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Research article

Sonochemical-assisted method for efficient synthesis of Cu-MOF and evaluating its antibacterial properties

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ABSTRACT

Sonochemical-assisted method was used to synthesize copper metal-organic frameworks (Cu-MOF) nanostructures. The final products were examined by related techniques such as XRD patterns, SEM image, BET N_2 adsorption/desorption technique and FTIR spectrum. Microtiter plates microbiological assay were used to investigate antibacterial properties and the results were analyzed using ANOVA and Tukey HSD tests. The results showed that Cu-MOF nanostructures have a mesoporous nature with an average particle size distribution around 60 nm. The final product had the property of preventing the growth of all tested bacteria in certain concentrations. Minimum Inhibitory Concentration (MIC) values were observed in the range of 30–100 ppm. It was also discovered that this nanostructure can not kill bacteria completely. In addition, the minimal inhibitory concentration for biofilm growth (MIC-B) of the nanostructure was investigated. The MIC-B analyzes demonstrated that the growth of bacterial biofilm decreased with increasing Cu-MOF concentration.

1. Introduction

In the last few decades, bacteria have lost their sensitivity to antibiotics in a wide range and even the structural modification of these drugs will not be a solution to the antibiotic resistance problem. Therefore, in order to solve the problem, there is an urgent need for an efficient class of antibacterial agents [1–3]. The formation of biofilm and the release of substances is an effective factor in the resistance of bacteria to antibacterial substances [4,5]. According to previous study, this procedure can reduce the penetration of antibiotic substances into the bacterial colony [6].

In recent decades, different nanostructures have been introduced for their outstanding antimicrobial structures. Compared to conventional porous materials such as zeolites and activated carbon, metal-organic framework (MOFs) have unique advantages such as high specific surface area, tunable pores, and tunable porous surfaces, and are formed through coordination bonds between organic ligands and metal ions [7,8]. The MOF compounds are produced through various processes including sonochemical, hydrothermal,

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Table 1
Bacterial species and related sources.

Bacterial species	Strain ID	Source
Pseudomonas aeruginosa (PAO1)	ATCC 15692	Pasteur Institute of Iran
Pseudomonas fluorescens	ATCC 13525	IBRC
Staphylococcus aureus	ATCC 6538	PTCC
Escherichia coli	ATCC 10536	PTCC
Bacillus cereus	ATCC 9634	IBRC
Bacillus subtilis	ATCC 6633	IBRC
Salmonella typhimurium	ATCC 14028	IBRC

ATCC = American Type Culture Collection.
IBRC = Iranian Biological Resource Center.
PTCC = Persian Type Culture Collection.

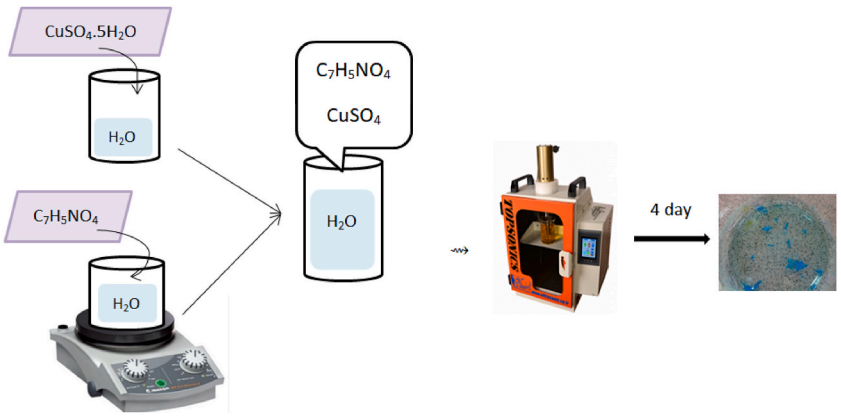

Fig. 1. sonochemical-assisted synthesis of Cu-MOF.

and reflux methods. The sonochemical-assisted methods are used more widely due to the production of high-yield products, as well as being less reactive in the environment [9,10].

Copper is one of the most attractive elements for use in the preparation of MOFs due to its abundant resources, availability, non-toxic properties, and most importantly, high complex strength [11]. Abdulmawati et al. synthesized copper metal-organic frameworks (Cu-MOF) with terephthalic acid (H_2BDC) and copper (II) nitrate ($Cu(NO_3)_2$). The results showed that Cu-MOF has significant anti-microbial activity against gram-negative bacterium *Escherichia coli* and *Salmonella enterica* [12]. Moreover, Cu-MOF with layered and bulk morphology showed excellent antibacterial properties against *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* bacteria [13].

In this study, Cu-MOF was produced by sonochemical-assisted method and characterized by related analysis. The antibacterial effects of Cu-MOF were evaluated in three scenarios, including Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and minimal inhibitory concentration for biofilm growth (MIC-B).

2. Materials and methods

2.1. Material characterization

$CuSO_4.5H_2O$ (Merck, 98 %) and 2, 6 pyridines dicarboxylic acid (Merck, 99 %) were used to synthesize Cu-MOF. Tryptic Soy Broth (TSB) and nutrient agar (Merk,99 %) media, along with a microtiter plate (corning flat-bottomed polystyrene; Nunc, Denmark) and an ELISA instruments Epoch model (Biotek, USA) were used for antimicrobial tests. Seven bacterial strains were obtained as mentioned in Table 1.

2.2. Sonochemical-assisted method

Aqueous solutions in a 1:1 ratio of $CuSO_4.5H_2O$ and 2,6-dicarboxylic acid pyridine were prepared. Then, the two solutions were mixed and sonicated for 21 min using an ultrasonic probe (with a temperature of 40 $^{\circ}C$ and a power of 175W). After keeping the solution at room temperature for 4 days and evaporating the solvent, blue MOF crystals related to the formation of Cu-MOF are formed. The process schematic for the synthesis of Cu-MOF is shown in Fig. 1.

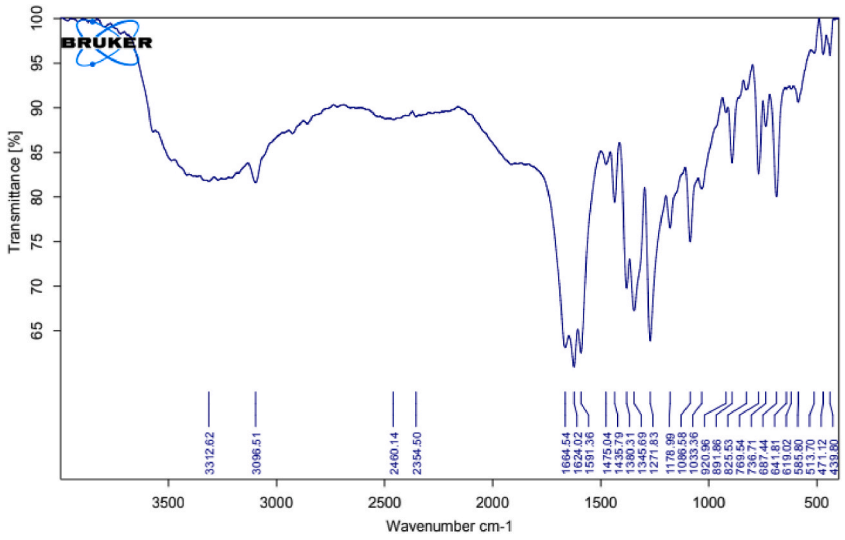

Fig. 2. FTIR spectrum of the Cu-MOF synthesized metal-organic nanostructure.

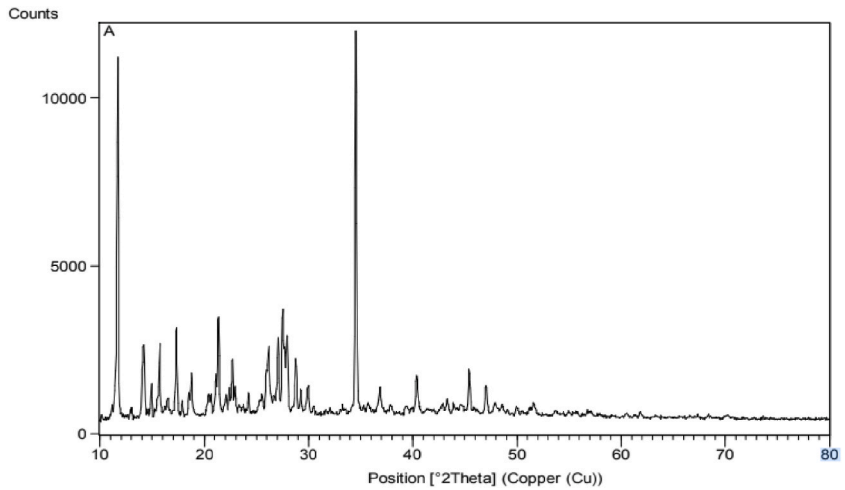

Fig. 3. XRD pattern of Cu-MOF prepared by sonochemical method.

2.3. Minimum Inhibitory Concentration (MIC) of Cu- MOF on selected bacteria

The MIC tests were performed as described in the previous study [14]. In summary, the wells of the microtiter plate were filled with the 0.5 McFarland bacterial inoculation from the logarithmic growth phase of each strain (Table 1), the designed concentrations of Cu-MOF nanostructure, and related volume of TSB culture medium (ultimate volume of 300 μl). The Cu-MOF concentrations in the 96-well design were adjusted to 200, 100, 60, 40, 30, 20 and 10 mg/l (3 replicates). The non-turbid wells after 24 h' incubation at 37 °C was interpreted as non-bacterial growth.

2.4. Minimum Bactericidal Concentration (MBC) of Cu- MOF on selected bacteria

Since the non-turbid wells imply that the Cu-MOF may only prevent the growth of bacteria, its bactericidal properties were also investigated. For this purpose, 5 μl of the wells content which were considered as no growth in the MIC test, were transferred to the nutrient agar plates and incubated for 24–48 h at 37 °C. The wells leading to no growth of bacterial colonies on the plates were considered as MBC.

2.5. Minimum Inhibitory Concentration for biofilm formation (MIC-B) of Cu- MOF on selected bacteria

The MIC-B tests were performed as described in the previous study [15]. In summary, the microbial suspension in the TSB (10^5

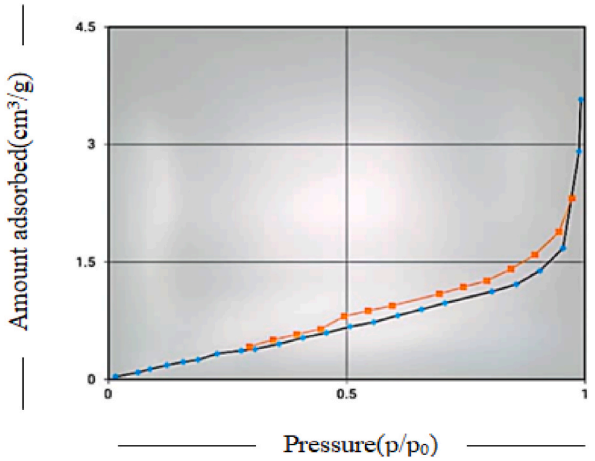

Fig. 4. Nitrogen adsorption/ desorption isothrmof Cu-MOF nanostructure synthesized by sonochemical method.

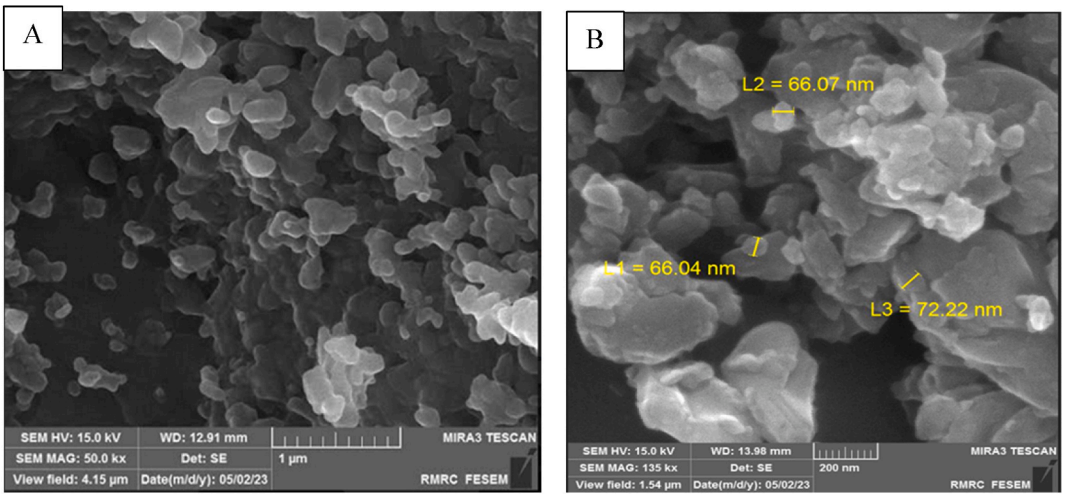

Fig. 5. SEM images of Cu-MOF synthesized by sonochemical method in different magnification (A:50kx nm and B: 135kx.(

cfu/mL) was prepared in the corning flat-bottomed polystyrene 96-well microplates (according to concentrations of part 2.3) and incubated on a shaker incubator for 48 h to form biofilm on the walls of the wells. Crystal violet solution was used to stain the attached bacteria and 200 µL of 33 % glacial acetic acid was used to dissolve the penetrating dye in the body of the biofilm. In the last stage, the dye concentration was measured by Epoch Elisa reader (Biotek Instruments; USA) after 15 min incubation at OD570. The results were analyzed using ANOVA and Tukey HSD tests (p-values less than 0.05 were considered significant values).

3. Results and discussion

3.1. Physicochemical properties of Cu-MOF

The Fourier transform infrared (FT-IR) spectrum of the Cu-MOF nanostructures is depicted in Fig. 2. The peak appearing in the range of 3312 cm^{-1} indicates the presence of coordinated water in the structure. The frequencies in the region of 3096 cm^{-1} examine the vibrations related to the aromatic C–H bond. In addition, the peaks observed at 2460 and 2354 cm^{-1} indicate the presence of COO groups, which are related to the ionized linker. Also, the absorption bands in the range of 447–619 cm^{-1} are dedicated to the formation of the Cu–O bond [16] and the vibrations at 641 cm^{-1} indicate the formation of the Cu–N bond between metals and nitrogen in the ligand [17].

Fig. 3 shows the X-ray diffraction (XRD) pattern of Cu-MOF nanostructures. Based on these patterns, samples were indexed in the crystallin lattice with high purity. According to the Debye Scherer equation ($D = 0.9\,\lambda/\beta\cos\theta$), the width of X-ray peaks (variable β) has an inverse relationship with the size of the crystals (variable D). In this sample, the size of the crystals was calculated to be 48 nm. The narrow crystalline size can be related to the effective effects of sonochemical methods on the final products of Cu-MOF.

Table 2
MIC values of Cu-MOF against various bacteria.

Bacteria	MIC values (mg/l)
E. coli	100
B. subtilis	30
P. aeruginosa	40
P. fluorescens	60
S. aureus	100
S. typhimurium	100
B. cereus	100

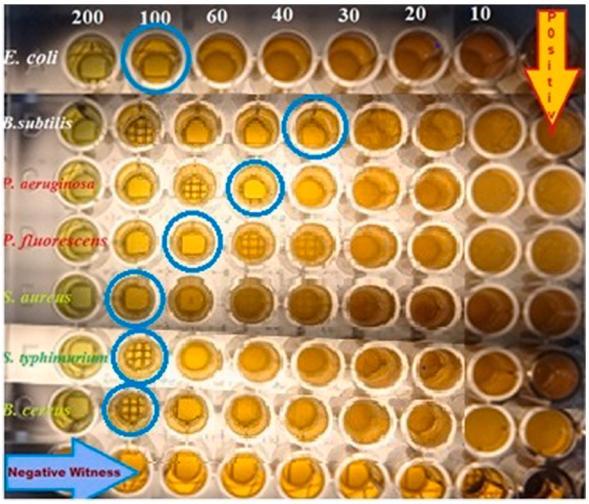

Fig. 6. MIC test for different concentrations of Cu-MOF (mg/L) against 7 bacterial strains. The MIC concentration of each bacterium is shown as bold blue circles (The microtiter plate is placed under the colony counter for imaging). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Nitrogen adsorption/desorption isotherm for Cu-MOF nanostructures is shown in Fig. 4. According to this isotherm, the synthesized samples have the same behavior as the fourth type of isotherms, which shows mesoporous distribution with an average pore size of 10 nm [18]. The mesoporous size distribution for final nanostructures causes surface interaction of molecules along the pore wall, which significantly affects on antibacterial capability of the Cu-MOF nanostructures.

Fig. 5A shows SEM image of the Cu-MOF nanostructure synthesized by sonochemical-assisted method in magnification of 50kx. Also, SEM image of Cu-MOF nanostructure in magnification of 135kx was exchibited in Fig. 5B. According to these images, the sonochemical-assisted process affects the synthesis of copper-based MOF with almost spherical morphology and an average particle size of 65 nm. These efficient physhicho-chemical features confirm the stable surface of final Cu-MOF and provide a desirable substrate for antibacterial properties.

3.2. MIC results for the Cu-MOF nanostructures

The results showed that from 49 wells containing different bacteria at various concentrations of Cu-MOF, bacterial growth was seen in 29 Wells (59 %) and 20 Wells (41 %) had no bacterial growth. Based on the MIC values stated in Table 2, this nanostructure has stronger antibacterial properties against *Bacillus subtilis* and *Pseudomonas aeruginosa* than other bacteria. Fig. 6 shows the actual location of the MIC test using a 96-well microtiter plate. These results were somewhat consistent with the findings of Rauf et al., who measured the MIC of micro and nano Cu-MOF against *E. coli* and *B. subtilis* bacteria [19]. The MIC of nano-MOF for *E.coli* in the two studies was close (100 mg/l in this study compared to 150 mg/l in Rauf et al. study), but the MIC for *B. subtilis* in our study was much lower (30 mg/l in this study compared to 150 mg/l in Rauf et al. study). This difference can be due to the structures created in the MOF and the ability to release the effective substance from it, the different bacterial sub-strains used, and other test conditions. Liu et al. synthesized a copper-based MOF using an electrospinning method and determined the antimicrobial effects of their nanocomposite against *E. coli* and *S. aureus*. As they reported, the inhibition zone of Cu-MOF as a fibrous membrane has the highest diameter and the inhibitory effect of this structure on *S. aureus* was a little better in comparison with *E. coli*. The findings of these researchers are consistent with the results of our study that the MIC of *S. aureus* is lower than that of *E. coli* (40 mg/l against 100 mg/l respectively) [20].

Table 3
Biofilm status of various types of bacteria at different concentrations of Cu-MOF.

Cu-MOF / Bacteria	C1 200ppm	C2 100ppm	C3 60ppm	C4 40ppm	C5 30ppm	C6 20ppm	C7 10ppm	Control +		Control -
E. coli	0/238	0/182	1/011	0/659	0/549	1/072	1/149	2/210	2/868	0/079
B. subtilis	0/158	0/127	0/120	0/132	0/135	0/145	0/188	0/404	0/412	0/049
P. aeruginosa	0/126	0/123	0/254	0/182	0/192	0/250	1/287	2/016	3/228	0/051
P. fluorescens	0/142	0/132	0/232	0/428	0/316	1/709	1/044	2/159	3/729	0/050
S. aureus	0/186	0/140	0/757	1/100	1/382	1/802	2/270	3/259	3/510	0/046
S. typhimurium	0/274	0/141	0/929	0/559	0/342	1/553	1/173	2/861	3/105	0/057
B. cereus	0/241	0/158	0/137	0/259	1/474	2/462	3/229	3/782	3/696	0/059
Negative control								Mean negative control:0/055		

Strong biofilm ☐

Medium biofilm ☐

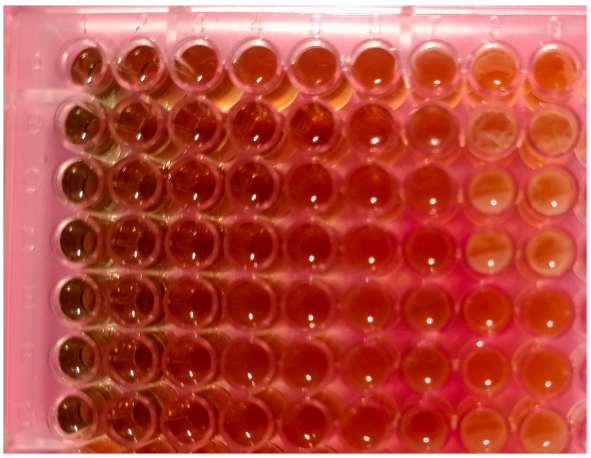

Fig. 7. Actual image of MIC-B test for different concentrations of Cu-MOF against 7 bacterial strains.

3.3. MBC results for the Cu-MOF sample

Bacterial growth was seen in all inoculated samples from negative MIC results. Therefore, Cu-MOF has not been able to kill any of the bacteria, and it is not possible to express a value for MBC. This finding could be due to the low Cu-MOF concentrations that were

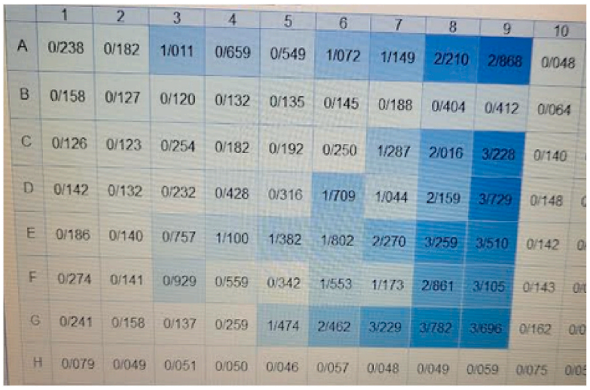

Fig. 8. Software image of MIC-B test for different concentrations of Cu-MOF against 7 bacterial strains.

Table 4
ANOVA statistical test results for biofilm inhibition in different concentration of Cu-MOF among microtiter plate wells.

Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	0.19500	0.21533	0.906	0.370313
ConcC2	−0.05171	0.30452	−0.170	0.865964
ConcC3	0.29643	0.30452	0.973	0.335910
ConcC4	0.27914	0.30452	0.917	0.364549
ConcC5	0.43214	0.30452	1.419	0.163248
ConcC6	1.08971	0.30452	3.578	0.000888 ***
ConcC7	1.28214	0.30452	4.210	0.000132 ***

Adjusted R-squared: 0.3734.
p-value: 0.0001903.

Table 5
The results of Tukey's test on the effect of nanostructure concentration on bacterial biofilm.

	P-value		P-value
C2–C1	0.9999977	C4–C3	1.0000000
C3–C1	0.9570058	C5–C3	0.9993359
C4–C1	0.9677954	C6–C3	0.1504215
C5–C1	0.7885407	C7–C3	0.0352044
C6–C1	0.0143425	C5–C4	0.9986883
C7–C1	0.0023438	C6–C4	0.1337776
C3–C2	0.9106942	C7–C4	0.0304683
C4–C2	0.9286331	C6–C5	0.3386799
C5–C2	0.6898375	C7–C5	0.01013070
C6–C2	0.0089659	C7–C6	0.9953216
C7–C2	0.0014041		

used in the study. However, studies that used higher concentrations found concentrations for MBC. For example, Abbasloo et al., who measured the properties of Cu-nanostructures on *P. aeruginosa*, observed MBC of 700 mg/L for the standard strain and 900 mg/L for the clinical strain [21].

3.4. MIC-B results for the Cu-MOF sample

The changes in biofilm formation of seven bacterial strains as a model organism for proteobacteria biofilms were investigated in this study. As a motile organism, all strains formed biofilms on the air-liquid surface of the wells. The average OD_{570} reading of each of the 3 replicate samples from all seven tested strains was compared with the mean OD_{570} of the negative control wells. The results in Table 3 showed that from 49 wells treated with Cu-MOF, the biofilm of 19 wells was at +2OD level and the rest of the wells had a biofilm production level higher than +4OD.

The Fig. 7 shows the actual image of MIC-B test for different concentrations of Cu-MOF against 7 bacterial strains. Also, Fig. 8 exhibits the software image of MIC-B test for different concentrations of Cu-MOF against 7 bacterial strains.

The ANOVA analysis in Table 4 shows a significant difference between the mean of biofilm growth in these concentrations (p-value = 0.00019).

Also, Tukey's test in Table 5 as a post-test of ANOVA showed that there is no significant difference in the average biofilm growth in

concentrations of 200, 100, 60, 40, and 30 ppm of this nanostructure.

On the other hand, by drawing a box-plot diagram, it was found that by increasing the concentration of nanostructure, less bacterial biofilm is formed.

It can be concluded that the concentrations of 1–5 of the synthesized Cu–MOF are effective in inhibiting the growth of bacterial biofilm. The findings of other studies have shown similar results. Wang et al. have developed an investigation on the effect of Cu-MOF nanosheets against *S. aureus* free and biofilm forms. As they have reported, a Cu-MOF concentration of 4 mg/L can eradicate *S. aureus* free bacteria up to 99 %. Biofilm eradication of *S. aureus* was observed in 512 mg/L with an efficiency as high as 97.6 %. Oxidation of surface proteins and lipids by high concentrations of Cu^{2+}/Cu^{+} has been reported as the dominant mechanism of bacterial death [22]. In another study by Zheng et al., with Cu-MOF-74 coating on polyvinylidene fluoride membranes, they increased on-membrane antifouling effects. As they reported, Cu-MOF can inhibit biofouling by *E. coli* bacteria for up to one week. The Cu-MOF enters the cell of bacteria attached to the membrane and releases Cu^{2+} and reactive oxygen species inside the cell that lead to destroying bacterial cells [23].

4. Conclusion

This study showed favorable antibacterial results against different bacterial strains so in addition to preventing the growth of bacteria, it was also able to reduce the growth and development of the bacterial biofilm. This issue can lead to the application of porous metal-organic nanostructures of copper in various fields, such as cleaning tiles, ceramics, and medical equipment, where biofilms have been responsible for many problems with cleaning and using them in medical centers. Therefore, by preparing suitable technology for the use of Cu-MOF, the growth of different types of bacteria can be prevented in these situations, or at least, it can weaken the development of biofilms. Consequently, this study supports the ability of the Cu-MOF nanostructure to deal with bacteria as a stable disinfectant, which can have a longer duration of antibacterial activity in the environment than common disinfectants.

CRediT authorship contribution statement

Nafiseh Abaszadeh: Writing – original draft, Software, Investigation, Formal analysis, Conceptualization. **Daryoush Afzali:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Ghasem Sargazi:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Abdolali Golpayegani:** Writing – original draft, Software, Investigation, Formal analysis.

Declaration of competing interest

This study introducess a new window of efficient materials with high performance of antibacterial properties. Initial reagents, synthesis method and improved properties are selected based on novel processes.

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