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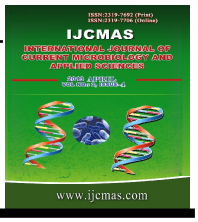

Original Research Article

The Antibacterial Activity of Activated Carbon, Silver, Silver Impregnated Activated Carbon and Silica Sand Nanoparticles against Pathogenic *E. coli* BL21

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Keywords

Antibacterial activity; activated carbon; silver nanoparticles; silica sand; *E. coli* BL21.

A B S T R A C T

The ability of the activated carbon, silver impregnated activated carbon, and silica sand to eliminate and destroy water borne *E. coli* BL21 were tested under plate assay and shake flask technique. Silver nanoparticles showed the highest antibacterial effect against E. *coli* BL21 with inhibition zone diameter 18 mm, on using the shake flask technique it was proved that bacterial count started to be reduced after one hour of incubation, while no bacterial growth was detected after 2,3 and 24 hours using the activated carbon. Bacterial growth was completely inhibited on using silver impregnated activated carbon at all the tested concentrations after one hour of incubation.

Introduction

One of the most widely used nanoparticles for water purification is activated carbon due to its large surface area and high adsorption capacity (Ortiz-Ibarra, *et al*., 2007). Activated carbon has proven to remove bacteria like *Pseudomonas aeruginosa* and *Escherichia coli* from fresh and potable water systems (Percival and Walker, 1999; Quinlivan, *et al*., 2005). Despite electrostatic repulsion between negatively charged microorganisms and carbon surfaces, microorganisms attach to activated carbon particles through strong Lifshitz–van derWaals forces (Jucker, *et al*., 1996). Potable water systems are considered low in ionic strength so electrostatic interactions can offer the possibly of enhancing the efficacy of activated carbon to remove microorganisms from water by positive charge modification of the carbon surfaces. Once there is a charge reversal, the electrostatic attraction between negatively charged microbial cell surfaces and positively modified carbon particles

will be strong (Bos, *et al*., 1999; Shi *et al*., 2007). Moreover, modification in the activated carbon particles by coating with a quaternary ammonium compound gives the activated carbon particles bactericidal properties (Shi *et al*., 2007) and decreases the possibility of biofilm growth. In addition to the microorganisms charge, the hydrophobicity of the surfaces that come in contact with microbes is important in adhesion (Bos, *et al*., 1999).

Nanoparticles have attracted great interest in their development as potential antibacterial drugs (Hsiao *et al*., 2006), which has also been reported that many biophysical interactions occur between silver nanoparticles and bacteria including biosorption, nanoparticles decomposition and cellular uptake, with effects bacterial cell membrane damage and toxicity (Priester, *et al*., 2009; Brayner, *et al*., 2006).

In Europe, silver has been used as a water disinfectant and has shown effectiveness against planktonic bacteria (Silvestry-Rodriguez, *et al*., 2007). Silver has gained this efficacy through its binding to disulfide or sulfhydryl groups present in the cell wall proteins (Feng *et al*., 2000). Silver has been shown to bind DNA in the nucleus thus causing cell death.

One draw back for the use of activated carbon is the lack of reversibility (Sheintuch and Matatov-Meytal, 1999), uncertainty when determining whether capacity has been reached. Silica based nanoparticles used as immobilizers have shown to enhance the non-selective capture of organic contaminants from wastewaters, thus increasing the time of contact between microorganisms and substrate resulting in enhanced biodegradation (Pedrazzani, *et al*., 2004)

The main objective of the present study is to evaluate the antibacterial efficacy of the activated carbon nanoparticles, silver impregnated activated carbon, and silica sand against waterborne pathogenic *E.coli* strain, where the combination of the activated carbon and silver would take an important antibacterial advantage, due to the strength of these two nanoparticles and these materials used widely for application in water purification.

Materials and Methods

Microorganism

A standard *Escherichia coli* BL21 was kindly provided by MIRCENC (Microbiological Resource Center, Ain Shams University-Egypt), maintained on Nutrient Agar slants and stored at 4°C with regular transfers at monthly intervals. For long preservation, the slants were folded with 25% glycerol.

Raw Materials

Activated carbon was washed with deionized water and modified by heat treatment (Yin 2007), Silver Nitrate, $AgNO_3$ (99%, Sigma Aldrich), sodium borohydride ($NaBH_4$, Sigma Aldrich), The M-Endo agar medium and Macconkey agar medium (Sigma Aldrich) Pure Sand (Sigma Aldrich), deionized water of high purity (Beirut Arab University) was used in all experiments. Silica sand was supplied by Soliver glass production company (Chweifat, Lebanon).

Preparation of seed culture

Transfers from single slant cultures (48 hours old) were taken into 50 ml aliquots of the seed medium containing (g/l): beef extract, 1; yeast extract, 2; peptone, 5; sodium chloride, 5 and 1 liter of distilled water. Dispensed in 250 ml Erlenmeyer flasks to initiate growth (OD<1). Standard inoculam of 2% (v/v) were taken from the latter liquid culture after growth for 18 hours at 30°C ± 2 on a reciprocal shaker to start growth in the fermentation flask which is equivalent to 3×10^8 colony forming unit (CFU/ml) according to McFarland scale 0.5.

Preparation of silica sand

Silica sand (50g) was rinsed using de ionized water and then treated with sodium acetate, sodium dithionate and sodium citrate to remove iron ions and hydrogen peroxide to remove organic matters. The silica sand was then saturated with Na^+ using 1M phosphate-buffered saline (pH 7.0), sterilized and stabilized by extensive washing with sterilized de-ionized water (Chen and Zhu, 2005).

Preparation of silver nanoparticles

Silver nanoparticles were prepared according to the chemical reduction method adapted by Fang, *et al*., (2005). 50 ml of $1x10^{-3}$ M silver nitrate was prepared, and then heated till boiling and 5 ml of 1% tri-sodium citrate added drop by drop. The solution was mixed vigorously and heated until the colour changed to pale brown followed by stirring until cooled to room temperature. The aqueous solution was air dried up to 4 days so as to obtain a powdered form of sliver nanoparticles.

Activated carbon impregnation with silver nanoparticles

Activated carbon (1g) was added to 20 ml of different $AgNO_3$ concentrations (a) 0.1; b) 1 and c) 1.5 mol.L^{-1}) one at a time. After 24 hour of impregnation in the dark, the powder samples were washed with water to remove loosely adsorbed $AgNO_3$, until no $AgNO_3$ was observed in the filtrate. The powder samples collected after decantation was air-dried until the next day. By adding 10 ml of 0.2 mol L^{-1} $NaBH_4$, impregnated $AgNO_3$ was chemically reduced (over 24h) to form Ag particles, then it was washed with water to remove the excess $NaBH_4$ followed by drying (Bandyopadhyaya, *et al*., 2008).

Antibacterial test

Impregnated activated carbon, silver nanoparticles and silica sand were tested for their antibacterial effect against waterborne pathogenic *E.coli* BL21 under test. If this organism is killed, as a standard, all other borne-disease-causing organisms are assumed killed.

(a) Plate Assay Method (qualitative test)

Melted M-Endo Agar medium was fortified with $3x10^8$ CFU/ml medium of *E .coli* BL 21equivalent to 0.5 Mcfarland. About 20 ml of the previously prepared seeded agar was then dispensed in petridishes, solidified by refrigerating for 4 to 6 hours. Seven mm diameter holes were made in the seeded agar using a sterilized cork borer. 25 mg of different nanoparticles under test were added one at a time in these holes, using one to two drops of sterilized water. They were left at 4°C for 1 hr then incubated at 37 □C for 24 hours and the antibacterial effect was

measured referring to the inhibition zone diameter.

(b)Shake flask test in saline (Quantitative test)

For the shake flask test, 50 ml of sterile saline (0.9% NaCl) was inoculated with 1 ml bacterial suspension ($3x10^8$ CFU/ml) equivalent to 0.5 Mc Farland. 50 mg of different nanoparticles were added to the flasks, one at a time and the contents were stirred on a rotary shaker at ambient temperature. The samples were drawn periodically (0, 1, 3 and 24 hours) from the flask and tested for the number of surviving *E.coli* by plate count method on M-Endo agar, using standard procedures. The percentage reduction of *E. coli* counts, were obtained after treatment with several nanoparticles according to this formula:

% Reduction of bacterial count=
((Viable count at $time_0$ - Viable count at time $_x$)/ Viable count at $time_0$)x100

Results and Discussion

Antibacterial test

(a) Plate Assay Method

Results in Table 1 showed the inhibitory effect of activated carbon, silver, silver impregnated activated carbon and silica sand. it was revealed that all the tested nanoparticles had an antibacterial effect exhibited by the diameters of the inhibition zones. Silver nanoparticles proved to be the most effective antibacterial agent against *E. coli* BL21 with an inhibition zone of 18 mm followed by silver impregnated activated carbon (AC-Ag $_a$) then AC-Ag $_b$, AC-Ag $_c$ and activated carbon. Silica sand showed the lowest antibacterial effect with an inhibition zone of 8mm. Waterborne *E. coli* BL21 adhere only weakly to different activated carbon particles, and the main difference between different types of activated carbons is the number of attractive sites revealed upon traversing of a carbon particle through the outer bacterial surface layer (Busscher, *et al*., 2008), while silica sand showed smaller inhibition zone which could be due to the texture and size of the sand granules since finer sand fractions were more efficient in bacterial removal than the coarse sand used in this test (Gargiulo, *et al*., 2007).

Table.1 Inhibitory effect of the nanoparticles under test against *E.coli* BL21, using plate assay method.

Nanoparticle used	Inhibitory zone diameter (mm)
Activated carbon	10.00
Silver	18.00
AC-Ag $_a$	11.00
AC-Ag $_b$	14.00
AC-Ag $_c$	16.00
Silica sand	8.00

In a trial to test the antibacterial effect of the tested nanoparticles against *E.coli* BL21 under shaken conditions, it was revealed that the number of non-adsorbed viable bacterial cells incubated with different nanoparticles reduced slightly within 1 hour after treatments with activated carbon, and silver impregnated activated carbon at different concentrations, and silica sand. However, after shaken for 3 hours, the total viable

E.coli count reached to zero with activated carbon and silica sand but with sand still have some bacterial colonies. Continuously, after contacting for 24 hours, the nanoparticles killed all bacterial cells and markedly proved to have bactericidal effect against pathogenic *E.coli* under test. Furthermore, As shown in Figure.2, The results showed slight difference in the percent reduction in *E. coli* count after treatment with the different nanoparticles under test, where 100% reduction in *E.coli* count was observed after 24 hours of contact with activated carbon and silica sand, as reported previously by Liu, *et al*., (2012). However, silica sand showed 100% reduction in bacterial count after 24 hours of contact (Figure. 2) as reported by Chen and Zhu (2005). For both materials, a large fraction of cell death occured in the first three hours of incubation.

The antibacterial effect of Silver impregnated activated carbon at different concentrations (a,b and c) against *E. coli* BL21 were tested. Results in table 2 showed no growth of bacteria starting one hour of incubation where the percentage reduction of the total viable count of *E.coli* was 100 % as shown in figure 2, while after 3 hours of incubation with activated carbon, 100% reduction in the bacterial count was reported. Therefore, the change after 1 hour of incubation with the nanoparticles under test is due to the fact that the loss of cell viability has approached the complete inactivation (Liu, *et al*., 2012). It was clear that the amount of silver particles on the activated carbon considered to be the main factor for the complete reduction in the total viable count of *E.coli* and responsible for the effective antimicrobial activity. The results are in agreement with that obtained by Zhou, *et al*., (2012), where silver nanoparticles inhibited *E. coli* and *Bacillus* sp. growth at low concentrations (0.1 µg/ml), as for: silver targets multiple components in the bacterial cell, and the mechanism behind its antibacterial activity is by weakening DNA replication and inactivating proteins, as a result, resistance to bacteria cannot easily develop (Zhou, *et al*., 2012).

Moreover, other researchers showed that activated carbon-Silver composite have a superior antibacterial activity towards *E. coli* due to the synergistic effect of activated carbon and silver nanoparticles (Sreeprasad and Pradeep, 2012).

In general, Most of the bacterial reduction and inactivation took place during the first three hours of incubation, and the mortality rate increases continuously with the increase of nanomaterial concentration and their antibacterial activities are time and concentration dependent (Liu *et al.*, 2012). Thus, Silver nanoparticles showed efficient antibacterial activity against pathogenic *E. coli* that was similar to that found by Rastogi, *et al.*, (2011).

The mechanism of inhibitory action caused by silver nanoparticles on *E.coli* BL21 is partially known, where some researchers reported that silver nanoparticles inhibit bacterial growth through binding to the thiol group leading to bacterial inactivation (Yan *et al*., 2012). Moreover, the Gram negative bacteria have a layer of lipopolysaccharides at the exterior that are composed of covalently linked lipids and polysaccharides; they lack strength and rigidity. Negative charges on the lipopolysaccharides are attracted towards the positive charges available on silver nanoparticles (Santoro, *et al*., 2007). The opposite charges attract

each other due to electrostatic forces. So once the nanoparticle comes in contact with the bacterial cell, it either inhibit the cell wall synthesis, damage the cytoplasmic membrane, inhibit nucleic acid and protein synthesis or inhibit specific enzyme systems which result in the complete bacterial inhibition (Sadeghi, *et al.,* 2010).The mechanism by which the nanoparticles are able to penetrate the bacteria is not understood completely, but previous studies suggested that when *E.coli* treated with silver, changes took place in its membrane morphology that produced a significant increase in its permeability affecting proper transport through the plasma membrane, leaving the bacterial cells incapable of properly regulating transport through the plasma membrane, resulting in cell death (Raffi, *et al*., 2008). Moreover, these studies showed that bacterial inhibition caused once silver nanoparticles penetrated inside the bacteria and caused damage by interacting with phosphorus and sulfur containing compounds such as DNA (Sadeghi, *et al*., 2010).

The antibacterial effect of activated carbon, silver nanoparticles, silver impregnated activated carbon and silica sand against waterborne pathogenic *E.coli* BL21 was obtained and compared. Plate assays and shake flask methods showed that silica sand had the lowest antibacterial activities compared to the other prepared nanoparticles.

However, impregnated activated carbon with silver at the highest concentration showed the maximum inhibitory effect against *E. coli* BL21. Therefore, higher concentrations of silver ions cause greater bactericidal effect and antibacterial activities are time and concentration dependant. Most of the bacterial cell number reduction occurred after three hours of incubation and the reduction rate was greatly influenced by the increase of silver ion concentrations and contact time. The present study demonstrates the potential of activated carbon composites for use in water purification and that silver impregnation is very effective in producing potable drinking water.

Table. 2 Total viable count as affected by the exposure time to different nanoparticles, using shake flask test.

Nanoparticles	Contact time (hr)	CFU/ml x10^4
Activated Carbon	0.0	97.0
	1.0	50.0
	3.0	23.0
	24.0	8.0
Silver	0.0	15.0
	1.0	4.0
	3.0	0.0
	24.0	0.0
AC-Ag $_a$	0.0	0.0
	1.0	0.0
	3.0	0.0
	24.0	0.0
AC-Ag $_b$	0.0	0.0
	1.0	0.0
	3.0	0.0
	24.0	0.0
AC-Ag $_c$	0.0	0.0
	1.0	0.0
	3.0	0.0
	24.0	0.0
Silica sand	0.0	56.0
	1.0	30.0
	3.0	15.0
	24.0	0.0

After contact for 24 hours, all nanoparticles killed all bacterial cells, proving their bactericidal effect against pathogenic *E. coli*.

Figure.1 Plate assay method for the detection of the Inhibitory effect of nanoparticles under test against *E.coli* BL2. **a:** activated carbon, **b:** AC-Aga, **c:** AC-Ag_b, **d:** AC-Ag_c and **e:** silica sand.

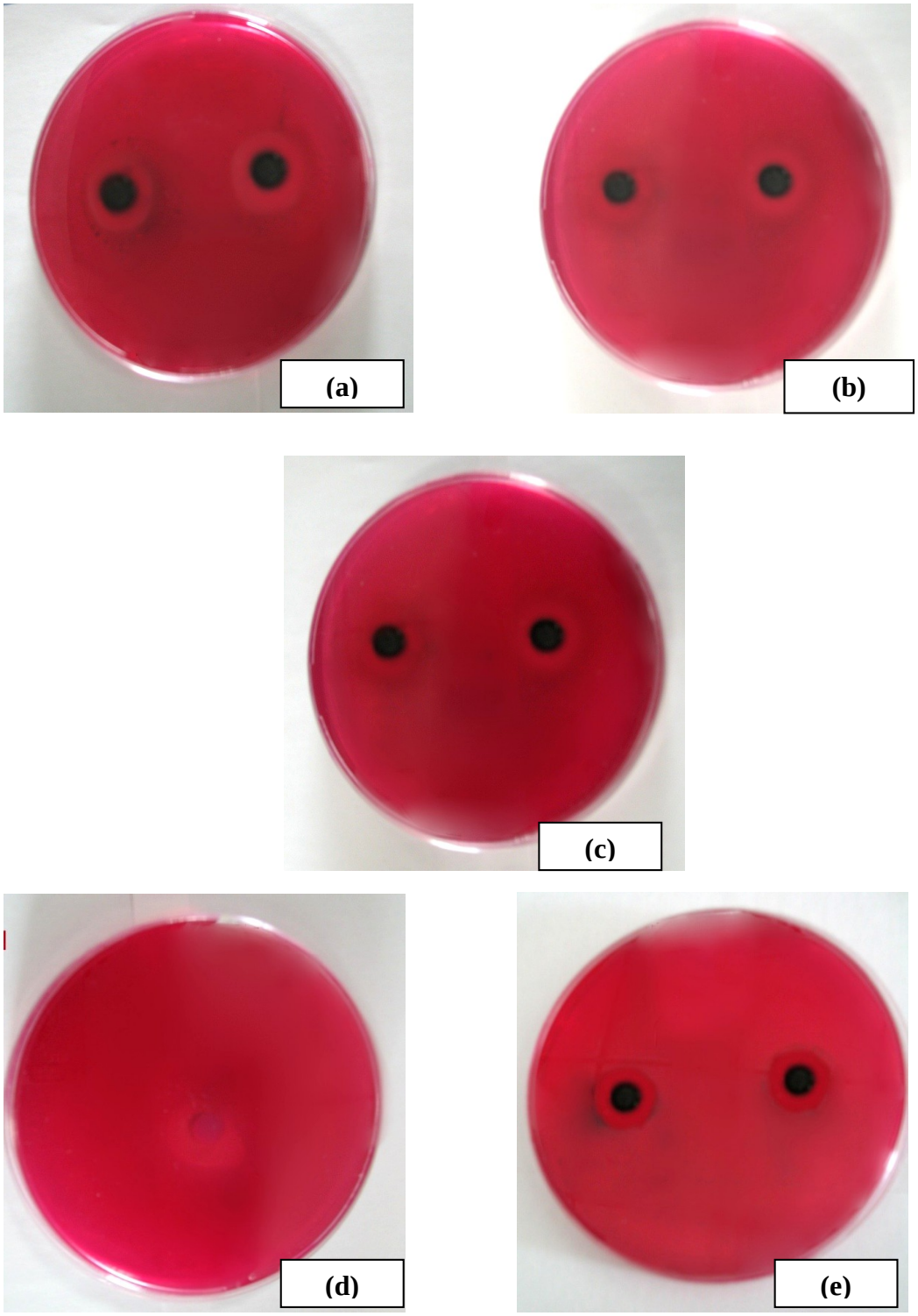

Figure.2 Reduction percentage of the total viable count of *E.coli* BL21 as affected by the exposure time to (a): activated carbon, (b): silver, (c): Ac-Ag $_a$, (d): Ac-Ag $_b$, (e): Ac-Ag $_c$ and (f): silica sand.

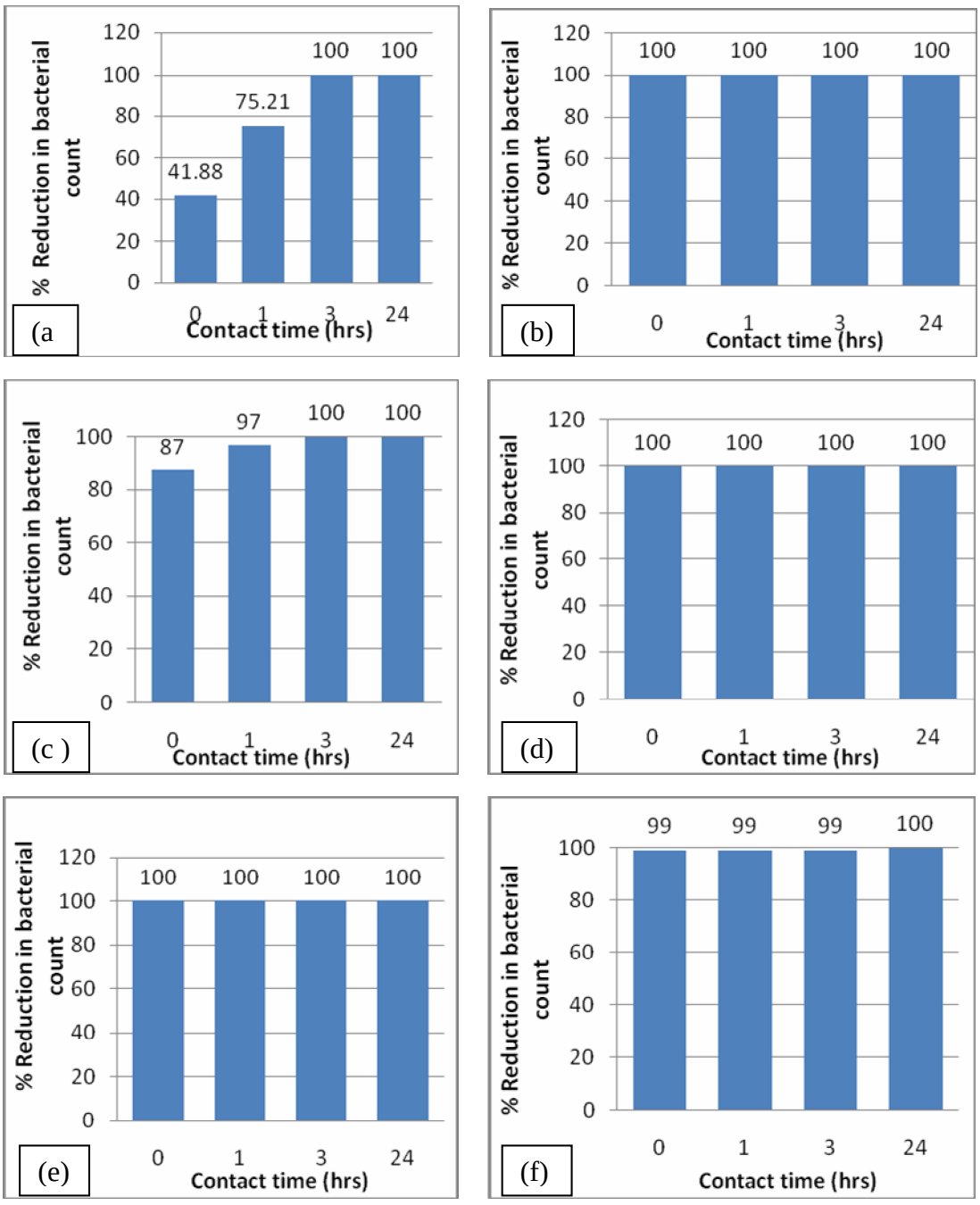

Figure 2 showed the differences in the percent reduction of *E. coli* cells after treatment with the different nanoparticles under test, where 100% reduction in *E.coli* count was observed after 24 hours of contact with GO and GO-Coated Sand, as reported previously by Shaobin *et al*., (2012).

However, sand showed 88.9% reduction in bacterial count after 24 hours of contact (Figure. 2). For both materials, a large fraction of cell death occured in the first three hours of incubation. GO coated sand showed higher antibacterial effect than GO nanoparticle at all incubation time intervals (Figure. 2). Therefore, the coated graphite oxide with sand have more bactericidal effect against *E. coli* than the uncoated nanomaterial as reported previously by Gao *et al.,* (2011).

Furthermore, the antibacterial effect of silver nanoparticles and impregnated graphite oxide with silver nanoparticles were tested against pathogenic *E. coli* BL21. Results in table 2 and figure 3 showed that no bacterial growth was observed after one hour of incubation with impregnated graphite oxide with silver at different concentrations, while after 3 hours of incubation with GO, 100% reduction in the bacterial count was reported.

Therefore, the change after 1 h of incubation with the nanoparticles under test is due to the fact that the loss of cell viability has approached the complete inactivation (Shaobin *et al.,* 2012). It was clear that the amount of silver particles on the graphite oxide seemed to be the main factor causing the complete reduction in the bacterial count and was responsible for the effective antimicrobial activity (Qi *et al*., 2011; Ma *et al*., 2011). Moreover, other researchers showed that GO-Silver composite have a superior antibacterial activity towards *E. coli* due to the synergistic effect of GO and silver nanoparticles (Sreeprasad and Pradeep, 2012).

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