

## Synergistic Effect of Copper Sulfate with some Traditional Biocides for Killing the Sulfate Reducing Bacteria in Oil Fields

<sup>1</sup>Ismail Aiad, <sup>2</sup>Abuzeid A. Hassanien, <sup>2</sup>Wessam N. El-Sayed, <sup>1</sup>Salah M. Tawfik, and <sup>3</sup>Ahmed Nazeif

<sup>1</sup>Egyptian Petroleum Research Institute, EPRI, Cairo, Egypt.

<sup>2</sup>Suez University, Faculty of Science, Chemistry Department, Suez, Egypt

<sup>3</sup>Qarun Petroleum Company, Egypt.

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### ABSTRACT

It is well known that sulfate-reducing bacteria (SRB) caused many problems in oil fields, for example, souring and increasing the corrosion rate, fouling and some deposits. In this study we target to struggle this type of bacteria in the oil fields. Three commercial biocides and some of their synergistic mixtures were tested. The synergistic mixtures were prepared via added different ratio of copper sulfate (1, 2.5, 5 and 7.5%) to glutaraldehyde (G<sub>0</sub>), cationic surfactant (C<sub>0</sub>) and tetrahydroxy methyl phosphonium sulfate (T<sub>0</sub>) to form G<sub>1</sub>, G<sub>2.5</sub>, G<sub>5</sub>, G<sub>7.5</sub>, C<sub>1</sub>, C<sub>2.5</sub>, C<sub>5</sub>, C<sub>7.5</sub>, T<sub>1</sub>, T<sub>2.5</sub>, T<sub>5</sub> and T<sub>7.5</sub>, respectively. These mixtures were tested as anti-sulfate reducing bacteria. Also, metals in the form of nanoparticles are one of the long-term challenges for the creation of a new class of antibacterial agents. The biological activity of nanoparticles of metals depends directly on their physical and chemical properties, therefore copper nanoparticles with glutaraldehyde were tested as anti-sulfate reducing bacteria. The contaminated water sample was collected from Qarun Petroleum Company (QPC) at western desert in Egypt using the dilution method. The results show good results as biocide for SRB in oil field.

**Key words:** Sulfate reducing bacteria (SRB), copper sulfate, Glutaraldehyde, THPS, copper nanoparticles, biocide

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### Introduction

SRB are one of the most common and problematic type of bacteria in oil and gas field systems (Cord-Ruwisch *et al.*, 1987; Ferris *et al.*, 1990; Agostini *et al.*, 1990; Hill *et al.*, 1990). Although strictly anaerobic, SRB can persist and survive in systems containing dissolved oxygen (Postgate *et al.*, 1984). Typically, they are found in quiescent water in dead legs of pipes, ratholes of wells, and under deposits of scale and other bacteria. They are also found as free-floating (planktonic) bacteria in turbulent waters. SRB tolerate a wide pH range of 5 to 9.5, but some oilfield brines reportedly are too saline to be conducive to active growth. Most strains of SRB grow best at temperatures between 25 and 35°C (77 and 95°F), but a few thermophilic strains function at temperatures higher than 60°C (140°F). *Desulfovibrio*, *Desulfobacter*, and *Desulfotomaculum* are three common genera of SRB. Other genera have also been identified (Postgate *et al.*, 1984). While SRB often differ in appearance or in the substances they metabolize, they all oxidize organic compounds to organic acids or CO<sub>2</sub> by reducing sulfate ions to sulfide ions through anaerobic respiration. In the absence of sulfate ions, SRB can also respire through reduction of sulfite and other sulfur-containing ions. Sulfide ions that are produced during the respiration process can react with dissolved iron to produce black deposits of iron sulfide, or with hydrogen ions to form poisonous hydrogen sulfide (H<sub>2</sub>S). The presence of either iron sulfide or H<sub>2</sub>S in field systems causes operational problems. When steel corrodes, a layer of atomic hydrogen builds up on the cathodic surface. If the hydrogen is not removed, it polarizes the surface and causes the corrosion rate to decrease (Lewanndowski *et al.*, 1997). Using hydrogen in their anaerobic respiration process, SRB remove the atomic hydrogen from the surface, causing the cathodic surface to depolarize and increasing the rate of corrosion. As a result, pit formation is accelerated. This corrosion process has been termed microbiologically influenced corrosion (MIC) and is the subject of many excellent reviews.

Reduce the numbers and types of the sulfate reducing bacteria (SRB) in the system using biocides to the bulk water to kill the organisms which entering the system, or reduce the growth rate of microorganisms within the biofilm, mechanical removal of biofilm from the substratum (sponge balls, brushes), and water treatments to decrease the numbers and types of organisms (aeration and deaeration) (Bryant *et al.*, 1991)

Our presented research is aimed to enhancing the activity of glutaraldehyde, cationic surfactants and tetrahydroxy methyl phosphonium sulfate by synergism with copper sulfate to become more effective biocides. The antimicrobial activities against sulfate-reducing bacteria in oil fields were determined.

*Experimental:**Materials:*

Commercially glutaraldehyde (50%), Benzalkonium (80%) and tetra hydroxyl methyl phosphoniumsulphate, THPS (75%) were purchased from Morgan Company, Cairo, Egypt. The copper sulfate was supplied from El Gomhoria Trade Pharmaceuticals & Chemicals Co. and Bacterial growth media were obtained from Intertekcompany.

Take water sample to Qarun base to perform water complete analysis for the separated water and from the report of water analysis it was clear by the following:-

- 1- No dissolved oxygen found.
- 2- The sulphate value is high that leads to an indication for the presence of SRB.
- 3- TDS and pH are suitable for SRB survive.

*Preparation of Mixtures:*

In this study we prepare four mixtures as the following:

*a. Gluteraldehyde/ Copper sulfate:*

Gluteraldehyde was supplied with concentration of 50 %. The copper sulfate was added with concentration of 0.0, 1.0, 2.5, 5.0 and 7.5%.

Gluteraldehyde mixture was labeled by G<sub>0</sub>, G<sub>1</sub>, G<sub>2.5</sub>, G<sub>5</sub> and G<sub>7.5</sub>.

*b. Cationic with Copper sulfate:*

Tetradecyl dimethyl benzyl ammonium chloride (Benzalkonium) was supplied with concentration of 50 %. The copper sulfate was added to Benzalkonium solution with concentration of 0.0, 1.0, 2.5, 5.0 and 7.5% to form C<sub>0</sub>, C<sub>1</sub>, C<sub>2.5</sub>, C<sub>5</sub> and C<sub>7.5</sub>.

*c. THPS with Copper sulfate:*

THPS is supplied with concentration of 50 %, The copper sulfate was added to THPS solution with concentration of 0.0, 1.0, 2.5, 5.0 and 7.5% to form T<sub>0</sub>, T<sub>1</sub>, T<sub>2.5</sub>, T<sub>5</sub> and T<sub>7.5</sub>.

*Synthesis of copper nanoparticles with gluteraldehyde:*

The four steps preparation scheme for copper nanoparticles starts with:-

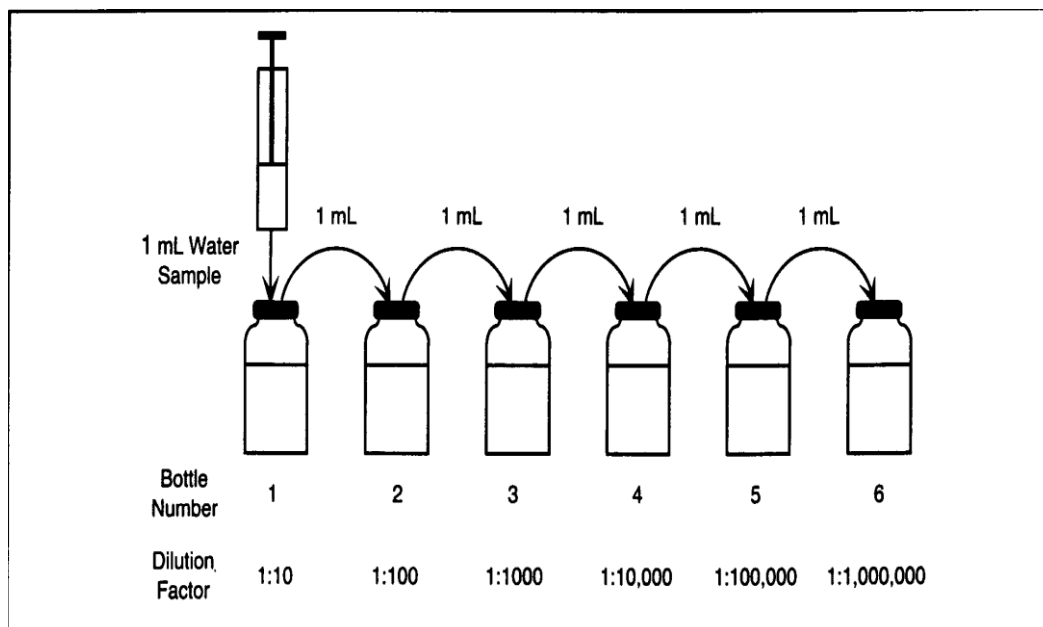
- 1-Dissolving copper (II) sulfate pentahydrate salts in deionized water to obtain a blue solution.
- 2- Gluteraldehyde added to the aqueous solution containing the copper salt while vigorously stirring, in this step the solution changed from blue to white.
- 3-Ascorbic acid (0.02M) and sodium hydroxide (0.1M) were dissolved in water and added to the synthesis solution, color change occurred in the aqueous solution from white to yellow.
- 4-A solution of NaBH<sub>4</sub> (0.1M) in deionized water was prepared and added to the solution under continuous rapid stirring, an instant color change occurred in the aqueous phase from yellow to black/red. The appearance of this dark color indicated that the reduction reaction had started. The source of electrons for the reaction was BH<sub>4</sub><sup>-</sup> the mixture was further stirred rapidly for around 10 min in ambient atmosphere, to allow the reaction to complete.

*Antimicrobial Activity Measurements:**I. Sample Collection:*

The samples (water-oil mixture) were collected from Qarun Petroleum Company (QPC) at western desert, Egypt. The sample collected from lagoon at karama field (this lagoon is the open drain pit for the main storage oil tanks at karama field) and the temperature ranged from 28 to 32 °C oil and water were separated from sample by keeping it at 40 °C for 60 min and allowing the water to settle at the bottom of the bottle. Samples were collected in 500 mL of anaerobic sterilized serum bottles. Samples were analyzed on site for pH, conductivity, dissolved oxygen and chlorine residual.

## II. Biocidal Test:

The inhibition activity on the growth of SRB at different doses of synergistic mixtures was measured and SRB counts were determined by the most probable number (MPN) technique using bacterial growth media Figure 1. SRB-contaminated water from Qarun Petroleum Co. (Western Desert, Egypt) was used for the test which conducted according to the ASTM D4412-84 (ASTM, 2009). The prepared water was subjected to growth of about 1,000,000 bacteria cell/ml. Three mixtures biocide samples were tested by dose of (200, 400, and 600, ppm by weight) and the system was incubated to contact time of 3.0 hrs; and system was cultured in SRB media for 21 days at 37 °C.

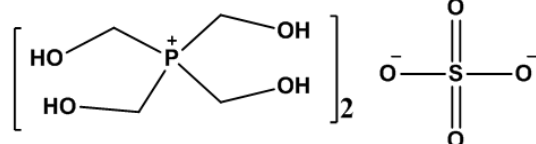
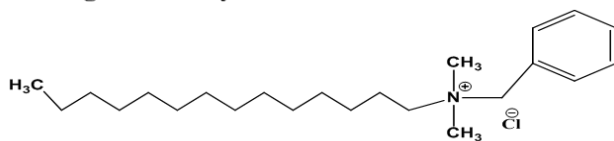
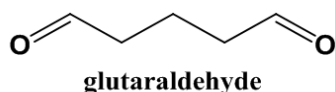


**Fig. 1:** Extinction dilution technique.

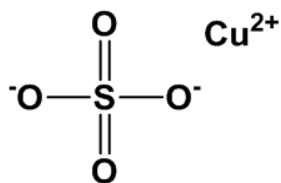
## Results and Discussion

### Structure:

The chemical structures of the used biocide compounds are showed below.



Tetrakis (hydroxymethyl) phosphonium sulfate (THPS)



### Copper sulfate

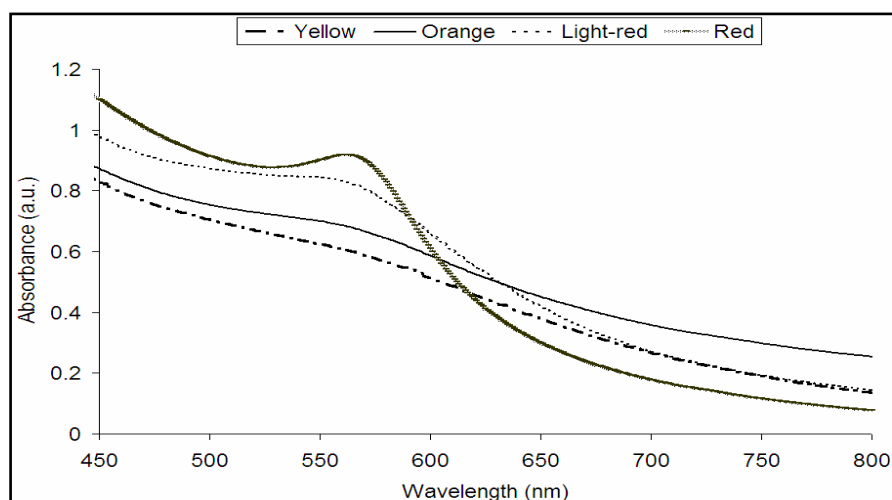
Characterization of the prepared nanoparticle of copper sulfate

#### i. Optical characterization:

Small metal nanoparticles exhibit the absorption of visible electromagnetic waves by the collective oscillation of conduction electrons at the surface (Link *et al.*, 2000). This is known as the surface plasmon resonance effect. The interest in this effect is the possibility of using it as a tracer for the presence of metal nanoparticles with a simple UV-visible spectrometer.

The size dependence of the Plasmon resonance for particles smaller than 20 nm (for gold) (Link *et al.*, 2000) is a complex phenomenon. One interesting feature is the increase in the bandwidth of the resonance with the decrease in the size of the particles due to electron scattering enhancement at the surface. The shift in the resonance and the variation in its bandwidth are thus interesting parameters to characterize the metal nanoparticles. Several samples were taken from the synthesis solution over time: one just after pouring the ascorbic acid solution, the second just before pouring the NaBH<sub>4</sub> solution and then at 5 and 10 min afterwards, as shown in Figure 2. Plasmon absorbance (562 nm) appears only when the solution is red (roughly 10 min after the strong reducing agent was added), although absorption already increased after 5 min, which suggests the appearance of small clusters or nanoparticles.

Before the addition of NaBH<sub>4</sub>, the yellow and orange solutions did not show plasmon resonance. Upon the addition of NaBH<sub>4</sub>, a quick increase in the absorbance at low wave lengths occurred that probably indicated the onset of particle formation (light red). The plasmon resonance of the Cu nanoparticles appeared at 562 nm when the solution turned red. The reaction was allowed to proceed in air. After the end of synthesis, the solution was kept under ambient atmosphere and the oxidation was qualitatively monitored with time by observation its color change. Within 8 h, the solution turned black to violet and ultimately blue particles appeared (Figure3)



**Fig. 2:** Plasmon resonance of copper sulfate nanoparticle



**Fig. 3:** (1) Freshly prepared red Cu sol -&(2) black &(3) violet and (4) upon onset oxidation

*ii. Effect of time:*

Time is a very important parameter in nanoparticle synthesis. As an empirical rule, the availability of a larger number of nuclei at a given time induces a decrease in the nanoparticle size, because smaller metal nuclei grow and consume metal ions at the same time. To study the effect of the reaction time during synthesis on the formation of product and the stability of copper nanoparticles, all of the samples in **Table 1** were prepared according to the procedure described, with the only variable being the duration of stirring with ascorbic acid before pouring the sodium borohydride.

**Table 1:** Plasmon resonance after synthesis and qualitative stability duration as a function of reaction time during synthesis

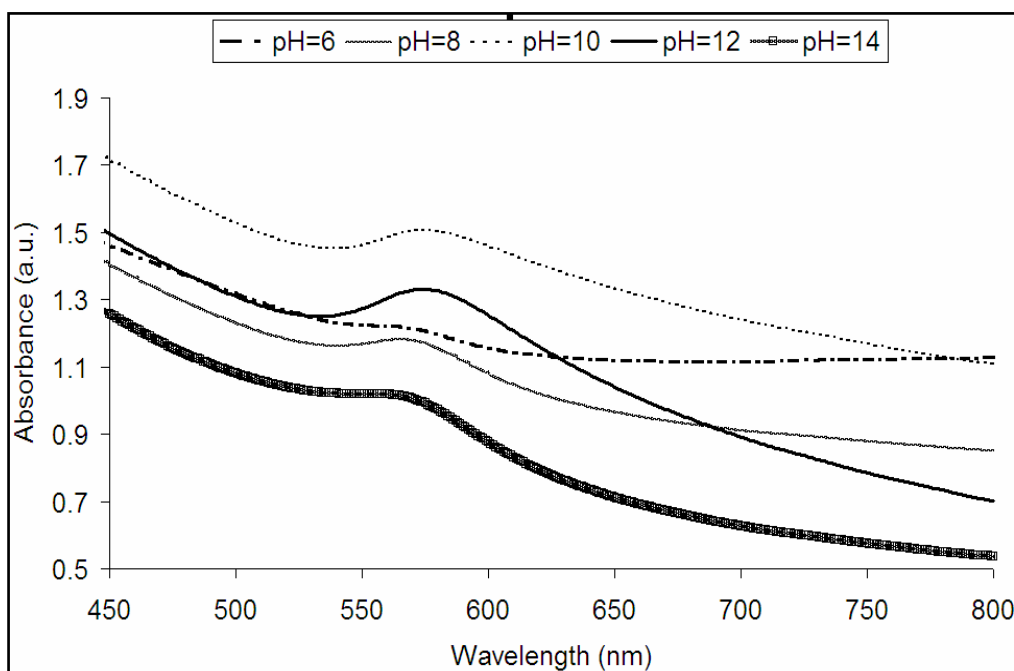
Sample	Reaction time (min)	$\lambda_{max}$ (nm)	Color of solution	Stability time*
1	15	572	Black	4 days
2	30	573	Black	2 days
3	60	562	Red	5 days
4	90	-	Black	4 days

\*Note: The stability time is the time when the solution turns from red/black to blue (oxidation).

At the moment, the mechanism associated with this phenomenon is not well understood. Ascorbic acid is well known to scavenge free radicals and thus provide an antioxidant action during copper nuclei formation. This provides the right conditions for subsequent rapid reduction by  $\text{NaBH}_4$  and copper nanoparticle completion. The red color characteristic of well-defined copper metal nanoparticles is essentially obtained at 60 min and is much darker at other times. It also appears that these particles present the longest time for stability under ambient atmosphere. The mechanism responsible for the change in color remains unclear: oxidation, redissolution of the particles, or both at the same time.

*iii. Effect of pH:*

The pH in aqueous media has an influence on the progress of the copper reduction reaction. The probable kinetic enhancement could also be conducive to a reduction in crystallite size because of the enhancement of the nucleation rate. The use of higher concentrations of ascorbic acid induced a reduction in the solution pH, which was adjusted back in the range from 6 to 14 with the drop wise addition of 0.1M NaOH solution. This probably indicates very small particles at this low pH. Plasmon resonance is clearly visible for pHs from 8 to 12. At pH 14, the peak is still detectable but much weaker. The measured values are 566, 575, 573 and 554 nm for pHs from 8 to 14, Figure 4.



**Fig. 4:** Plasmon resonance of copper sulfate nanoparticle at different pH

*Biocidal Activity of the studied biocide/ copper sulfate mixtures:*

Antimicrobial activity of the studied mixtures against sulfate-reducing bacteria (SRB) sessile bacteria accelerate corrosion processes in several ways. They accelerate the pitting corrosion by removing the corrosion by product atomic hydrogen from the cathode surface. In removing hydrogen, bacteria depolarize the surface and allow corrosion reactions to continue. Sulfate reducing bacteria (SRB) produces  $H_2S$  which increases the corrosiveness of brine, causing metals to crack and blister. In addition, bacterially produced  $H_2S$  reacts with iron that is solubilized from the anode, thereby removing another corrosion by product to accelerate the corrosion process. Acid-producing bacteria produce acids that remove passivating oxide films from surfaces. Operational problems that are typically caused by bacteria are an increasing frequency of corrosion failures,  $H_2S$  concentrations, reservoir souring, rapid production decline, metal sulfide scales, failure of down hole equipment due to metal sulfide deposits, inefficiency of oil/water separation, In efficiency of heat exchange, black water, black powder in gas transmission lines, filter plugging and loss of injectivity. Bacterially produced  $FeS$  causes plugging problems in production wells, down hole equipment, pumps, surface facilities, filters, and at sand faces in injection wells. As discussed above, SRB produce  $H_2S$  that can react with dissolved metals such as iron, zinc, and lead to the production of insoluble metal sulfides. These *et al* sulfide particles collect throughout production facilities, but are not able for being responsible for failures of down hole pumps, frequent replacements of cartridge and sand filters in surface facilities, and loss of the quantity of injected and disposal water. Plugging of injection and disposal wells is a problem that is often underestimated by many production personnel. Bacterial cells, biofilm fragments, and metabolic by products, such as iron sulfide, constitute a large percentage of the total suspended solids that are filtered or injected into a formation. The result is a solid mass of organic and inorganic matter that can significantly reduce injectivity (Gonzalez *et al.*, 2002). As a result of the numerous problems caused by bacteria in oil and gas production operations, energetic measures have been taken to monitor and control bacterial populations.

Measures to monitor bacteria are not usually considered until after corrosion failures point to MIC (microbially induced corrosion). By the time that MIC is discovered, extensive and costly damage to the operating systems has often already occurred. Monitoring is often conducted in sweet systems with no hint of bacterial contamination to ensure that bacterial populations are under control and that operating costs and risks (health, safety, environmental, and mechanical failures) cannot be lowered by initiating an effective biocide program (Down *et al.*, 2002). The antimicrobial activity of the three studied biocides against SRB was determined by a serial dilution method at dosages of (200, 400 and 600 ppm by weight) and the results are listed in Tables (2-4). The three studied synergistic mixtures were applied as biocides against sulfate-reducing bacteria (*Desulfomonas pigra*) showed imposing results due to their relatively high efficiency against this type of bacteria. Tested of glutaraldehyde ( $G_0$ ) and glutaraldehyde-copper sulfate formulations ( $G_1$ ,  $G_{2.5}$ ,  $G_5$  and  $G_{7.5}$ )

as biocide at different doses (200, 400 and 600) was presented in Table (2) we observed that glutaraldehyde kill the all strain of SRB within 3h at dose 600 ppm (Figure 5) and has low efficiency at the lowest concentrations (200, 400 ppm by weight). while in case of synergism between glutaraldehyde and four ratio of copper sulfate (1, 2.5, 5 and 7.5%) we observed that there are more effect on the growth of SRB bacteria with 1% copper sulfate at different doses where in case 2.5% and 5% from copper sulfate we observed high effect on the killing all SRB strain at low and high doses (200, 400 and 600) the high efficiency produced from copper sulfate because the biocidal action of copper is thought to relate to damage to plasma membranes, site-specific Fenton reaction-mediated damage to nucleic acids, and disruption of essential sulphhydryl group-containing proteins. More recent work also demonstrates the biocidal activity of solid copper surfaces for both HAI organisms and mycobacteria (Borkow *et al.*, 2005; Wilks *et al.*, 2005; Faundez *et al.*, 2004; Noyce *et al.*, 2006). While in high ratio from copper sulfate (7.5%) has little effect on the struggle of SRB this because in high concentration from copper sulfate the mixtures become in soluble and effect on inhibition efficiency of biocide.

In case second mixtures (cationic surfactants and copper sulfate) Table (3) the cationic surfactant has weak little effect on killing of SRB strain at different doses (200, 400 and 600) see Figure (6) and the biocidal efficiency improved gradually with increase additives from copper sulfate and reach to the maximum efficiency which kill all SRB bacteria within 3 h at all tested doses (200, 400 and 600) at ratio 7.5%.

The results of the third mixtures (THPS and copper sulfate) presented in Table 4. The effect of THPS on the killing of SRB bacteria illustrated in Figure 7, The THPS has little effect at low dose (200 and 400 ppm) and has high effect and ability to killing all SRB bacteria at high dose (600 ppm) his effect produced from chemical structure of THPS and the positive charges in the molecules neutralize the negative charges on the bacterial cell membranes. Accordingly, the selective permeability which characterizes the outer cellular membrane is completely deactivated hence; the vital transportation of essential components for cell bioreactions and activities is disturbed, causing death to these microorganisms (Jamora *et al.*, 2001; Shaban *et al.*, 2013). Enhancement the effect of THPS at low concentration (200 and 400 ppm) by synergism with copper sulfate by different percent (1, 2.5, 5, and 7.5 %) and the results of synergism declare that there are enhancement in the biocidal effect of THPS at low doses and reach to the high efficiency at percent 7.5% from copper sulfate which kill all SRB strain.

**Table 2:** Evaluation of the studied mixtures ( $G_0$ ,  $G_1$ ,  $G_{2.5}$ ,  $G_5$  and  $G_{7.5}$ ) as antimicrobial against sulfate reducing bacteria

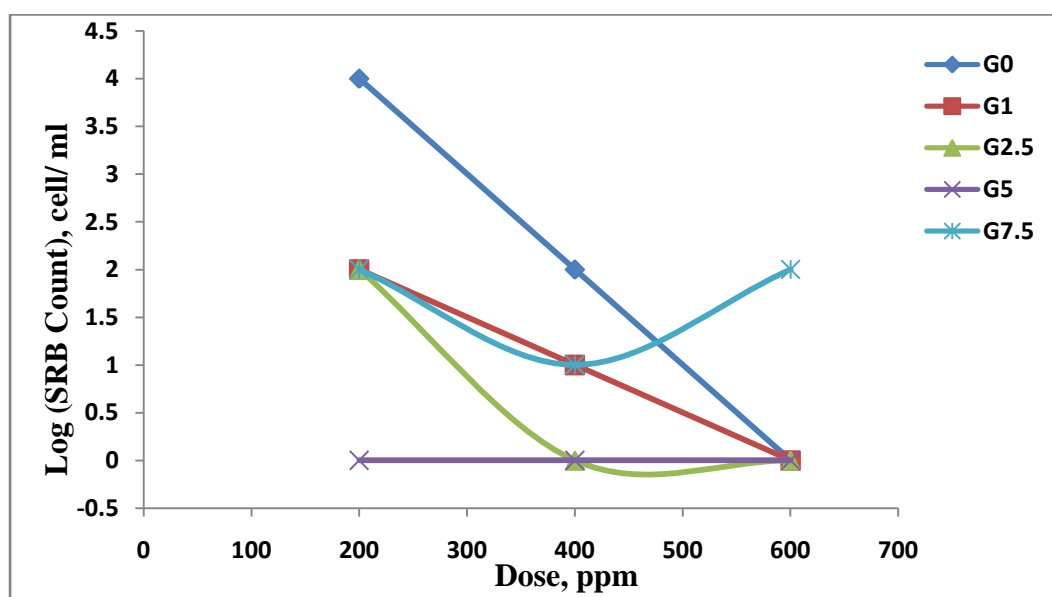
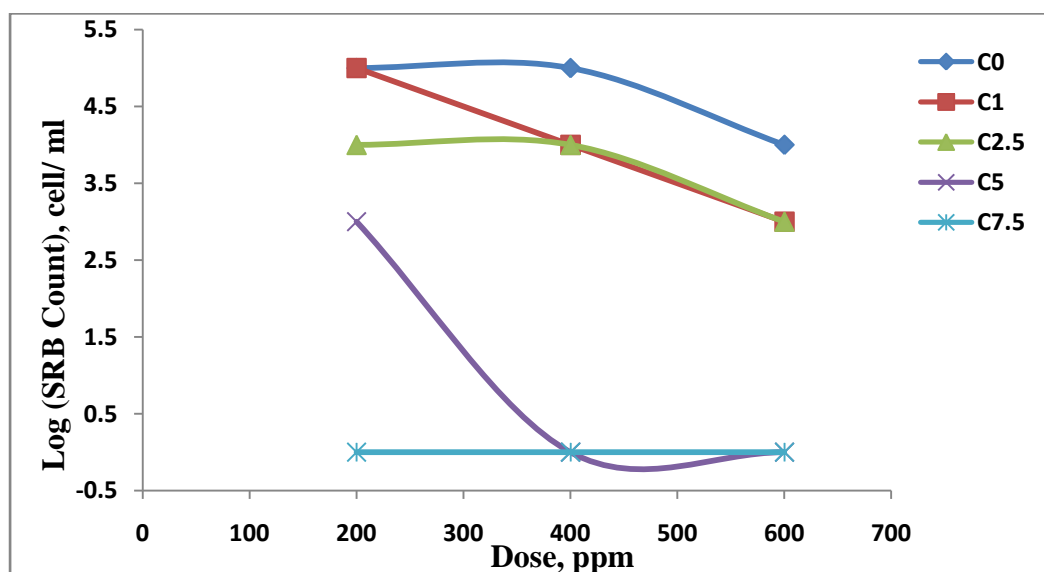
Type	Dose, ppm		
	200	400	600
Glutaraldehyde ( $G_0$ )	$10^4$	$10^2$	Nil
Glutaraldehyde with 1% $\text{CuSO}_4$ ( $G_1$ )	$10^2$	$10^1$	$10^1$
Glutaraldehyde with 2.5% $\text{CuSO}_4$ ( $G_{2.5}$ )	Nil	Nil	Nil
Glutaraldehyde with 5% $\text{CuSO}_4$ ( $G_5$ )	Nil	Nil	Nil
Glutaraldehyde with 7.5% $\text{CuSO}_4$ ( $G_{7.5}$ )	$10^2$	$10^1$	$10^2$

**Table 3:** Evaluation of the studied mixtures ( $C_0$ ,  $C_1$ ,  $C_{2.5}$ ,  $C_5$  and  $C_{7.5}$ ) as antimicrobial against sulfate reducing bacteria

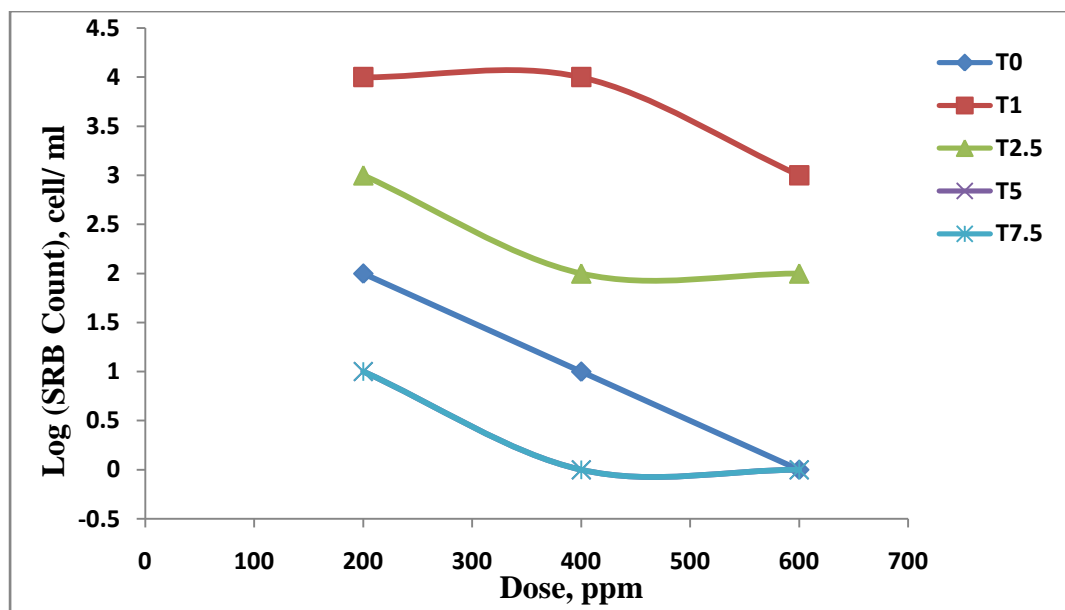
Type	Dose, ppm		
	200	400	600
Cationic ( $C_0$ )	$10^5$	$10^5$	$10^4$
Cationic surfactant with 1% $\text{CuSO}_4$ ( $C_1$ )	$10^5$	$10^5$	$10^4$
Cationic surfactant with 2.5% $\text{CuSO}_4$ ( $C_{2.5}$ )	$10^4$	$10^4$	$10^3$
Cationic surfactant with 5% $\text{CuSO}_4$ ( $C_5$ )	$10^3$	Nil	Nil
Cationic with 7.5% $\text{CuSO}_4$ ( $C_{7.5}$ )	Nil	Nil	Nil

**Table 4:** Evaluation of the studied mixtures (T<sub>0</sub>, T<sub>1</sub>, T<sub>2.5</sub>, T<sub>5</sub> and T<sub>7.5</sub>) as antimicrobial against sulfate reducing bacteria

Dose, ppm	200	400	600
Type			
Tetrahydroxy methyl phosphonium sulfate (T <sub>0</sub> )	10 <sup>2</sup>	10 <sup>1</sup>	Nil
Tetrahydroxy methyl phosphonium sulfate with 1% CuSO <sub>4</sub> (T <sub>1</sub> )	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>3</sup>
Tetrahydroxy methyl phosphonium sulfate with 2.5% CuSO <sub>4</sub> (T <sub>2.5</sub> )	10 <sup>3</sup>	10 <sup>3</sup>	10 <sup>2</sup>
Tetrahydroxy methyl phosphonium sulfate with 5% CuSO <sub>4</sub> (T <sub>5</sub> )	10 <sup>1</sup>	Nil	Nil
Tetrahydroxy methyl phosphonium sulfate with 7.5% CuSO <sub>4</sub> (T <sub>7.5</sub> )	10 <sup>1</sup>	Nil	Nil

**Fig. 5:** Effect of mixtures dose (G<sub>0</sub>, G<sub>1</sub>, G<sub>2.5</sub>, G<sub>5</sub> and G<sub>7.5</sub>) on sulfate reducing bacteria growth**Fig. 6:** Effect of mixtures dose (C<sub>0</sub>, C<sub>1</sub>, C<sub>2.5</sub>, C<sub>5</sub> and C<sub>7.5</sub>) on sulfate reducing bacteria growth





**Fig. 7:** Effect of mixtures dose (T<sub>0</sub>, T<sub>1</sub>, T<sub>2.5</sub>, T<sub>5</sub> and T<sub>7.5</sub>) on sulfate reducing bacteria growth

*Biocidal Activity of copper nanoparticles with glutaraldehyde:*

Metal nanoparticles, due to their special properties and also small dimensions, find important applications in optical, magnetic, thermal, electronic and sensor devices, SERS (surface enhanced Raman scattering), catalysis, etc. Almost all properties of nanoparticles are due to their small sizes. Over the past few decades, inorganic nanoparticles, whose structures exhibit significantly novel and improved physical, chemical, and biological properties, phenomena, and functionality due to their nanoscale size, have elicited much interest. Nanophasic and nanostructure materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity, especially in biological and pharmaceutical applications (Theivasanthi *et al.*, 2011).

Nanomaterials are the leading in the field of nanomedicine, bionanotechnology and in that respect nanotoxicology research is gaining great importance. The US Environmental Protection Agency (EPA) has approved registration of copper as an antimicrobial agent which is able to reduce specific harmful bacteria linked to potentially deadly microbial infection. In addition, no research has discovered any bacteria able to develop immunity to copper as they often do with antibiotics. The emergence of nanoscience and nanotechnology in the last decade presents opportunities for exploring the bactericidal effect of metal nanoparticles.

Copper nanoparticles with glutaraldehyde were applied as biocides against sulfate-reducing bacteria (*Desulfomonas*) showed good results compared to normal copper with glutaraldehyde at different doses (200, 400 and 600) Table (5) we observed that glutaraldehyde kill the all strain of SRB within 3h at dose 600 ppm (Figure 8) and has low efficiency at the lowest concentrations (200, 400 ppm by weight). The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution. A cell wall is present around the outside of the bacterial cell membrane and it is essential to the survival of bacteria. It is made from polysaccharides and peptides named peptidoglycon. There are broadly speaking two different types of cell wall in bacteria, called gram-positive and gram negative Figure 9. The names originate from the reaction of cells to the gram stain, a test long-employed for the classification of bacterial species. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan. In contrast, gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan. Surfaces of copper nanoparticles affect interact directly with the bacterial outer membrane, causing the membrane to rupture and killing bacteria.

**Table 5:** Evaluation of the studied copper nanoparticles with glutaraldehyde as antimicrobial against sulfate reducing bacteria growth

sample	Dose, ppm	SRB Count, cell/ ml
copper nanoparticles with glutaraldehyde	200	10 <sup>3</sup>
	400	10
	600	Nil

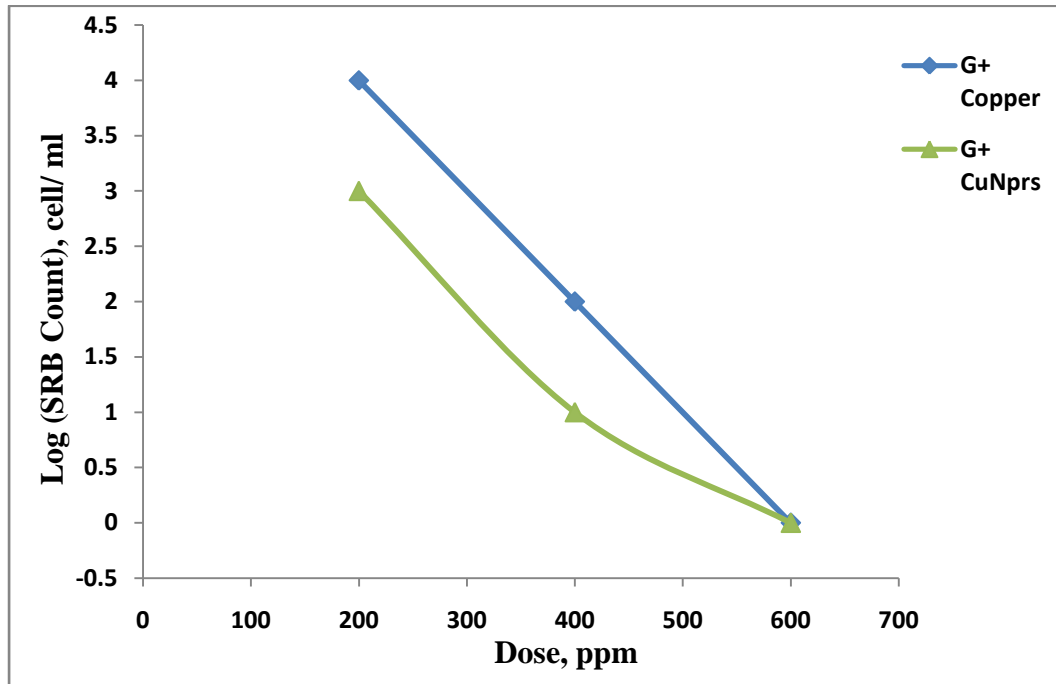


Fig. 8: Effect of copper and copper nanoparticles on sulfate reducing bacteria growth

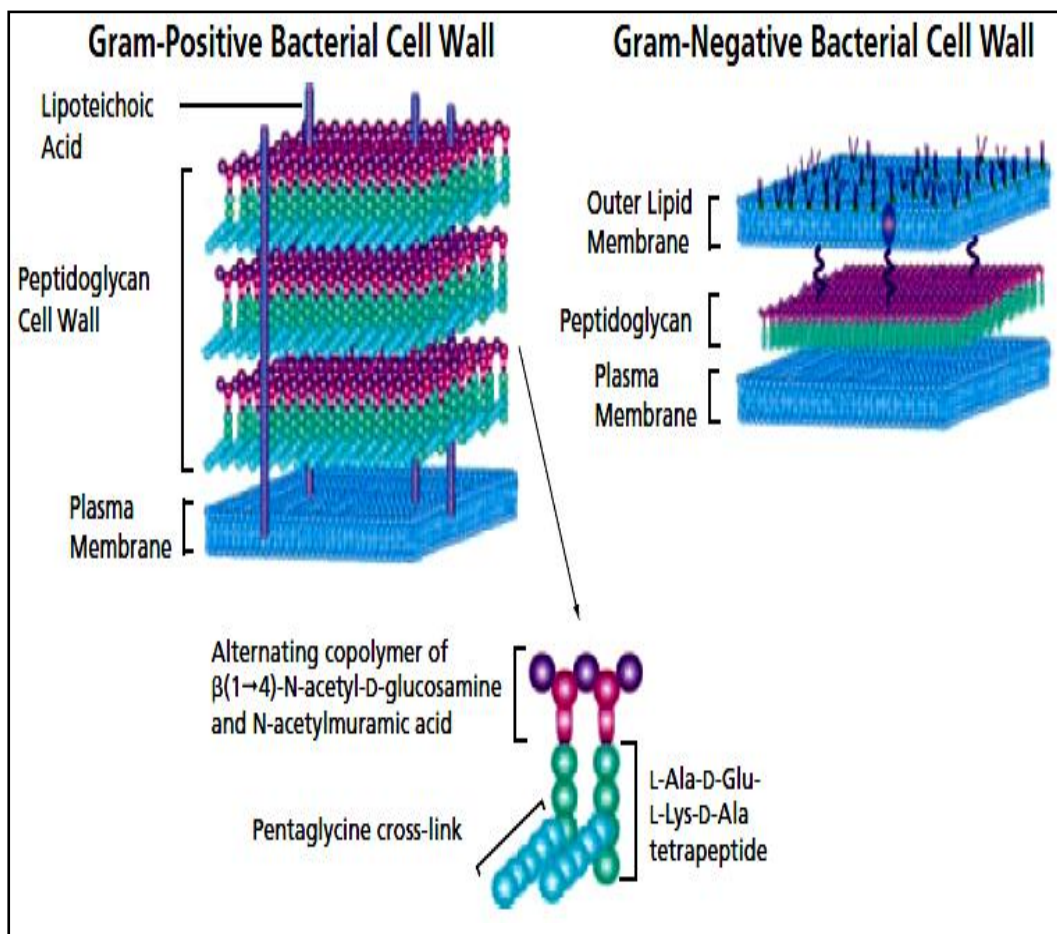


Fig. 9: Structure of the bacterial cell walls

**Conclusion:**

The antimicrobial activity of the formulated biocide mixtures showed high killing for sulfate reducing bacteria at different doses and contact time. The biological activity of nanoparticles of metals depends directly on their physical and chemical properties, therefore copper nanoparticles with glutaraldehyde are good results as biocide.

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