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Wastewater Disinfection by Synthesized Copper Oxide Nanoparticles Stabilized with Surfactant

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Abstract
CuO NPs were prepared by a quick precipitation method in the absence and presence of tetraoctylammonium bromide (TOAB) that was used as a stabilizer to control the nanoparticles size. X-Ray Diffraction (XRD) and Scanning electron microscope (SEM) were used to characterize CuO NPs. The NPs average size was from 7-12 nm with rod-like shape that was controlled by the change of preparation temperatures and the presence of TOAB surfactant.The antibacterial activity of the prepared CuO NPs were evaluated using total coliform (TC), fecal coliform (FC) and *Enterococcus faecalis* (*E. faecalis*) bacteria counts in wastewater. Different parameters were studied to obtain the optimum wastewater disinfection conditions, these parameters are nanoparticlessize, and concentration, TOAB surfactantstabilization, contact time, pH, shaking and temperature of wastewater.

Keywords **:** Nanoparticles; Coliform; Waste Water;

1. Introduction

Due to increasing demands on clean water for many purposes such as drinking, industrial and irrigation purposes, with the shortage of clean water sources to meet population growth, increasing industrial demands and other reasons. Nowadays, wastewaterusage is one of the most appropriate alternatives available to meet the clean water demands. However; health effects of pathogens and hazard chemicals found in wastewater is the most important issues that need treatment to fulfill the hygiene and health standards [1, 2].

An effective method for wastewater treatment is the usage ofnanoparticles to disinfect the wastewater from the different pathogens. Nanomaterials, like nanoparticles, nanotubes, nanowires and thin films, are defined as very small aggregate of atoms with less than 100 nm dimension [3].The importance of nanoparticles is due to the unique different physical, chemical and biological characteristics compared to the bulk scale, due to their high surface-to-volume ratio [4]. Wastewater treatment using nanoparticles is one of the areas of concentration in nanotechnology among the various applications for the nanotechnology such as fuel cells, hydrogen storage and various clinical antibacterial activity applications [3, 5].

Copper(II) oxide is semiconducting compound that belongs to monoclinic structure systems. It has many useful physical and chemical properties such as superconductivity at high temperature, photovoltaic properties, relatively stable, low cost and has antimicrobial activity[6]. CuO nanoparticles also have various technology applications such as catalysis [7], batteries due to high electrochemical capacity [8], and gas sensors [9].CuO nanoparticles can be synthesized for by different methods such as sonochemical technique [10], electrochemical method [11], high temperature combustion [12] and novel quick precipitation method [13].

CuO nanoparticles synthesis by novel quick precipitation (salt reduction) method is very interesting because it is safe, simple, environmentally friendly method and gives large scale of nanoparticles [14, 15]. Zhu *et al*. prepared highly dispersed CuO nanoparticles using copper acetate aqueous solution as a precursor and sodium hydroxide (NaOH) as reducing agent. The average size of CuO NPs product was 6 nm [13]. Wu *et al.* prepared well dispersed CuO nanoparticles by dissolving $Cu(NO_3)_2 \cdot 3H_2O$ in N,N-Dimethylacet amide (DMAC) and using NaOH solid as reducing agent, different size of CuO NPs were obtained by using NaOH at different temperatures [14].

Using a fast and simple quick precipitation method,Fathima *et al.* prepared a stabilized CuO nanorods with sodium dodecylsulfate (SDS), dodecyltrimethylammonium bromide (CTAB) and Triton X-100 as anionic, cationic and neutral surfactants, respectively. The ionic SDS surfactant has the strongest interaction with the cationic CuO NPs according to negative charge of SDS surfactant. However, other results showed that the surfactants play important roles in the shape and applications of the nanomaterials [16].

Antimicrobial activity of CuO NPs studies are very limited. Among the few studies, Ren *et al.* reported that CuO NPs has antibacterial activity against a range of gram-positive and gram-negative bacteria such as *S. aureus*, Epidemic MRSA-15 and *E .coli* NCTC 9001 [17]. Baek *et al.* studied the antibacterial effect of CuO, NiO, ZnO, and Sb_2O_3 nanoparticles, and showed that CuO nanoparticles is the most toxic of these metal oxide nanoparticles against *E. coli* as gram-negative bacteria and *B. subtilis* and *S. aureus* as gram-positive bacteria. On the other hand, CuO NPs showed higher activity against *E. coli* more than against gram-positive bacteria [18]. Heinlaan *et al.*studied the effects of bulk and nano CuO on *Vibrio fischeri*, crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. The results showed that CuO NPs have higher antibacterial activity than bulk CuO [19].

In this study, size selective copper oxide nanoparticle were prepared with and without tetraoctyl ammonium bromide (TOAB) surfactant matrix. The size and the morphology of the samples were investigated using XRD and SEM. Then, the prepared CuO NPs anti-bacterial effect were investigated using the wastewater bacterial indicators; total coliform (TC), fecal coliform (FC) and *Enterococcus faecalis* (*E. faecalis*). Many parameters on the antibacterial activity of CuO NPs were studied including NPs size, TOAB stabilization, concentrations, contact time, pH, shaking and temperature of wastewater. Finally, flow up test was carried out as a practical application of the prepared CuO NPs for water disinfection.

2. Materials and Methods

2.1. Materials

Copper(II) sulfate pentahydrate ($CuSO_4.5H_2O$)was purchased from Alfa Aesar, a Johnson Matthey Co.,sodium hydroxide (NaOH) was purchased from Frutarom Co., sodium phosphate dibasic(Na_2HPO_4)was purchased from Merck Co., Citric acid was purchased from Frutarom Co., tetraoctylammonium bromide (TOAB) 98% purity was purchased from Sigma Co. enterococcus agar was purchased from BD Co., violet red bile agar CM0107 purchased from OXOID Co.Wastewater samples were collected from western region of Nablus city sewage system by water department at Nablus municipality.

2.2. Copper oxide nanoparticles (CuO NPs) preparation

Quick precipitation method was used to prepare two types of copper oxide nanoparticles with and without tetraoctylammonium bromide (TOAB) surfactant. CuO NPs stabilized with TOAB surfactant (CuO-TOAB) were prepared by dissolving 15.00 g of $CuSO_4.5H_2O$ with 2.34g of TOAB surfactant in 150 mL of distilled water.The different CuO NPs sizes were prepared at 65, 75 and 85 °C using the reflux condenser. After 15 min of heating and shaking at 150 rpm, 100 mL of 2.0M sodium hydroxide, as reducing agent, were rapidly added to the solution. The black precipitate was collected, washed with distilled water and then dried.Copper oxide nanoparticles without surfactant were prepared the same as copper oxide nanoparticles with surfactant prepared steps but without presence of TOAB surfactant.

2.3. Characterization of copper oxide nanoparticles

Shape and size characterization of CuO NPs were conducted with XRD and SEM techniques. XRD technique was used to determine the structure and size of the CuO nanoparticles, using Philips-X'Pert Model-98 XRD machine with Cu source (Cu-Kα1 line, λ=1.5045 Å). While the shape and the morphology of all six CuO nanoparticles samples were characterized by SEM, the images obtained using JEOL, JSM-6360LV SEM.

2.4. Culture
Violet red bile agar was used for TC and FC count. In 1L conical flask, 19.25 g of violet red bile agar was dissolved in 500 mL distilled water; the solution was heated on heater until the solution become clear. Then tempered at 48 ºC until use. *E. faecalis* media were prepared by dissolving 21.00 g of *Enterococcus* agar in 500 mL distilled water into 1 L conical flask, the solution was heated on heater until the solution became clear and then stored at 48 ºC until used.

2.5. Plate counting method
The antibacterial activity of the CuO nanoparticles was determined using the pouring plate counting method.The antibacterial activity of CuO NPs were studied using TC, FC and *E. faecalis*counts in wastewater samples. The count of TC, FC and *E. faecalis* in these samples was found to be at the range of 10^3-10^4 colony forming unit CFU and pH of 6.8. Pour plate method was used to measure the concentrations of viable TC, FC and *E. faecalis* in wastewater samples for all treated and control samples that obtained in all studied parameters using a sterile pipettes, 1.00 mL of each sample was pipetted into the center of 3 empty petri dishes and about 20.0 mL of tempered TC, FC and *E. faecalis* agars were poured on the samples, after that the petri dishes were rotated 20 times clockwise and anticlockwise to spread the samples throughout the agar and allowed for about 5 minutes to solidify, then the petri dishes were inverted before incubation. The dishes that have TC and *E. faecalis* agar were incubated at 37.0 °C using Incubator (Selecta Incubator model no.0345944), while that contain FC agar were incubated at 44.5 °C for 24 h. Then the colonies in all dishes were counted using electronic colony counter (Electronic Colony Counter, catalog no. 37862-0000).

2.6. Different parameters effect on the NPs antibacterial activity
Antibacterial activity and wastewater disinfection using CuO NPs was studied using different parameters. The parameters were NPs size effect, the presence and absence of TOAB surfactant, nanoparticles concentrations, contact time, pH, shaking and temperature of wastewater effect.

2.6.1. Concentration effect
The antibacterial activity of CuO NPs with different concentrations was studied. The chosen concentrations were 10, $1x10^2$, $3x10^2$ and $5x10^2$ µg/mL, for CuO-TOAB NPs and $1x10^2$, $1x10^3$, $3x10^3$, $5x10^3$ and $7x10^3$ µg/mL for CuO non-stabilized NPs. All samples, in addition to control sample were shaked at 150 rpm for 2 h at 25 °C.

2.6.2. NPs size and surfactant antibacterialactivity
In 100 mL conical flasks,CuO nanoparticles, that prepared at 65, 75 and 85 °C, without (0.010 g; 10^3 µg/mL)and with TOAB (0.001g; 10^2 µg/mL) surfactant were added to 10.0 mL of wastewater samples. All samples, in addition to control sample were shaked at 150 rpm for 2 h at 25 °C.

2.6.3. Contact time effect
CuO-TOAB and CuO non-stabilized NPs that prepared at 75 °C and used throughout this study as CuO-TOAB(3) and CuO(4),respectively,where chosen for their better activity as seen from their size effect. The study carried out with the concentrations; 10^2 and 10^3 µg/mL of CuO-TOAB(3) and CuO(4), respectively. All samples, in addition to control sample were shaked at 150 rpm for 0, 1, 2 and 24 h at 25 °C.

2.6.4. Temperature effect
CuO-TOAB(3) and CuO(4) were used at 10^2 and 10^3 µg/mL of concentrations, respectively. The samples, in addition to control sample were shaked at 150 rpm for 2 h at 15, 25, 35 °C.

2.6.5. pH effect
CuO-TOAB(3) and CuO(4) were used in different pH; 6.0, 7.0 and 8.0, of wastewater were investigated. Phosphate-citrate buffer solution was used to adjust the pH values of wastewater samples. All samples, in addition to control sample were shaked at 150 rpm for 2 h at 25 °C.

2.6.6. Shaking effect

As in the above experiments; 10^2 and 10^3 μg/mL of CuO-TOAB(3) and CuO(4) were used to investigate the shaking effect at 0 and 150 rpm for 2 h at 25 °C.

2.7. Flow test

As a practical application of the prepared CuO NPs for water disinfection, flow test were used to investigate CuO NPs antibacterial activity. Sterile syringe column (L: 44 X D: 12 mm) was used to flow 4.00 mL of wastewater through CuO-TOAB(3) and CuO(4) layer of about 1.0 mm thickness at constant flow rate of 10 mL/min. TC, FC and *E. faecalis* bacterial indicators were investigated before and after passing the wastewater through the CuO NPs layer using the previously mentioned plate counting method.

3. Results and Discussion

In this study, CuO NPs were prepared with and without TOAB surfactant and were investigated in a real wastewater samples. Different parameters that may affect the antibacterial activity were studied to obtain the optimal conditions to have NPs with high activity, low cost and low cytotoxicity to be used as wastewater disinfectant.

3.1. Synthesis and characterization of CuO NPs

CuO NPs were synthesized using quick precipitation method (eq. 3.1), in which $CuSO_4.5H_2O$ was used as Cu sources, NaOH solution and TOAB as reducing agent and stabilizer, respectively, after few seconds a black precipitate was observed as indication to CuO NPsprecipitation. The precipitate was collected and dried.To control the size and the shapes of the nanoparticles, the synthesis was carried out at different constant temperatures (65, 75 and 85 °C), in the presence and absence of the stabilizer to obtain CuO NPs.

$$CuSO_{4(aq)} + NaOH_{(aq)} \xrightarrow{\text{Stabilizer}} CuO\ (NPs) \qquad eqn.(3.1)$$

Zhu et al. prepared highly dispersed CuO nanoparticles by mixing copper acetate aqueous solution as a precursor with glacial acetic acid at 100 °C, black precipitate of CuO was observed by adding NaOH as reducing agent, the average size of CuO NPs produced was 6 nm [8]. Fathima et al. prepared stabilized CuO nanorods with sodium dodecylsulfate (SDS), cetyltrimethylammonium bromide (CTAB) and triton X-100 as anionic; cationic and neutral surfactants, respectively by fast and simple quick precipitation method. They found that, the ionic SDS surfactant has the strongest interaction of its negative charge with the cationic CuO NPs [16].

The advantage of our approach for CuO NPs synthesis is that it can be considered as a green synthesis in which all materials used as reactant and all products are environmentally friendly.The size and the shape of the obtained nanoparticles were characterized using XRD and SEM.

3.1.1. XRD characterization

XRD characterizations were carried out for the prepared CuO NPs, the obtained X-Ray diffractogram for all CuO with and without TOAB surfactant are shown in Fig. 1 to Fig. 3.

Based on three different XRD peaks analysis, the mean size was found to be 11.5, 9.9 and 7.8 nm for CuO-TOAB stabilized NPs and 12.4, 11.4 and 9.1 nm for CuO non-stabilized NPs thatwere prepared at 65, 75, 85 °C, respectively. Obviously, CuO non-stabilized NPs were larger than the sizes obtained in the presence of the surfactant (Table 1).

These results show that increasing the temperature during nanoparticles preparation lead to decrease in nanoparticle size. This can be explained as the high temperature break the hydrogen bonds of the metastable copper hydroxide ($Cu(OH)_2$) to transform into CuO. This complex was formed when $CuSO_4.5H_2O$ dissolved in water to produce hexaaqua copper(II) ion $[Cu(H_2O)_6]^{+2}$ followed by formation of $Cu(OH)_2$ after adding NaOH to the solution [14, 20].

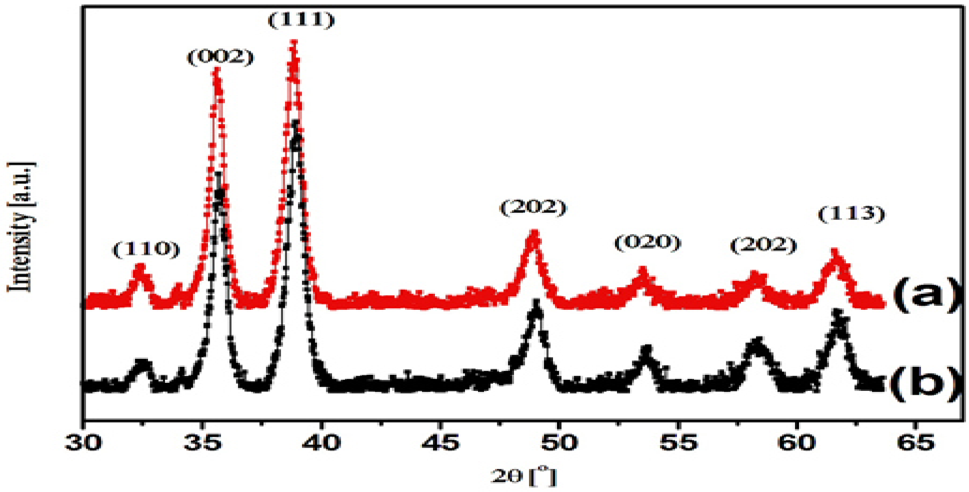

Fig. 1: XRD pattern of CuO nanoparticles prepared at 65 °C a) without TOAB surfactant b) with TOAB surfactant.

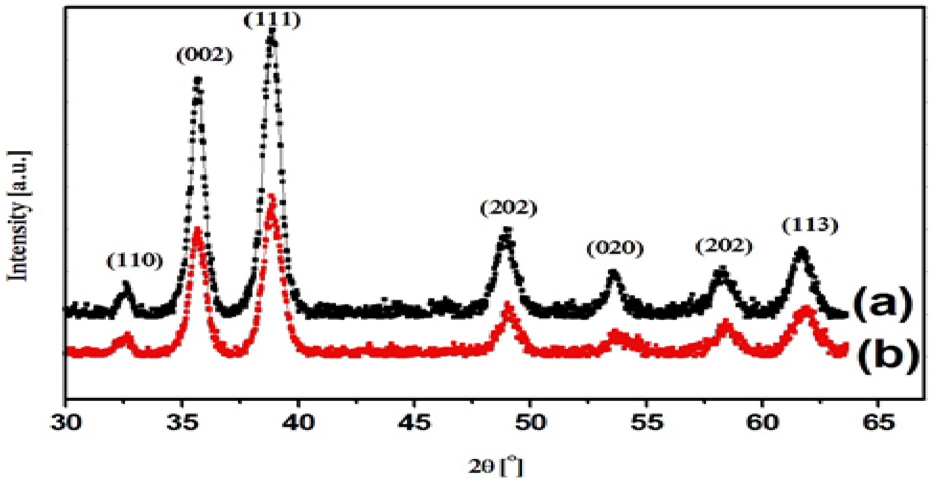

Fig. 2: XRD pattern of CuO nanoparticles prepared at 75 °C a) without TOAB surfactant b) with TOAB surfactant.

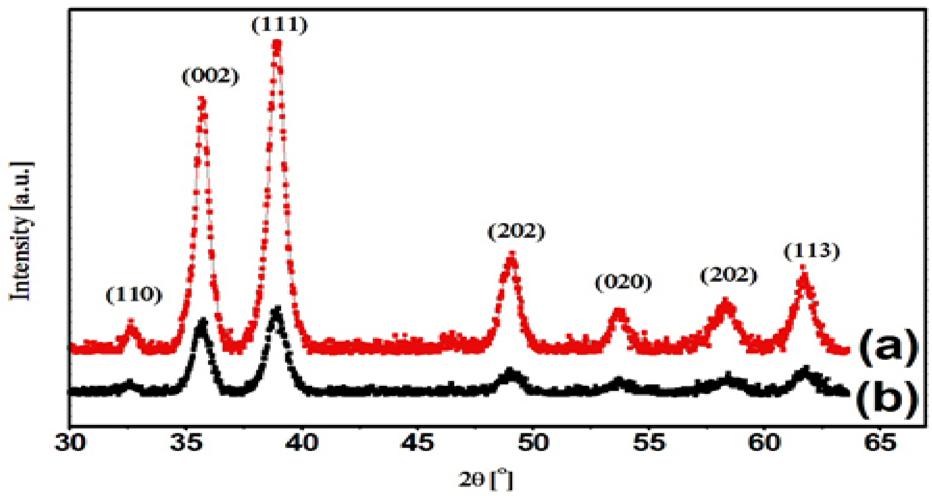

Fig. 3: XRD pattern of CuO nanoparticles prepared at 85 °C a) without TOAB surfactant b) with TOAB surfactant.

The size of CuO-TOAB stabilized NPs are smaller than CuO non-stabilized NPs that were prepared at the same temperature. Therefore, the surfactant have a role in controlling the CuO NPs size by preventing the undesired aggregation of atoms during nanoparticles preparation [21]. As reported in previous studies, X-ray structural analysis of the prepared samples (Fig 1 to Fig 3) confirmed copper oxide CuO nanoparticles monoclinic structures [22].

Table 1: The size of CuO-TOAB stabilized NPs and CuO non-stabilized NPs prepared at different temperatures.

Temperature (°C)	**Stabilized Sample**		**Non-stabilized Sample**	
	Sample number	**Size (nm)**	**Sample number**	**Size (nm)**
65	CuO-TOAB(1)	11.5	CuO(2)	12.4
75	CuO-TOAB(3)	9.9	CuO(4)	11.4
85	CuO-TOAB(5)	7.8	CuO(6)	9.1

3.1.2. SEM characterization

The shape and size of all CuO nanoparticle samplesprepared with and without surfactant at different temperature that were investigated by SEM techniques are shown in Fig 4 through Fig 6.

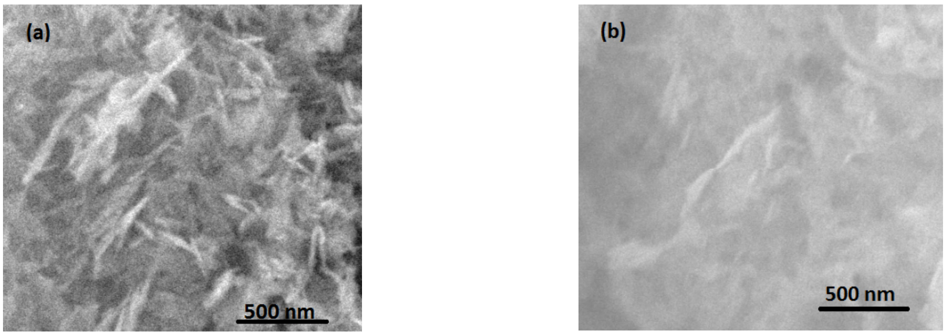

Fig. 4: SEM images of CuO nanoparticles prepared at 65 °C a) with TOAB surfactant b) without TOAB surfactant

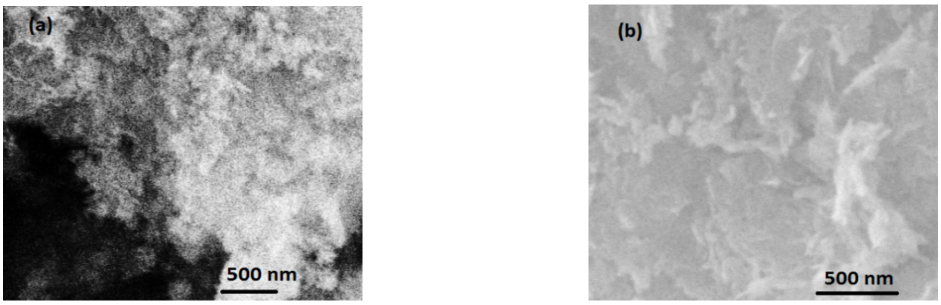

Fig. 5: SEM images of CuO nanoparticles prepared at 75 °C a) with TOAB surfactant b) without TOAB surfactant.

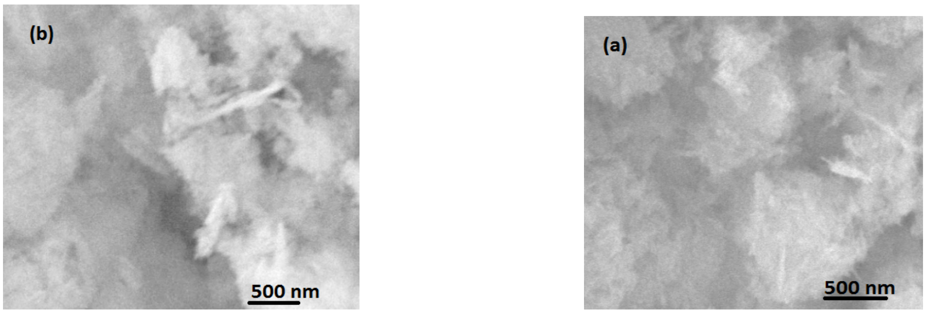

Fig. 6: SEM images of CuO nanoparticles prepared at 85 °C a) with TOAB surfactant b) without TOAB surfactant.

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Rody-stick shape was recorded for all CuO NPs with and without surfactant samplesprepared at different temperatures; 65, 75 and 85 ºC. However, it looks more regular for CuO-TOAB stabilized NPs.

Antibacterial activity study

3.1.3. Antibacterial activity of different concentrations CuO NPs with and without TOAB

Antibacterial activity of the different concentrations of CuO nanoparticles without stabilization were investigated against the selected bacterial indicators. The results of TC, FC and *E. faecalis* bacterial degradation by the different concentrations CuO(4) treated wastewater are listed in Fig 7 (a) to (c).

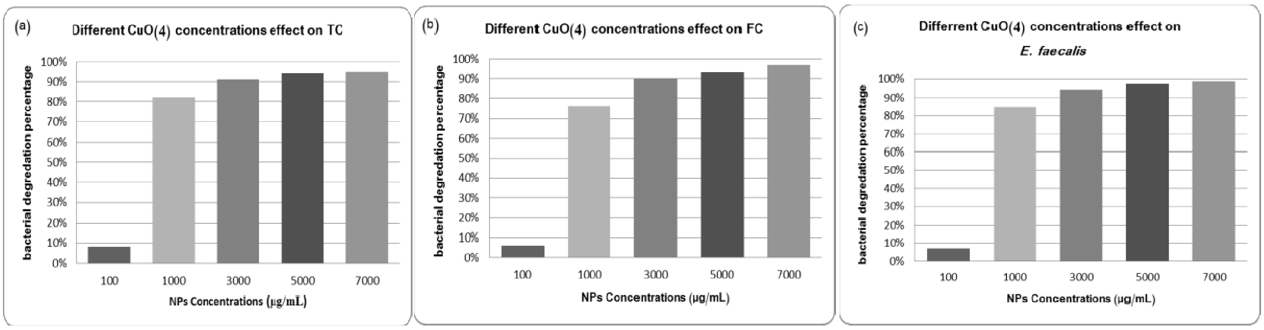

Fig. 7: CuO(4) NPs different concentrations bacterial growth degradation percentage using a) TC, b) FC and c) *E. faecalis* bacteria.

Bacterial degradation was consistent for the used bacterial degradation indicators; TC, FC and *E. faecalis* and all were less than 1000 µg/mL. At 100 µg/mL, TC, FC, *E. faecalis*bacterial degradation was 8%, 6% and 7%, respectively. While at 1000 µg/mL, TC, FC, *E. faecalis*bacterial degradation was 82%, 76% and 85%, respectively. More than 90% degradation for all of the bacterial indicators was recorded for the higher concentrations (Fig 7a, b, c). The noticed percentage degradation was very close for all used indicators. However, there is a slightly higher degradation percentage for *E. faecalis* in comparison to TC and FC, that could be correlated to the bacterial cell wall structure of gram-positive *E. faecalis* bacteria and the gram-negative bacteriaTC and FC indicators (Fig 7).An example for the results, Fig 8 shows theeffect of CuO(4) on TC bacteria indicator at 0 µg/mL (Fig 8a) and$7x10^3$ µg/mL (Fig 8b) concentrations.

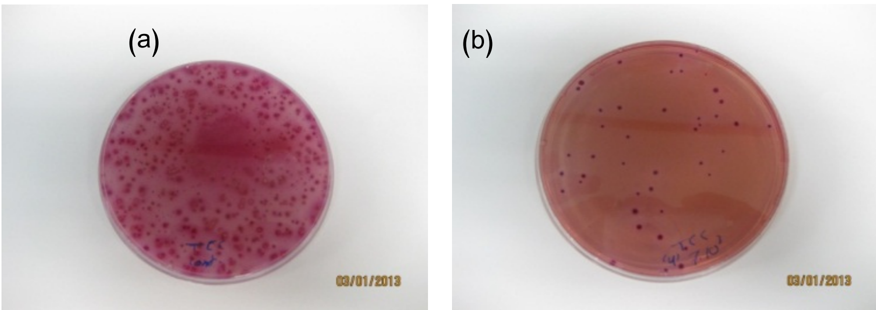

Fig.8: Effect of CuO(4) NPs on TC with the concentrations of a) 0 µg/mL b) $7x10^3$ µg/mL.

The antibacterial activity of the stabilized CuO nanoparticles were investigated forCuO-TOAB(3). The results of bacterial growth inhibition percentage for TC, FC and *E. faecalis* treated with different concentrations of CuO-TOAB(3) are shown in Fig 9a to c.

As indicated in the bacterial degradation for CuO NPs, bacterial degradation of CuO- TOAB stabilized NPs were consistent for the used bacterial degradation indicators that were less than 100 µg/mL in comparison to 1000 for CuO(4). At 10 µg/mL, TC, FC, *E. faecalis*bacterial degradation was 8%, 7% and 9%, respectively.

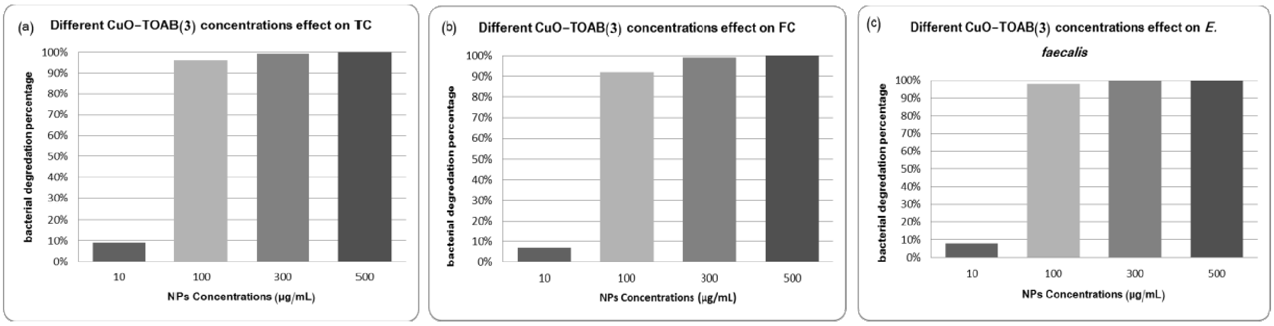

Fig. 9: The different CuO-TOAB(3) concentrations effect on a) TC, b) FC and c) *E. faecalis*.

While at 100 µg/mL and the higher concentrations the noticed degradation were more than 92% degradation for all of bacterial indicators. A 100% degradation for all of the used indicators at 500µg/mLconcentration (Fig 9a, b, c).As shown for the CuO(4), CuO-TOAB stabilized NPs bacterial degradation percentage were again higher in gram positive bacteria against *E. faecalis* in comparison with gram negative bacteria.An example for the TC indicator is shown in Fig 10 at0 µg/mL (Fig 10a) and 3x10^2 µg/mL (Fig 10b) concentrations.

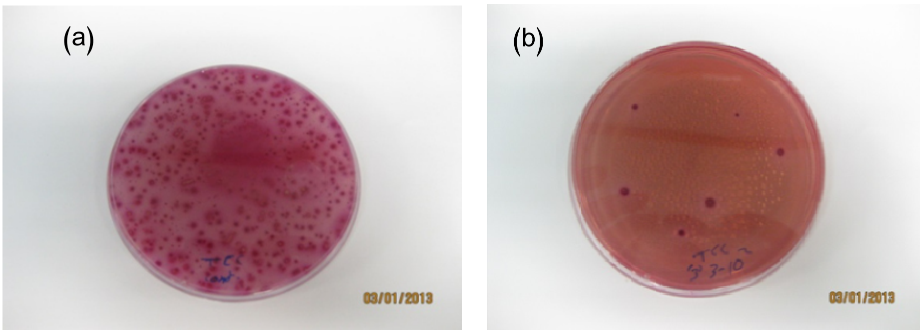

Fig.10: Effect of CuO-TOAB(3) NPs on TC with the concentrations of a) 0 µg/mL b) 3x10^2 µg/mL.

In conclusion,the bacterial degradation rate begin at about 100 and 10 µg/mL for CuO(4) and CuO-TOAB(3), respectively, that indicate the effect of the addition of the surfactant as antibacterial activity. Even though, the antibacterial activity of the used indicators in this study, TC, FC and *E. faecalis*, showed a very close NPs concentration response for both CuO with and without TOAB surfactant. As well as, antibacterial activity were increased with increasing NPs concentrations.

3.1.4. CuO NPs size and TOAB surfactant antibacterial effect

Different particles size with and without TOAB surfactant were used to studytheir wastewater disinfection through investigating TC, FC and *E. faecalis* bacteria indicators degradation percentage. Fig.11 show the bacterial degradation percentage of CuO non-stabilized NPs (10^3 µg/mL); CuO(2), CuO(4) and CuO(6) that represent 12.4, 11.4 and 9.1 nm NPs size, respectively. CuO(2), the largest NP of 12.4 nm, shown the lowest antibacterial activity of 73%, 64% and 75%, followed by CuO(6), the smallest NP of 9.1 nm, with 77%, 72% and 78%for TC, FC, *E. faecalis*bacterial indicators, respectively. The highest antibacterial activity were noticed for the medium NP size, CuO(4) NP of 11.4 nm, that ranged from 76% to 85% (Fig 11).

As expected from the previous indication, the stabilized CuO NP shown the higher antibacterial activity. Fig 12 show the bacterial degradation percentage of CuO-TOAB stabilized NPs (10^2 µg/mL); CuO-TOAB(1), CuO-TOAB(3) and CuO-TOAB(5) that represent 11.5, 9.9 and 7.8 nm NPs size, respectively.CuO-TOAB(1), the largest NP of 11.5 nm, shown the lowest antibacterial activity of 86%, 77% and 89%, followed by CuO-TOAB(5), the smallest NP of 7.8 nm, with 90%, 86% and 92%for TC, FC, *E. faecalis*bacterial indicators, respectively. As shown for the non-stabilized CuO, the highest antibacterial activity were noticed for the medium NP size, CuO-TOAB(3) NP of 9.9 nm, that more than 92% (Fig 12).

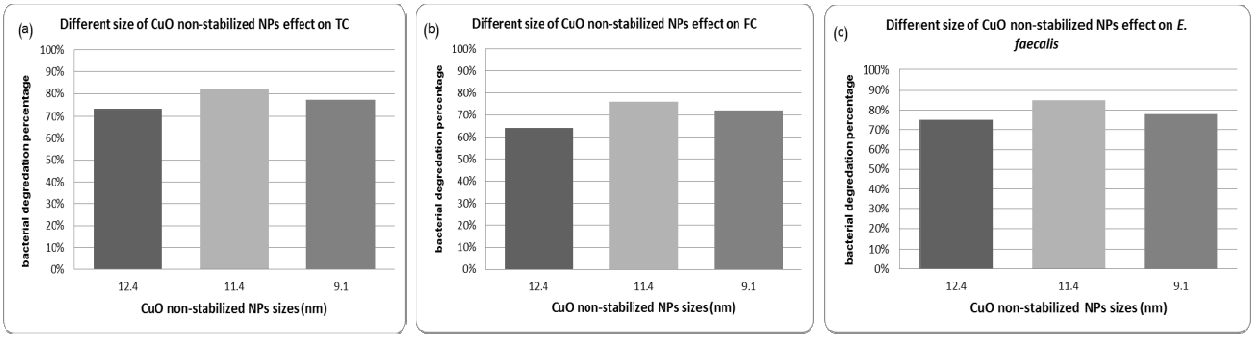

Fig. 11: CuO non-stabilized NPs size effect on a) TC b) FC and c) *E. faecalis* bacteria.

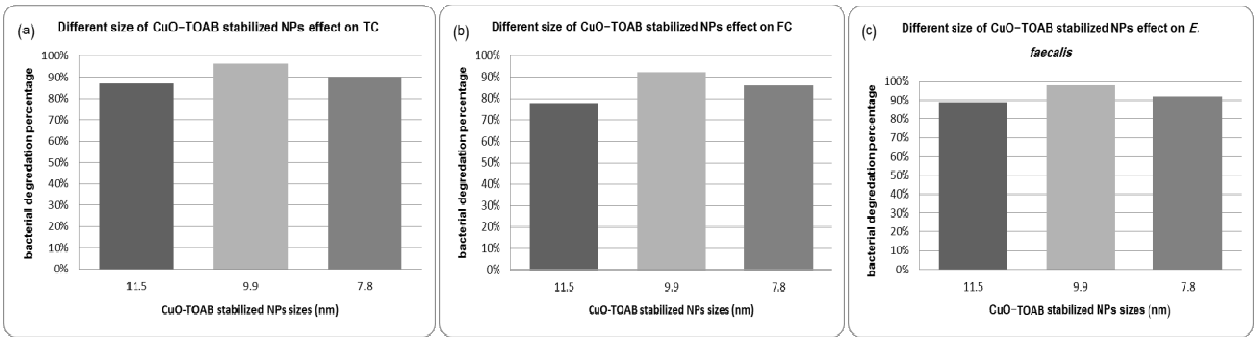

Fig.12: CuO NPs size with surfactant effect on a) TC b) FC and c) *E. faecalis*.

Interestingly, this study results showed that the highest antibacterial activity were for the medium size for both CuO with TOAB (9.9 nm) and without TOAB surfactant (11.4 nm). To the best of our knowledge the only available article that studied the size effect of CuO reached only 20 nm CuO NPs size with the highest antibacterial activity among the studied NPs size that goes with the notion of decreasing NPs size will increase the antibacterial activity [23]. However, our work reached a smaller size of NPs, less than 12.4 and 11.5 of CuO without TOAB and CuO with TOAB, respectively.

3.1.5. Antibacterial activity of CuO NPs with different contact time

The antibacterial activity of CuO NPs was studied to determine the most appropriate contact time. Antibacterial activity was studied at different contact time of 1, 2 and 24h. The effect was studied for two samples type: CuO(4) without surfactant and CuO-TOAB(3) with TOAB.

Antibacterial effect for CuO NPs without TOAB surfactant (10^3 µg/mL),CuO(4) NPs, are shown in Fig 13. Antibacterial activity after 1, 2, 24 hours contact time were 80, 82 and 95% for TC (Fig 13a); 75, 76 and 87% for FC (Fig 14b); and 80, 85 and 100% for *E. faecalis* (Fig 13c), respectively.

CuO-TOAB(3) NPs with TOAB surfactant(10^2 µg/mL) antibacterial activity after 1, 2, 24 hours contact time of TC were 90, 96 and 99% (Fig 14a); FC were 88, 92 and 99% (Fig 14b); and *E. faecalis* were 91, 98 and 100% (Fig 14c), respectively.

CuO-TOAB stabilized NPs showed a higher antibacterial activity at all studied contact times 1, 2, 24h, in comparison to the CuO non-stabilized NPs (Fig 13 and 14). The results showed that, the longer contact time has increased the antibacterial activity of both CuO NPs with and without TOAB surfactant. Generally, the difference in contact time antibacterial effect was small in both CuO NPs with and without TOAB. However, the antibacterial activity difference was slightly higher in CuO NPs without TOAB, which give the TOAB a better advantage to be used for wastewater treatment at shorter contact time.

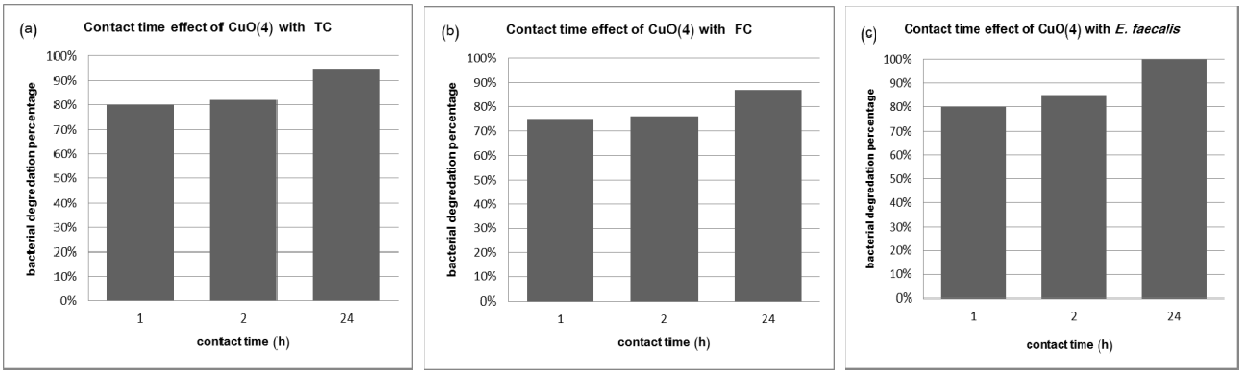

Fig.13: Contact time of 1, 2 and 24 h effect for CuO(4) on a) TC b) FC c) *E. faecalis*.

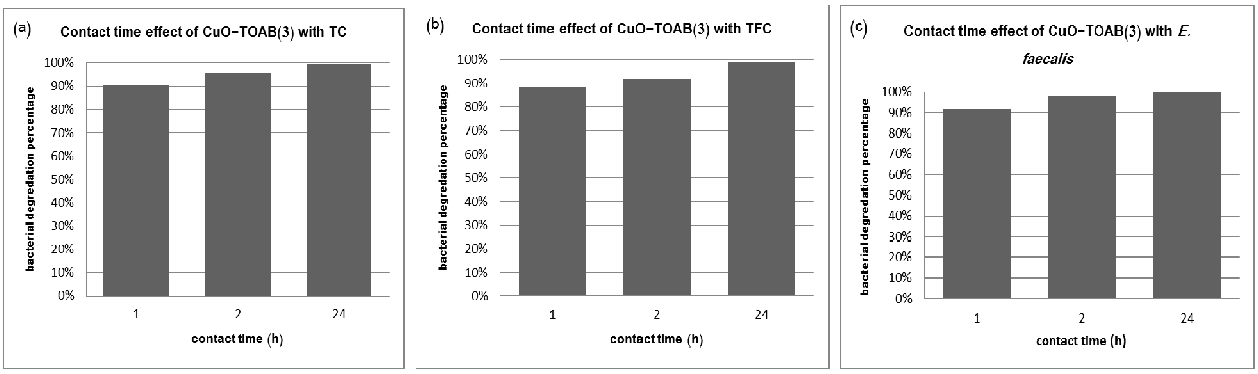

Fig.14: Contact time of 1, 2 and 24 h effect for CuO-TOAB(3) with a) TC b) FC c) *E. faecalis*

3.1.6. Temperature effect

Temperature is one of the most important factors affecting bacterial growth in wastewater. Palestine annual weather temperature almost range from 10-35 °C, that may reflect the wastewater temperature, however the wastewater temperature will probably have much narrower temperature range, as a reflection of the slow water response to the weather temperature and the wastewater samples are of human sources with constant body temperature. Therefore, the effect of different incubation temperature; 15, 25 and 35 °C, were studied as a factor in the antibacterial activity of CuO NPs with CuO-TOAB(3)(10^2µg/mL)and without TOAB surfactantCuO(4) (10^3µg/mL).

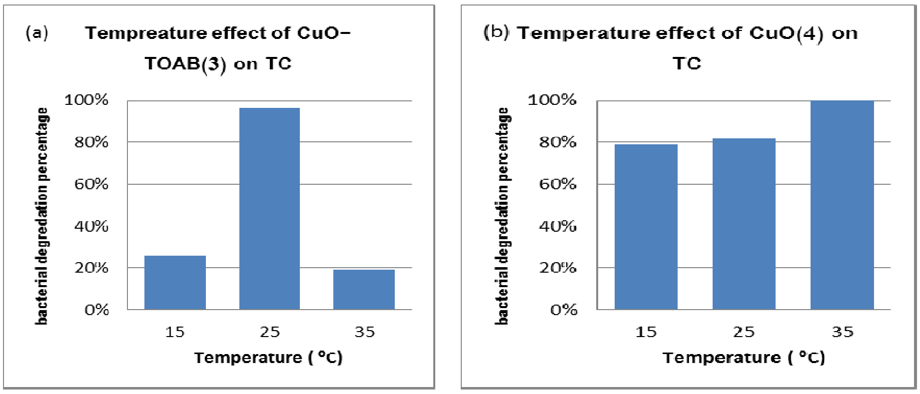

Fig.15: The effect of wastewater temperature (15, 25 and 35 ºC) on degradation percentage of TC using a) CuO-TOAB(3) and b) CuO(4).

As shown in Fig 15, the maximum TC bacterial degradation percentage of 100% was seen in CuO NPs without TOAB surfactant stabilization CuO(4) at the 35 °C incubation temperature. However, the bacterial count of untreated original wastewater samples, which were used as control, at 35 °C incubation temperature was of about 50%of 25 °C, i.e, the count at 25 °C was double of the 35 °C. Interestingly, this is in contrary to our preliminary investigation that the indicated maximum bacterial growth was 25 ºC, that were used as the optimal treatment temperature throughout the previous sections. The other temperature of 15 ºC and 25 ºC of CuO NPs without TOAB showed a lower effect but a closer result, however it was higher at 25 ºC of 82% and 79% at 15 ºC. On contrary, CuO-TOAB(3) showed the highest effect at 25 ºC of 96% degradation and a much lower effect at 15ºC of 26% and 35ºC of 19% bacterial TC degradation effect.

A similar temperature effect, as seen for TC, was seen for the FC (Fig 16) and*E. faecalis* (Fig 17) indicators. A very close effect was seen for FC and TC indicators. There is a similar effect of CuO NP without stabilization against the three indicators including *E. faecalis* but the stabilized CuO-TOAB(3) showed also a similar with a higher effect at 15ºC and 35 ºC as well as 25 ºC (Fig 15, 16, 17).

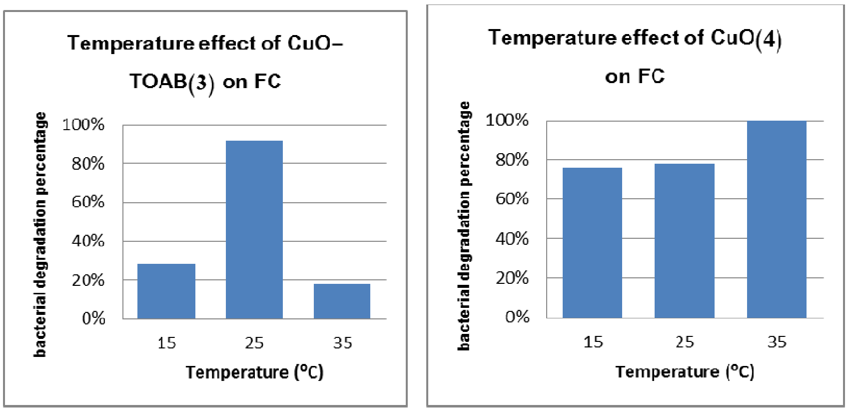

Fig.16: The effect of wastewater temperature (15, 25 and 35 ºC) on degradation percentage of FC using a) CuO-TOAB(3) and b) CuO(4).

Unexpectedly, stabilized CuO NP in comparison to CuO NPs without TOAB surfactant, showed a noticable reduction in the bacterial degradation percentage at 15 ºC and 35 ºC. CuO NPs stabilized with TOAB (Figure 15-17) showed the maximum bacterial degradation percentage.This result could be explained by that the surfactant highest effect with maximum metabolically active bacterial count at 25 ºC.

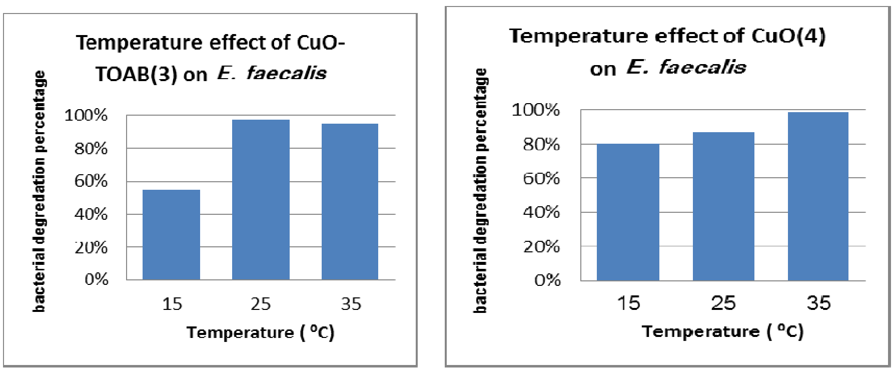

Fig.17: The effect of wastewater temperature (15, 25 and 35 ºC) on degradation percentage of *E. faecalis* using a) CuO-TOAB(3) and b) CuO(4).

3.1.7. pH effect

Different pH values were investigated to determine the optimal pH for antibacterial activity of CuO NPs with and without TOAB. Phosphate-citrate buffer was used to prepare pH values of 6, 7 and 8 (acidic, neutral and basic media) that represent the optimal range for bacterial growth and were used as test medium for the bacterial degradation percentage. CuO non-stabilized NPs (10^3 µg/mL) and CuO-TOAB stabilized NPs (10^2 µg/mL) at different pH medium showed slight difference in the TC, FC, *E. faecalis* indicators (Fig 18 (a) to (c) and Table2).

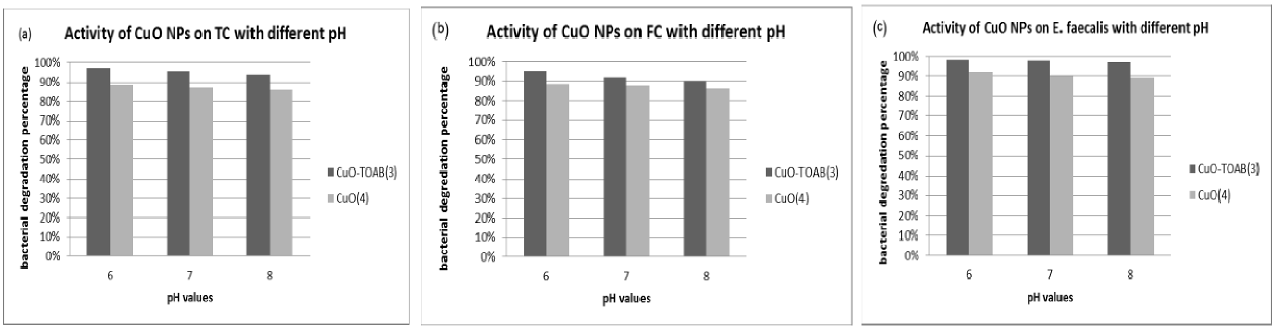

Fig.18: The effect of different pH (6, 7 and 8) of CuO(4) and CuO-TOAB(3) on a) TC b) FC c) *E. faecalis* bacteria.

Table 2: Bacterial inhibition growth rate by different pH using CuO(4) NPs (10^3 µg/mL) and CuO-TOAB(3) NPs (10^2 µg/mL).

Bacteria/ pH	6		7		8	
	CuO(4)	CuO-TOAB(3)	CuO(4)	CuO-TOAB(3)	CuO(4)	CuO-TOAB(3)
TC	88%	97%	87%	96%	86%	94%
FC	89%	95%	88%	92%	86%	90%
E. faecalis	92%	98.5%	91%	98%	89%	97%

The antibacterial activity properties of CuO NPs with and without TOAB surfactant showed increasing degradation percentage of all studied indicators with decreasing pH values with 5% maximum effect (Fig 18 and Table 2). The slight difference in the bacterial degradation of different pH, exclude the pH effect as an important factor in controlling CuO NPs antibacterial activity. Even though, the maximum effect was seen at pH 6 and 7 that where the pH of the wastewater fell to 6.8, that mean no any further pH treatment is needed for the wastewater.

3.1.8. Shaking effect on the antibacterial activity

The bacterial inhibition growth rate using CuO(4) after 2 h without shaking was 58%, 51% and 65% for TC, FC and *E. faecalis*, respectively. While the bacterial degradation percent by CuO-TOAB(3) was 90%, 87% and 88% for TC, FC and *E. faecalis*, respectively (Table 3). Even antibacterial activity without shaking was prominent; there was a significant increase in the antibacterial activity when used with shaking for CuO NPs without stabilization, while there is much smaller shaking effect for CuO NPs stabilized with TOAB (Table 3).

In conclusion, shaking may gave a higher chance for bacterial contact effect with the CuO NPs. Higher antibacterial activity noticed with shaking lead us to build the filtration system as a model for wastewater treatment plan.

Table 3: Antibacterial activity of CuO(4) and CuO-TOAB(3) on TC, FC and *E. faecalis* with and without shaking.

NP	CuO(4) (10^3 µg/mL)		CuO-TOAB(3)(10^2 µg/mL)	
	Without shaking	With shaking	Without shaking	With shaking
TC	58%	82%	90%	96%
FC	51%	76%	87%	92%
E. faecalis	65%	85%	88%	98%

3.2. Flow up test

Flow up test at constant flow rate of 10 mL/min was applied to investigate the antibacterial activity of CuO NPs with and without TOAB surfactant using the optimum parameters investigated through this study including, size, pH and temperature. The bacterial degradation percentage results were 100% for all used bacterial indicators when the wastewater sample passed through CuO-TOAB(3) stabilized NPs layer. However, when the wastewater sample passed through CuO(4) non-stabilized NPs layer; TC, FC and *E. faecalis* bacterial degradation percentage were 85, 78 and 87%, respectively.

The above results are consistent with the investigated criteria of CuO NPs stabilized with TOAB is more effective in bacterial degradation than that shown in CuO NPs without stabilization. As CuO-TOAB(3) showed complete destruction of all bacterial indicators prove its applicability as a novel wastewater bacterial disinfection technique.

Conclusions

CuO NPs were prepared by a quick precipitation method in the absence and presence of tetraoctylammonium bromide (TOAB) that was used as a stabilizer to control the nanoparticles size. X-Ray Diffraction (XRD) and Scanning electron microscope (SEM) were used to characterize CuO NPs. NPs average size was from 7-12 nm with rod-like shape that was controlled by the change of preparation temperatures and the presence of TOAB surfactant.

The antibacterial activity of the prepared CuO NPs were evaluated using total coliform (TC), fecal coliform (FC) and *Enterococcus faecalis* (*E. faecalis*) bacteria counts in wastewater.

Different parameters were studied to obtain the optimum wastewater disinfection conditions, these parameters are size of nanoparticles with and without TOAB surfactant, nanoparticles concentration, contact time, pH, shaking and temperature of wastewater.

CuO-TOAB stabilized NPs showed higher antibacterial activity more than that without TOAB surfactant, where it was less than 100 and 1000 µg/ml for CuO NPs with and without TOAB surfactant, respectively. The effect of NPs size were studied where the sizes for CuO-TOAB stabilized NPs was found to be 11.5, 9.9 and 7.8 nm, while the sizes of CuO non-stabilized NPs was found to be 12.4, 11.4 and 9.1 nm, however, medium size for both CuO-TOAB stabilized NPs (9.9 nm) and CuO non-stabilized NPs (11.4 nm) have the highest antibacterial activity of other sizes. Contact time effect was small as there was slight difference in the antibacterial activity of both CuO NPs with and without TOAB. Noticeable high activity of CuO NPs with and without TOAB surfactant occurred when wastewater samples were treated at 25 ºC and 35 ºC, respectively. The antibacterial activity of CuO NPs with and without TOAB surfactant slightly increased by decreasing wastewater pH values. Antibacterial activity of CuO NPs without shaking showed lower activity of about 70 and 90% for CuO NPs without and with TOAB surfactant in comparison to the antibacterial activity with shaking. In all parameters were studied, the antibacterial activity of both CuO NPs with and without TOAB surfactant were higher against gram positive bacteria (*E. faecalis*) compared to the activity against gram negative (TC and FC). Flow up test proved the applicability of CuO-TOAB NPs as a novel wastewater disinfection technique.

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