



Bioaccumulation, resistance, and remediation of Mn(II) and Cu(II) and their impacts on antioxidant enzymes of *Anoxybacillus flavithermus*

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Abstract

In this article, the effects of copper (Cu(II)) and manganese (Mn(II)) ions on the superoxide dismutase (SOD) and catalase enzymes (CAT) of the thermophilic *Anoxybacillus flavithermus* (*A. flavithermus*) were thoroughly examined. Results from experiments using the minimum inhibitory concentration (MIC) showed that *A. flavithermus* was less tolerant of ions in the liquid media than in the solid medium. In comparison to the control, the greatest percentage of bacterial growth was determined to be 20.4% including the addition of Mn(II) and 17.6% including the addition of Cu(II). Mn(II) bioaccumulation increases the total weight of the bacteria, while Cu(II) bioaccumulation specifically increases the weight of dry *A. flavithermus* (MDBW). The ability of *A. flavithermus* cell membrane to bioaccumulate ions was examined. At various ion concentrations, the CAT and SOD activities were examined. Any possible morphological alteration following the encounter with ions. The findings suggested that *A. flavithermus* may be used to extract and remove hazardous metals from industrial wastewaters. *A. flavithermus* is a potential bioindicator of hazardous metal-contaminated waters, according to antioxidative enzyme activity. Additionally, the activities of the antioxidant enzymes CAT and SOD increased with increasing ion concentrations up to 10.0 mg/L over a 48 h period. This indicates a protective response against oxidative stress caused by Cu(II) and Mn(II) ions.

Keywords Antioxidant enzyme · *Anoxybacillus flavithermus* · Bioaccumulation · Cu(II) · Mn(II) · Thermophilic bacteria

Introduction

Pollution from toxic metals has increased exponentially in recent decades due to technological advances and urbanization. These are at the center of human life due to wastes from technological tools that come

into our lives with the development of technology and industry. Vehicle emissions caused by the increasing number of vehicles, acidic batteries commonly used in vehicles, agricultural fertilizers, all kinds of paints, industrially treated trees, aging sewage infrastructure, and microplastics floating in all global water sources are some of the sources of heavy metals in our lives (Suja et al. 2009).

The studies carried out for the remediation of toxic metals, which are important for environmental safety, have become important. Studies in this area can be classified as chemical, physical and biological methods. There is an excessive amount of chemical and energy consumption when applying physical and chemical methods. Therefore, biological methods, which are economical and environmentally friendly methods should be used instead of these methods. Removal of various heavy metals using accumulation, mobilization or immobilization methods and changing their oxidation steps are done in many countries by using microorganism (Duprey et al. 2014; Mateos et al. 2016). Numerous studies have been realized on this subject during

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the last years. For example, the biomass of fungi and algae has been studied for their ability to absorb or remove metal ions (Costa and Tavares 2017; Flouty and Estephan 2012; Anahid et al. 2011). Another approach to removing toxic metals from various sources is through studies with bacteria. Bacteria isolated from soil, water, plants, etc., can be used for metal removal under various working conditions. There have been numerous studies conducted in this field (Deng et al. 2003; Nanda et al. 2019; Matyar and Kaya 2008; Özdemir et al. 2012; Özdemir et al. 2017). Most studies on the bioaccumulation and removal of toxic metals have been done using mesophilic microorganisms (Cai et al. 2019; Santhiya et al. 2011; Jardine et al. 2019; Ozdemir et al. 2022; Nonaka et al. 2014). In the literature, there are few studies on the use of thermophilic bacteria in toxic metal removal and bioaccumulation studies (Özdemir et al. 2012; Özdemir et al. 2013).

A. flavithermus, a thermophilic bacterium, has gained significant attention in recent years for its potential applications in the bioaccumulation, resistance, and remediation of toxic metals, such as Hexavalent chromium. The increasing industrialization and technological advancements have led to a significant rise in environmental pollution caused by these toxic metals, posing substantial risks to ecosystems and human health (Li et al. 2019; Yang et al. 2022). The purpose of this study is to determine the percentage of removal and bioaccumulation capacities of manganese and copper, as well as to assess the activities of SOD and CAT enzymes using the thermophilic bacterium *A. flavithermus*. The effects of Mn(II) and Cu(II) ions on the growth of the thermophilic bacterium *A. flavithermus* were also examined by conducting minimum inhibitory concentration analyses and studying the effects of two types of ions on α -amylase enzyme production.

Materials and methods

Instrumentation

Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES) was used to quantify the amounts of Cu(II) and Mn(II) in samples (Perkin Elmer Optima TM 2100 DV). A Mettler Toledo MPC 227 was used to measure pH. The Fourier Transform Infrared Spectroscopy (FTIR) spectra of the KBr pellet samples were collected using a Perkin-Elmer Spectrum 400 spectrometer. LEO 440 SEM (20 kV acceleration voltage) device was used for scanning electron microscope (SEM) analysis. All of the samples had Au/Pd coatings before being subjected to the SEM investigation.

Growth of *A. flavithermus*

The thermophilic *A. flavithermus* was extracted from mud samples, Gecek spa in Afyonkarahisar, Turkey. The accession number of *A. flavithermus* is KJ434792. After isolation and identification procedures (morphological, physiological, biochemical, and molecular analysis), it stored in laboratory at $-72\text{ }^{\circ}\text{C}$ freezer in glycerol. Preparation procedures for experiments related to the growth of bacteria at different metal concentrations were carried out as described in the literature (Özdemir et al. 2013; Korkmaz et al. 2020; Tiri et al. 2022; Aygün et al. 2020; Ozdemir et al. 2020).

Determination of minimum inhibitory concentration

The minimum inhibitory capacity experiments of thermophilic *A. flavithermus* in solid and liquid environments with Cu(II) and Mn(II) ions were performed according to the method in the literature (Özdemir et al. 2013). *A. flavithermus* was cultivated, and a UV–vis spectrophotometer operating at a wavelength of 540 nm was used to measure the growth of the bacteria at 4 and 24 h. All of the analysis were performed at least three times. The results were presented with their standard deviations.

Determination of Cu(II) and Mn(II) ions bioaccumulation, removal, and the performance on α -amylase generation

Pre-experimental preparations for the bioaccumulation, removal and determination of the α -amylase enzyme released by thermophilic *A. flavithermus* bacteria were carried out as mentioned in the literature (Özdemir et al. 2013). The metal removal percentage efficiency of thermophilic bacteria (*A. flavithermus*) at different metal concentrations was calculated. In this process, the initial concentration for each metal and the residual metal concentrations in the fermented culture at equilibrium were taken into account. The percentage of removal was calculated using the formula from previous studies (Özdemir et al. 2013). The supernatant was also used to determine the activity of α -amylase using the Bernfeld process (Bernfeld 1955). When calculating the enzyme activity according to this method, the amount of α -amylase that hydrolyses 1 mmol/minute of maltose during fermentation was considered as one unit of α -amylase enzyme activity. All analyses were performed at least three times, and the results were presented with their standard deviations.

Mn(II) and Cu(II) ions bioaccumulation on the cell membrane

Bioaccumulation of Mn(II) and Cu(II) ions with *A. flavithermus* bacteria was performed according to the methods in our previous study and reported in the literature (Özdemir et al. 2013). All of the analysis were performed at least three times. The results were presented with their standard deviations.

Determination of antioxidant enzyme (superoxide dismutase and catalase) activities

The experiments of determining the activity of antioxidant enzymes with thermophilic *A. flavithermus* were performed according to our previous study in the literature (Özdemir et al. 2013). The behaviours of SOD and CAT enzymes of *A. flavithermus* were measured spectrophotometrically at 550 nm and 240 nm, respectively. All of the analysis were performed at least three times. The results were presented with their standard deviations.

Results and discussion

Inhibitory minimum concentration (MIC)

The MIC (Minimum Inhibitory Concentration) values of two types of ions in solid and liquid media were determined to assess the resistance of bacteria to various metal ions under different conditions. The results revealed that thermophilic *A. flavithermus* exhibited lower resistance in liquid media compared to solid media. The MIC values for manganese and copper ions in the solid medium were determined as 5500 µg/mL and 2600 µg/mL, respectively (Table 1), while in the liquid medium, they were found to be 225 µg/mL and 40 µg/mL, respectively (Table 2).

A. flavithermus exhibited approximately 65.0 times higher sensitivity to Cu(II) in liquid media compared to agar media, as indicated by the MIC data presented in the tables. Similarly, a value of 24.4 was determined for Mn(II). Since metal ions behave differently in solid and liquid forms, and metals are less soluble in solid environments, bacteria may have more interactions with metal ions in such conditions. This could explain the lower resistance of the bacteria to liquid media compared to solid media. Several studies, including (Chaudhary et al. 2017, Barboza et al. 2017, Mihdhir and Assaeedi 2016, and Singh et al. 2017), have investigated the resistance of various bacteria to different metal ions, including Mn(II) and Cu(II). In contrast to their findings, our results demonstrated that thermophilic *A. flavithermus* exhibited greater tolerance. These findings highlight the

Table 1 MIC of Mn(II) and Cu(II) ions in solid media

Concentration (µg/ mL)	<i>A. flavithermus</i> 17		
	Cu(II)	Concentration (µg/ mL)	Mn(II)
1	+	1	+
2.5	+	2.5	+
3	+	3	+
5	+	5	+
7.5	+	7.5	+
10	+	10	+
15	+	15	+
20	+	20	+
30	+	30	+
40	+	40	+
50	+	50	+
60	+	60	+
70	+	70	+
80	+	80	+
90	+	90	+
100	+	100	+
110	+	110	+
120	+	120	+
130	+	130	+
140	+	140	+
150	+	150	+
200	+	200	+
250	+	250	+
300	+	300	+
400	+	400	+
500	+	500	+
750	+	750	+
1000	+	1000	+
1500	+	2000	+
1600	+	3000	+
1750	+	4000	+
2000	+	5000	+
2250	+	5250	+
2500	+	5400	+
2550	+	5450	+
2600	–	5500	–
2650	–	5600	–

potential of thermophilic *A. flavithermus* in the bioremediation of toxic metal ion contamination.

The effects of the quantities of two type ions on *A. flavithermus*

In the concentration range of 0–10 µg/mL, the impact of two types of ions on the growth of *A. flavithermus* was investigated (Ozdemir et al. 2023). The results are shown in Fig. 1a

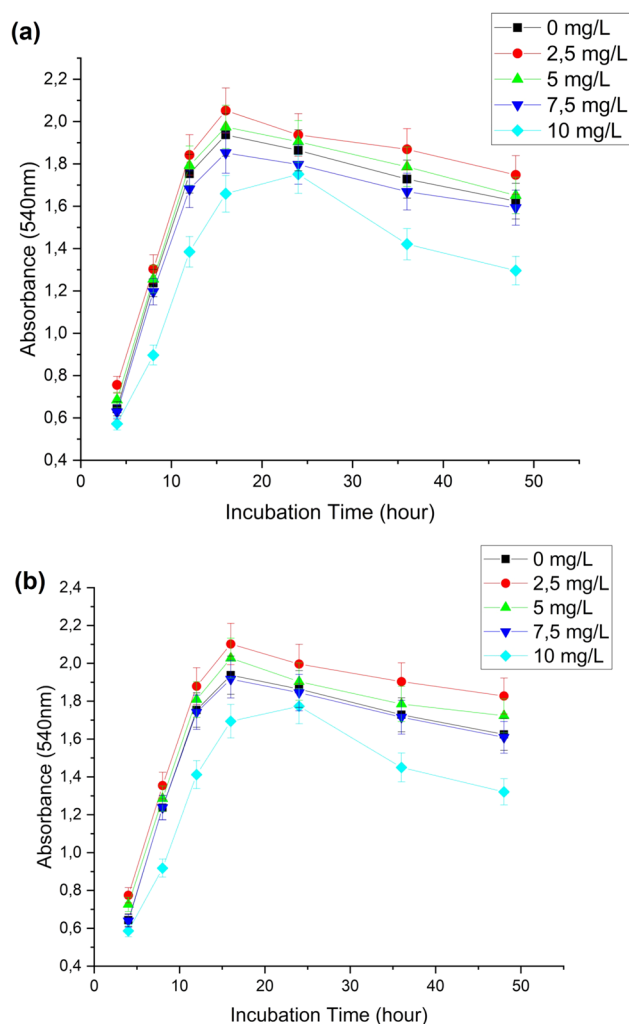


Table 2 MIC of two type ions in Liquid Medium

Concentration ($\mu\text{g}/\text{mL}$)	<i>A. flavithermus 17</i>		
	Cu(II)	Concentration ($\mu\text{g}/\text{mL}$)	Mn(II)
1	+	1	+
2.5	+	2.5	+
3	+	3	+
5	+	5	+
7.5	+	7.5	+
10	+	10	+
15	+	15	+
20	+	20	+
30	+	30	+
40	-	40	+
45	-	50	+
50	-	60	+
55	-	70	+
60	-	80	+
		90	+
		100	+
		110	+
		120	+
		130	+
		140	+
		150	+
		155	+
		160	+
		170	+
		180	+
		190	+
		200	+
		210	+
		220	+
		225	-
		230	-
		235	-

for Cu(II) ions and Fig. 1b for Mn(II) ions. The results demonstrated distinct growth patterns of thermophilic *A. flavithermus* in media containing varying concentrations of Cu(II) and Mn(II). Both Mn(II) and Cu(II) exerted detrimental effects on thermophilic *A. flavithermus*, with Cu(II) being more dangerous than Mn(II). Mn(II) and Cu(II) are essential minerals that living cells require from their environment and play crucial roles. However, excessive intake of these metals beyond the required amounts can have toxic effects.

In the trials using two types of ions, bacterial growth rose in comparison to control levels at 2.5 and 5.0 $\mu\text{g}/\text{mL}$ but decreased at 7.5 and 10.0 $\mu\text{g}/\text{mL}$ concentrations. The maximum bacterial growth, when compared to controls, was

**Fig. 1** Effect of heavy metals concentrations on *A. flavithermus* growth a Cu(II) and b Mn(II) ($n=3$)

observed at 2.5 $\mu\text{g}/\text{mL}$ ion concentrations at 16 h, and at 4 h with a 2.5 $\mu\text{g}/\text{mL}$ ion concentration for both ions.

In contrast to the control, *A. flavithermus* was favourably impacted by 2.5 $\mu\text{g}/\text{mL}$ manganese ion density in the following time intervals: 20.4% (4 h), 9.5% (8 h), 7.2% (12 h), 6.8% (16 h), 10.1% (24 h), 12.5% (36 h), and 7.1% (48 h).

The results demonstrate a reduction in cell viability at concentrations of 5.0 $\mu\text{g}/\text{mL}$ for both ions in comparison to control. For instance, at 7.5 $\mu\text{g}/\text{mL}$ copper ion density, the increase of the bacteria was demonstrated to be lower than the control by 2.3% (4 h), 3.3% (8 h), 4.1% (12 h), 4.4% (16 h), 3.6% (24 h), 3.4% (36 h), and 1.9% (48 h) (Fig. 1a). The maximum decreases occurred at a metal concentration of 10 $\mu\text{g}/\text{mL}$. In the presence of 10 $\mu\text{g}/\text{mL}$ copper ions, the decreases were 11.0% (4 h), 27.5% (8 h), 21.0% (12 h), 14.4% (16 h), 6.1% (24 h), 17.8% (36 h) and 20.2% (48 h) (Fig. 1a) compared to the control.

A. flavithermus was adversely impacted by 7.5 µg/mL Mn(II) ion concentration as shown in Fig. 1b, with negative effects of 0.6% (4 h), 0.6% (8 h), 0.7% (12 h), 1.1% (16 h), 1.0% (24 h), 0.8% (36 h), and 0.9% (48 h). Maximum reductions happened at 10 µg/mL of metal concentration. In comparison to the control, there were decreases of 8.9% (4 h), 25.8% (8 h), 19.5% (12 h), 12.6% (16 h), 4.9% (24 h), 16.1% (36 h), and 18.7% (48 h) in the presence of 10 µg/mL Mn(II) ions. Compared with the control, the maximum reductions occurred at 8 h at a concentration of 10 µg/mL in Cu(II) and Mn(II). The reduction was 27.5% and in presence of copper ions and 25.8% in the presence of Mn(II) ions.

The different possible mechanisms such as redox reactions, creation of complexes with different components, extra and intra-cellular sequestration for resistance to toxic metal ions, were indicated by some researchers (Malik 2004; Veglió et al. 1997; Liu et al. 2004). Also several heavy metals may be used as final electron acceptors throughout anaerobic respiration (Gadd 1992). The cell membrane was harmed by Mn(II) and Cu(II), which also disrupted nutrient transport. This has led to the growth of microorganisms. A prolonged lag phase period was monitored with increasing metal ions at higher tested levels by *A. flavithermus*. These results showed similarity with result of Anahid et al. (2011).

Removal and bioaccumulation of Mn(II) and Cu(II) ions by *A. flavithermus*

The clearance percentages of *A. flavithermus* were examined, and the impacts of various concentrations of Cu(II) and Mn(II) ions are depicted in Fig. 2a and b, respectively.

The clearance percentages were investigated using Cu(II) and Mn(II) ions at concentrations ranging from 2.5 to 10.0 µg/mL over a 0–48 h incubation period. The percent removal values for Cu(II) ions increased within the first 24 h at a concentration of 2.5 µg/mL and within the first 36 h at concentrations of 5.0 µg/mL, 7.5 µg/mL, and 10.0 µg/mL when compared to control. The highest removal values of Cu(II) were 100% at 24 h at 2.5 µg/mL and at 36 h at 5.0 µg/mL. However, at concentrations of 7.5 µg/mL and 10.0 µg/mL, the removal values were 98.4% and 95.2% at 36 h, respectively, compared to the control.

Up to 16 h and 24 h at a concentration of 10 µg/mL, the percent removal values with Mn(II) ions increased compared to the control, but they dropped in measurements made beyond this point. The percent removal values with Mn(II) ions at 16 h were found to be 100%, 100%, 98.1%, and 86.5% in the concentration range of 2.5, 5.0, 7.5, and 10.0 µg/mL respectively, compared to the control. The percent removal tended to decrease and then increase after 16 h at all concentrations. For example, the percent removal values at 24 h were calculated as 95.2% (2.5 µg/mL), 92.7% (5.0 µg/mL), 94.7% (7.5 µg/mL) and 93.4% (10.0 µg/mL)

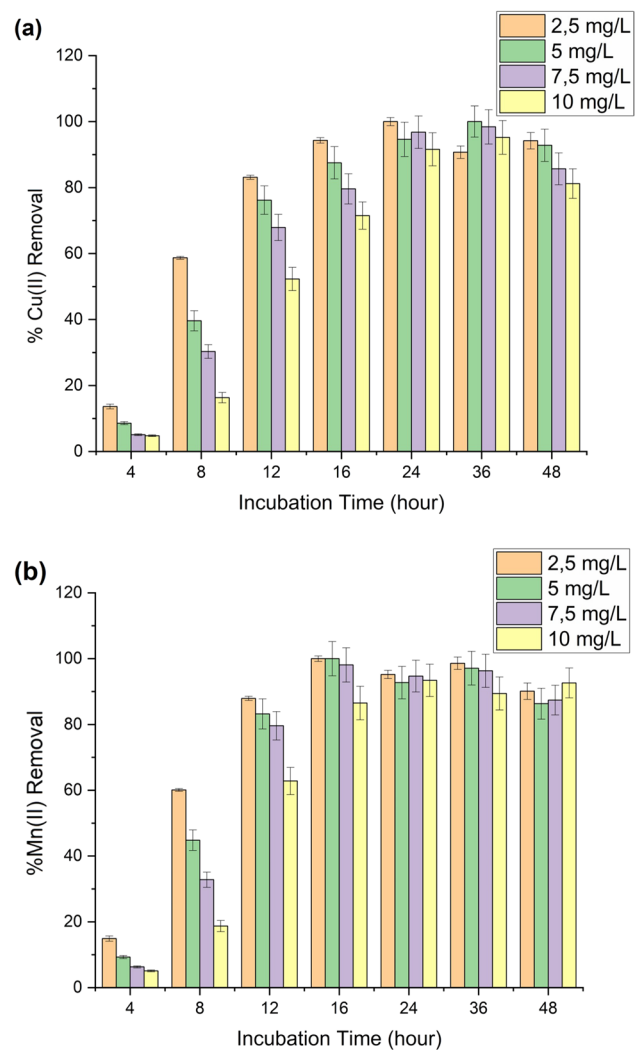


Fig. 2 Heavy metals removal by *A. flavithermus* **a** Cu(II) and **b** Mn(II) ions, ($n=3$)

compared to the control. Additionally, at a concentration of 2.5 µg/mL of Cu(II). The removal performance dropped from 100 to 90% after 36 h before rising again from 90 to 98% at 48 h. In 2018, 7 different types of bacteria have been isolated from the mining sites by Gbemisola et al. (2018). These strains have been used in the bioaccumulation and removal of 10 different heavy metals, including copper and manganese. Among these bacterial strains, *L. macroides* did not grow in the presence of copper and other bacteria showed more sensitive to copper ions than manganese ions. Similar results were obtained with *A. flavithermus* in this study. Gbemisola et al. (2018) also found that, *B. cereus* and *A. spanius* absorbed 0.81 and 0.79 wt% in Cu-enriched media. In Mn media, *A. spanius*, *P. mosselii* and *B. cereus* retained 4.76, 2.19 and 1.91 wt% Mn, respectively while *B. kochii*, *K. pneumoniae* and *P. nitroreducens* removed 1.54,

1.40 and 0.90 wt% respectively. Our results showed higher removal percentages than their findings.

The bioaccumulation capacity of *A. flavithermus* is affected by different concentrations of both cations, as illustrated in Fig. 3a and b. As can be observed in Fig. 3a and b, there was no appreciable increase in the biological accumulation of Cu(II) and Mn(II) ions during the initial four hours.

However, between the values of 2.5 and 7.5 µg/mL, there was an increase in the bioaccumulation of manganese and copper cations at approximately 12 h. From the fourth to the eighth hour, the bioaccumulation of Cu(II) increased from 6.4 µg/mL metal/dry body weight (MDBW) of *A. flavithermus* to 23.8 MDBW at a concentration of 2.5 µg/mL Cu(II) ions, while the bioaccumulation of Mn(II) increased from the seventh to the eighth hour at a concentration of 2.5 µg/mL Mn(II) ions. The highest values for Cu(II) bioaccumulation were found to be 23.8 MDBW at 2.5 µg/mL for 48 h, 47.6 MDBW at 5 µg/mL for 36 h, 74.6 MDBW at 7.5 µg/mL for 36 h, and 102.4 MDBW at 10 µg/mL for 36 h.

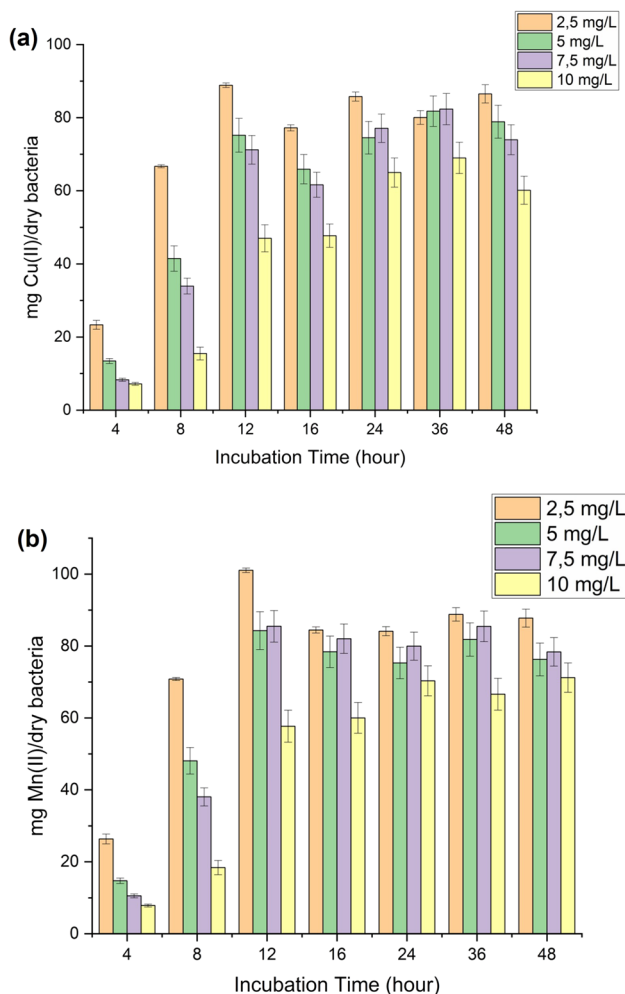


Fig. 3 Heavy metals bioaccumulation by *A. flavithermus* a Cu(II) and b Mn(II) ions ($n=3$)

Similarly, the maximum Mn(II) bioaccumulation capacities were determined to be 24.4 MDBW at 2.5 µg/mL for 36 h, 49.1 MDBW at 5 µg/mL for 12 h, 77.4 MDBW at 7.5 µg/mL for 12 h, and 105.7 MDBW at 10 µg/mL for 48 h. The bioaccumulation of Cu(II) decreased after 16 h to doses of 2.5 and 5 µg/mL, then it increased again at 24 h. The copper ion bioaccumulations also decreased from 64.5 MDBW to 55.8 MDBW and from 102.4 MDBW to 89.2 MDBW at 7.5 and 10 µg/mL, respectively, from 16 to 48 h. Similarly, after 16 h, the bioaccumulation of Mn(II) increased again at doses of 2.5, 5.0, and 7.5 µg/mL. Additionally, from 24 to 36 h at a concentration of 10 µg/mL, the biomagnification of manganese cations fell from 104.3 MDBW to 98.9 MDBW, and then increased again to 105.7 MDBW after 48 h. Several researches (Volesky et al. 1993) and (Macaskie and Dean 1984), have drawn attention to the fact that the amount of metals that the bacterial cells bioabsorbed varied depending on their growth phases. It is clear that the different amounts of bioaccumulation of copper and manganese ions are consistent with the literature data (Özdemir et al. 2013). The differences in the production of metal-binding proteins and the difference in the variations in the cell wall structure can also contribute to the disparities in the amount of metal accumulated by different cells (Flouty and Estephane 2012; Huang et al. 2018; Máthé et al. 2012).

FTIR and SEM characterization of *A. flavithermus* morphology

SEM(scanning electron microscope) and EDX(Energy dispersive X-ray) images of *A. flavithermus* with Cu(II) and Mn(II) were presented in Fig. 4

Figure 4 illustrates the excellent inhibition observed when exposed to Cu(II) and Mn(II) ions. The response upon metal cell entry varies depending on the differences in cell morphology, as observed in the SEM results depicting the effects of metals on cell morphology (Fig. 4a, b, c). No changes in size or overall surface properties were observed in cells exposed to metals. The presence of Cu and Mn metals was confirmed by EDX analysis (Fig. 4d, e). Figure 5 presents the FTIR spectra of *A. flavithermus*, both in the absence and presence of metals.

The hydroxyl groups in the structure of *A. flavithermus* bacteria can be identified by sharp peaks around 2902–2971 cm^{-1} and 3268–3676 cm^{-1} in the FTIR spectrum. The presence of sulfone and sulfoxide groups in the bacterial biosorbent, as well as carboxyl groups, is indicated by peaks at approximately 1736–1623 cm^{-1} , 1231 cm^{-1} , and 1056 cm^{-1} respectively. After Cu(II) bioaccumulation, hydroxyl group peaks in the range of 2902–3676 cm^{-1} were observed in the FTIR spectra, along with carbonyl, sulfone, and sulfoxide group peaks at 1633 cm^{-1} , 1229 cm^{-1} , and 1066–1057 cm^{-1} respectively. Similarly,



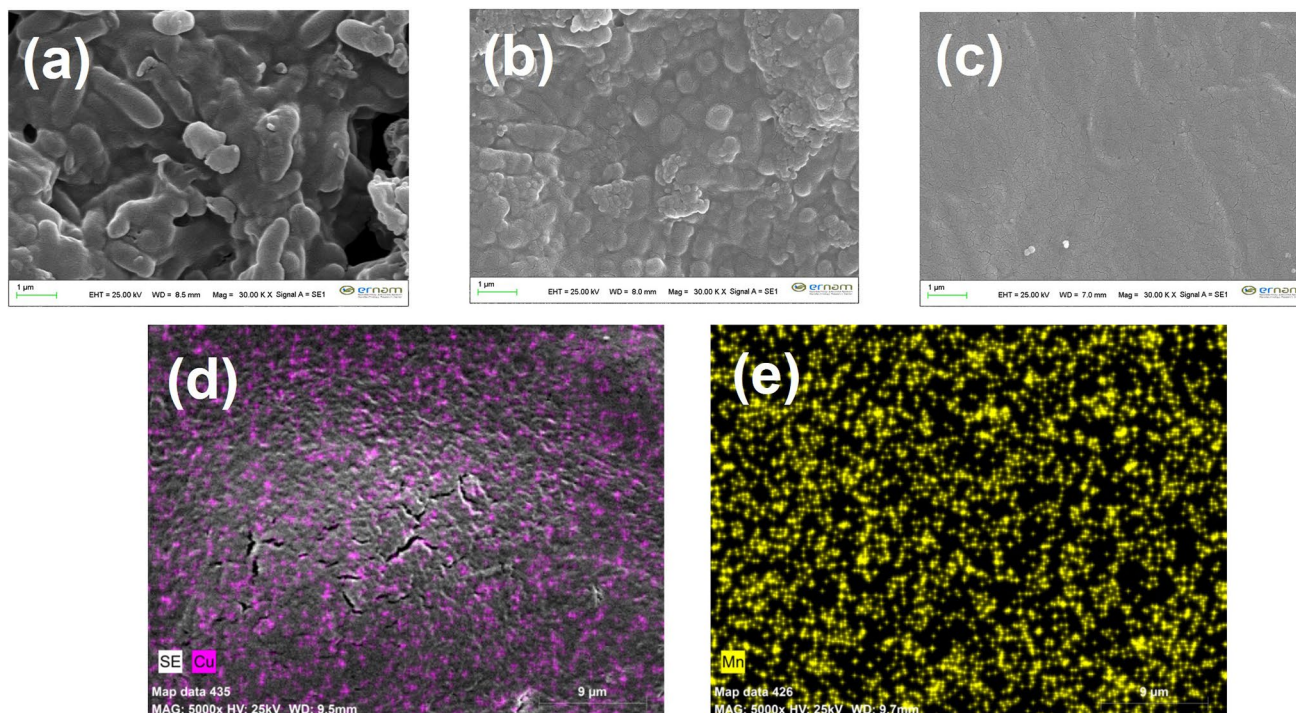


Fig. 4 SEM images of **a** *A. flavithermus*, **b** *A. flavithermus* + Cu(II), **c** *A. flavithermus* + Mn(II), and EDX images of **d** *A. flavithermus* + Cu(II) and **e** *A. flavithermus* + Mn(II) ($n = 3$)

following Mn(II) bioaccumulation, the peaks were identified as $2902\text{--}3676\text{ cm}^{-1}$ (OH), 1735 cm^{-1} , 1250 cm^{-1} , and 1057 cm^{-1} (–COOH, sulfone, sulfoxide).

The FTIR spectra show changes in peaks after the bioaccumulation of copper and manganese cations, indicating complexity. The interaction between these cations and functional groups *A. flavithermus* bacteria is evident in the FTIR spectra. Copper and manganese cations are classified as soft and borderline acids according to the hard and soft acid/base theory. This suggests that the surface of *A. flavithermus* contains various functional groups, such as primers, secondary amines, carbonates, nitrates, phosphates, sulfates (as hard bases), and sulfides, nitrites, and aromatic amine groups. The FTIR results, along with the theory, contribute to our understanding of the metal–organic coordination as a bio-mechanism. In conclusion, the bioaccumulation of copper and manganese ions by *A. flavithermus* has been confirmed based on the FTIR spectral results.

Determination of the effect of Cu(II) and Mn(II) on α -amylase generation and bioaccumulation capacity

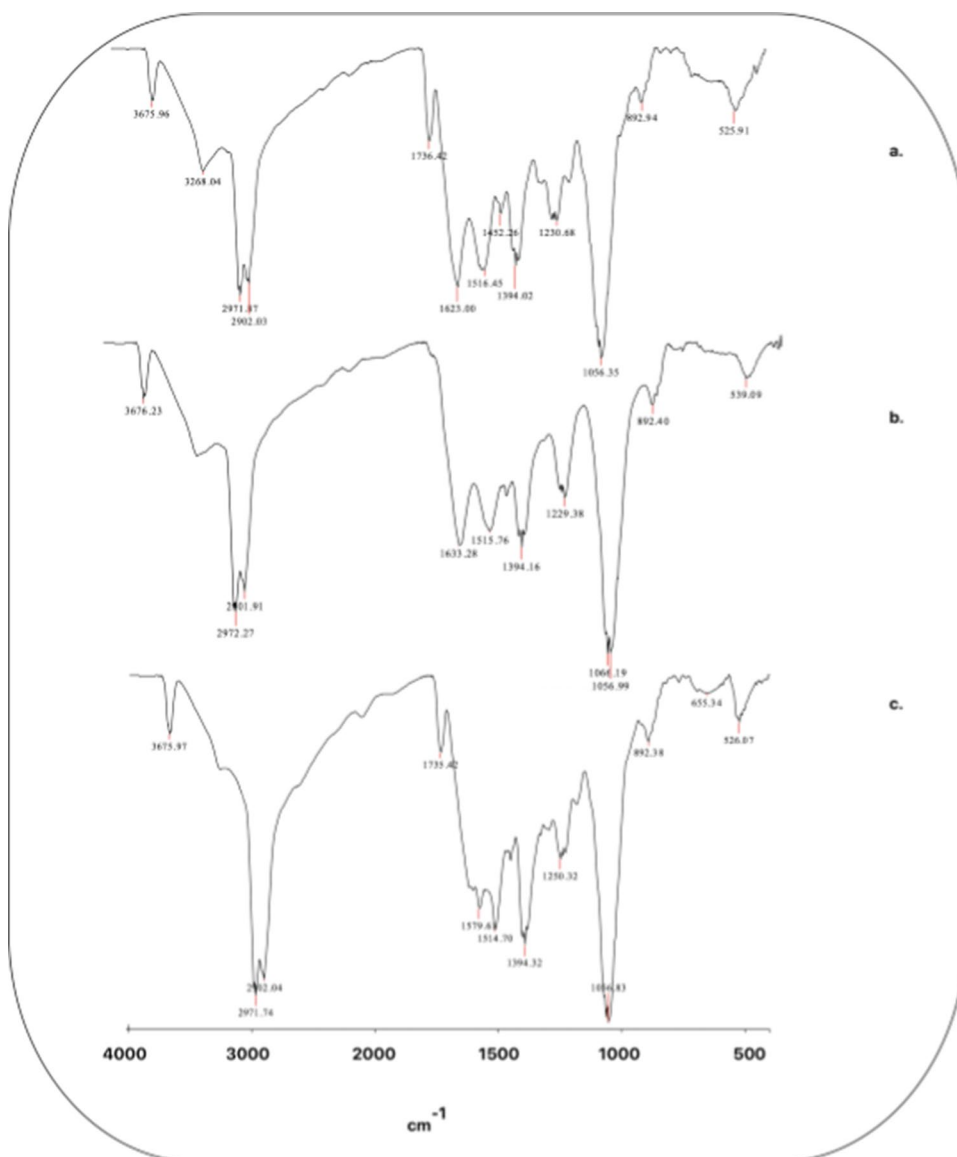
α -Amylase is a highly important substance in the industry, finding applications in various sectors such as bread production, glucose and fructose syrup production, beer brewing, starch processing, textile manufacturing, paper production,

detergent industry, as well as medical and clinical chemistry analysis with biotechnology applications (Bernfeld 1955). The production of this vital enzyme for industrial purposes is one of the main objectives of this study, which also aims to expedite the bioaccumulation process. Additionally, investigations were conducted to examine the impact of varying metal ion concentrations on enzyme synthesis. The findings of these investigations are presented in Fig. 6a and b.

In the initial 8 h of Cu(II) ion experimentation, no significant enzyme synthesis was observed. However, starting at 12 h, α -amylase production increased compared to the control, ranging from 2.5 to 5.0 $\mu\text{g}/\text{mL}$. The highest increase was seen at 5.0 $\mu\text{g}/\text{mL}$ after 24 h (3657.2 U/mg), representing a 12.9% increase compared to the control. For Mn(II) ions, enzyme synthesis was not observed in the first 8 h, but it increased after 16 h at concentrations between 2.5 and 5.0 $\mu\text{g}/\text{mL}$. The maximum increase was observed at 5.0 $\mu\text{g}/\text{mL}$ after 24 h (3698.1 U/mg), showing a 14.2% increase compared to the control at the same time and concentration. However, both ions resulted in lower enzyme production at doses of 7.5 and 10 $\mu\text{g}/\text{mL}$. Cu(II) ions at 10.0 $\mu\text{g}/\text{mL}$ reduced enzyme production by 4.06% at 4 h, 9.1% at 8 h, 6.6% at 12 h, 7.6% at 16 h, 4.7% at 24 h, 6.6% at 36 h, and 2.6% at 48 h, compared to the control. Similarly, at 10 $\mu\text{g}/\text{mL}$, Mn(II) ions caused a reduction in enzyme synthesis by 3.1% in the first 4 h, 6.7% in the next 8 h, 7.3% in the next 12 h, 6.6% in the next 16 h, 4.6% in the next 24 h, 6.4% in



Fig. 5 Comparison of FTIR spectra of **a** *A. flavithermus*, **b** *A. flavithermus* + Cu(II) and **c** *A. flavithermus* + Mn(II)



the next 36 h, and 1.5% in the last 48 h. Despite these reductions, significant decreases in enzyme production were not observed.

Previous studies investigating the metal bioaccumulation capacities of bacterial cell membranes at different metal concentrations have shown that the amount of metal accumulated increases with higher metal concentrations (Özdemir et al. 2012). Consistent with these findings, thermophilic *A. flavithermus* bacteria were incubated in a culture medium containing Cu(II) and Mn(II) at concentrations ranging from 2.5 to 10 µg/mL. The Cu(II) membrane bioaccumulation capacities in this concentration range were calculated as 53.4, 130.12, 189.2, and 251.1 mg Cu(II)/g wet cell membranes, respectively. Similarly, the Mn(II) ion membrane bioaccumulation capacities in the

same concentration range were determined to be 73.1, 145.7, 203.1, and 308.7 mg Mn(II)/g wet cell membranes, respectively. These results indicate that the binding of metals to the cell membrane of thermophilic bacteria is highly effective and rapid, while its effectiveness and speed in the cell cytoplasm are lower. Other studies, such as El-Helou et al. (2000) have also highlighted the potential of bacterial cell membrane binding as an effective tool for the biological clean-up of metal-contaminated environmental and industrial areas. The findings of this study regarding the removal of manganese and copper ions through this mechanism align with similar publications in the literature (Özdemir et al. 2013; Özdemir et al. 2012; Dönmez and Aksu 2001; Augusto Da Costa and Duta 2001).



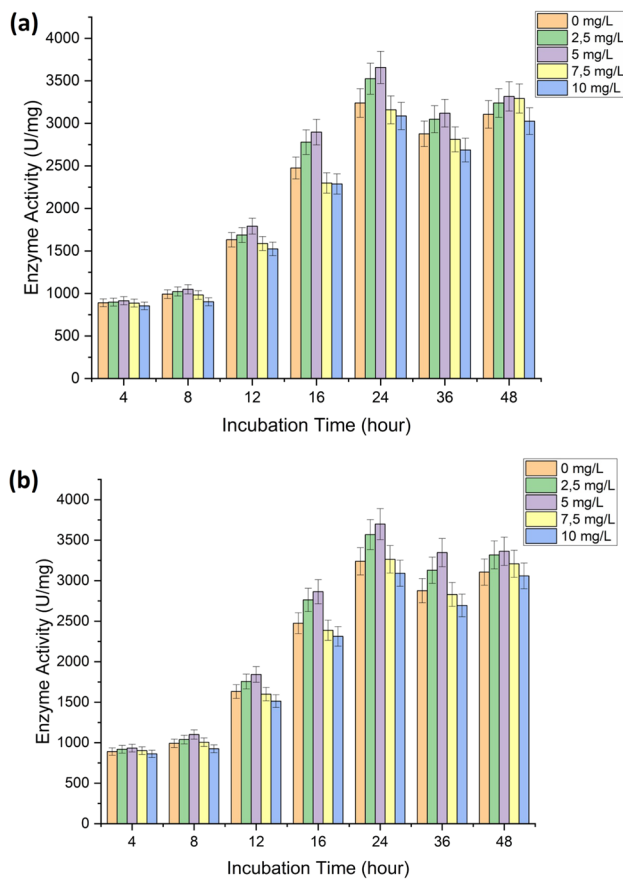


Fig. 6 Influence of heavy metals amounts on α -amylase production **a** Cu(II) and **b** Mn(II) ($n=3$)

SOD and CAT enzyme activities of *A. flavithermus*

While many metals are essential components of enzymes that play important functions in our body, metals such as copper and manganese ions also serve vital biological roles (Uauy et al. 1998; Fraga 2005). Cu(II) is crucial for various enzymes such as peroxidase, cytochrome oxidase, catalase, monoamine oxidase, lactase, ascorbic acid oxidase, superoxide dismutase (SOD), and tyrosinase. Additionally, copper is involved in numerous metabolic reactions due to the presence of a wide range of enzymes. For instance, SOD containing copper facilitates the conversion of superoxide to hydrogen peroxide and oxygen (Angelova et al. 2011; Uauy et al. 1998). Mn is an essential component of enzyme systems, including those involved in oxygen processing. It is a vital part of the antioxidant SOD, which helps combat free radicals (Treiber et al. 2012; Law et al. 1998). The key antioxidant enzymes include SOD and CAT (Lin et al. 2009). The activity of antioxidant enzymes can vary depending on the presence of pollutants from different sources. Therefore, organisms can be used as indicators to assess environmental contamination levels. Changes in antioxidant enzyme

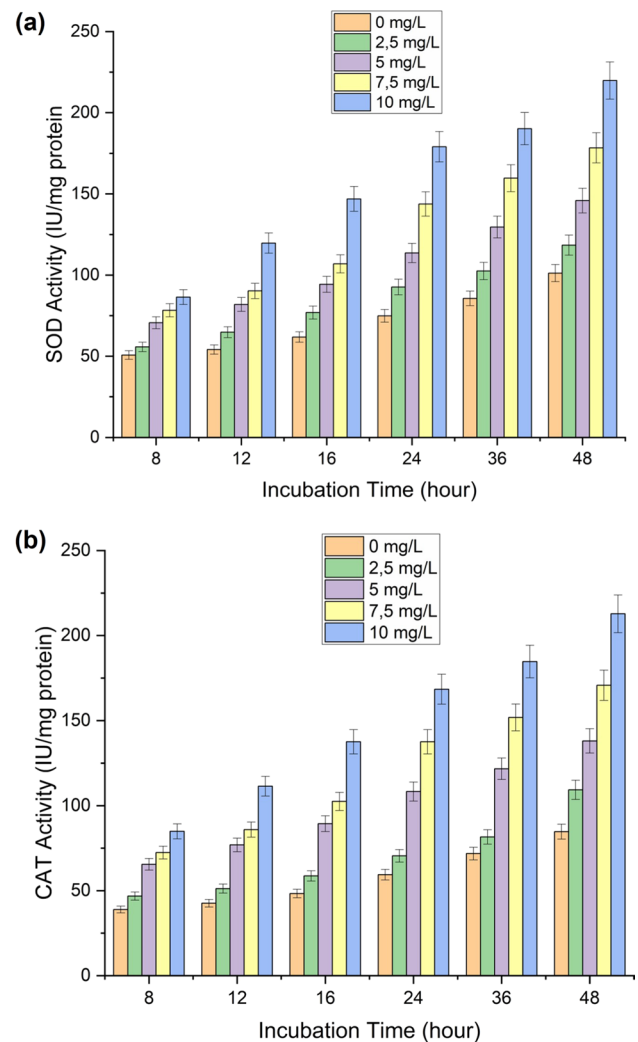


Fig. 7 Effect of various amounts of copper Cu(II) ions (the range of 0.0–10.0 $\mu\text{g}/\text{mL}$) on **a** SOD activity and **b** CAT activity of *A. flavithermus* ($n=3$)

activities (SOD and CAT) were analysed after exposing *A. flavithermus* to manganese and copper ions over a range of 8–48 h, and the results are presented in Fig. 7a, b and Fig. 8a, b.

The superoxide dismutase (SOD) and catalase (CAT) levels in *A. flavithermus* were measured at different Cu(II) ion concentrations (ranging from 2.5 to 10.0 $\mu\text{g}/\text{mL}$). The enzyme activity showed a linear increase with increasing copper ion concentration over the 48-h period ($p < 0.05$). The highest levels of SOD (197.30 ± 0.43 IU/mg protein) and CAT (219.80 ± 0.91 IU/mg protein) were observed at 48 h ($p < 0.05$). Similarly, for Mn(II) ions at various doses (from 2.5 to 10.0 $\mu\text{g}/\text{mL}$) over a 48 h period, the maximum levels of SOD (181.91 ± 1.6 IU/mg protein) and CAT (209.61 ± 1.03 IU/mg protein) were observed at 48 h and 10.0 $\mu\text{g}/\text{mL}$ ($p < 0.05$).

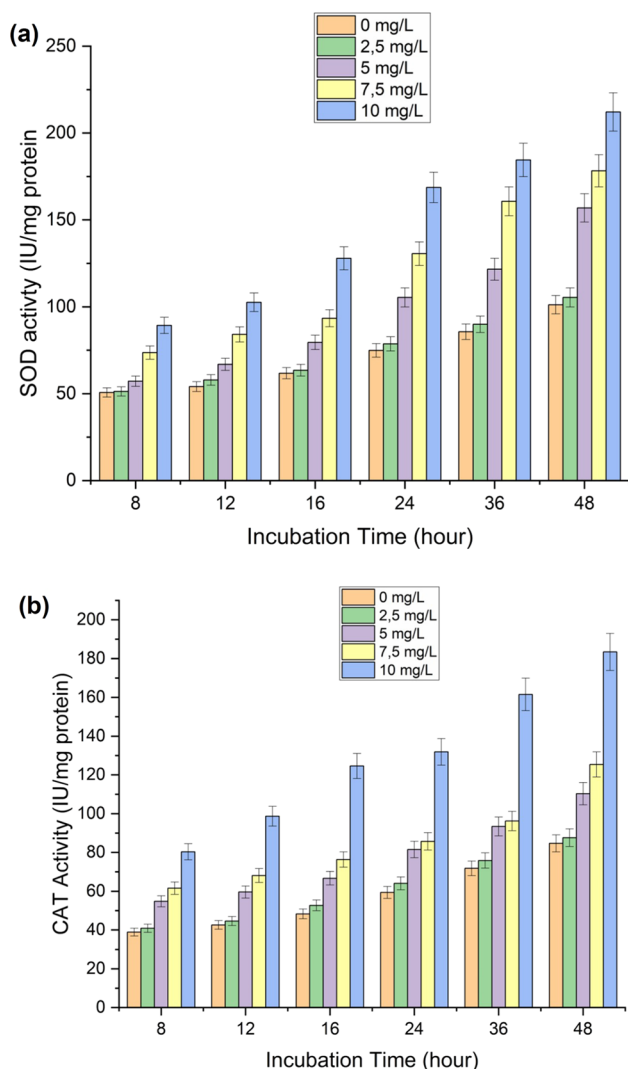


Fig. 8 Effect of various amounts of manganese Mn(II) ions (the range of 0.0–10.0 $\mu\text{g/mL}$) on **a** SOD activity and **b** CAT activity of *A. flavithermus* ($n=3$)

The SOD activities between (8–48) hours after treatment with 10 $\mu\text{g/mL}$ of Cu(II) ions were 86.4 ± 1.13 (8 h), 119.7 ± 1.41 (12 h), 146.9 ± 1.49 (16 h), 179.1 ± 1.03 (24 h), 190.2 ± 0.91 (36 h), and 219.8 ± 1.14 (48 h) IU/mg protein, respectively. The corresponding CAT activities at the same time periods and concentrations of Cu(II) ions were 84.9 ± 0.85 (8 h), 111.4 ± 1.24 (12 h), 137.6 ± 2.22 (16 h), 168.5 ± 1.68 (24 h), 184.7 ± 0.45 (36 h), and 212.8 ± 2.05 (48 h) IU/mg protein. In these time periods, treatment with 10 $\mu\text{g/mL}$ of Cu(II) ions resulted in a positive increase of 70.4% (8 h), 121.3% (12 h), 137.7% (16 h), 139.1% (24 h), 122.2% (36 h), and 117.2% (48 h) in SOD activities compared to the control. Similarly, there were positive increases of 118.3% (8 h), 161.5% (12 h), 185.0% (16 h), 183.7% (24 h), 157.2% (36 h), and 151.2% (48 h)

in CAT enzyme activities after treatment with 10 $\mu\text{g/mL}$ of copper(II) in the same time periods.

When different concentrations of Mn(II) ions (2.5, 5.0, 7.5, 10 $\mu\text{g/mL}$) were assessed for their impact on enzyme activities in *A. flavithermus*, antioxidant enzyme activities increased compared to the control as Mn(II) concentrations increased ($p < 0.05$). For example, the SOD activities between (8–48) hours after treatment with 10 $\mu\text{g/mL}$ of Mn(II) ions were 89.3 ± 1.06 (8 h), 102.6 ± 0.79 (12 h), 127.9 ± 0.81 (16 h), 168.7 ± 1.22 (24 h), 184.5 ± 1.92 (36 h), and 212.1 ± 0.99 IU/mg protein, respectively. The CAT activities at the same time period measured after the same concentration of Mn(II) treatments were found as 80.3 ± 0.28 (8 h), 98.7 ± 0.51 (12 h), 124.6 ± 0.83 (16 h), 131.9 ± 1.08 (24 h), 161.5 ± 0.95 (36 h) and 183.4 ± 1.07 (48 h) IU.mg⁻¹ protein, respectively. In these time periods (8–48 h), 10 $\mu\text{g/mL}$ of Mn(II) treatments was resulted in %76.1 (8 h), %89.7 (12 h), %107.0 (16 h), %125.2 (24 h), %116.0 (36 h) and %109.6 (48 h) positively increase in SOD behaviours compared to the check, respectively. Similarly, following treatment with 10 $\mu\text{g/mL}$ of Cu(II) in the corresponding time periods, there were positive increases in the CAT enzyme activities of 106.4 (8 h), 131.7 (12 h), 158.0 (16 h), 122.1 (24 h), 125.0 (36 h), and 116.5 (48 h). Studies on antioxidant enzymes on different microbes are often conducted to lessen or remove the impacts of metals and herbicides. This study indicates that high levels of CAT and SOD enzyme activity against copper and manganese exposure can be stated as an essential liability of *A. flavithermus* against oxidative stress.

Conclusion

The present study provides an initial assessment of the thermophilic bacterium *A. flavithermus* for bioaccumulation, remediation, and tolerance of Mn(II) and Cu(II) ions. Bacterial growth was successful at a concentration of 2.5 $\mu\text{g/mL}$ for both copper and manganese ions, reaching its peak at the 16th hour. However, a decrease in bacterial growth was observed at concentrations above 5.0 $\mu\text{g/mL}$ for both ions. The remediation potential of copper and manganese ions by *A. flavithermus* was also investigated, and it was found that *A. flavithermus* accumulated less Cu(II) ions compared to Mn(II) ions. This study demonstrates that thermophilic *A. flavithermus* has the potential to remove and recover toxic metals from industrial wastewater. Additionally, the bioremediation process by thermophilic *A. flavithermus* can also lead to the production of α -amylase, making it valuable in biotechnology. The activities of antioxidant enzymes (CAT and SOD) increased with increasing concentrations up to 10.0 $\mu\text{g/mL}$ over a 48 hour period. The changes observed in CAT and SOD enzyme activities in *A. flavithermus* can be considered as a protective response against oxidative stress

caused by Cu(II) and Mn(II) ions. Based on the enzyme activity results of *A. flavithermus*, it can be potentially used as a natural indicator for detecting metal pollution in natural waters.

Data availability All data generated or analysed during this study are included in this published article.

Declarations

Conflict of interest The authors declare that there have no conflicts of interest.

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