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Synthesis of nanocomposites and its antimicrobial and adsorptive characteristics

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ABSTRACT : **The study investigated the effect of nanocomposites on water treatment. The modifications of sodium aluminum silicate hydrate by 3-bromo-5-chlorosalicylaldehyde to form a new composite. The synthesized composite was utilized for the removal of Acinetobacter baumannii cells from water samples. The maximum adsorption capacity of the synthesized composite towards Acinetobacter baumannii cells is 98.7%. These results suggest that nanocomposites could be a good adsorbent system for the removal of bacteria.The highest inhibition was at 50 % concentration. Acinetobacter baumannii were reduced in nanocomposite treated water sample, because of adsorption of bacteria on the nanocomposite surface.studies suggested physical disruption of bacterial membrane and oxidative stress as the major mechanisms. Therefore, the cells in contact with this nanomaterial were probably inactivated by one or both mechanisms.Nanocomposite is effective in treating microorganisms from water. These nanomaterials can be used to improve water quality and the availability and viability of water resources, such as through advanced filtration, which enables sustainable water reuse.**

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I. INTRODUCTION

Water contamination is one of the key problems in today's world. Water contamination affects the biosphere (humans, atmosphere, aquatic media…) with its associated high costs. The contaminants produced from numerous domestic discharges and industries such as paper, agriculture, textile, etc., continuously degrade water quality. Despite all the achievements, there is a universal need for efforts to ensure protected access to clean water worldwide. According to the reported data from the World Health Organization (Pandey et al., 2017), approximately 11% of people still have a deficiency in safe drinking water. WHO estimated that one in three people lack access to adequate sanitation, and approximately 263 million people spend over 30 min per trip collecting water from an improved source. Access to basic safe drinking water in most less-developed countries is a serious issue that must be addressed. Around 56% of the freshwater used for industrial, municipal, and agricultural purposes is released as wastewater, and 80% of the world's produced wastewater is not properly treated before being released into the environment, according to the United Nations World Water Development Report of 2017. (U. Hani and Alex. Eng. J., 2023)

Nanoparticles with finely controlled properties with sizes in the range of 1–100 nm can be effortlessly synthesized. They offer exclusive thermal, structural, and mechanical properties compared to bulk materials as the quantum size effect becomes considerable at the nanoscale. (Khodakarami M. and Clean. Prod. J., 2021)

In recent years, nanocomposites have attracted the attention of scientists and technologists in water purification due to improved processability, surface area, stability, tunable properties, and cost effectiveness. Nanocomposites have showed fast decontamination ability with high selectivity to remove various pollutants. The importance of nanocompostes in the removal of microorganism from polluted water. (Ambaye T.G. et al., 2021)

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II.Materials and Methods

2.1. Sampling collection: Acinetobacter baumannii strains, was donated by Dr. G. Eldaydamoni, Department of Botany and microbiology.

2.2. Synthesis of composite: Firstly, sodium aluminum silicate hydrate sample was synthesized as described by Ehab et al. (abdelrahman et al., 2021). The reaction between sodium aluminum silicate hydrate and (3-aminopropyl) trimethoxysilane was carried out as described by Khalifa at al. but slight modifications (Altowayti et al., 2021). 2 gm of sodium aluminum silicate hydrate and 2.20 ml of (3-aminopropyl) trimethoxysilane was

refluxed for 24 hrs using 50ml toluene. The obtained product was separated utilizing a centrifuge, washed with ethanol and dried at 65oC for 24 hrs. Besides, 1gm sodium aluminum silicate hydrate/(3-aminopropyl) trimethoxysilane sample, 1 gm of 3-bromo-5-chlorosalicylalhyde, 50 ml of ethanol were mixed then transferred to 100ml Teflon-lined stainless steel autoclaved for hydrothermal treatment at 120oC for 3hrs utilizing a microwave oven. The product was separated with a centrifuge, washed with ethanol, and then died at 55oC for 24 hrs.

2.3. Microbiological assessment of water samples: Acinetobacter baumannii was cultured in nutrient broth at 37oC overnight with shaking. Then inoculate water sample with 0.2 ml of cells suspension, approximately, 107cells/ml of Acinetobacter baumannii and incubation was carried out at 37o C for 24hrs. Membrane filter method was described by Hirsch for selective isolation of filamentous actinomycetes. Nutrient agar medium supports the growth of bacterial culture and suitable for actinomycetes isolation.

Overlay of nutrient agar medium with 0.45µm pore size membrane filter followed by inoculation of filter surface and incubation allow the growth of bacteria. Actinomycetes possess highly branched mycelial networks and hence they have ability to penetrate the pores of membrane filter to the underlying agar medium, whereas growth of non-actinomycetes bacteria is restricted to the filter surface. Removal of membrane filter and incubation of agar medium allow the development of the isolated actinomycetes colonies (Hirsch et al., 1983).

2.4. Identification of bacterial isolates: A pure culture for each isolate was grown on nutrient agar medium for maintenance, as well as, for cultural and morphological characterization and then placed into genera or groups, after that they will be subjected to a scheme of biochemical tests either to complete or confirm their identification. (Holt, et al., 1994)

2.5. Congo red dye agar test (CR Test): The test was carried out as per the technique of (Berkhoff and Vinal,1986). The colonies were streaked on Congo red agar; from hand book of microbiological media, USA (Soybean-casein digest agar; 890.0 mL, Hemoglobin solution; 100.0 mL, Suupplement solution; 10.0 mL and Congo red (0.01% solution)) and incubated for 72 hours at 250C.

Reaction was recorded at 18, 24, 48 and 72 hours. Appearance of red colonies within 72 hours was recorded as a positive reaction. Negative colonies did not bind the dye and remained white or grey even after 72 hours and were declared negative.

2.6. Treatment of water using nanocomposite: The nanocomposite was added to the tested water sample by 10% w/v for 3h with aeration using air supply. Then sample was tested for detected microorganisms. (Sharshar, 2011)

III.RESLUTS

3.1. Detection, isolation and purification of pathogenic bacteria

In the study, the isolate *Acinetobacter baumannii* was subjected to cultures and microscopic examination.

The nanocomposite was added to the tested water sample by 10% w/v for 3h with aeration using air supply.

Table (1): Evaluation quantity of *Acinetobacter baumannii* counted in untreated water sample and water sample treated with nanocomposite

Pathogenic bacteria	Untreated	log	Treated	log	LSD at 0.05 level	Treatment percentage
Acinetobacter baumannii	2.513×10^{5a} $\pm2.8\times10^{3}$	5.40	$3.2\times10^{3\ b}$ $\pm3.4\times10^{2}$	3.50	$7.22x10^{2}$	98.7 %

3.2. Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

From these results, it can be concluded that the minimal inhibitory concentration (MIC) as a result of nanocomposite using, was at 5g/100mL concentration (Table 3). While minimum bactericidal concentration (MBC) was at 50g/100mL concentrate of nanocomposite (Table 3).

Table (2): Determination of protein leakage for bacterial cells treated with nanocomposite

nanocomposite Conc. (%)	Protein leakage of *Acinetobacter baumannii*(µg\ml)
5	3.85
10	7.25
30	10.95
50	18
control	0

3.3. Effect of the nanocomposite on ultrastructure of *Acinetobacter baumannii*

From the previous experiment, nanocomposite was the most effective against *Acinetobacter baumannii* in a concentration of 50g /100ml.

So, its effect was tested on the ultrasturucture of *Acinetobacter baumannii*.

The obtained transmission electron micrographs illustrated that nanocomposite had effect on cell wall and caused the formation of less dense area in the cytoplasm and cells shrinked (Fig. 1,2). The micrographs of control without nanocomposite demonstrated clear cell wall and homogenous cytoplasm (Fig. 3, 4).

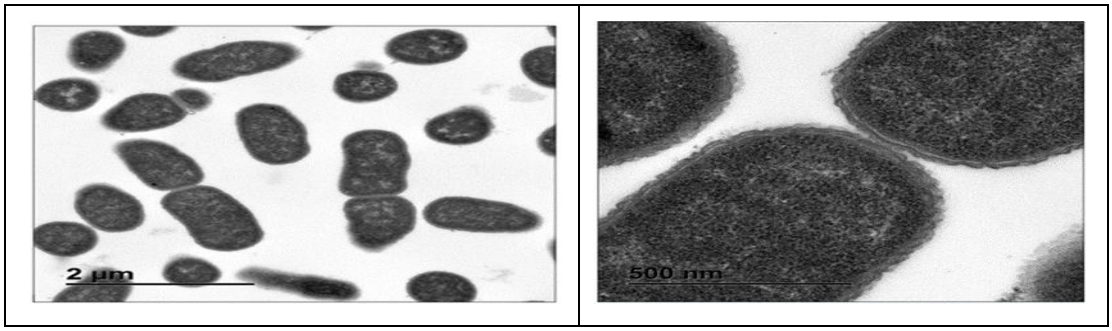

Fig (1): (A) and (B) are TEM of control bacterial cell with clear cell wall and dense cytoplasm at 2µm and 500 nm.

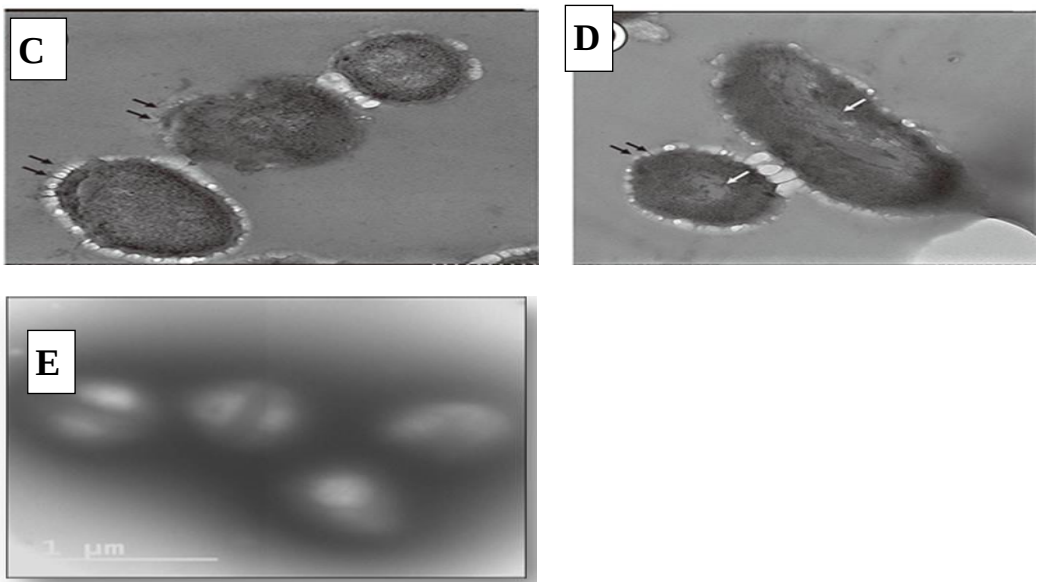

Fig (2): (C), (D) and (E) are TEM of treated bacterial cell demonstrated shrinking cells, disrupture of the cell wall and less dense cytoplasm at 1µm.

IV.Discussion

The study was to evaluate the effectiveness of nanocomposite in water treatment. The obtained results indicated that the values of Acinetobacter baumannii was reduced in nanocomposite treated water sample. The inhibition ability increased with increasing the concentration of nanocomposite in the treated water sample. The highest inhibition was at 50 % concentration. Acinetobacter baumannii were reduced in nanocomposite treated water sample, because of adsorption of bacteria on the nanocomposite surface. The used nanocomposite has been reported to significantly reduce the bacterial load in water due to its antimicrobial properties. This occurred by bacterial adhesion on the surface and then bacterial cells disrupted and cell wall broken down, so the protein leakage outside the cell and caused shrunk. So the protein leakage increased with increasing nanocomposite concentration and with increasing the inhibition of bacterial cells. So the Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) could be detected by using the protein leakage concentration. MIC was detected at the lowest amount of nanocomposite caused highest leakage of protein from the cells. It was a at 5g/100mL concentration, as appeared in (Table 3). While MBC was the concentration of nanocomposite that caused completely dead of cells and it was at 50g/100mL concentrate of nanocomposite. These results are in agreement with those reported by (Kang et al., 2007, 2008, 2009; Brady-Este´vez et al., 2008).

Several studies suggested physical disruption of bacterial membrane and oxidative stress as the major mechanisms. Therefore, the cells in contact with this nanomaterial were probably inactivated by one or both mechanisms. (Aslan et al., 2010; Kang et al., 2007; Rodrigues and Elimelech, 2010; Schiffman and Elimelech, 2011). Bacterial cells disrupted and shrunk on nanocomposite filter surfaces. (Ahmed et al., 2013).

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