

Original Research

Cold-Shock Resistance of Activated Sludge Microorganisms Strengthened by a Static Magnetic Field

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Abstract

We studied the anti-cold shock performance of activated sludge microbes (ASM) with magnetic field (MF) strengthening under slow and rapid cooling and rewarming. From stage S3 to S4, the TTC-DHA (triphenyltetrazolium chloride dehydrogenase) of reactor A₂ and B₂ were 98.5% and 72.5% higher than that of reactor A₁ and B₁, which showed that MF had a better strengthening effect on TTC-DHA under slow temperature variation than that under rapid temperature variation. MF had a definite strengthening effect on relief and inhibition of cell membrane lipid peroxidation and low-temperature injury, which was indicated by SOD (superoxide dismutase), CAT (catalase), and MDA (malonaldehyde), while the MF strengthening effect on SOD and CAT activity was not stable under rapid cooling and rewarming. The MF had little effect on phospholipid fatty acid (PLFA) diversity of ASM under slow cooling, but had higher PLFA diversity on that under rapid cooling. Optimal MF strengthening application under different temperature variation mode is supposed to be a potential pathway to strengthen the activity and cold resistance of ASM and improve the efficiency of wastewater treatment in low temperatures.

Keywords: temperature variation, activated sludge, magnetic field strengthening, phospholipid fatty acid, microbial diversity

Introduction

Low temperature (0-10°C) is a key limiting factor for biochemical wastewater treatment methods [1]. The fact that varying cooling speed rate can cause different degrees of microbial cell damage has been studied.

Rapid cooling causes intracellular ice damage, while slow cooling leads to microbial solution loss (solution damage) [2]. In a biological wastewater treatment process, the temperature variation rate is relative to the microbe, which has great influence on wastewater treatment efficiency [3-4].

Under normal temperatures, the generation and elimination of active oxygen in vivo keeps balance, while the active oxygen tends to excessive accumulation

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under an extreme low-temperature environment (0–10°C). The main forms of active oxygen contain superoxide anion radicals, hydrogen peroxide (H₂O₂), and oxygen free radical (·OH). The reactive oxygen will react with membrane lipids, proteins, DNA [5], and other biological large molecules, resulting in the peroxidation of cell membrane, the accumulation of membrane lipid oxidation product malondialdehyde (MDA), damage to the membrane structure, the increase of membrane permeability, lysosomal disruption and cell lysis, the decrease of cell membrane fluidity, and abnormality of cell physiological function [6]. Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) are the main tools for eliminating reactive oxygen. The main function of SOD and CAT is to transform the superoxide anion free radical to H₂O₂ [7] as the protective enzyme defense system within the organisms. The activity of SOD and CAT indirectly reflects the ability of microbes to remove oxygen free radicals, and the MDA content indirectly reflects the severity of microbe attacked by free radical.

Increasing attention has been directed to the possibility of improving wastewater treatment processes by applying static magnetic fields (MF), which is a new method to intensify the elimination of wastewater pollution. The MF does not require extensions of existing plants or constructing expensive bioreactors [8], and can be directly applied on the original wastewater treatment structures. MF strengthening on activated sludge microbes (ASM) has been applied to wastewater treatment at low temperature, and the intensity of 30 mT has proven to be more effective [9]. The microbial membrane's fluidity is weakened and the enzyme activity is inhibited at low temperatures, and MF strengthening has the potential to cover the shortage of low-temperature limitation through affecting the orientation rearrangement of lipid bilayer and the activity center site of an enzyme [10]. Meanwhile, phospholipid fatty acid (PLFA) as the component of cell membrane will have corresponding variation at low temperatures, and analysis of this constituent makes it possible to obtain biochemical fingerprints of communities and yield information about the taxonomy, functions, physiology, and abundance of community members [11].

This experiment mainly studied the anti-cold shock performance of ASM activity with MF strengthening under slow and rapid cooling and rewarming. The variation laws of TTC-DHA, SOD, MDA, and CAT were researched. While PLFA and 16S rRNA analysis are combined to analyze the community structure and diversity of activated sludge at low temperatures, which reflect the MF strengthening effect on microbial resistance to cold-shock [12].

Material and Methods

Bioreactor

Four simulated sequencing batch reactors (SBR; reactors A₁, A₂, B₁, and B₂) made of plexiglass were operated with the working volume of 2 L. The radii and heights of the reactors are 5 cm and 30 cm, respectively. The operational state of the four reactors is listed as Table 1.

Two heteropolar magnetic plates were placed in parallel beside reactors A₂ and B₂ in order to generate a static MF. The plate intervals were adjusted to assure that the center MF intensities were 30 mT, while reactors A₁ and B₁ were set as the control variable without exposure to any MF. The MF intensities were detected by a TM-701 Tesla Meter, Kanetec.

After adaptation at 25°C of the four reactors for 25 d in the temperature-controlled incubators, reactors A₁ and A₂ experienced slow cooling and rewarming process (temperature variation rate = 1.2°C/h), and reactors B₁ and B₂ experienced rapid cooling and rewarming (instantaneous transfer). The process experienced seven stages: S1 to S7. Stage S1 was running at 25°C, stages S2 to S4 were cooling stages running at 0°C to simulate the most extreme low-temperature environment of wastewater treatment, and stages S5–S7 were rewarming stages running at 25°C. Every stage lasted 5 days.

The synthetic wastewater consisted of C₆H₁₂O₆ (1000 mg/L), NH₄Cl (191 mg/L), and KH₂PO₄ (44 mg/L), diluted to a final COD of 200 mg/L at domestication stage and 400 mg/L at the experimental stage; the mass ratio of C:N:P was 100:5:1. Trace elements were included as well. The cycle time of the reactors was 12 h with 1000 mL wastewater and 1000 mL activated sludge aerated with an air pump (3.0 L/min). The hydraulic retention time (HRT) was 10h with a solid retention time (SRT) of 10 days. This process was operated in batch with filling, reaction, and settling times of 0.2 h, 10 h, and 1.8 h, respectively. The oxygen concentration during this time was around 8.65 mg/L for the four reactors and no external pH control was applied during this study. The sludge for the inoculation in the reactors was collected from a local wastewater treatment plant in Nanjing, China and its concentration was about 4800 mg/L.

Table 1. Reactor operation state.

Reactor code	A ₁	A ₂	B ₁	B ₂
Magnetic field	without	with	without	with
Temperature variation	slow	slow	rapid	rapid
Stage 1	25°C	25°C	25°C	25°C
Stage 2	25 to 0°C	25 to 0°C	25 to 0°C	25 to 0°C
Stage 3-4	0°C	0°C	0°C	0°C
Stage 5	0 to 25°C	0 to 25°C	0 to 25°C	0 to 25°C
Stage 6-7	25°C	25°C	25°C	25°C

Enzyme Activity and Malonaldehyde Content

The TTC-DHA of microorganisms was analyzed according to Tracey's, with slight modification [13]. TTC as the artificial hydrogen acceptor will translate into TF (triphenyl formazan), which can be measured at 486 nm. The yield was defined as 1 ug TF per hour as a unit of enzyme activity.

Protein, MDA, SOD, and CAT contents were determined by the reagent kits provided by Nanjing Jiancheng bioengineering institute. Crude enzyme was extracted as follows: activated sludge was collected by centrifugation, added with normal saline, and broken in an icewater bath by ultrasonic. Protein content was determined by the method of Coomassie brilliant blue. SOD was detected by the method of WST-1 (water soluble tetrazolium salt). WST-1 and super oxide anion created by Xanthine oxidase catalysis react to form formazan dye, which can be inhibited by SOD. The SOD activity can be calculated by colorimetric analysis of the WST-1 product. CAT activity was measured by the visible light method. The reaction of CAT decomposing H_2O_2 can be stopped quickly by ammonium molybdate. The residual H_2O_2 and ammonium molybdate react to form light yellow complex, which can be detected at 405 nm to calculate CAT activity. MDA content was determined by the TBA (thiobarbituric acid) method. The MDA in the degradation products of lipid peroxide can be condensed with TBA, and generates red product, which has the maximum absorption peak at 532 nm.

Phospholipid Fatty acid Extraction and Nomenclature

The PLFA extraction method was modified based on Balsler's [14-16]. Briefly, 5 mL activated sludge was collected randomly from the middle level of the sedimental activated sludge layer in the reactors for three times and mixed evenly, and the mixture was stirred then centrifuged for 5 minutes with the supernatant discarded. Through separation, elution, saponification, methylation, and extraction, the resulting fatty acid methyl esters were prepared according to MIDI protocol and detected with an Agilent 7890 GC, and the results were analyzed using the MIDI Sherlock Microbial Identification System (MIDI, Newark, DE) [17]. Fatty acids were designated in terms of total number of carbon atoms, with the number of double bonds given after a colon, following the form A:B ω C, where A is the number of carbon atoms, B is the number of double bond, and C is the position of the first double bond from the ω or the molecule's aliphatic end. In branched chain fatty acids, the suffixes marked iso and anteiso present homotype and heterotype fatty chains, respectively.

Statistical Analysis

The enzyme activity values were expressed as the mean standard deviation. The data was statistically

analyzed using SPSS Version 18.0 for Windows. The differences between the control and experimental groups were analyzed using one-way analysis of variance and LSD test. Values of $p < 0.05$ were considered significant, and values of $p < 0.01$ were accepted as having high statistical significance. Meanwhile, *a*, *b*, and *c* presented that A_2 relative to A_1 , B_2 relative to B_1 and B_2 relative to A_2 had significant differences.

The Shannon-Wiener index was generally defined as:

$$H = -\sum_{i=1}^s p_i \lg(p_i)$$

...where *H* was Shannon-Wiener index, *s* was the total number of PLFA in each sample, and p_i was the percentage of the peak area of PLFA to the total area of each sample. The GC assay peak areas were used to calculate values of p_i for each PLFA, which were inserted into the equation above.

Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis Analysis for Bacterial Community

Polymerase chain reaction (PCR) was performed with the primers 518r and 338f (with GC-clamp) under the following conditions: 94°C /5 min denaturation step; 30 cycles of 94°C /30 s, 58°C /30 s, and 72°C /45 s; and a final extension step at 72°C /10 min. Denaturing gradient gel electrophoresis (DGGE) was carried out in a DGGE system for the PCR products. Gels were photographed using Kodak 1D image analysis software after being stained with ethidium bromide. For further identification of DGGE bands in each sample, several representative DGGE bands were cut from the DGGE gel and amplified with the primers 518r and 338f (without GC-clamp), and then were sent for sequencing by Genscript, Nanjing.

Results and Discussion

Triphenyltetrazolium Chloride Dehydrogenase

The TTC-DHA of microbes can reflect the efficiency of substrate degradation under different temperature variation conditions [18-19]. Fig. 1 reflects the TTC-DHA variation of four reactors under different periods of cooling and rewarming. After cooling, the TTC-DHA of each reactor was significantly reduced at stage S2. From stage S3 to S4, the TTC-DHA of reactor A_2 and B_2 increased, and were 98.5% and 72.5% higher than that of reactors A_1 and B_1 , respectively. Reactor A_2 relative to A_1 had positive significant differences at stage S2 ($p = 0.001$) and negative significant differences at stages S3 ($p = 0.001$) and S4 ($p = 0.002$). The TTC-DHA of reactor B_2 relative to B_1 did not vary too much, and reactor B_2 relative to A_2 had negative

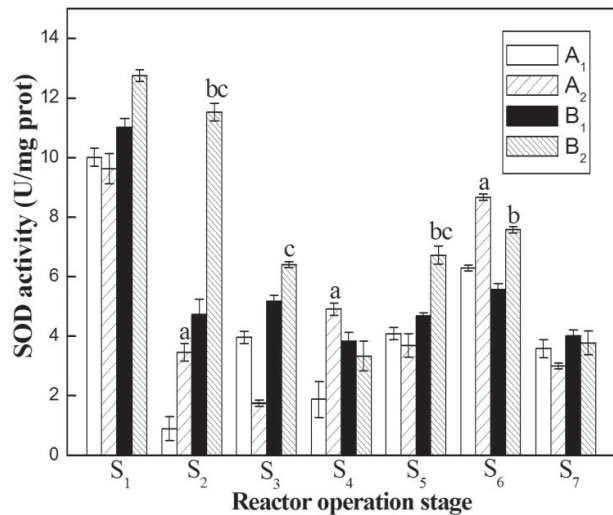


Fig. 1. TTC-DHA of activated sludge reactors in the process of temperature variation;

significant difference at stage S3 ($p = 0.001$). At stage S5, reactor B₂ ($p = 0.001$) relative to A₂ had negative significant difference, and the TTC-DHA of MF strengthening reactors were higher than that of control groups. At the whole stage of rewarming, the TTC-DHA all improved and recovered to a certain extent, and reached the level before cooling, in which the TTC-DHA variation ranges of reactors A₁ and A₂ were larger than those for reactors B₁ and B₂.

The four reactors were greatly affected by low temperature at the initial cooling stage of S2, but the TTC-DHA increase of reactors A₂ and B₂ at stages S3 and S4 compared to the control group indicates that the MF had a strengthening effect on TTC-DHA of ASM at cooling phase. The significant difference of reactor A₂ relative to A₁ showed that the MF improved the TTC-DHA under slow cooling, while the low activity of reactor B₂ after cooling demonstrated that rapid cooling greatly inhibited the TTC-DHA, and MF had poor strengthening effect on it [20]. After rewarming, the variations showed that rapid temperature change greatly affected TTC-DHA – with even the temperature returning to 25°C, and the TTC-DHA cannot restore to normal state immediately from low-temperature injury [21]. The significant difference of reactor A₂ relative to B₂ at stage S5 showed that MF had a better strengthening effect on ASM under slow temperature variation than that under rapid temperature variation.

Superoxide Dismutase Activity

SOD is the most important enzyme for organism resistance to clear free radicals, which mainly scavenge *vivo* active oxygen ($O_2^{\cdot-}$) [22]. Fig. 2 shows the SOD activity of activated sludge reactors in the process of cooling and rewarming variation. As can be seen, stage S1 has high SOD activity of four reactors at 25°C. The SOD activity was all reduced after the start of

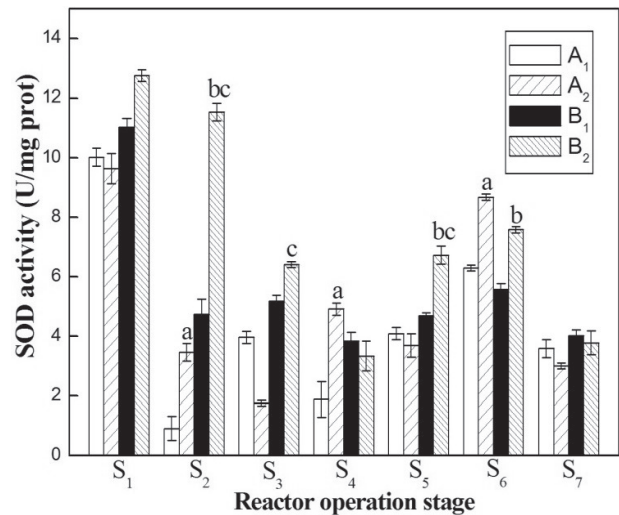


Fig. 2. SOD activity of activated sludge reactors in the process of temperature variation.

cooling, while the decline ranges of reactors A₁ and A₂ were larger and reactor B₂ was the smallest. Compared to reactors A₁ and B₁, the SOD activity of reactors A₂ ($p = 0.006$) and B₂ ($p = 0.001$) had significant differences, as well as reactor B₂ ($p = 0.001$) relative to A₂. At the stage of continuous low-temperature condition (stage S2 to S3), reactor B₂ ($p = 0.001$) had significant difference with respect to reactor A₂ at stage S3, as well as reactor A₂ ($p = 0.005$) relative to A₁ at stage S4. At the stage of rewarming (stage S5 to S7), reactor B₂ had significant differences to B₁ ($p = 0.03$) and A₂ ($p = 0.01$) at stage S5. At the continuous rewarming phase of stage S6, MF strengthening reactors A₂ ($p = 0.022$) to A₁ and B₂ ($p = 0.029$) to B₁ all had significant differences.

Reactors A₁ and A₂ were under slow cooling, and exposure to low temperature for more than 24 h at stage S2, while reactors B₁ and B₂ were under rapid cooling, and transferred to the environment of 0°C instantaneously through adjusting the incubator temperature. The microbial cells of reactor A₁ and A₂ were damaged more severely, and biomass in reactor B₁ and B₂ were less affected, though they were all under dramatic temperature drop [23]. The results indicated that it is an accumulated process of low-temperature injury for microbial cells and SOD activity [24]. From stage S3 to S4, the SOD activity of reactor A₂ remained stable, while reactor B₂ showed a gradual decline in the overall trend, which demonstrated that rapid temperature variation and continual exposure time at low temperature increased the injury of microbial cells, and inhibited the activity of SOD. In the three phases of low temperature, the average SOD activity of reactor A₁ and B₁ were 45.0% and 52.4% lower than that of reactor A₂ and B₂, respectively. It showed that the MF had a definite strengthening effect on SOD activity at low temperature [25]. After warming, the SOD activity of four reactors were all improved and the significant differences of stages S6 and S7 indicated that the SOD

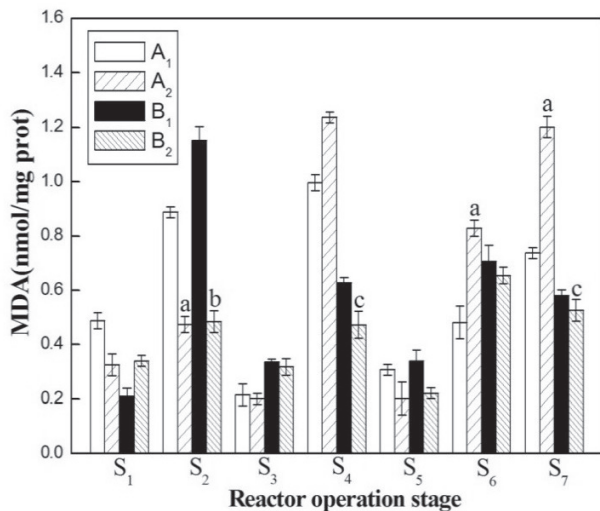


Fig. 3. MDA content of activated sludge reactors in the process of temperature variation.

activity recovery time was longer for a slow-cooling reactor, but the activity will become higher than that of under rapid cooling gradually, which meant that rapid temperature variation had a greater impact on SOD activity recovery [26].

Malonaldehyde Content

MDA is the product of lipid peroxidation, and the higher the MDA content, the greater damage degree the microbial membrane endures [27-28]. Fig. 3 shows the MDA content variation of different reactors during temperature changes. After cooling, the MDA content at stage S2 all increased for four reactors, in which reactors A₁ and B₁ increased rapidly, by 82% and 449.1% respectively compared to stage S1, while reactors A₂ and B₂ increased only by 45.5% and 42.2% respectively, and reactors A₂ ($p = 0.042$) and B₂ ($p = 0.046$) had significant differences compared with the control groups. At the phase of stage S2 to S4, the MDA contents of reactors A₂ and B₂ increased gradually, and B₂ ($p = 0.028$) had significant differences at stage S4 compared to A₂. During the rewarming period, the MDA content of A₂ greatly increased, and had significant differences at stages S6 ($p = 0.027$) and S7 ($p = 0.026$) relative to the control group (A₁). However, the MDA content of B₂ changed little, and was lower than that of A₂ during S5 to S7. In addition, B₂ ($p = 0.006$) had significant differences with A₂ at S7.

At the initial cooling stage, the decreasing temperature aggravated lipid peroxidation of cell membrane, causing damage to the microbial cell, and the membrane lipid oxidation was more serious under rapid cooling [29]. From S2 to S4, the significant differences of A₂ and B₂ compared with the respective control group indicated that the MF had a strengthening effect on the resistance to cell low-temperature injury. The MDA contents of four reactors decreased at stage

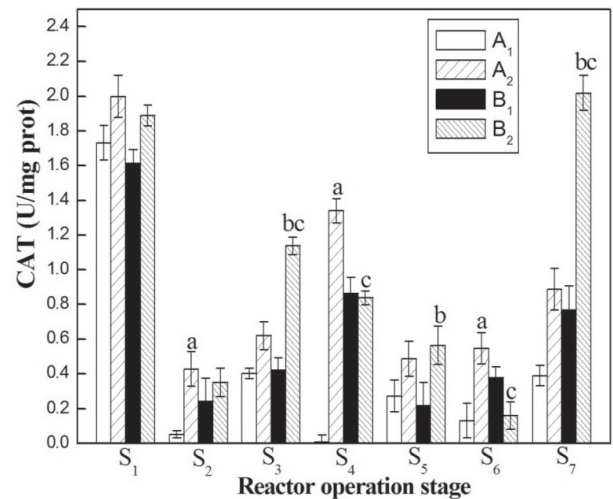


Fig. 4. CAT activity of activated sludge reactors in the process of temperature variation.

S5, but all increased from S6 to S7, and exceeded that of the initial reactor operation phase of S1 under moderate temperature. The reason is that the metabolism of microbial cells become stronger to repair the damaged cells, simultaneously the quantity of dead cell membrane was transformed to MDA [30]. The MDA content of reactor B₂ was lower than that of B₁ for the whole operation phase with the significant differences of MDA content of B₂ to A₂ at S4 and S7, which demonstrated that the MF had a better strengthening effect on the relief and inhibition of cell membrane lipid peroxidation and low-temperature injury [31].

Catalase Activity

CAT is an important endogenous active oxygen scavenger that can remove H₂O₂ and ·OH in vivo [32]. Fig. 4 shows the CAT activity variation of the four reactors during cooling and rewarming. At the initial stage of cooling, the CAT activity of four reactors all greatly decreased compared to that at 25°C, but the CAT activities of A₂ and B₂ with MF were higher than that in the control group. A₂ relative to A₁ at S2 ($p = 0.006$) and S4 ($p = 0.001$), B₂ relative to B₁ at S3 ($p = 0.003$), and B₂ relative to A₂ at S3 ($p = 0.017$) and S4 ($p = 0.018$) all had significant differences. At S5, the CAT activity of the four reactors did not restore to the initial state of S1, but increased at S7. The CAT activity of A₂ ($p = 0.003$) over the whole rewarming phase and B₂ at S5 ($p = 0.027$) and S7 ($p = 0.001$) all had significant differences compared to respective control groups. And B₂ at S6 ($p = 0.004$) and S7 ($p = 0.001$) had significant differences, but it was a negative significant difference at S6.

After cooling, the CAT activity of A₂ and B₂ were higher than that of A₁ and B₁, which indicated that the MF had a strengthening effect on CAT activity of ASM. During the whole cooling phase, the significant difference of A₂ relative to A₁ showed that MF had

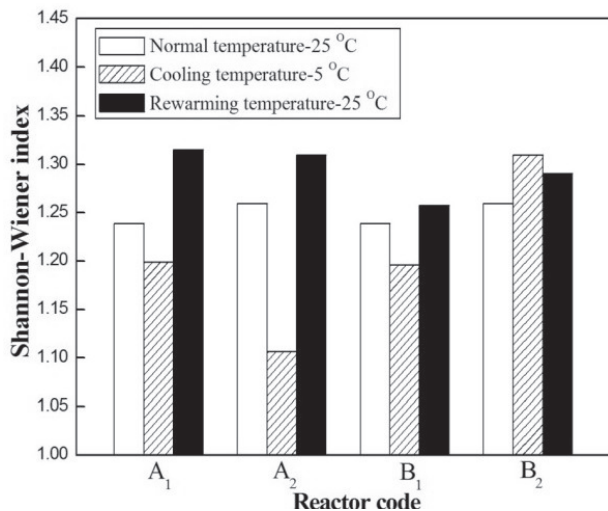


Fig. 5. Shannon-Wiener diversity indices of PLFA in the process of temperature variation.

a strengthening effect on CAT activity under slow cooling [33-34]. At the rewarming phase, the CAT activity of every reactor could not recover, which demonstrated that the CAT activity was inhibited by low temperature, and it needed more time to restore after rewarming. The significant difference of A₂ relative to A₁ after rewarming showed that MF had improved the CAT activity of slow cooling and rewarming. While the positive and negative significant difference of B₂ relative to B₁ explained that the MF strengthening effect was not stable under rapid cooling and rewarming [35-36].

Shannon-Wiener Diversity Analysis of Phospholipid Fatty Acid

At every stage of THE cooling and warming processes there were a total of 42 species of PLFA counted by carbon chain distribution from C10 to C20. In order to investigate the richness and evenness of PLFA, which was the reflection of community diversity, Shannon-Wiener diversity analysis was applied [37].

The PLFA Shannon-Wiener diversity indices of the four reactors are shown in Fig. 5. As can be seen, the indices of A₁, A₂, and B₁ were lower than that under medium temperature, and the decline range of A₂ was the largest at the end-cooling stage, while the index of B₂ increased compared with that at 25°C. At the end warming stage, the indices of the four reactors all recovered to the level before cooling.

Low temperature will inhibit the growth and activity of mesophilic microbes, leading to dormancy and death, and cold adapted microorganism gradually becomes the dominant bacteria group [38]. But due to the slow growth rate and long generation, the quantity of microbes at low temperature is difficult to reach the level of mesophilic microorganism, which results in overall activity reduction, species number, and PLFA diversity decrease. After rewarming, microbes began to multiply

quickly, the biomass and species of ASM gradually recover, and the species containing specific PLFA also increase, which increase the diversity of PLFA [39]. The PLFA diversity index of reactor A₂ dropped and reactor B₂ remained at a high level, indicating that the MF had little affect on PLFA diversity of ASM under slow cooling, but had a better strengthening effect on that under rapid cooling. Meanwhile, rapid cooling probably stimulated the cell membrane to produce new PLFA to enhance the cold adaptability [40], which could increase the PLFA diversity of reactor B₂.

Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis Analysis Comparison of the Four Runs from Denaturing Gradient Gel Electrophoresis

From Fig. 6, 10 representative bands in four lanes were obtained, and had distinguishing distributions in different lanes. It can be obtained from band quantity that the band quantity with MF strengthening (A₂ and B₂) were more than the control groups (A₁ and B₁), and the band quantity of slow cooling and rewarming with MF (A₂) were more than that under rapid temperature change conditions with MF (B₂).

Bands 1, 4, and 6 belonged to four reactors, and were more intense by comparison, which indicated that this species of bacteria was enriched under the corresponding culture condition. Bands 2, 3, and 8 did not exist in Lanes 1 and 3, but existed in Lanes 2 and 4, which indicated that the bacteria corresponding to Bands 2, 3, and 8 did not exist in A₁ and B₁, but

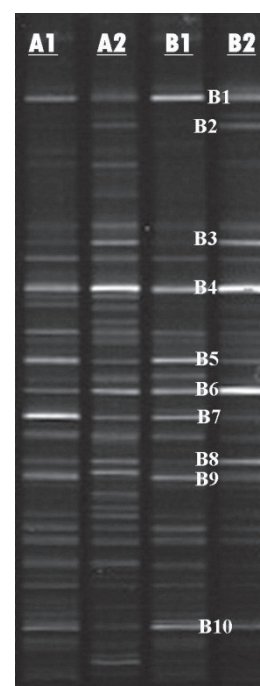


Fig. 6. 16S rRNA analysis of bacterial community structure on different activated sludge reactors for the four runs by PCR-DGGE.

existed in A₂ and B₂. The situation of Band 5 was opposite of Bands 2, 3, and 8, which showed that the bacteria in Band 5 did not exist under low temperature and MF strengthening. Bands 7 and 9 existed in Lanes 1, 2, and 3, but disappeared in Lane 4, so the bacteria in Bands 7 and 9 did not enrich in rapid temperature variation conditions with MF. On the contrary, Band 10 just existed in Lanes 1, 3, and 4, which meant that the bacteria in Band 10 was only enriched under rapid cooling and rewarming with MF.

Analysis of Microbial Community

The ten representative bands were sequenced and blasted with National Center for Biotechnology Information (NCBI).

Band 1 was closely related to *Clostridium cellulovorans*, in the family of *Clostridiaceae*. Band 4 was closely related to *Dehalogenimonas* sp., in the phylum of *Chloroflexi*. Band 6 was closely related to *Flavobacterium indicum*, in the family of *Flavobacteriaceae*. The above-mentioned three kinds of bacteria were common bacteria for four lanes, belonging to dominant bacteria in the reactor operation process.

Band 2 was closely related to *Delftia* sp., belonging to the *Comamonadaceae* family, and studies had reported the MF strengthening effect on *Delftia* to maintain the cell physiological function and activity control [41]. Band 3 was related to *Clostridium cellulovorans*, in the family of *Clostridiaceae*. Krauss separated the species by magnetic separation technology in the analysis of the enhancement effect of crystalline cellulose on bacteria activity [42]. Band 8 was related to *Flavobacterium branchiophilum*, in the family of *Flavobacteriaceae*, belonging to Gram negative bacteria. Ryumae studied the rapid detection of immune magnetic separation of this bacteria by flow cytometry [43]. The above species all had magnetic biological effect reported by corresponding research, as a result they were enriched under MF strengthening condition. While Band 5 was related to *Wolinella succinogenes*, in the family of *Helicobacteraceae*, the disappearance of Band 5 in Lanes 2 and 4 indicated that the bacteria growth was inhibited by MF condition at low temperature.

Band 7 was similar to *Novosphingobium* sp., in the family of *Sphingomonadaceae*. Jung et al. found that the low-temperature activity was 40% higher than that of other species when they studied the epoxide hydrolase activity of this species [44]. Band 9 was related to *Burkholderia phytofirmans*, belonging to the *Burkholderiaceae* family. Essaid et al. studied the cold resistance of this species in plant inoculation and found that it could reduce the cold sensitivity of a plant to improve cold resistance ability [45]. The experimental results showed that although the two species had certain cold resistance, they were inhibited by rapid cooling and rewarming under low temperature. While Band 10 was similar to *Klebsiella oxytoca*, in the family of

Enterobacteriaceae it was not enriched as affected by MF under slow cooling and rewarming.

Conclusions

The MF had a better strengthening effect on ASM under slow temperature variation than rapid temperature variation. Rapid temperature variation and continual exposure time at low temperature increased the injury of microbial cells, and inhibited the activity of SOD and CAT. Meanwhile, MF had a definite strengthening effect on SOD activity at low temperature. The MF had a better strengthening effect on the relief and inhibition of cell membrane lipid peroxidation and low-temperature injury indicated by MDA. The MF had little affect on PLFA diversity of ASM under slow cooling, but had better strengthening effect on that under rapid cooling. Optimal MF strengthening application under different temperature variation mode can strengthen the activity and cold resistance of ASM and improve the efficiency of wastewater treatment under low temperature.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

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