BASIC AND CLINICAL CANCER REPORTS | Volume 1, Issue 1 | Summer 2026

Production of copper nanoparticles through a consortium of microorganisms and investigation of their antimicrobial properties

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Abstract

Nano includes materials with particle size in the range of 1-100 nm. Metal nanoparticles have been used in various scientific and industrial fields. Recently, the biosynthesis of nanoparticles by microbes has been considered as a suitable alternative for the mass production of nanoparticles. This study aimed to investigate the biosynthesis of copper nanoparticles using a group of microorganisms and their antimicrobial effects. In this study, four types of microorganisms, Fusarium oxysporum, Saccharomyces cerevisiae, Lactobacillus fermentum, and Lactobacillus acidophilus, were used to produce copper nanoparticles. The microorganisms were grown in their own culture medium. To biosynthesize copper nanoparticles, a one millimolar copper sulfate solution was used. We then checked for color change. In this study, the antimicrobial effects of E. coli (PTCC 1330) and S. aureus (PTCC 1112) were investigated. Our findings showed that copper nanoparticles synthesized with Lactobacillus fermentum, Fusarium oxysporum, Saccharomyces cerevisiae, and Lactobacillus acidophilus had the smallest grain size and the highest purity, respectively. In addition, disinfection was better for samples with higher purity and grain size.Copper nanoparticles can be a suitable substitute for antibiotics; however, it should be noted that the resistance level of clinical strains is higher than that of standard strains. Therefore, before prescribing nanoparticles, the resistance of bacteria to them should be investigated.

Keywords: copper nanoparticles, Lactobacillus fermentum, Fusarium oxysporum, Saccharomyces cerevisiae, Lactobacillus acidophilus

1.Introduction

Nano refers to materials with particle sizes between 1 nm and 100 nm (1). Metal nanoparticles are widely used in various scientific and industrial fields (2, 3). For example, copper nanoparticles (CuNPs) have received considerable attention in recent years because of their good conductivity, chemical stability, and catalytic, photonic, and optoelectronic properties. In addition, CuNPs are used as bactericidal materials in biological science research (4). In addition, CuNPs have recently been used for DNA detection (5).

Different chemical methods have been proposed for the synthesis of NPs, including the microemulsion method, synthesis with the help of microwaves, the method of using templates, chemical regeneration methods, electrochemical regeneration, irradiation method, chemical regeneration in aqueous solution, chemical regeneration without aqueous solution, light-induced regeneration or photocatalytic regeneration, regeneration with the help of ultrasonic waves, and biochemical methods (6). According to studies, the use of chemical methods for the production of nanoparticles, on the one hand, can have potential risks for human health and the environment, and on the other hand, they are usually expensive (7, 8); As a result, recently, biological methods of nanoparticle synthesis have been developed to produce environmentally friendly and inexpensive nanoparticles (9). In this regard, the biosynthesis of nanoparticles by microbes has recently been considered a suitable alternative for the mass production of nanoparticles (10, 11).

Studies have shown that one of the important parameters in the production of nanoparticles by biosynthesis is the use of different bacteria during the process (12). Lactobacillus acidophilus, Pichia pastoris yeast, and Fusarium oxysporum are among the most important bacteria for the biosynthesis of CuNPs (13, 14). In addition to the type of bacteria, other parameters such as temperature and pH can affect the size and morphology of the produced copper particles (15).

Because the production of nanoparticles by the biosynthetic method results in better compatibility with the environment and reduces pollution, we conducted a study to investigate the effect of the microorganisms Fusarium oxysporum, Saccharomyces cerevisiae, Lactobacillus fermentum and Lactobacillus acidophilus on the size and morphology of CuNPs produced by the bioleaching method.

2.Material and method

2.1.Ethics Statement

The study was approved by the ethics committee of Islamic Azad University, Tehran Central Branch . In the present study, the microorganisms Fusarium oxysporum (PTCC 5115), Saccharomyces cerevisiae (PTCC 5269), Lactobacillus fermentum (PTCC 1638), and Lactobacillus acidophilus (PTCC 1643) were obtained from the website of the Iran Scientific and Industrial Research Organization.

2.2.Culture of microorganisms

To culture the microorganisms, each Lactobacillus species, including Lactobacillus acidophilus and Lactobacillus fermentum, was transferred to an Erlenmeyer flask containing 100 ml of MRS Broth and incubated at 37 °C for 48 h. In addition, to culture Saccharomyces cerevisiae and Fusarium oxysporum, PDA culture medium (agar + potato extract + water) was used.

2.3.Biosynthesis of copper nanoparticles

Copper sulfate solution (1 M.mol) was used to perform the experiments. Next, the supernatant of each microorganism was added and placed in a shaker incubator at 30 °C and 150 RPM.

2.4.Color change assay

The color of the solution changed from golden to blue at the beginning, and finally, a red color was found from the copper particles.

2.5.Spectrophotometric assay

A spectrophotometer was used to measure the optical absorption of the prepared samples at wavelengths of 300-650 nm. Distilled water was used as a blank. According to previous studies, the synthesis of CuNPs results in a strong peak in the 325 nm range (16).

2.6.Measuring by electron microscope

The surfaces of some samples were examined using a PC3-WDX scanning electron microscope (SEM). Transmission electron microscopy (TEM) was used to determine the size and shape of the NPs produced. The samples prepared in the experiments were placed in a sonicator for 10-15 minutes, and then a drop of the solution was placed on grids coated with Formvar and placed under UV light to dry the grids. The prepared grids were examined using TEM.

2.7.Atomic Absorption Assay

We used an Atomic Absorption Assay device to determine the concentration of the biosynthesized CuNPs. Specific concentrations of standard CuNPs were read using the device, and a standard curve was drawn. The samples were examined using this device, and the concentration of biosynthesized nanoparticles was determined.

2.8.Antimicrobial effects assay

First, we cultured standard strains of E. coli (PTCC 1330) and S. aureus (PTCC 1112) on nutrient agar culture media and kept them in an incubator at 37 °C for 24 h. After incubation, the bacteria were used to prepare the bacterial suspension. We collected some of the bacterial colonies using a needle to prepare a microbial suspension and added the activated microorganism to a sterile test tube containing 15 ml of sterile physiological serum and stirred it with a shaker. Then, we measured the light absorption of this suspension at a wavelength of 600 nm using a spectrophotometer. In addition, we observed the turbidity visually with a 0.5 McFarland tube (1.5×108 cfu/ml). Next, we used discs impregnated with different concentrations of the Cu nanoparticles. Then, we placed the discs in Mueller Hinton agar culture medium, on which the bacteria under investigation were uniformly cultured, and incubated for 24 h at 37 °C. Finally, we examined the diameter of the halo around the disc.

2.9.MIC assay

The MIC is the lowest concentration of an antimicrobial substance that inhibits the growth of microorganisms. For this purpose, we prepared various concentrations of CuNPs in a serial manner and added 100 µL of the desired bacterial suspension to all the tubes, followed by incubation for 24 h at 37 °C. Finally, the concentration at which growth was not observed was determined as the MIC.

2.10.MBC assay

The MBC is the lowest concentration of an antimicrobial agent required to kill 99.9% of primary microorganisms. In this method, we cultured the concentrations at which growth was not observed (MIC obtained) along with the concentration before and after on Mueller-Hinton agar culture medium and investigated the growth and colony formation.

2.11.Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 25 was used to analyze the data. Group differences were assessed using one-way analysis of variance (ANOVA), with statistical significance set at a P-value of < 0.05. Graphs were created using Microsoft Excel.

3.Result

In our study, during the synthesis of CuNPs, we observed a change in color from gold to red, which was caused by the composition of CuNPs, which have a red color (**figure 1**).

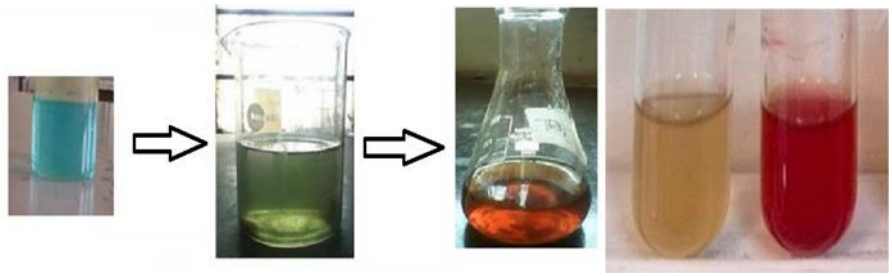


Figure 1. Color change due to the formation of CuNPs

3.1.Synthesis with Fusarium oxysporum

In the current study, parameters such as pH (or the change in the concentration of the supernatant) and the duration of the process were very important.

The effect of pH and time on the mean size of copper grains synthesized with the help of Fusarium oxysporum following 2.7 : 66.85 , 4.7 ; 51.69 , 5.7 ; 50.93 , and 24 ; 59.97 , 48 ; 48.83 , 72 ; 60.69 , respectively .According to the results obtained from this study, the best time for the formation of smaller particles was 48 h, but the times of 24 and 72 h did not show a statistically significant difference.

According to **Table 1**, the best conditions for creating samples with the smallest grain size in the presence of Fusarium oxysporum microorganisms were obtained under the two conditions. First, when the pH was approximately 7.4, the duration was 24 h, and second, when the pH was approximately 7.5, the duration was 48 h. The sizes of the particles in the first and second conditions were estimated to be approximately 46.28 nm and in the second condition about 45.63 nm.

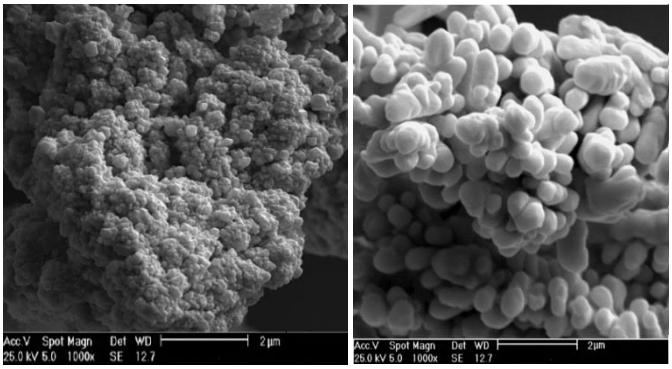
PH	Time	Mean
2.7	24	87.44
2.7	48	52.15
2.7	72	60.98
4.7	24	46.28
4.7	48	48.70
4.7	72	60.10
5.7	24	46.19
5.7	48	45.63
5.7	72	60.99

Table 1. Comparison of mean effects of pH and time on CuNPs size.

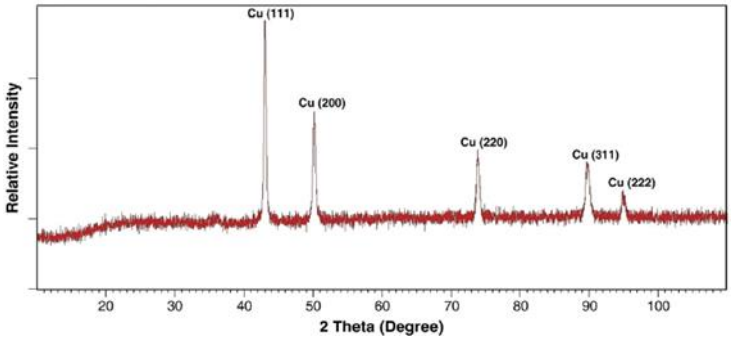
Figure 2A shows the powder created in the presence of Fusarium oxysporum under two different optimal conditions. According to the images obtained from the scanning electron microscope, the morphology of the particles did not change significantly with changes in the optimal conditions, and these particles had a coaxial morphology.

EDS and XRD analyses showed that the resulting powder was pure copper. However, with the passage of time and exposure of the powder to air, the color of the powder gradually darkened and turned black, indicating the oxidation of the powder (**Figure 2B and 2C**).

2A



2B



2C

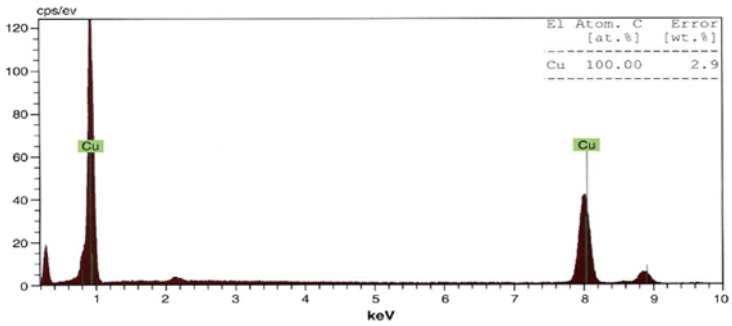


Figure 2. SEM image for the first and second samples with optimal conditions(A).XRD analysis of the optimal sample in the presence of Fusarium oxysporum microorganisms (B) .EDS analysis of the optimal sample in the presence of Fusarium oxysporum microorganisms (C) .

3.2.Synthesis with Saccharomyces cerevisiae

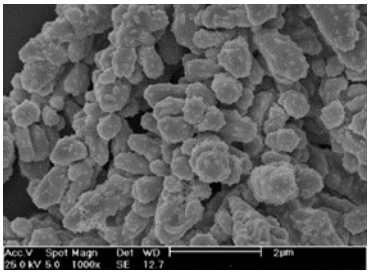
According to the results obtained from this study, the best time for the formation of smaller particles was 48 hours, but the times of 24 and 72 hours did not have a statistically significant difference following . According to **Table 2**, the best conditions for creating samples with the smallest grain size in the presence of the microorganism Saccharomyces cerevisiae were a pH of approximately 5.7, duration of 24 h, and particle size estimated to be approximately 59. 93 nm.

PH	Time	Mean
2.7	24	77.49
2.7	48	62.25
2.7	72	70.88
4.7	24	66.38
4.7	48	68.60
4.7	72	62.60
5.7	24	60.09
5.7	48	59.93
5.7	72	60.99

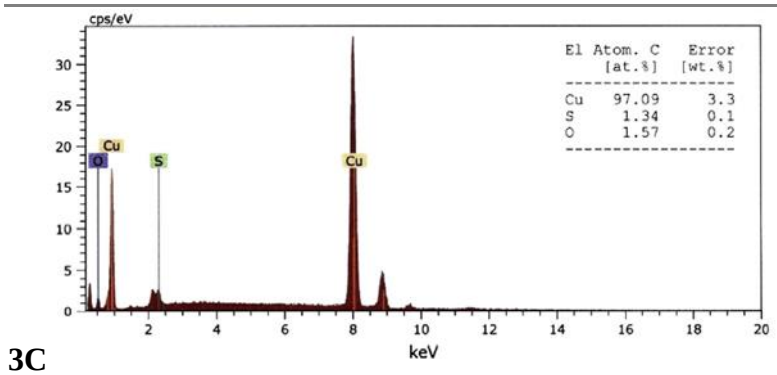
Table 2. Comparison of mean effects of pH and time on CuNPs size.

Figure 3A shows the powder created in the presence of the microorganism Saccharomyces cerevisiae. According to the image, the morphology of the particles did not change significantly with changes in the concentration, and these particles had a planar morphology. EDS and XRD analyses showed that the resulting powder was not pure Cu. In the compositions presented by XRD, the sample contained copper oxide, and EDS results showed that the amount of synthesized copper was less than 98**% (Figure 3B and 3C) .** Therefore, one of the most important problems in using this microorganism, in addition to its large particle size, is the high impurity **.**

3A



3B



3C

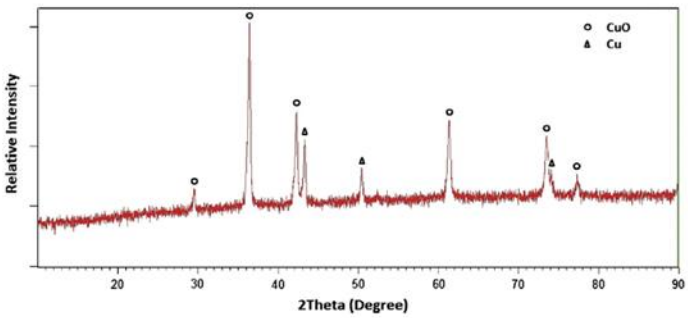


Figure 3. SEM image of the sample with Saccharomyces cerevisiae microorganisms(A) .XRD analysis of the optimal sample in the presence of Saccharomyces cerevisiae (B) .EDS analysis for the optimal sample in the presence of Saccharomyces cerevisiae(C) .

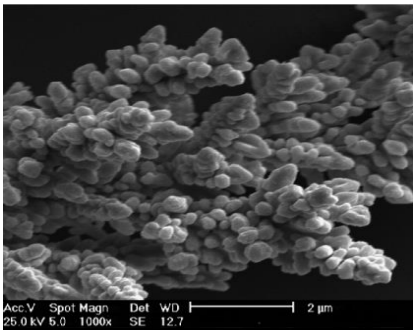
3.3.Synthesis with Lactobacillus fermentum

According to the results obtained from this study, the best time for the formation of smaller particles was 24 h, but the times of 48 and 72 h did not show a statistically significant difference **.** According to **Table 3**, the best conditions for creating samples with the smallest grain size in the presence of the microorganism Lactobacillus fermentum were a pH of approximately 5.7, a duration of 24 h, and an estimated particle size of be about 52.41 nm. **Figure 4A** shows the powder created in the presence of Lactobacillus fermentum microorganisms. According to the image, the morphology of the particles did not change significantly with changes in the concentration, and these particles had a dendritic and elongated morphology. EDS and XRD analyses showed that the resulting powder was pure copper. However, with the passage of time and exposure of the powder to air, the color of the powder gradually darkened and turned black, indicating the oxidation of the powder. According to the accuracy of EDS and XRD, it can be said that the purity of the powder is more than 99.5% (**Figure 4B and 4C)** .

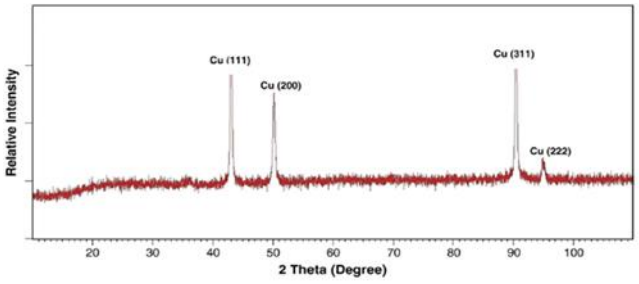
PH	Time	Mean
2.7	24	57.59
2.7	48	56.75
2.7	72	55.38
4.7	24	29.41
4.7	48	30.60
4.7	72	32.50
5.7	24	31.39
5.7	48	29.83
5.7	72	31.69

Table 3. Comparison of the mean effects of pH and time on the size of CuNPs

4A



4B



4C

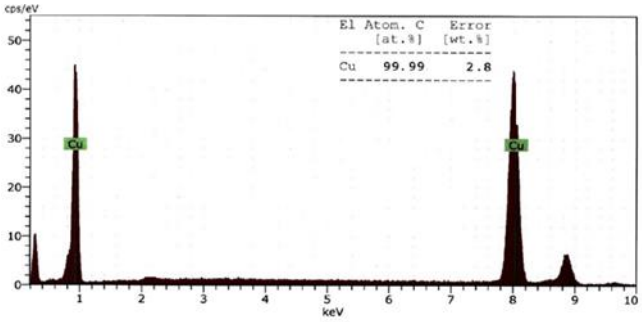


Figure 4. SEM image for the sample with Lactobacillus fermentum microorganism (A) .XRD analysis of the optimal sample in the presence of Lactobacillus fermentum (B) .EDS analysis for the optimal sample in the presence of Lactobacillus fermentum (C) .

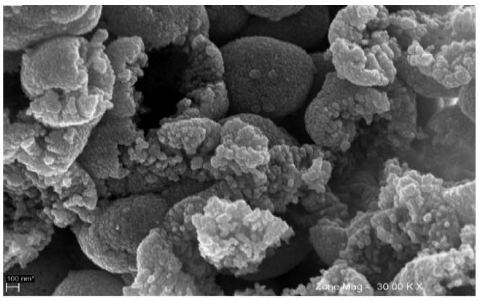
3.4.Synthesis with Lactobacillus acidophilus

The comparison mean of the simple effects of pH on the size of CuNPs made in the presence of this microorganism showed that the most suitable pH in terms of the smallest particle size was 7.5 and according to the obtained average, the largest particle size was pH = 7. According to the results obtained from this study, the best time for the formation of smaller particles was 24 h, but the times of 48 and 72 h did not show a statistically significant difference **.** According to **Table 4**, the best conditions for creating samples with the smallest grain size in the presence of the microorganism Lactobacillus acidophilus were a pH of approximately 5.7, a duration of 24 h, and an estimated particle size of be about 77.83 nm. **Figure 5A** shows the powder created in the presence of Lactobacillus acidophilus. The morphology of the particles did not change significantly with changes in concentration, and these particles had a planar morphology. EDS and XRD analysis showed that the resulting powder is not pure copper; Because in the compositions provided by the XRD, the sample has amounts of copper oxide and copper sulfide, and the EDS results also showed that the amount of synthesized copper was less than 98% **(Figure 5B and 5C) .** Therefore, one of the most important problems of using this microorganism, in addition to the large particle size, is the high impurity. Since, in addition to the oxide composition, there was also a sulfide composition in it, this microorganism had the weakest performance.

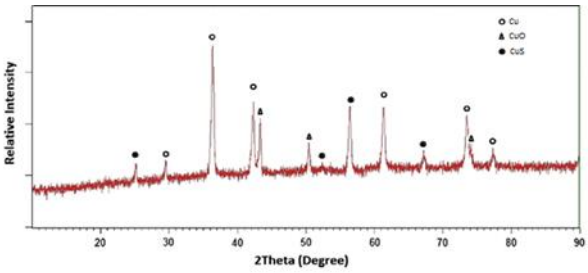
PH	Time	Mean
2.7	24	106.65
2.7	48	95.15
2.7	72	90.25
4.7	24	86.36
4.7	48	88.70
4.7	72	89.82
5.7	24	97.84
5.7	48	89.63
5.7	72	77.83

Table 4. Comparison of the mean effects of pH and time on the size of CuNPs.

5A



5B



5C

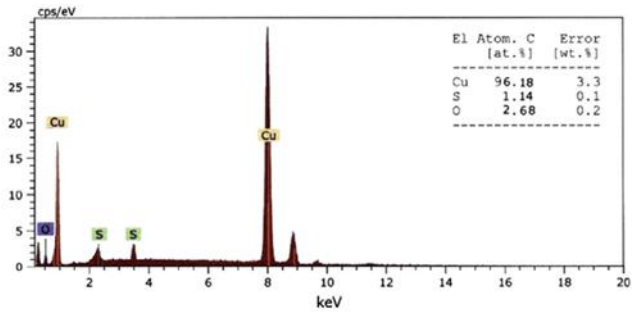
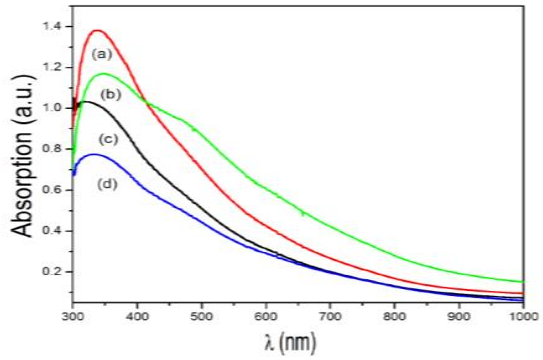


Figure 5. SEM image of the sample containing Lactobacillus acidophilus (A) .XRD analysis of the optimal sample in the presence of Lactobacillus acidophilus (B) .EDS analysis of the optimal sample in the presence of the microorganism Lactobacillus acidophilus (C) .

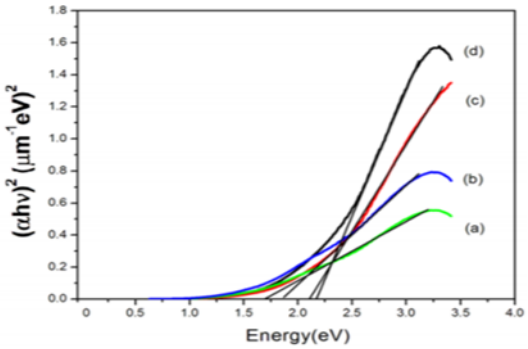
3.5.Measuring the concentration with UV-Vis

Figure 6A shows the optical absorption spectra of the samples at room temperature in the wavelength range of 300-1000 nm. The optical gap of thin copper layers is larger than its bulk state, which is due to the quantum limitations caused by the size of the particles. As shown in **Figure 6A**, the optical absorption edge slowly changed with the increase in impurity in the copper structure and moved to longer wavelengths; thus, from the wavelength of 327 nm corresponding to the spectrum of pure copper to the wavelength of 341 nm corresponding to the sample with 5% impurity. The decrease in absorption observed in the peaks of the spectra can be attributed to the increase in the participation of impurities in the copper structure. **Figure 6B** shows the energy gap diagrams of the layers. The increase in the energy gap with the increase in the amount of impurity can be attributed to the decrease in the size of the nanostructures. This shift of the energy gap towards higher energies and shorter wavelengths leads to an increase in the crystal order, a decrease in scattering, and an increase in the density of electrons in thin-layer nanostructures. **Figure 6C** shows the effect of increasing the amount of impurity in increasing the energy gap of the layers. In addition, in our study, we examined copper purity according to pH and time for all microorganisms. The results are shown in the table below.

6A



6B



6C

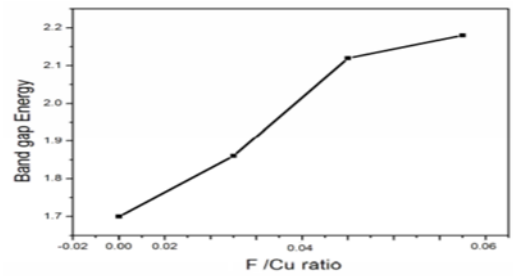


Figure 6. Optical absorption curve a: Fusarium oxysporum, b: Saccharomyces cerevisiae, c: Lactobacillus fermentum, d: Lactobacillus acidophilus(A) .Graph (αhν)2 in terms of energy (B) .Energy gap changes with increasing impurity (C).

3.6.Antimicrobial assays

Figure 7 shows the zones of inhibition of E. coli and aureus bacteria by CuNPs synthesized with Lactobacillus acidophilus, Lactobacillus fermentum, Saccharomyces cerevisiae, and Fusarium oxysporum. The results showed that the zone of inhibition of Staphylococcus aureus for Lactobacillus

acidophilus was the largest, and in other cases, the zone of inhibition was almost the same. For E. coli, the largest zone of inhibition was related to Fusarium oxysporum and Saccharomyces cerevisiae, and the other two microorganisms had smaller halos than the other two microorganisms. The following table (**Table 5**) shows the MIC and MBC values for CuNPs synthesized using Fusarium oxysporum, Saccharomyces cerevisiae, Lactobacillus fermentum, and Lactobacillus acidophilus. Among the microorganisms, the best results were obtained for Lactobacillus acidophilus, which was able to remove the S. aureus bacteria well and also to remove the E. coli microorganism to some extent.

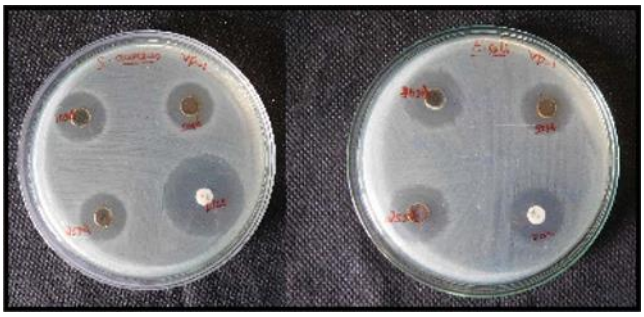


Figure 7. Zone of inhibition of CuNPs.

Type of microorganism	S. aureus		E. coli	
	MIC (mm)	MBC (LogCFu/ml)	MIC (mm)	MBC (LogCFu/ml)
Lactobacillus acidophilus	8	3.1	8	6.7
Lactobacillus fermentum	8	6.5	8	6.6
Saccharomyces cerevisiae	8	6.8	8	4.8
Fusarium oxysporum	8	5.4	8	4.6

Table 5. MIC and MBC values for CuNPs synthesized with studied microorganisms

4.Discussion

In nanotechnology, it is possible to obtain nanostructures using two methods: top-down and bottom-up. The top-down method is related to the conversion of materials from the macro size to the nano size, while the bottom-up method refers to the production of products from individual molecules or atoms to larger structures and molecules. Today, nanotechnology includes five major fields: nanoelectronics, nanomaterials, nanomolecular technology, nanobiological technology, and microscopy (with separation at the nanoscale and nanoscopy). Although the boundaries are unknown, each of these cases show different paths in terms of creating new opportunities (17).

Generally, particles between 1 and 100 nm are called nanoparticles. In the current research, all the particles produced were less than 100 nm; therefore, the results of the current research are consistent with the general definition of nano. On the other hand, the production of nanoparticles with the help of biological materials is called nano biosynthesis, and in current research, biological materials such as bacteria and fungi are used; therefore, the production method of our research is consistent with the general definition of biosynthesis (18). In this study, a color change from gold to red was observed during copper synthesis. In this regard, Caroline et al. conducted a study in 2015, and the results of our study were consistent with them(19).

In this study, we showed that in the presence of Fusarium oxysporum, the most appropriate pH in terms of the smallest particle size was pH = 4.7, and the largest particle size was pH = 2.7. In 2004, Armendariz et al. conducted a study in this direction. They reported in their study that the tendency of copper ions to oat biomass depends on pH; therefore, larger nanoparticles with size was 25-85 nm were formed at pH = 2, whereas smaller nanoparticles of approximately 5-20 nm were formed at pH = 4.3. Finally, the results obtained in our study are in accordance with those of Armendariz et al. 's study (20). In addition, we showed that in the presence of the microorganisms Saccharomyces cerevisiae and Lactobacillus acidophilus, the most appropriate pH in terms of the smallest particle size was 5.7, and the largest particle size was 2.7. The results of our study were consistent with those of a study conducted by Roy et al. In their study, they showed that they were able to produce nanoparticles at pH values of 5, 7, and 9 at 4 °C and room temperature. They indicated the production of stable nanoparticles at pH 7 and 9, which remained intact for 2 months, whereas nanoparticles produced at a pH of approximately 5 accumulated in 24 h (21). The size of CuNPs made in the presence of Lactobacillus fermentum microorganisms showed that pH = 4.7 is suitable for the production of small nanoparticles. Caroling et al. (2015)

investigated the biosynthesis of CuNPs by Phyllanthus emblica. In their study, they showed that the synthesized copper particles had a size of 15-30 nm, and these biological CuNPs helped in controlling the effective growth of human pathogens such as Staphylococcus aureus and Escherichia coli (19).

In general, gram-negative bacteria are more sensitive to CuNPs than gram-positive bacteria are. In addition, clinical strains were less sensitive to CuNPs than standard strains. Although the concentration of CuNPs in tube number 5 containing CuNPs created with Lactobacillus acidophilus, Saccharomyces cerevisiae, and Fusarium oxysporum microorganisms was higher, they could not remove Aureus ATCC 25923 and Enterococcus faecalis. The sample created with Lactobacillus fermentum at lower concentrations succeeded in removing these bacteria. Therefore, it can be concluded that disinfection is related to the grain size and purity of the sample particles because the purest sample was related to Lactobacillus fermentum. In addition, in this study, we found that CuNPs synthesized by the chemical regeneration method were more effective against gram-negative bacteria, such as Escherichia coli, while they had less effect on gram-positive bacteria, especially Staphylococcus aureus. Various studies have been conducted on this topic. Alshareef et. showed in their study that among metals such as gold, silver, and copper, silver nanoparticles showed strong antibacterial activity against E. coli and E. faecium. They pointed out that this is due to the production of reactive oxygen species (ROS) in the cell wall, which affect metabolic processes by damaging DNA replication (22). Guzman et al showed a very high effect of CuNPs on Gram-positive and Gram-negative bacteria. They stated that the size of CuNPs has a great effect on antibacterial properties, and that particles with a size of 9-19 nm had the greatest effect on bacteria. This can be due to the increase of the surface to the volume of copper particles and the larger surface available for them to react with bacterial proteins, or due to the greater penetration of smaller nanoparticles into the bacterial cell (23). Copper biosynthesis using bacteria is a low-cost method; however, it creates polydisperse nanoparticles. Considering their good antibacterial activity, CuNPs can be a suitable substitute for antibiotics; however, it should be noted that the resistance level of clinical strains is higher than that of standard strains. Therefore, before prescribing nanoparticles, the resistance of bacteria to them should be investigated. The results of the present study show that copper synthesis with the microorganisms Lactobacillus fermentum, Fusarium oxysporum, Saccharomyces cerevisiae, and Lactobacillus acidophilus has the smallest grain size and highest purity, respectively. In addition, the disinfection rate was better for samples with higher purity and grain size.

Acknowledgments

It must be noted that acknowledgments are not applicable in the given context.

Ethics

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflicts of interest

The authors declare no conflicts of interest**.**

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References

1. Joudeh N, Linke D. Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists. Journal of Nanobiotechnology. 2022;20(1):262.
2. Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry. 2019;12(7):908-31.
3. Mody VV, Siwale R, Singh A, Mody HR. Introduction to metallic nanoparticles. J Pharm Bioallied Sci. 2010;2(4):282-9.
4. Hussain S, Pal A. Incorporation of nanocrystalline silver on carbon nanotubes by electrodeposition technique. Materials Letters. 2008;62(12-13):1874-7.
5. Park KW, Lee CY, Batule BS, Park KS, Park HG. Ultrasensitive DNA detection based on target-triggered rolling circle amplification and fluorescent poly(thymine)-templated copper nanoparticles. RSC Adv. 2018;8(4):1958-62.
6. Sajadi G, Shojaei A, Fazeli M, Amini J, Jamalifar H. Extracellular synthesis of silver nanoparticles by Fusarium exispurium in laboratory scale. Donyay-e-Microbeha J. 2010;1:44-7.
7. Ahmed SF, Mofijur M, Rafa N, Chowdhury AT, Chowdhury S, Nahrin M, et al. Green approaches in synthesising nanomaterials for environmental nanobioremediation: Technological advancements, applications, benefits and challenges. Environmental Research. 2022;204:111967.
8. Hristozov D, Malsch I. Hazards and risks of engineered nanoparticles for the environment and human health. Sustainability. 2009;1(4):1161-94.
9. Singh P, Kim Y-J, Zhang D, Yang D-C. Biological synthesis of nanoparticles from plants and microorganisms. Trends in biotechnology. 2016;34(7):588-99.
10. Tsekhmistrenko S, Bityutskyy V, Tsekhmistrenko O, Horalskyi L, Tymoshok N, Spivak M. Bacterial synthesis of nanoparticles: A green approach. Biosystems Diversity. 2020;28(1):9-17.
11. Yang Y, Waterhouse GI, Chen Y, Sun-Waterhouse D, Li D. Microbial-enabled green biosynthesis of nanomaterials: Current status and future prospects. Biotechnology Advances. 2022;55:107914.

12. Singh NA, Narang J, Garg D, Jain V, Payasi D, Suleman S, et al. Nanoparticles synthesis via microorganisms and their prospective applications in agriculture. Plant Nano Biology. 2023;5:100047.
13. Koul B, Poonia AK, Yadav D, Jin J-O. Microbe-mediated biosynthesis of nanoparticles: Applications and future prospects. Biomolecules. 2021;11(6):886.
14. Vaseghi Z, Nematollahzadeh A, Tavakoli O. Green methods for the synthesis of metal nanoparticles using biogenic reducing agents: a review. Reviews in Chemical Engineering. 2018;34(4):529-59.
15. Kumar CG, Mamidyala SK. Extracellular synthesis of silver nanoparticles using culture supernatant of Pseudomonas aeruginosa. Colloids and Surfaces B: Biointerfaces. 2011;84(2):462-6.
16. Fukushima RS, Hatfield RD. Extraction and isolation of lignin for utilization as a standard to determine lignin concentration using the acetyl bromide spectrophotometric method. J Agric Food Chem. 2001;49(7):3133-9.
17. M J. Molecular nanotechnology and biological nanotechnology: interaction between nature and technology. first ed: University of Mazandaran; 2007. 444 p.
18. Wang J. Nanoparticle-based electrochemical DNA detection. Analytica Chimica Acta. 2003;500(1-2):247-57.
19. Caroling G, Vinodhini E, Ranjitham AM, Shanthi P. Biosynthesis of copper nanoparticles using aqueous Phyllanthus embilica (Gooseberry) extract-characterisation and study of antimicrobial effects. Int J Nano Chem. 2015;1(2):53-63.
20. Armendariz V, Gardea-Torresdey J, editors. Jose Yacaman M, Gonzalez J, Herrera I, Parsons JG. Gold nanoparticle formation by oat and wheat biomasses. Proceedings of Conference on Application of Waste Remediation Technologies to Agricultural Contamination of Water Resources; 2002.
21. Roy K, Sarkar CK, Ghosh CK. Fast colourimetric detection of H2O2 by biogenic silver nanoparticles synthesised using Benincasa hispida fruit extract. Nanotechnology Reviews. 2016;5(2):251-8.
22. Alshareef A. An investigation into the effects of shape and structure on the antibacterial efficacy of metal nanoparticles: De Montfort University; 2019.
23. Guzman M, Dille J, Godet S. Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. Nanomedicine: Nanotechnology, Biology and Medicine. 2012;8(1):37-45.