 kg/m^3, 60 kg/m^3, and 80 kg/m^3 BPFS when the optimal conditions are pH value of 1.8, circulation flow rate of 0.28–0.30 L/h, and reaction temperature of 28 ± 1°C. When the prepared BPFS and SPFS (solid biopolymeric ferric sulfate) were used to dispose Songhua River water, the removal rate of turbidity and COD_{Mn} of BPFS was slightly better than that of SPFS. The removal efficiencies of turbidity and COD_{Mn} by BPFS could reach 93.9% and 79.7%, respectively. The result suggests that the BPFS has good flocculating activity.

1. Introduction

An important inorganic macromolecule flocculant, polymeric ferric sulfate (PFS) is mostly prepared by direct oxidation of ferrous sulfate using strong oxidants such as H_2O_2, $KClO_3$, NaClO, and HNO_3 or by catalytic oxidation of ferrous sulfate using $NaNO_2$ or NaI as a catalyst in the acid media [1–7]. These production methods are under harsh production conditions, long period, high cost, and oxidant residue resulting in serious corrosion on the dosing system [8]. In order to overcome these shortcomings, biotechnology was introduced into the preparation of PFS. The product called biopolymeric ferric sulfate (BPFS) was prepared by catalytic oxidation of microorganisms. BPFS has good turbidity and COD removal, its preparation conditions are mild, production costs are low, the process is no pollution, and microbial secretions can play a secondary flocculation [9].

Thiobacillus ferrooxidans (*T. f*) is Gram-negative chemolithotrophic bacterium. *T. f* with CO_2 as the carbon source and nitrogen and phosphorus as nutrients can obtain energy from the oxidation of ferrous iron and sulfur [10, 11]. *T. f* has been widely utilized in the processes of bioleaching, the desulphurization of coal and environmental pollution control. In recent years, there are many researches on their leaching capacity and their own metabolic activities. Arsenic (As) leaching was conducted from highly concentrated As mine tailings using the iron-oxidizing bacteria, and the pH range should be controlled to prevent the formation of jarosite [12]. More recently, *T. f* has been widely utilized in the processes of preparation of BPFS at normal temperatures and pressures [13]. At present, the methods of preparation of BPFS by biocatalytic oxidation have many advantages such as wide sources of raw materials and easy operation, but the easy loss of bacteria leads to the low utilization rate of bacteria and production efficiency and the long period.

In order to further improve the reaction rate and shorten the reaction cycle, the preparation of BPFS by the microbial immobilization technology was studied [14]. In this study, BPFS was prepared by the microbial immobilization technology on the polyurethane as the support

matrix. The influence of aeration and nutrient factors on the oxidation rate of Fe^{2+} was also investigated. The growth of *T. f* in low concentration of nutrient conditions was studied to optimize the preparation of BPFS. At the same time, its application in water treatment was also investigated. This study provides the theoretical basis for the further industrialization of BPFS and the expansion of application fields.

2. Materials and Methods

2.1. Culture Media. The enrichment and culture by *T. f* were studied in the 9K medium. *T. f* was collected from strains preserved in our lab. Solid medium Z (1 L) consisted of 500 mL 9K basal salt media (pH 2, autoclaved at 121 ± 1°C), 200 mL Ø0.45 μm filter-sterilized $FeSO_4{\cdot}7H_2O$, and 300 mL 2% (w/v) agarose (autoclaved at 121 ± 1°C). These three solutions were autoclaved/sterilized separately and mixed when the temperature of the autoclaved solution was cooled to 45 ± 1°C [15].

2.2. Enrichment and Isolation. *T. f* was enriched in the solid medium Z successively and incubated in a 30 ± 1°C incubator. The pure isolate was obtained after many times of sequential respreadings [16].

2.3. The Molecular Biology Identification of Thiobacillus ferrooxidans. The genomic DNA was extracted from the bacterial genome, amplified by PCR, and amplified. The recovered fragment was sent to Ming Chen Zhiyuan Biotech Company for sequencing. The sequencing primers were 16S PCR primers. Sequence identification was performed at the BLAST facility of the National Center for Biotechnology Information (NCBI) to identify microorganisms.

2.4. Culture of the Strain. *T. f* was enriched in the 9K medium at 30 ± 1°C under shaking condition (150 rpm). Short rod-shaped bacterium was observed into the 9K liquid medium after logarithmic growth. The influence of pH and concentration of nitrogen and phosphorus in solution on catalytic oxidation of Fe^{2+} activity was also investigated.

2.5. Preparation of BPFS. In this experiment, a bioreactor with reticulated polyurethane as the carrier was used to carry out the immobilization experiment of the microbe and the preparation experiment of the polyferric sulfate by repeated batch operation [17, 18]. The preparation apparatus is shown in Figure 1.

Based on breeding selection and domestication, *T. f* was selected as the biocatalyst to prepare BPFS. Cycling batch operation results in the preparation of 40 kg/m^3, 60 kg/m^3, and 80 kg/m^3 BPFS when the optimal conditions are circulation flow rate 0.28–0.30 L/h and reaction temperature 28 ± 1°C.

2.6. Flocculation Experiments. To evaluate the flocculation effect of 40 kg/m^3, 60 kg/m^3, and 80 kg/m^3 BPFS and SPFS, flocculation experiments tested winter Songhua river (T = 1 ± 1°C, pH = 7.52, turbidity = 2.61 NTU, COD_{Mn} = 6.21 mg/L) in a jar test apparatus. Afterwards, the suspension was agitated at speed of 150 r/min for 60 s and at speed of 70 r/min for 20 min; then, it was left undisturbed for 25 min, and the supernatant sample was collected for further analysis.

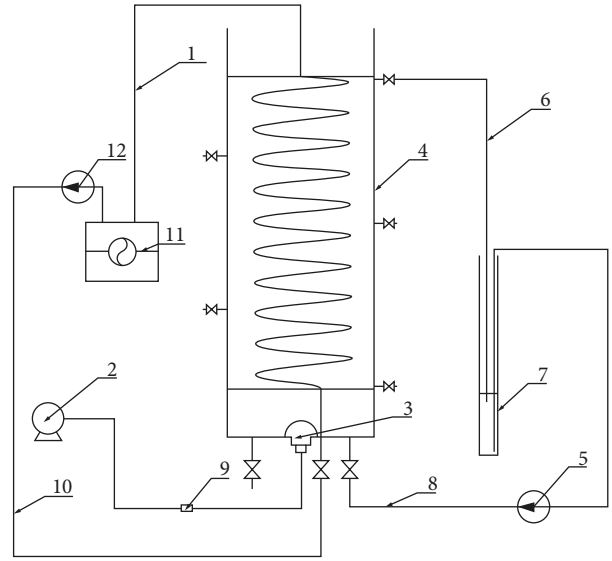

Figure 1: BPFS preparation device. 1, circulating water outlet pipe; 2, electromagnetic air pump; 3, microporous aerator; 4, bioreactor; 5, electromagnetic diaphragm pump; 6, drainpipe; 7, circulation collector; 8, liquid inlet tube; 9, rotameter; 10, circulating water inlet pipe; 11, thermostatic waterbath; 12, immersible pump.

3. Results and Discussion

3.1. Isolation and Molecular Biology Identification of the Strain. Round red-brown colonies on the culture were observed on the surface of the solid medium Z after two weeks. And then after single-layer coating on the separation, a large colony was selected and repeatedly purified in the 9K liquid medium. The agarose single-layer plate method was used for 16S rDNA sequence analysis and identification, the sequence length of 1469 bp (Figure 2).

Thiobacillus ferrooxidans is strictly autotrophic organisms, and most of the chemoautotrophic organisms are highly sensitive to organic compounds, such as polysaccharides and other components in agar. The small molecules are produced by the agar under acidic condition. However, saccharomycetes can metabolize the small molecules produced by the agar and inhibit the growth of *T. f* on the solid medium. Therefore, the modified solid medium used agarose instead of agar to gel solidify the medium, and this can support the growth of most strains [19, 20].

The sequence homology was compared with that of NCBI by Blast. The results showed that the strain had high homology with Acidithiobacillus sp. E6-1, 3–8 HM769771.1 (>99.5%), and it can be concluded that the strain belongs to the genus *Thiobacillus acidophilus*. The strain was fermented

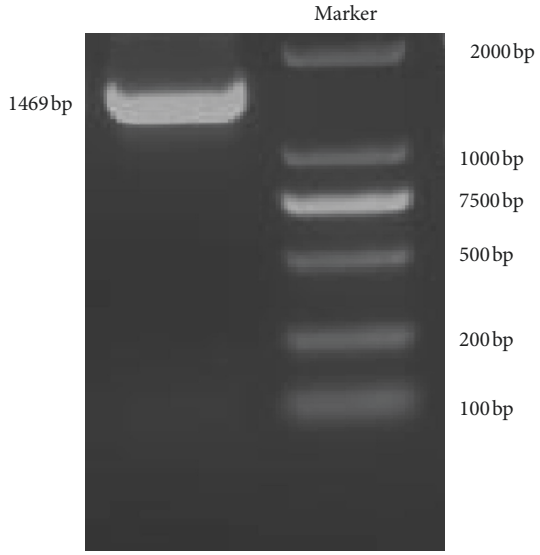

Figure 2: 16S rDNA amplification product electrophoresis.

with iron as energy and strictly autotrophic, according to "Berger's Bacteria Identification Manual" to identify it as *T. f*.

3.2. Culture of *Thiobacillus ferrooxidans*

3.2.1. Influence of the Initial pH. pH is very important for microbial growth and activity of microbial enzymes. To investigate the effect of pH on culture of *T. f*, experiments were conducted at five different pH values. The results are shown in Figure 3. The produced precipitates were dried and weighed, and the obtained precipitates were 0, 0.33, 1.92, 3.64, and 4.78 g/L, respectively. The preliminary analysis of the precipitate showed yellow potassium ferrite and some metabolites of microorganisms, during the process of microbial growth and reproduction. Due to the increasing Fe^{3+} content, it easily reacts with M^+ (where M may be K^+, NH^{4+}, Na^+, etc.), HSO_4^-, and H_2O and induces the precipitation of jarosite $[MFe_3(SO)(OH)_6]$.

According to Figure 3, the decrease in biooxidation is related to the inhibition of bacterial activity by the pH value in a certain range. When $pH \le 1.2$, Fe^{2+} content keeps steady, due to partial enzyme of *T. f* inactivation, making the thallus not to carry out the complete life activities, grow, and multiply. Therefore, Fe^{2+} cannot be oxidized to Fe^{3+}, and the reaction cannot continue to occur. When $1.2 < pH < 1.6$, it can inhibit the growth of bacteria, and the average oxidation rate of Fe^{2+} decreases. When $1.6 \le pH \le 2.0$, with the increase of catalytic activity of *T. f*, the average oxidation rate of Fe^{2+} increases. As pH increases, more precipitation is generated. In addition, considering reagent usages and generating precipitation, the initial pH value of 1.8 is considered to be appropriate for the preparation of BPFS.

3.2.2. Influence of Nitrogen and Phosphorus Source Concentration. The effects of two nutrient contents on

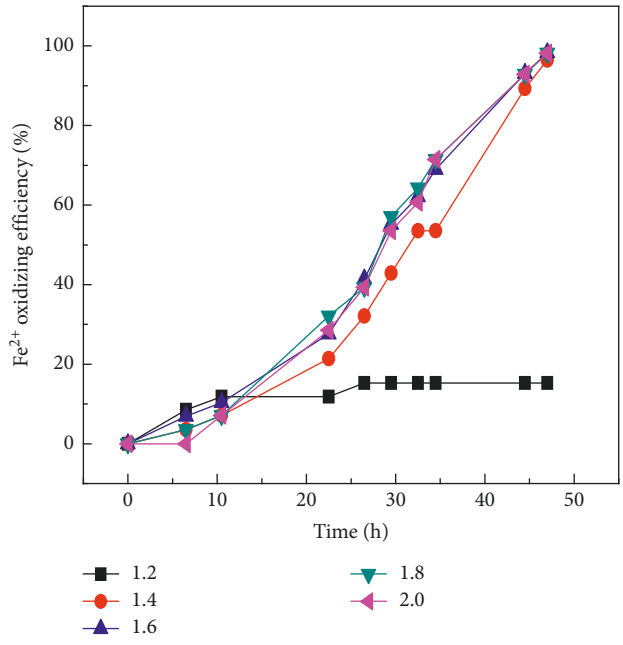

Figure 3: Fe^{2+} oxidizing efficiency vs. time at different initial pH values.

microbial growth and the Fe^{2+} oxidizing efficiency were investigated. The results are shown in Figure 4.

As shown in Figure 4, under the catalysis of microbes, reddish-brown BPFS was synthesized through 9 g/L and 40 g/L of industrial ferrous sulfate solution as the energy source, both at low concentration of nutrients and normal concentration of nutrients. It is illustrated that, under low concentration of nutrients, the concentration of nutrients does not have much impact on the conversion of Fe^{2+} and keeps good oxidative activity of bacteria. Thus, reducing the nitrogen and phosphorus concentration for the preparation of BPFS is chosen.

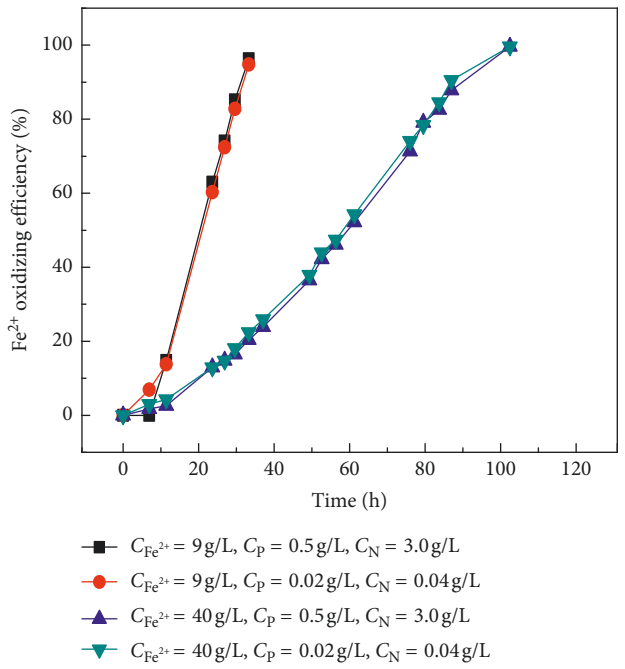

Figure 4: Fe^{2+} oxidizing efficiency vs. time at different nitrogen and phosphorus concentrations.

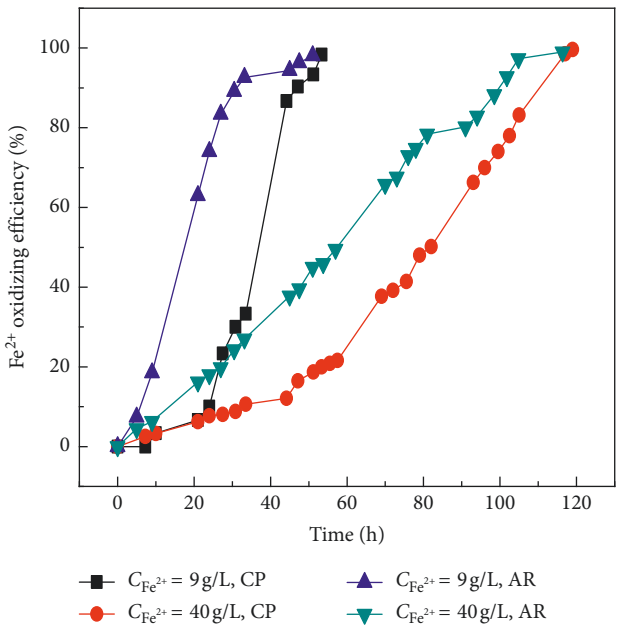

Figure 5: Fe^{2+} oxidizing efficiency vs. time at a different grade ferrous sulfate.

3.2.3. Adaptability of Thiobacillus ferrooxidans to Industrial Ferrous Sulfate. The effect of industrial ferrous sulfate as an energy source in the preparation of BPFS on microbial growth and the Fe^{2+} oxidizing efficiency was investigated. The results are shown in Figure 5.

As shown in Figure 5, under the condition of low concentration of nutrients, with the increase of Fe^{2+} concentration, the average oxidation rate of Fe^{2+} decreases. Low Fe^{2+} concentration cannot provide sufficient energy to the growth of bacteria. However, too much Fe^{2+} can inhibit the growth of bacteria, making microbial catalytic oxidation capacity to be reduced. Ferrous sulfate solution with high or low Fe^{2+} content has a negative effect on the activity of microbial catalytic oxidation, which is due to industrial sulfuric acid iron solution containing copper, zinc, manganese, and other impurities [21], inhibiting the growth of bacteria, making microbial catalytic oxidation capacity to be reduced. Based on the comparison of the growth of bacteria under different $FeSO_4$ conditions, considering reagent usages and production costs, it is concluded that industrial ferrous sulfate has a higher practical value.

3.3. Preparation of BPFS by Immobilized Microorganisms

3.3.1. Microbial Immobilization with Reticulated Polyurethane. The adsorption and immobilization process of microorganisms in the reticulated polyurethane bioreactor was shown by the average oxidation rate of Fe^{2+} in the previous 18 cycles. The results are shown in Figure 6.

As shown in Figure 6, the average oxidation rate of Fe^{2+} increased with the reaction period in the immobilization (biofilm culturing) stage. When the average oxidation rate of Fe^{2+} stabilized in two adjacent periods, the number of

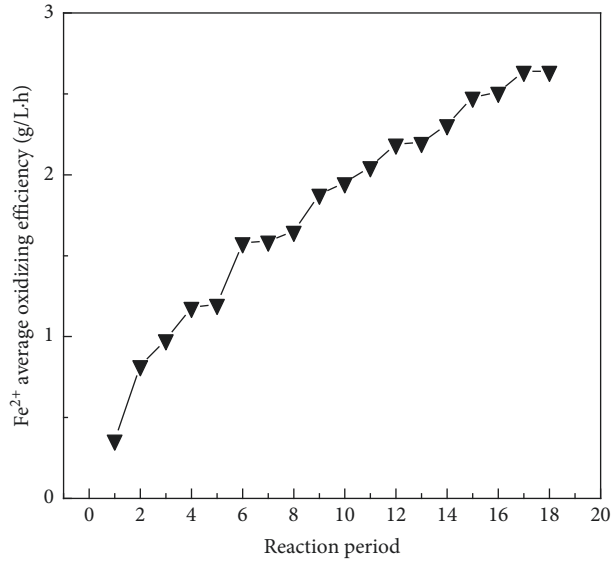

Figure 6: The trend of the average oxidation rate of Fe^{2+}.

immobilized microorganisms was saturated. When the biofilm was formed completely, the average oxidation rate of Fe^{2+} is up to 2.64 g/L·h and BPFS with good performance can be obtained. To optimize the preparation conditions of BPFS, experiments were conducted with different total iron concentrations of BPFS.

3.3.2. Preparation of BPFS

(1) The Effect of Aeration Rate. In order to improve the production efficiency, the effect of aeration rate on the

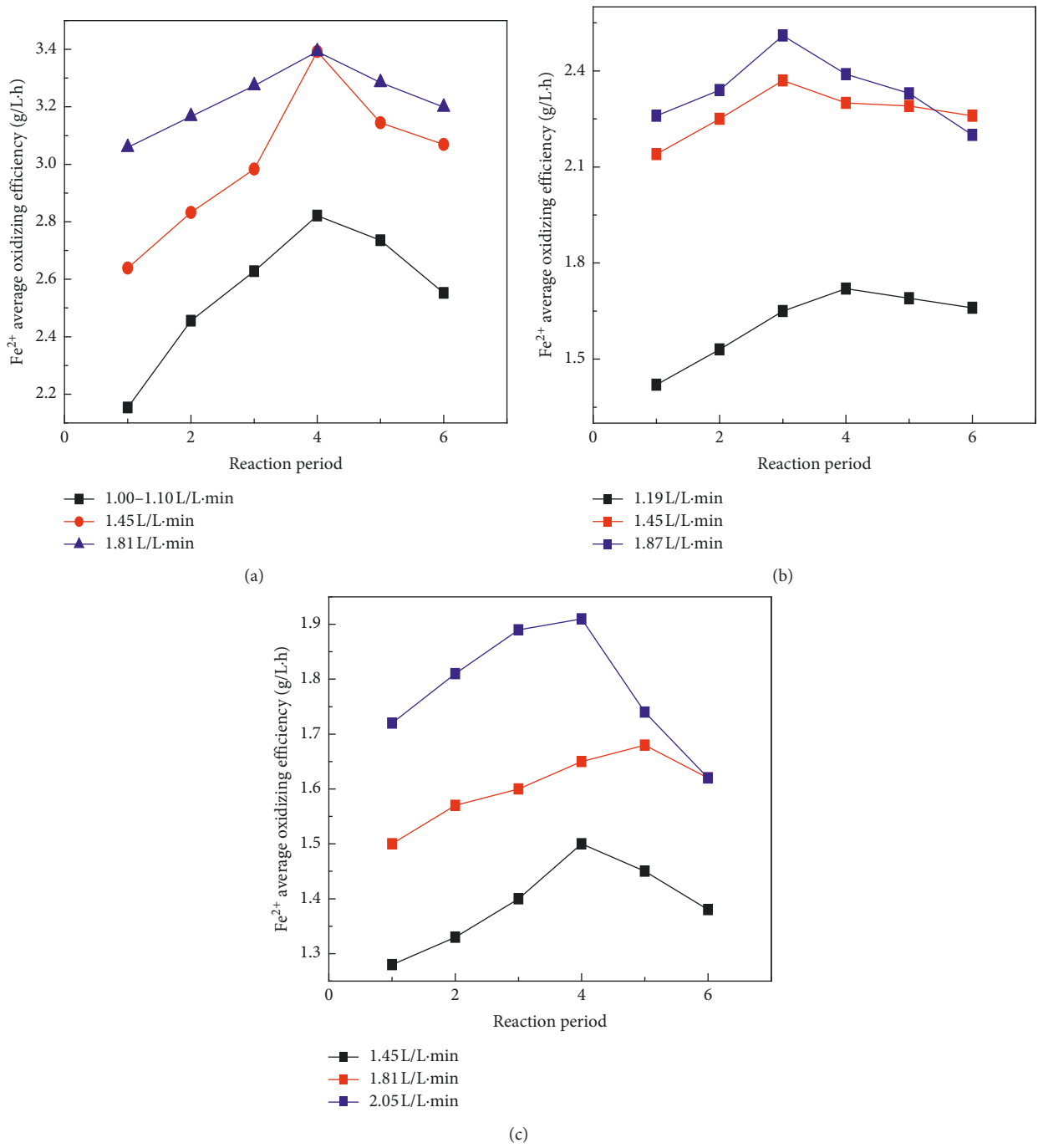

Figure 7: Effect of aeration on the production efficiency of BPFS: (a) 40 kg/m^3 BPFS; (b) 60 kg/m^3 BPFS; (c) 80 kg/m^3 BPFS.

production efficiency was studied when the total iron concentration was 40 kg/m^3, 60 kg/m^3, and 80 kg/m^3 BPFS. The results are shown in Figure 7.

Figure 7 shows that the 40 kg/m^3, 60 kg/m^3, and 80 kg/m^3 BPFS were obtained by the cycling batch operation, the optimum aeration rate were 1.40–1.50 L/L·min, 1.80–1.90 L/L·min, and 2.00–2.10 L/L·min, respectively, and the average oxidation rates of Fe^{2+} were 3.35 g/L·h, 2.51 g/L·h, and 1.91 g/L·h, respectively. With the increase of aeration amount, the average oxidation rate of Fe^{2+} increases, and the production efficiency of BPFS is also improved. High concentrations of BPFS have a higher viscosity; in the low aeration, the oxygen cannot break through the liquid membrane, resulting in the microbial lack of adequate oxygen; growth and reproduction are limited, and the average oxidation rate of Fe^{2+} decreased. In the high aeration, the reactor was filled with a certain amount of air so that a large number of microorganisms can grow and multiply, and the average oxidation rate of Fe^{2+} was improved.

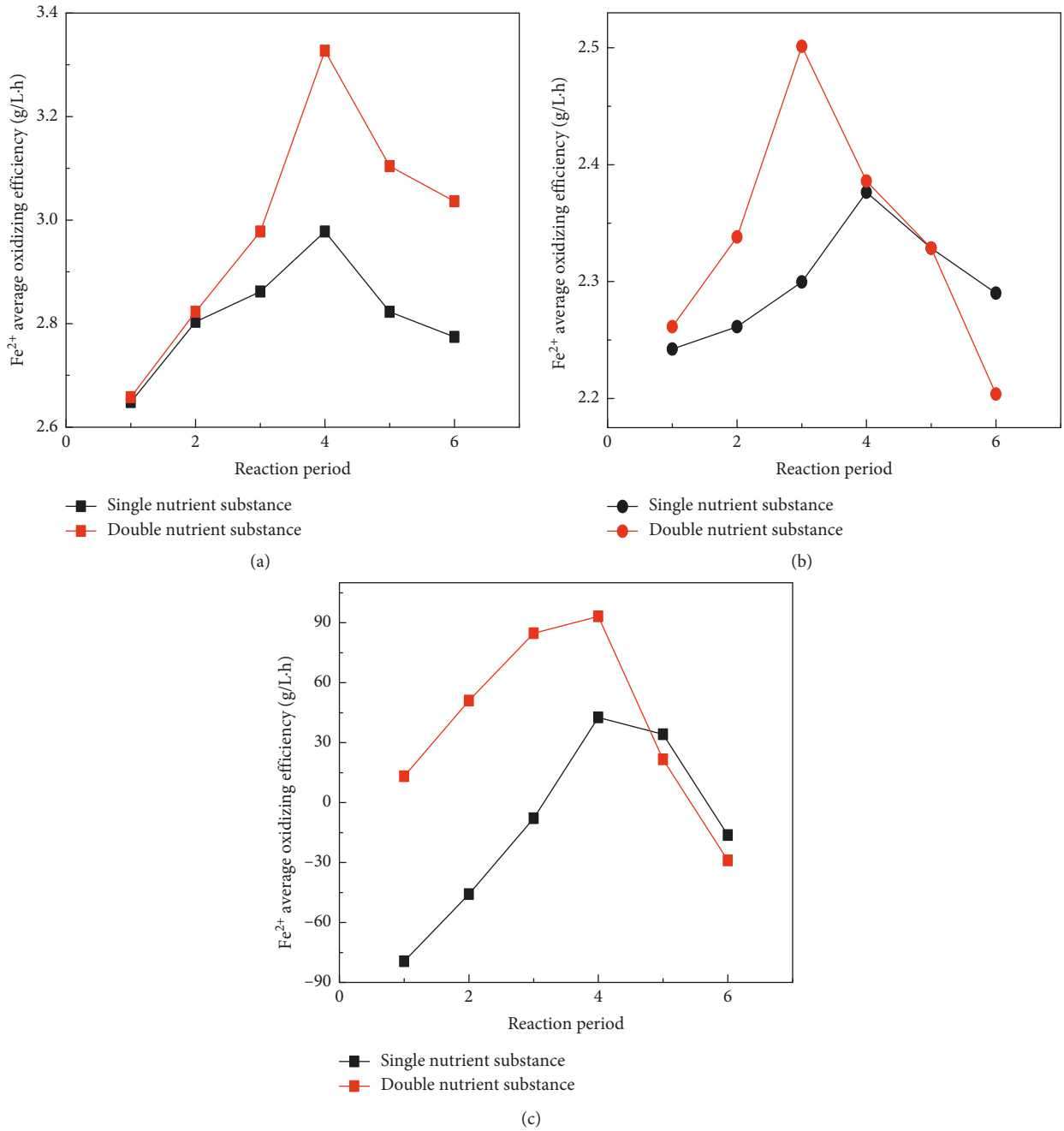

Figure 8: Effect of nutrient content on production efficiency of BPFS: (a) 40 kg/m^3 BPFS; (b) 60 kg/m^3 BPFS; (c) 80 kg/m^3 BPFS.

(2) Effect of Nutrient Substances. To investigate the effect of the nutrient substances on the BPFS preparation, experiments were conducted with single- and double-nutrient substances. Single nutrient composition is as follows: 1 g/m^3 KCl, 40 g/m^3 $(NH_4)_2SO_4$, 20 g/m^3 K_2HPO_4, 20 g/m^3 $MgSO_4$, and 1.5 g/m^3 $Ca(NO_3)_2$. The content of double-nutrient substances is twice the single nutrients. The results are shown in Figure 8.

According to Figure 8, with the increase of multiple nutrients, the catalytic oxidation capacity of microorganisms in the reactor increased, and the production efficiency of BPFS was improved. Under the conditions of single nutrients, microbial growth is limited because of insufficient nutrient requirement for microbial growth and reproduction. Thus, the optimum nutrient content is double-nutrient substances.

(3) Reaction of Different Sampling Ports in the Bioreactor. Under optimal operating conditions, the Fe^{2+} content in the four samples connection and outlet of the bioreactor was monitored simultaneously. The distribution of the results is shown in Figure 9.

Figure 9 shows that the Fe^{2+} contents of the four sampling ports and the liquid outlet are close at the same

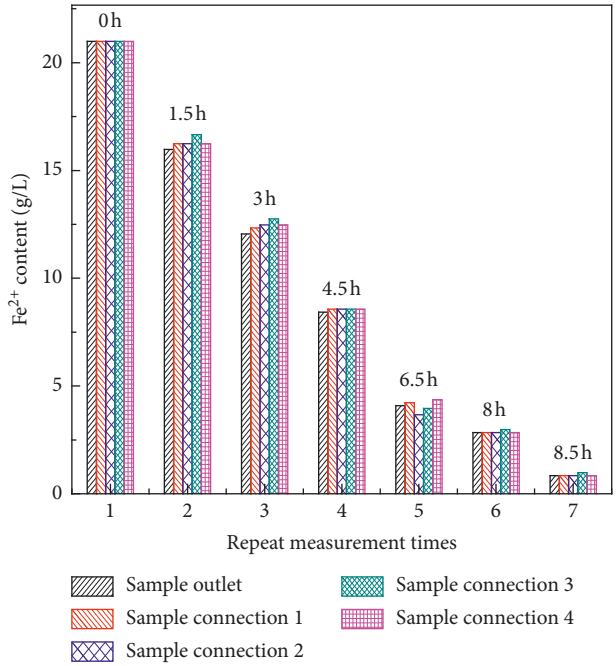

Figure 9: Fe^{2+} content in various parts of the bioreactor column.

time. It is shown the mass transfer of gas and liquid during the operation of the reactor makes the Fe^{2+} content uniform in all parts of the reactor. The catalytic oxidation of Fe^{2+} is mainly dependent on the catalytic activity of microorganisms. Therefore, microbial distribution in the bioreactor is very uniform.

When the polyurethane material is used as the carrier to immobilize microorganism, the average oxidation rate of Fe^{2+} is higher than that of the best experimental results in the early stage of the study. At the early stage, in the preparation of BPFS of 40 kg/m^3, 60 kg/m^3, and 80 kg/m^3 by ceramic raschig ring as the carrier, the microbial concentration of the immobilized microorganism on the ceramic raschig ring was $1.35 * 10^8$ cells/mL, $1.96 * 10^8$ cells/mL, and $1.11 * 10^8$ cells/mL, respectively. The average oxidation rates of Fe^{2+} were 3.35 g/L·h, 2.51 g/L·h, and 1.91 g/L·h, respectively, and the concentration of the microbes with polyurethane immobilization was $2.31 * 10^8$ cells/mL, $2.10 * 10^8$ cells/mL, and $1.55 * 10^8$ cells/mL. Therefore, the use of polyurethane as a carrier to immobilize microorganisms can enhance catalytic oxidation of Fe^{2+} and solve the problem of low utilization of bacteria to improve the production efficiency of BPFS.

3.4. Application of BPFS. The flocculating effect of BPFS and SPFS is compared. The results are shown in Figures 10 and 11.

Figures 10 and 11 demonstrate that the BPFS prepared in this study is superior with respect to the turbidity and COD_{Mn} removal. When the dosage of BPFS was 13–20 g/m^3, turbidity removal rate (above 90%) and significant COD_{Mn} removal efficiency (above 70%) were found, as shown in Figure 10. This is because BPFS not only has a high degree of polymerization but also contains microorganisms, which can catalyze the oxidation of organic matter as a condensation nucleus during the flocculating deposition process. Thus, it is sticky and can improve the coagulation efficiency, resulting in the adsorption of big molecular organic matter [22].

On the contrary, the figure shows that there is a sharp decrease in the treatment efficiency as the coagulant dosage increased to 20 g/m^3. The reason for the worsening performance with the increase of coagulant's concentration is the overdosing effect, which is based on the chain bridging mechanism [23–26]. With the addition of flocculant, the surface zeta potential of the particles decreases, and the particles in the water begin to flocculate. As the flocculant dosage increases, there is a gradual increase in the zeta potential value, and it almost reaches the point of zero charge (0 mV) [27]. Continuing to increase the amount of flocculant dosage, the adsorption surface of the colloid is coated by flocculant, which lost the opportunity to combine with other colloidal particles. So the surface zeta potential begins to rise, and the repulsion of colloidal particles increases [28–30]. Therefore, achieving another relatively stable state, the emergence of the antistabilization phenomenon is hard to agglomerate, which results in deterioration of the flocculation effect [31–34]. Less dose of flocculant is not enough to form a bridge, and the excess dose of flocculant deteriorates the function of the polymer flocculant. Therefore, in the water treatment process, adding appropriate amount of flocculant improves its flocculation effect [35].

4. Conclusion

The immobilization of *T. f* on the polyurethane as the support matrix was investigated to improve the utilization

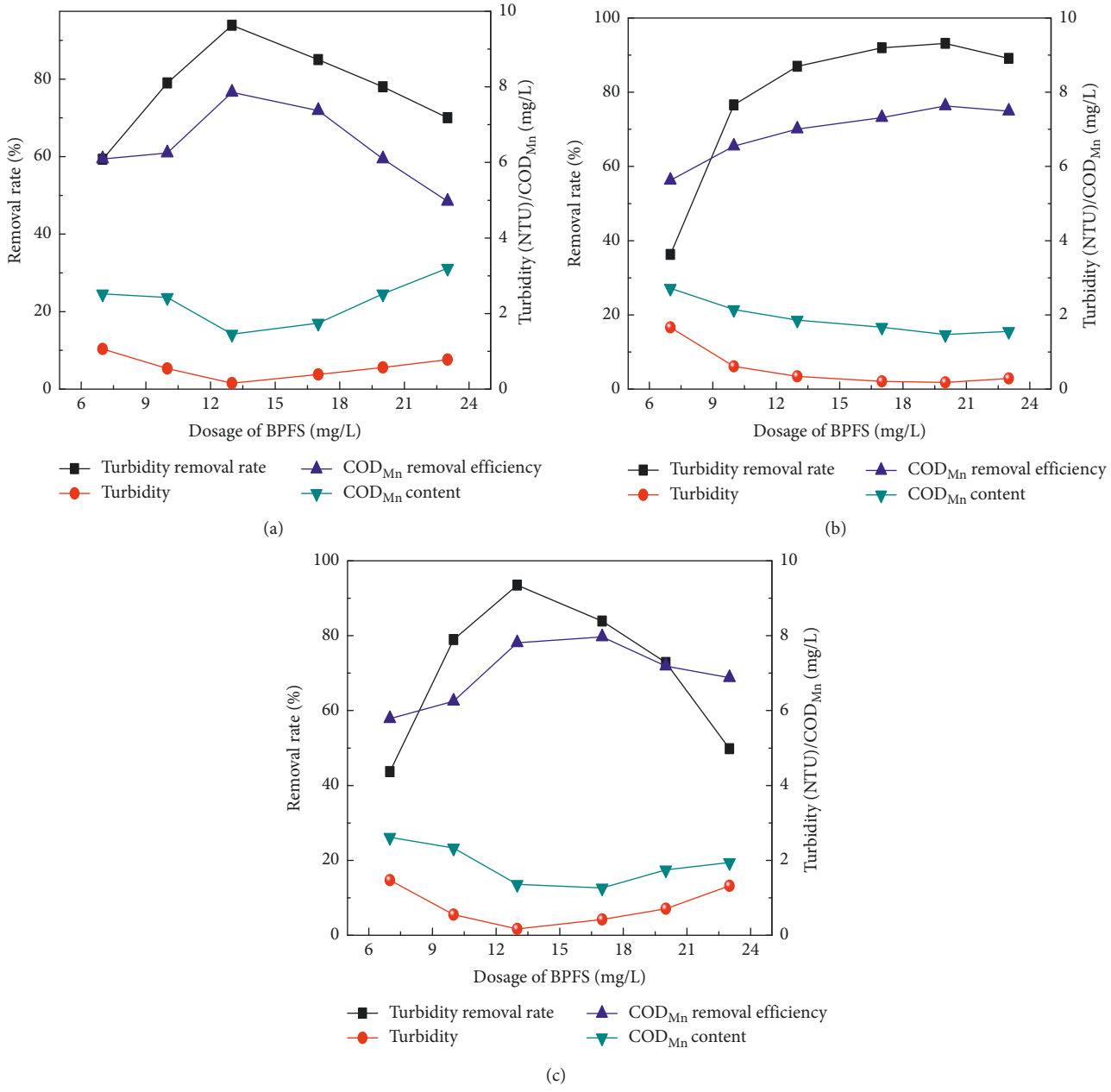

Figure 10: Impact of BPFS dosage on turbidity and COD$_{Mn}$ removal efficiency: (a) 40 kg/m^3 BPFS; (b) 60 kg/m^3 BPFS; (c) 80 kg/m^3 BPFS.

rate of bacteria and increase the production efficiency of BPFS.

(1) Strain *T. f* was isolated on the agarose single-plate medium. Based on 16S rRNA sequence data, the bacterium was identified as *T. f*

(2) A new preparation method of PFS was conducted using a *T. f* pH value of 1.8, circulation flow rate of 0.28–0.30 L/h, double nutrient material, and reaction temperature of 28 ± 1°C. Under the optimal conditions, the 40 kg/m^3, 60 kg/m^3, and 80 kg/m^3 BPFS product was obtained by cycling batch operation, the optimum aeration rate was 1.40–1.50 L/L·min, 1.80–1.90 L/L·min, and 2.00–2.10 L/L·min, respectively, and the average oxidation rates of Fe^{2+} were 3.35 g/L·h, 2.51 g/L·h, and 1.91 g/L·h, respectively.

(3) The BPFS is an effective flocculant for water treatment, and the removal efficiencies of COD$_{Mn}$ and turbidity by the BPFS reach above 79.7% and 93.9%, respectively.

In this study, due to the limitation of time and conditions, with the increase of total iron content, the increase of raw liquor viscosity gradually becomes the main factor restricting the oxidation rate of Fe^{2+}. This is a question that

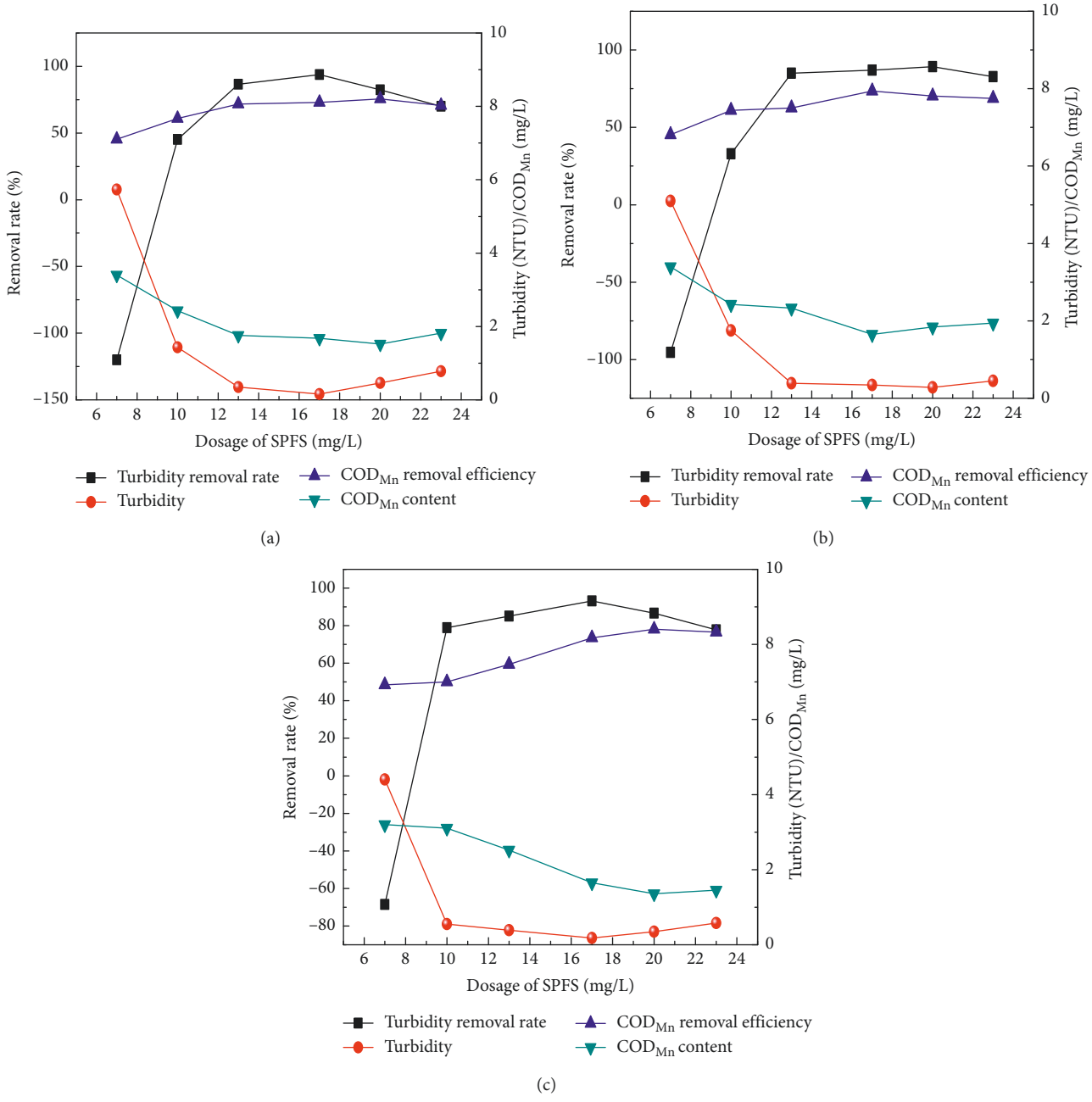

Figure 11: Impact of SPFS dosage on turbidity and COD$_{Mn}$ removal efficiency: (a) 40 kg/m^3 SPFS; (b) 60 kg/m^3 SPFS; (c) 80 kg/m^3 SPFS.

needs to be studied in the future. BPFS has excellent coagulation properties, and it will be of significant importance for the further research on the fields of water and wastewater treatment.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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