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Research on microbial community structure and treatment of dye wastewater with the enhancement of activated sludge by magnetic field at low temperature

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This study characterized the effect of different magnetic field (MF) intensities (10–40 mT) on the degradation of dye wastewater by activated sludge and the diversity of the microbial community at a low temperature (5 °C). The examined MF range promoted the degradation of dye wastewater by the microorganisms in the activated sludge at a low temperature. It was found that the optimal degradation performance was achieved at 30 mT. Additionally, the maximum degradation efficiency of COD and chromaticity (66.30% and 60.87%, respectively) were also achieved at 30 mT and the peak TTC-dehydrogenase activity (TTC-DHA) was 9.44 mg TF g^{-1} SS. Furthermore, it was revealed that MF enhancement increased the richness and diversity of activated sludge microorganisms, thus promoting the growth and reproduction of activated sludge microorganisms at low temperatures. Bacterial taxa known to effectively participate in the degradation of pollutants by activated sludge were enriched at 30 mT. The dominant bacteria under 30 mT were *Flavobacterium*, *Hydrogenophaga*, *Gemmatimonadaceae*, *Zoogloea*, *Saprospiraceae*, *Pseudomonas*, and *Geothrix*.

1. Introduction

It is reported that the highest amount of dyes used every year in the world is in the textile industry.[1,2] However, some dyes are not effectively utilized, resulting in a large amount of dye wastewater, which is ultimately discharged into natural waters.[3,4] Due to their complex composition, difficult biodegradability, and low recycling rate, the removal of dyes from wastewater is an ongoing challenge. In most countries, biochemical methods are commonly and extensively utilized to treat dye wastewater. Currently, the process of treating dye wastewater at room temperature is relatively mature, and extensive research has been conducted on the decolorization and degradation of printing and dyeing wastewater, and the performance of sewage treatment plants is generally considered to be excellent.[1,2,5,6]

However, low temperatures are known to inhibit the microbial activity of activated sludge.[7] In turn, low temperatures may also reduce the utilization of substrates by activated sludge microorganisms, as well as the adsorption and sedimentation capacity of activated sludge.[8] Furthermore, microbial community structure and species richness are significantly affected by temperature.[9,10] Specifically, low temperature can reduce the diversity and species richness of microbial communities, which may deteriorate activated sludge performance.[11] As a result, a drop in wastewater temperature will unavoidably have an impact on microbial metabolism and the removal of pollutants.[12] Current biochemical methods cannot effectively meet the requirements for the treatment of dyeing wastewater at low temperatures, making it difficult to comply with discharge standards. In turn, this has serious repercussions on water environment pollution. Nevertheless, previous studies have reported that activated sludge contains cold-adapting microorganisms at low temperatures, which can effectively degrade organic pollutants in sewage.[8] Thus, magnetic field enhancement technology has been applied to the field of wastewater biological treatment to more effectively utilize these cold-adapted microorganisms.

Given the effects of low temperature on the biological treatment of wastewater, recent studies have focused on the enhancement of activated sludge by magnetic fields, which can improve wastewater treatment efficiency and maintain operational stability at low temperatures.[13] Previous studies have demonstrated that static magnetic fields (SMF) with different intensity strengths (from 1 mT to 1 T) can affect many biological systems to some extent,[14] and may directly affect the growth and metabolism of activated sludge microorganisms.[15] Activated sludge biomass growth and dehydrogenase activity are positively correlated under low-intensity magnetic fields.[16] Moreover, magnetic fields affect the enzyme activity and biofilm

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formation capacity of microorganisms, in addition to flocculation activity and microbial biomass.[17] Therefore, magnetic fields can enhance the degradation of wastewater treatment performance by increasing dehydrogenase activity.[18] Another study reported that exposing an activated sludge reactor to a weak magnetic field significantly increased its COD removal efficiency.[19] Furthermore, previous studies have also demonstrated that exposing activated sludge to 5.0 and 20.0 mT magnetic fields increased the bacterial growth rate compared to the blank control group (0 mT), whereas the 200.0 and 500.0 mT bacterial growth lag period was prolonged and the growth rate was reduced, meaning that a low-intensity magnetic field promoted microorganism growth.[20] Furthermore, the dehydrogenase activity of microbes can be stimulated by a magnetic field intensity of 20–40 mT to adapt to the cold environments, with cold-adapted Gram-negative bacteria being substantially enriched at 30 mT.[21]

This study focuses on the use of magnetic fields in conjunction with dye wastewater treatment technology, as well as the relationships between magnetic field intensity and treatment efficiency of dye wastewater, and dehydrogenase activity. Furthermore, high-throughput sequencing technology was used to study microbial community structure and diversity in activated sludge systems, as well as the effects of different magnetic field intensities on dye degradation performance. Thus this findings provided partial insights into the physiological mechanisms of microorganism-mediated dye degradation. Therefore, it provides a basis for future studies on the biological treatment of dye wastewater at low temperatures.

2. Materials and methods

2.1 Source of activated sludge

The activated sludge was obtained from a wastewater treatment plant in Nanjing, China, and contained an activated sludge concentration of approximately 3320 mg L^{-1}.

2.2 Lab-scale reactor system and simulation of dye wastewater

The activated sludge reaction system consisted of a reactor with a 5 cm radius and a 30 cm height. Two plexiglass cylinder reactors (R_1 and R_2) were utilized, each with a working volume of 2 L. Each reactor was equipped with three drain valves, and the oxygen-filled aeration component consisted of an electromagnetic aeration pump, an aeration hose, and an aeration sand head. The amount of aeration in the reactor could be freely controlled using a knob. To generate a static magnetic field, two parallel heteropolar magnetic plates were attached to R_2, as shown in Fig. 1, and the magnet size was 15 cm × 10 cm × 2.5 cm. A Tesla meter was used to measure the central magnetic field strength of the inner wall of the activated sludge reactor in this experiment, which was characterized by the intensity of the reactor's magnetic field. The magnetic field intensity was then controlled by adjusting the distance between the parallel magnets, and operation of entire process of the reactor was artificially controlled, and the period was operated for 24 hours.

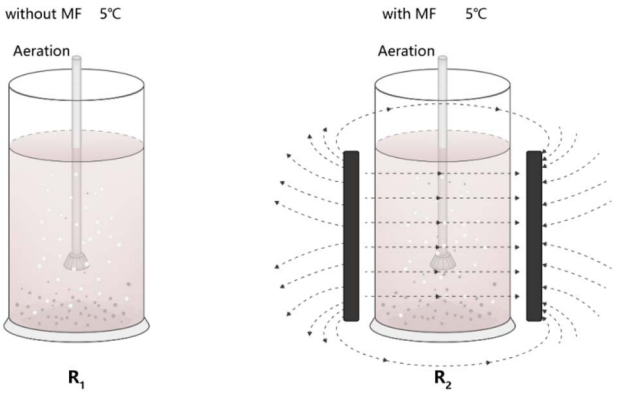

Fig. 1 Schematic diagram of experimental setup (R_1 refers to reactor #1 kept at 5 °C without magnetic field treatment; R_2 refers to reactor #2 kept at 5 °C with different magnetic field intensities).

Each MF intensity was administered for 7 days, and both the R_1 and R_2 reactors were operated at 5 °C. The oxygen concentration in the two reactors was roughly 8 mg L^{-1} throughout the entire experimental period.

The whole experimental design was divided into four stages. In the sludge acclimation stage, the inoculated sludge was cultured and acclimated to stabilize under low-temperature conditions. The second stage was the application of the magnetic field treatment, whereas R_1 was the blank control without magnetic field, R_2 was the experimental group with magnetic field, and then MF intensities (the magnetic field intensities were adjusted to 0, 10, 20, 30, and 40 mT, respectively). The third was the stable operation stage, activated sludge was adapted to different MF intensities and the effluent quality of the system remained stable. Finally, after identifying the most suitable magnetic field intensity for degrading dye wastewater, the reactor was operated under the selected magnetic field intensity for one month, after which high-throughput sequencing was conducted to characterize the microbial community structure in the activated sludge.

The dye wastewater used in this experiment was prepared in the laboratory. Acid red B (molecular formula, $C_{20}H_{14}N_2O_7S_2Na_2$) was the dye selected to conduct this experiments. The synthetic wastewater consisted of acid red B, NH_4Cl, and KH_2PO_4, the COD concentration was 120 mg L^{-1} (Table 1), and the mass ratio of C : N : P was 100 : 5 : 1. The composition of the synthetic dyeing wastewater is summarized in Table 1.

2.3 TTC dehydrogenase activity (TTC-DHA)

Triphenyl tetrazolium chloride-dehydrogenase activity-dehydrogenase activity (TTC-DHA) is an important indicator

Table 1 Composition of the dye wastewater

Dye	COD (mg L^{-1})	Composition	Concentration (mg L^{-1})
Acid red B	120	NH_4Cl	18.3
		KH_2PO_4	4.5

of microbial metabolism and organic degradation capacity. Dehydrogenase is an extracellular enzyme produced by microbial cells, which can effectively participate in the degradation of organic matter. It is an essential enzyme for microbial biodegradation of organic pollutants in wastewater. Therefore, dehydrogenase is not only an indicator of microbial activity,[22] but also reflects the ability of microbial cells to degrade substrates.[23] The TTC-DHA analysis was conducted as described previously with minor modifications.[23,24] Centrifuge tube containing the activated sludge (15 mL) and a stopper were centrifuged at 4000 rpm for 5 minutes, after which the supernatant was discarded. Next, 15 mL of distilled water was added, the mixture was stirred, and the supernatant was discarded once again after a 5 minute centrifugation. The samples were then washed three times and diluted with distilled water to the original volume for use. Next, 2 mL of activated sludge solution was transferred to a stoppered test tube, followed by 1.5 mL of Tris–HCl buffer solution, 0.5 mL of 0.1 mol L^{-1} glucose solution, 0.5 mL of 4% TTC solution, and 0.5 mL of 0.36% Na_2SO_3 solution. The samples were then immediately placed in a 5 °C incubator for 2 h. The reaction was terminated by adding 0.5 mL of formaldehyde, and 5 mL of toluene was rapidly added after every termination reaction. The sample was extracted by shaking at room temperature for 10 min, centrifuged at 4000 rpm for 5 min, and the absorbance of the supernatant was measured at 485 nm. Under the aforementioned conditions, an enzyme activity unit was defined as the production of 1 mg of triphenyl formazan (TF) in 1 h.

2.4 Microbial community structure

Based on the results of the above-described experiments, this study selected the magnetic field intensity that was most suitable for strengthening the treatment of dye wastewater by activated sludge. Both the R_1 (without MF) and R_2 (with MF) reactors were placed in a biochemical incubator at 5 °C and only R_2 was exposed to the selected magnetic field intensity. The reactors were then stably operated for 30 days and sludge samples were recovered at 15 and 30 days. Next, the 1–1.5 mL activated sludge samples from R_1 and R_2 were centrifuged, after which genomic DNA was extracted using the OMEGA Soil DNA kit. The isolated DNA was then examined using 1% agarose gel electrophoresis. The extracted DNA was PCR amplified by using 16S rRNA gene V3–V4 region universal primers with 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). The acquired PCR products were sequenced by Biozeron Co., Ltd using a Miseq Illuminasequencer (Shanghai, China). Based on a 97% identity, the resultant sequences were grouped into operational taxonomic units (OTUs). The OTU representative sequences were compared to the matching database's template sequence, the taxonomic information for each OTU was acquired, and the four diversity indexes of Chao1, Coverage, Shannon, and Simpson diversity indices were calculated. Based on the OTU classification and classification status identification results, the composition of the microbial community of the activated sludge and the number of microorganisms were analyzed.

2.5 Statistical analysis

Statistical analysis was performed using SPSS version 22.0 for Windows. One-way analysis of variance was used to examine the differences between the control and experimental groups. Values of $p < 0.05$ were considered significant, and values of $p < 0.01$ considered highly significant. OTUs with 97% similarity were selected and analyzed by high throughput sequencing using the Illumina PE250 platform. Normalized data were used to calculate the alpha diversity index of different samples using Mothur (*e.g.*, Chao, Shannon, and Simpson indices).[25] Based on the taxonomic analysis, the community structure composition at different classification levels (*e.g.*, phylum, genus) was obtained. Beta diversity analysis was carried out using R language and STAMP for statistical analysis and mapping.

3. Results and discussion

3.1 Bioreactor efficiency

3.1.1 COD and chromaticity degradation efficiency. At low temperature, the COD degradation efficiency of dye wastewater in the stable operation stage of the activated sludge reactor exhibited different variation patterns depending on the magnetic field intensities. The average COD degradation efficiencies of R_2 under the four examined MF intensities (10, 20, 30, 40 mT) were 44.61%, 52.34%, 64.28%, and 60.94%, respectively (Fig. 2). In contrast, the R_1 reactor without magnetic field exhibited values of 38.94%, 38.04%, and 38.12%, 38.70%. The COD degradation efficiency of R_1 fluctuated greatly, whereas the COD degradation efficiency of reactor R_2 tended to increase steadily with time. Previous studies have demonstrated that the COD degradation efficiency of low-temperature reactors can be significantly improved if the process and reaction time are optimal.[26] With higher magnetic field intensity, the biodegradation efficiency of activated sludge increase until it reaches a peak at 30.0 mT, after which it rapidly decreases as magnetic field intensity increases further.[20]

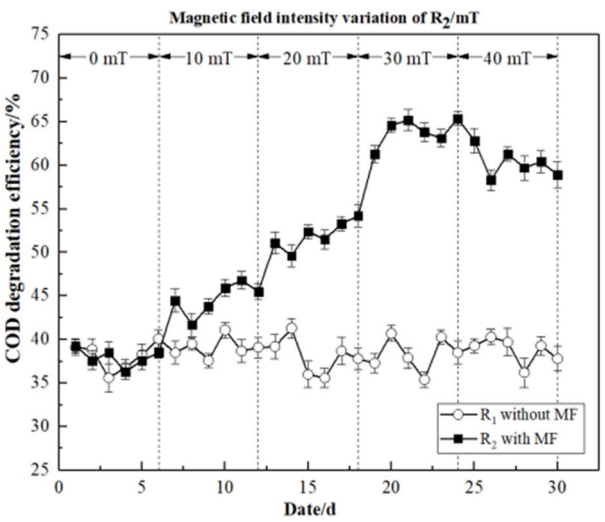

Fig. 2 COD degradation efficiency of the whole stable operation stage.

In this study, the COD degradation efficiency of R_1 and R_2 and the performance of the activated sludge were close to synchronization when the magnetic field intensity was 0 mT. Additionally, both the low temperature and the intensity of the magnetic field had an impact on the initial performance of R_2 at 10 mT. Compared with R_1, microbial activity was temporarily inhibited in a more complex environment. Therefore, the degradation efficiency of dye wastewater by activated sludge was not obvious. After adjusting MF intensity of R_2, the COD degradation efficiency increased with higher magnetic field intensity, and reached its maximum at 30 mT (66.30%). However, as the MF intensity increased further, the COD degradation efficiency dropped until it reached a stable state. These findings suggested that magnetic fields can increase the permeability and cold adaptation of the microbial membranes enhanced.[27] Therefore, magnetic fields can improve the COD degradation efficiency of activated sludge in dye wastewater.

As illustrated in Fig. 3, the average degradation efficiencies of R_2 under four different magnetic field intensities (10, 20, 30, 40 mT) were 41.56%, 47.60%, 58.90%, and 56.76%, respectively. In contrast, R_1 without magnetic field exhibited values of 35.97%, 35.81%, 36.53 and 39.67%, respectively. As the effect of the MF on microorganisms was cumulative, some time was required for it to exert a reinforcing effect.[23] The degradation efficiency of activated sludge began to increase at 20 mT. The degradation efficiency of R_2 exhibited an interesting trend. Particularly, we observed a positive correlation between degradation efficiency and MF intensity, and the peak value was 60.87% at 30 mT, which decreased slightly with a higher MF intensity. These findings indicated that the activity of the activated sludge microorganisms was inhibited by 40 mT to some extent. However, activated sludge microorganisms may still be strengthened by the 40 mT magnetic field to degrade dye wastewater. Therefore, it demonstrated that the magnetic field was beneficial to the activated sludge, thus improving the chromaticity degradation efficiency of dye wastewater.

Based on these findings, it was concluded that the reactor with MF improvement was able to adapt to the low-temperature environment more quickly, and achieved a greater COD and chromaticity degradation efficiency.

3.1.2 TTC-DHA. The activity of activated sludge microbial dehydrogenase may indicate the degradation efficiency of substrates at low temperatures under varied magnetic field intensities. As illustrated in Fig. 4, once the reactors reached a stable operation stage, the TTC-DHA in R_1 fluctuated slightly around the mean value of 6.97 mg TF g^{-1} SS, and the overall trend was relatively stable. The values of TTC-DHA in R_2 at MF intensity of 0, 10, 20, 30, and 40 mT were 6.73, 7.16, 8.26, 9.44 and 8.65 mg TF g^{-1} SS, respectively. The TTC-DHA activity of R_2 generally increased with the higher magnetic field, reached the maximum value of 9.44 mg TF g^{-1} SS at 30 mT, and the TTC-DHA value of the 40 mT treatment (8.65 mg TF g^{-1} SS) was greater than that of the 20 mT treatment (8.26 mg TF g^{-1} SS). Niu *et al.* reported the TTC-DHA activity levels of approximately 1.2 to 1.5 mg TF g^{-1} SS at 5 °C, which was significantly lower than the results with magnetic field strengthening.[23] Other studies have also confirmed that activated sludge may achieve the highest efficiency at 17.9 mT, whereas higher MF intensities (46.6 mT) had a negative impact on substrate degradation and microbial growth rate.[28] The effects of magnetic fields on enzymatic reaction kinetics may be attributed to changes in the conformation and activity of the enzyme, resulting in macroscopic changes in the physiological and biochemical properties of the organism.[29] It indicated that weak magnetic fields could increase enzymatic reaction at low temperatures, thus improving the substrate degradation efficiency of microorganisms. Additionally, a magnetic field intensity of 30 mT markedly increased the dehydrogenase activity of the activated sludge microbiota.

These findings thus demonstrated that the magnetic field intensities of 10–40 mT could improve degradation efficiency. However, the most significant magnetic field strengthening effects were observed at 30 mT.

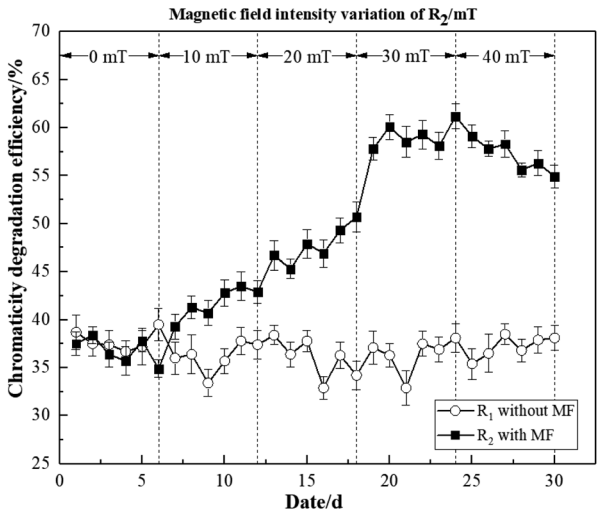

Fig. 3 Chromaticity degradation efficiency of the whole stable operation stage.

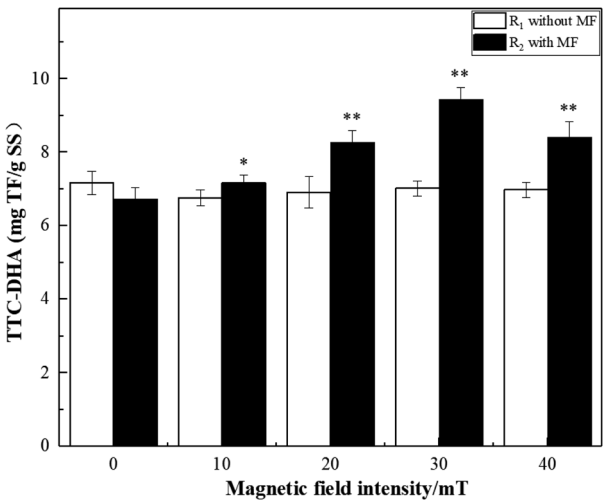

Fig. 4 TTC-DHA of activated sludge microorganisms in two reactors.

3.2 Microbial community structure

Several studies have demonstrated that temperature is among the environmental factors with the strongest influence on the composition of microbial communities in activated sludge.[30,31] In turn, the compositions and dynamics of bacteria have a major impact on the treatment performance of activated sludge.[32] Therefore, we next analyzed the effects of magnetic field treatment on the microbial community structure of the activated sludge samples.

3.2.1 Venn diagram and principal component analysis. The overlap and similarity between four samples from the two reactors were graphically visualized using Venn diagrams for this time.[33] The shared and distinct bacterial genera in all biomass samples were thus identified. Specifically, 45 genera were found to be common among all experimental conditions, 79 were shared between the R_1 and R_2 (15 day) samples, and 66 were shared between the R_1 and R_2 (30 days) samples. There were 10, 28, 16, and 34 unique genera in the R_1 and R_2 (15 days) and the R_1 and R_2 (30 days) samples, respectively. Additionally, after 30 days, 100 genera were found in the R_1 samples, whereas 136 were found in the R_2 samples. Therefore, with more prolonged exposure to the magnetic field, more genera were found in R_2 than in R_1, whereas the number of genera shared by the R_1 and R_2 biomass declined (Fig. 5).

As illustrated in Fig. 6, PCA was conducted to observe the overall variation in bacterial communities among the four activated sludge samples. PC1 and PC2 represent 87.7% and 10.8% of the variations between the samples, respectively. PCA revealed the four samples had significant variance, with the variance between R_1 and R_2 (30 days) being larger than that between R_1 and R_2 (15 days). Furthermore, the variance between R_1 (15 days) and R_1 (30 days) and that between R_2 (15 days) and R_2 (30 days) was explained by both on PC1 and PC2, whereas the variance between R_1 and R_2 (15 days) and that of R_1 and R_2 (30 days) both were only explained by PC2. This indicated that the similarity of the microbial community structure between two given samples was significant, when the distance between two sample points was closer. The magnetic field was one of the

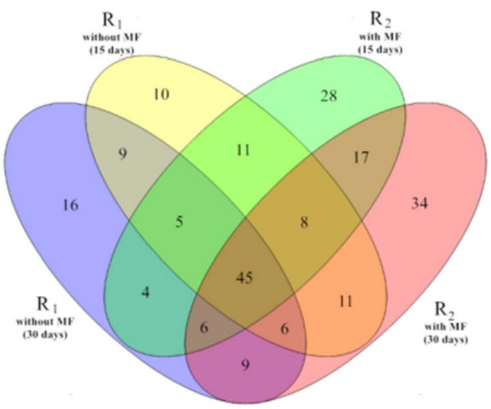

Fig. 5 Venn diagram of four samples at the genus level (R_1 refers to reactor #1, without magnetic field; R_2 refers to reactor #2, kept at 5 °C at a magnetic field intensity of 30 mT).

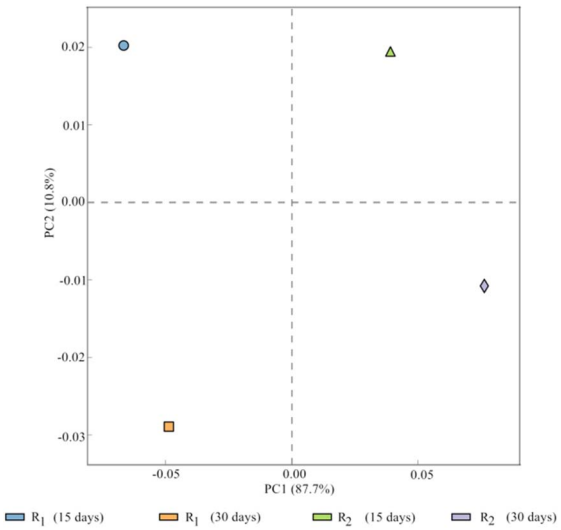

Fig. 6 Principle component analysis (PCA) of four samples at the OTU level.

primary factors that influenced microbial growth and contributed to the variation in microbial composition. Moreover, the variances of R_1 and R_2 were represented in PC1. Similarly the factor of time contributed substantially to PC2.

Therefore, magnetic field treatment was identified among the primary environmental elements that directly affect microbial diversity, which may influence the cold adaptability and physiological function of microorganisms in activated sludge. Thus, the microbial community structure of activated sludge changed significantly at a MF intensity of 30 mT.

3.2.2 Microbial richness and diversity. Bacteria are the major component of activated sludge and play an important role in the degradation of pollutants. There are several approaches for measuring microbial community diversity using different indices. Microbial diversity research and single-sample diversity analysis (alpha diversity) can reflect the abundance and diversity of microbial communities, including a range of statistical analytic indices that assess the species abundance and ecological community diversity.

The obtained sequence number, coverage, and alpha indices are summarized in Table 2 and Fig. 7. The coverage of the activated sludge samples in R_1 and R_2 was above 99%, which indicates that the probability of the sequence being detected in the two sludge samples was high. Therefore, the sequencing results were sufficient to reflect the true status of the microorganisms in the sample, meaning that the samples were representative.[34] The Shannon and Simpson indices were employed

Table 2 Alpha index of microbial diversity after 30 days

Sample	Quality sequence number	Chao	Coverage	Shannon	Simpson
R_1	38 615	1214	0.995	4.61	0.051
R_2	57 801	1215	0.997	5.13	0.022

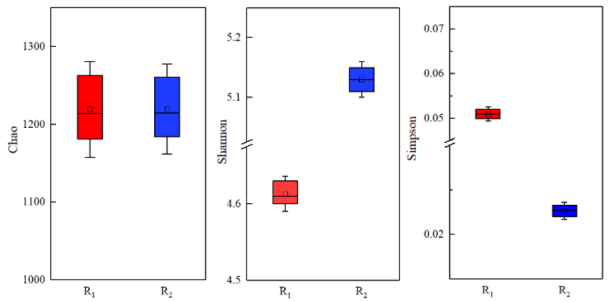

Fig. 7 Comparison of microbial richness and diversity after 30 days.

to assess the diversity of the microbial community, whereas the Chao index was utilized to assess the microbial richness.[35] In this study, the total number of sequences detected in the R_2 sample exposed to a MF intensity of 30 mT was as high as 57 801, whereas that of R_1 without magnetic field treatment was 38 615. Although there was no significant difference in the Chao indices of the two reactors, the microbial diversity in R_2 was significantly higher than in R_1. As summarized in Table 1, the Shannon and Simpson indices of R_2 were 5.13 and 0.022, respectively.

These findings suggest that magnetic field could increase the richness and diversity of activated sludge microorganisms, and thus promote the growth and reproduction of activated sludge microorganisms at low temperatures.

3.2.3 Microbial community composition at the phylum level. At the phylum level, the relative abundances of the activated sludge bacteria differed among samples taken in the presence or absence of magnetic field. The classified phyla with an abundance greater than 1% of the overall biomass community in R_1 and R_2 are illustrated in Fig. 8, *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Planctomycetes*, *Acidobacteria* and *Gemmatimonadetes* were the six most abundant phyla in all samples.

After 30 days of cultivation of the activated sludge, the samples exhibited unique patterns of microbial diversity. *Proteobacteria* and *Bacteroidetes* were the bacterial phyla with the highest abundance in the activated sludge of the two reactors. Studies have shown that *Proteobacteria* was widely distributed in activated sludge treatment systems, which was the main type population of activated sludge systems in urban sewage plants.

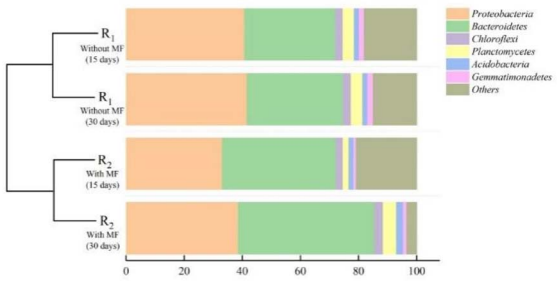

Fig. 8 Microbial relative abundance (%) with cluster tree at the phylum level.

These systems contain a variety of bacteria with unique metabolic types, mainly for the degradation of organic pollutants, as well as for nitrogen and phosphorus removal.[36–38] The relative abundance of *Proteobacteria* in R_1 and R_2 was 41.61%, and 38.57%, respectively. It suggested that the bacteria were widely distributed and highly diverse, including many bacteria that survived at low temperatures.[37] *Bacteroidetes* have a strong metabolic capacity to metabolize complex organic matter, proteins, and lipids. The members of this phylum can decompose complex macromolecular substances into simple compounds, and play an important role in the ecosystem.[39] *Bacteroidetes* accounted for 33.09% and 46.97% of the bacterial diversity of the activated sludge samples from R_1 and R_2, respectively. Interestingly, the abundance of *Bacteroidetes* was higher in R_2 exposed to the magnetic field compared to R_1, indicating that the magnetic field enhances the survival of *Bacteroides*. Compared with R_1, the relative abundance of *Chloroflexi* in R_2 exposed to the magnetic field was relatively high (2.96%). A previous study reported that *Chloroflexi* was mostly composed of filamentous bacteria, and existed in the form of a floc skeleton within the sludge microbial floc, which promoted the flocculation of sludge and was capable of degrading macromolecular organic matter and removing biological phosphorus.[40] *Planctomycetes* are small aquatic bacteria or aerobic bacteria, which play an important role in sewage treatment.[41,42] The relative abundance of *Planctomycetes* in R_1 and R_2 was 3.97% and 4.56%, respectively, suggesting that the bacteria were more likely to survive in an activated sludge system treated with a magnetic field. Moreover, the relative abundance of *Acidobacteria* in R_1 and R_2 was 1.82% and 2.36%, showing that the magnetic field was beneficial to maintain the reproductive activity of *Acidobacteria* in activated sludge. *Acidobacteria* is an important class of microorganisms in activated sludge, which could degrade macromolecular organic matter,[43] and significantly contribute to the stabilization of the ecological environment.[44] *Gemmatimonadetes* is a type of Gram-negative bacterium that greatly contributes to biological phosphorus removal and contaminant degradation,[45] and its relative abundance in R_1 and R_2 was 1.88% and 1.14%, respectively.

It demonstrated that the structure of the activated sludge's microbial community changed noticeably under a 30 mT magnetic field intensity, and the continuous culture time of activated sludge exposed to magnetic field was also *a* factor affecting the changes in the microbial communities. As the activated sludge culture time increased, the relative abundance of the six dominant phyla in all samples with an magnetic field intensity of 30 mT, *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Planctomycetes*, *Acidobacteria* and *Gemmatimonadetes* were increased by 46.97, 2.96, 4.56, 2.36, and 1.14, respectively.

3.2.4 Microbial community composition at the genus level. Fig. 9 illustrates the dominant bacteria with abundances above 1% at the genus level. *Flavobacterium*, *Hydrogenophaga*, *Gemmatimonadaceae*, *Dechloromonas*, *Zoogloea*, *Saprospiraceae_uncultured*, *Pseudomonas* and *Geothrix* were the eight most abundant genera in all samples. These results suggest that these microorganisms play a key role in the biodegradation capacity of activated sludge treatment systems.

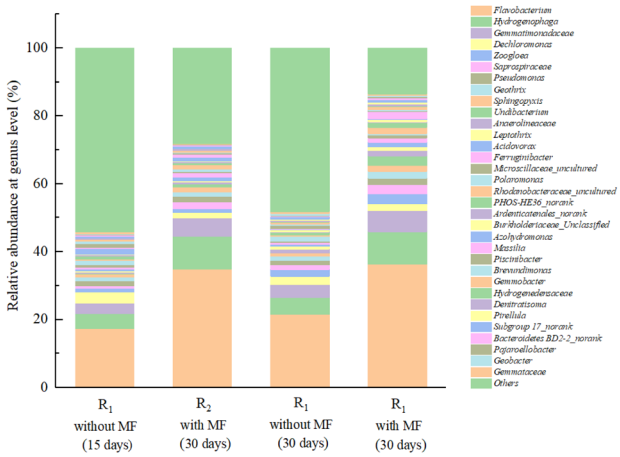

Fig. 9 Relative abundance of bacteria at the genus level.

As illustrated in Fig. 9, the bacterial community had undergone significant changes, as the magnetic field continued to contribute to the acclimation of the activated sludge in the reactors. *Flavobacterium* was the most abundant genus in the activated sludge in R_1 and R_2 accounting for 21.47% and 36.33% of the overall abundance, respectively. Moreover, the biomass produced by proliferation of R_2 under the magnetic field was significantly higher than that of R_1. Previous studies have reported that *Flavobacterium* is strictly aerobic, and it cannot produce acid or gas in the reaction solutions containing carbohydrates. Additionally, the members of this genus were the main contributors to micelle formation, which not only has the function of degrading macromolecular organic matter such as high molecular substances, proteins, lipids, and cellulose, but also participates in nitrification,[46] and could be involved in nitrogen removal from sewage.[47] Therefore, *Flavobacterium* became a dominant genus in the R_2 with magnetic field enhancement. *Hydrogenophaga* was the second most abundant genus in R_2, accounting for 9.59% of the relative abundance. Which was reported to be facultative bacterium that was able to survive in a carbon source environment and was capable of denitrification.[48] *Gemmatimonadaceae*, accounting for 3.85% and 6.17% of the bacterial abundance in R_1 and R_2 respectively, were assigned to *Gemmatimonadetes*, which is an important genus in the process of biological phosphorus removal, and consumed substantial amounts of organic matter during this period.[47] *Dechloromonas* accounted for 2.51%, 1.92% in R_1 and R_2, and played an important role in the biological phosphorus removal process. However, these bacteria are autotrophic bacteria,[49,50] and do not use external organic carbon sources, meaning that there is no significant effect on the degradation of organic matter. Moreover, *Zoogloea* was also to be found in the samples, accounting for up to 1.98%, 2.95% of the total bacterial abundance in R_1 and R_2, was capable of degrading refractory organics, including phenols and NHCs,[51] and some of the fungi in *Zoogloea* can promote the flocculation of activated sludge. Combined with the experimental observations, a suitable magnetic field intensity could effectively promote the flocculation and sedimentation performance of activated sludge. *Saprospiraceae* was reported to be frequently detected in activated sludge flocs, which may degrade proteins by producing extracellular enzymes. In this study, these bacteria were more abundant in R_2 (2.81%) higher than R_1 (1.33%). Furthermore, *Pseudomonas* and *Geothrix* were also detected in all samples. *Pseudomonas* is a common genus in the natural environment, which are an aerobic heterotrophic microorganisms that can metabolize various organic materials, including some complex organic compounds that are not easily degraded by other microorganisms. These microorganisms can also decompose proteins and fats,[52] and some of them can participate in the process of biological nitrogen removal.[53] Furthermore, *Geothrix* belongs to the phylum *Acidobacteria* and the members of this genus accounted for 1.36% and 2.01% in R_1 and R_2, respectively, which may be benefit the degradation of organic pollutants.

It revealed that an appropriate magnetic field intensity of 30 mT encouraged the growth of a wide variety of microbial taxa in the activated sludge community, including *Flavobacterium*, *Hydrogenophaga*, *Gemmatimonadaceae*, *Dechloromonas*, *Zoogloea*, *Saprospiraceae_uncultured*, *Pseudomonas* and *Geothrix*.

3.2.5 Significant differences in bacterial abundance. Based on Fisher's exact test results, the differences in the abundance of the dominant bacterial genera of the R_1 and R_2 samples exposed to an optimal magnetic field for 30 days were shown in Fig. 10. The relative abundance of each genus is shown on the left abscissa, whereas the difference between genus abundances is shown on the right plot within a 95% confidence intervals (** indicates $0.001 < P$ value ≤ 0.01 and *** indicates P value ≤ 0.001).

The genus abundances in the R_1 and R_2 samples differed significantly ($P < 0.05$), meaning that the microbial communities were highly susceptible to magnetic field treatment. The abundance of *Dechloromonas*, *Zoogloea*, *Geothrix*, and *Anaerolineaceae* under magnetic field varied only slightly ($0.01 < P$ value

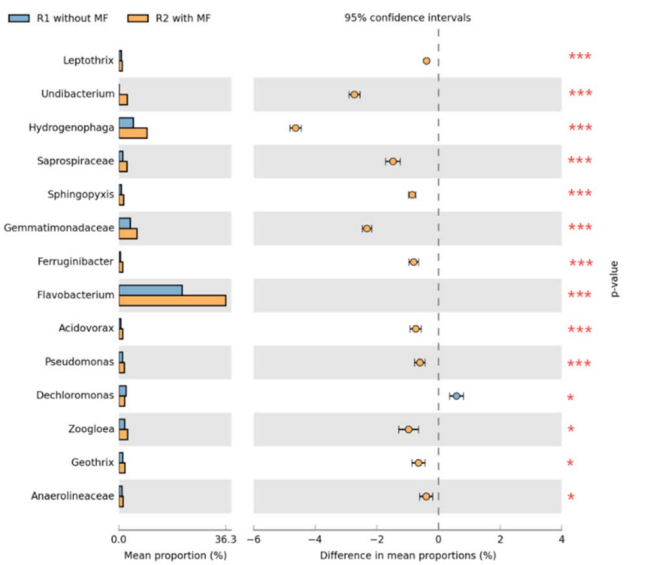

Fig. 10 Differences in the abundance of bacterial genera between samples of R_1 and R_2 based on Fisher's exact test.

≤ 0.05). In contrast, the abundance of *Flavobacterium*, *Hydrogenophaga*, *Gemmatimonadaceae*, *Undibacterium*, *Saprospiraceae*, *Leptothrix*, *Acidovorax*, *Ferruginibacter*, *Pseudomonas*, and *Sphingopyxis* showed highly significant differences (P value $\leq$ 0.001). Studies have shown that these bacteria were widely present in activated sludge, and may effectively degrade pollutants in wastewater.[45,46,48,49,51,52] Moreover, *Zoogloea* can promote the flocculation of activated sludge.[51]

Collectively, it demonstrated that magnetic field treatment increased the abundances of the aforementioned genera, which enabled the reactor to adapt more easily to low-temperature environments and maintain a high pollutant degradation performance in the activated sludge. Moreover, these results were consistent with the results of the bioreactor efficiency.

4. Conclusions

In this study, it demonstrated that magnetic field intensities of 10–40 mT could promote microorganism growth in activated sludge, thus promoting the degradation of dye wastewater at low temperatures. Particularly, a 30 mT magnetic field intensity at a low temperature enhanced the microbial activity in the activated sludge, thus improving the efficiency of activated sludge degradation for dye wastewater. More importantly, our findings revealed that magnetic field treatment effectively enriched bacterial communities, thus enabling the reactor to more easily adapt to low-temperature environments and maintain the pollutant removal performance of the activated sludge reactor. The dominant microbial taxa in the activated sludge reactor treated with a 30 mT magnetic field, which effectively participate in the degradation of pollutants, included *Flavobacterium*, *Hydrogenophaga*, *Gemmatimonadaceae*, *Zoogloea*, *Saprospiraceae*, *Pseudomonas*, and *Geothrix*.

Conflicts of interest

There are no conflicts to declare.

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