Nanocomposition Bentonite Algenate for Bacterial Treatment

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Abstract

The use of polymer nanocomposites as novel materials for water remediation has emerged as a promising alternative for disinfection of bacteria contaminated water. Bentonite clay technique was successfully applied to improve wastewater quality and upgrade its use in agricultural and industrial purposes. Bentonite is a natural clay characterized by possessing excess of negative charges on its lattice and swell into very large surface area when dispersed in water. Sodium alginate, a natural biopolymer has been investigated in this study by encapsulating antimicrobial zinc oxide nanoparticles supported bentonite. The confirmation of the alginate nanocomposites was done by use of TEM, FESEM and XRD. The antimicrobial activity of the bentonite alginate nanocomposites alginate nanocomposites was investigated by batch studies using wastewater outlet. The effect of bentonite ZNO-nanocomposite beads ZNO-nanocomposite beads, bentonite on total count of bacteria and count of enterobacteriacea has been studied. The inactivation results indicated that the nanocomposite effectively inactivated bacteria in wastewater. With an amount of 0.5 g of the bentonite,0.2 g, 0.5g,1g from nanocomposites beads, the decreased in number of bacterial counts have an expulsion relationship with the increased of nano composition beads of bentonite. Therefore, the results of this study have indicated that the alginate nanocomposites can be deemed as a potential antimicrobial agent for water disinfect. Sodium alginate immobilized microorganisms were found to be an environmentally friendly and cost-effective alternative for treatment of municipal and industrial wastewater outlet.

Keywords: bentonite, zn0-nan0partical, sodium alginate

1. Introdaction

The presence of bacteria in water is of major concern to the global economy due to the threat they pose to humans and animal life, although water is an essential part of life, in developing countries, some communities are still largely dependent on the use of water from open sources such as rivers, streams and dams. Often times this water is not clean and safe for human consumption as it is infested with bacteria. The World Health Organisation (WHO) has reported that almost a million people die each year in developing countries, due to lack of clean drinking water (1, 2).

Biopolymers such as chitosan, cellulose, starch and alginates have found their way in various applications including water remediation in this study, sodium alginate was selected as the host polymer to encapsulate ZnO-NPs loaded bentonite to form polymer nanocomposites.

Bentonite is readily available around the world and is inexpensive. The clay, with its high surface area and chemical stability is often used as a supporting material for nanoparticles, which tend to aggregate when used alone due to their small sizes. Hence, bentonite was used in this study to support ZnO-NPs for this purpose (3).

2. Material and Method

The following instrument which used in the present study are listed in the table (1).

Table. 1 Laboratory instrument:

No.	Instrument	Company
	Autoclave	Korea
	Sensitive Balance	Germany
	Microwave	Germany
	Oven	Memert/Germany
	Incubater	Germany
	Centerifuge	Germany
	Magnetic Stirrer	France
	VITEK-2 Compact System	France
	Burner	Turkey
	Plain tube plastic	Japan
	Loop	England
	Petri Dish, Beaker, Cylinder, Bottles, Spatula, Filter Paper, Washing Bottle	Different Origins
	Transmission electron microscopy (TEM)	China
	Field Emission Scanning electron microscopy (FESEM)	Japan
	XRD (X-ray diffraction analysis	Japan
	Fourier Transform Infrared (FTIR)Spectrometry	China
	HOTPLATE STIRRER	China
	VORTEX MIXER	China

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3.5 Chemical Material

Table. 2 Chemical material used in present study:		
NO.	MATERIAL	ORIGIN
	Ethanol	Thomas baker (India)
	Sulfuric acid (98%)	Thomas baker (India)
	bentonite	INDIA
	Cacl2	Thomas baker (India)
	Sodium alginate	Germany (sigma –aldrich)
	Zinc oxied –nano particle	American

3.8. A- Synthesis of Bentonite supported ZnO

nanoparticles

1. Bentonite graoundand sieved to a size <150 μm. The clay was then chemically activated by acid treatment with 3 M sulphuric acid and washed for 5 time with distilled water.
2. Zinc oxide nanoparticles dispersed in ethanol were calcined at 500 C for 5 h to obtain the powdered nanoparticles.
3. Bentonite supported ZnO-NPs was obtained by weighing (0.1g) of the nanoparticles into 1.5 g of the acid activated clay and heated under reflux at 60 C for 20 min in a microwave at a power setting of 500 W. The resulting suspension was filtered and washed 4 time with distilled water.

The clay composite was then dried on filter papear overnight in air at 60 C and after crushed and sieved to 150mm for later use.

3.8. B - Synthesis of ZnO-Bent nanocomposites encapsulated alginate beads

1. The alginate solution was prepared by dissolving 3 g sodium alginate salt into 300 ml distilled water and stirred thoroughly until a clear solution was obtained. On the other hand,0.5g from the ZnOBent nanocomposite was added into 5ml distilled water and stirred.
2. Added 10ml from alginate solution to ZNOBent nanocomposite solution and 15ml from alginate solution to ZNOBent nanocomposite solution and 20 ml from alginate solution to ZNOBent nanocomposite solution and 25ml from alginate solution to ZNOBent nanocomposite solution.
3. Each two solutions were then mixed together and stirred to obtain a homogeneous dispersion. The resulting alginate dispersion was extruded using a peristaltic pump into the gelation medium.
4. The gelation medium was 0.2 M CaCl2. The extrusion was done under slow stirring to improve the bead formation and also to prevent the aggregation of the formed beads. The formed alginate beads were then left in the solution for 10 min. Afterwards, the CaCl2 solution was decanted, and the beads washed several times with water and then freeze-dried for 3 days.

3.8.C. Antibacterial studies

1. To determine the antibacterial activity of the Bentonite and Bentonite alginate nanocomposites, wastewater contain bacteria was investigated through batch experiments. (1ml) of waste water sample after maked second dilution was pipetted into center of 3 empty petri dishes and about 20 ml of nutrient, blood and macconkey agar were poured on the samples,after that the petri dishes were rotate clockwise and anticlockwise to spread the samples throughout the agar and allowed for about 5 minutes to solidify,then petri dishes were inverted before incubation . these dishes incubated at 37 C for 24 h. where nanoparticles stayed 24h in wastewater for treatment. According to (4) the number of colony was determined N= N1-N2/N1 where N1 and N2 number of colony before and after treatment .

The following table for different Weight of bentonite zno nanocomposites beads and khawaclay zno nanocomposites beads:

Table. 3 Alginate Concentration and weight beads	
Alginate Concentration	bentonite zno beads
10 ml	(0.2 g, 0.5g, 1g)
15 ml	(0.2 g, 0.5g, 1g)
20 ml	(0.2 g, 0.5g, 1g)
25 ml	(0.2 g, 0.5g, 1g)

2. Sterilized bottles were filled with 50 ml of the wastewater and the alginate nanocomposites were added. To evaluate the minimum amount of the nanocomposites that will be required to inactivate the bacteria, the nanocomposites were weighed in 0.2 g, 0.5 g and 1.0 g.
3. The sampel culture on nutrient,blood and macconkey ager before and after treatment with bentonite and bentonite zno nanocomposites beads with different concentration with different weight and incubat before and after treatment at 37c for 24h.

3.9. Characterization of the alginate n anocomposite

1. The morphology of ZnO-NPs and ZnO-Bent nanocomposite was investigated by TEM instrument, operated at 200 kV.
2. The crystallinity of the samples was determined by X-ray diffraction
3. The surface morphology and dispersion of ZnOBent nanocomposites in the alginate matrixwas analysed using FE SEM.

3. Results and Discussion

bacteria before treatment was 4200, and the removal efficiency of the bentonite ZNO- nanocomposite beads, used different whight (0.2 g,0.5 g, 1 g). the removal efficiency for 0.2 g from beads was(12%, 23%, 30%,41%) for the concentrations of sodium alginate (10, 15, 20, and 25), respectively, and with the same concentrations, the removal efficiency for (0.5g) from beads of bentonite ZNO- nanocomposite (47%, 58%, 65%,68%) with the same concentration of sodium alginate and the removal efficiency for(1g)) from beads of bentonite ZNO- nanocomposite(57%, 76%, 77%, 81%) The following figure shows the percentage of removal efficiency of the total count from beads of bentonite ZNO- nanocomposite:

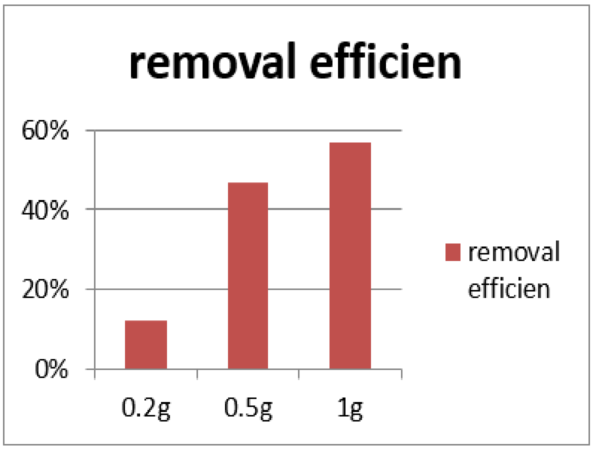


Figure 1: removal efficiency for bentonite –zno nanocomposite beads in 10ml alginate

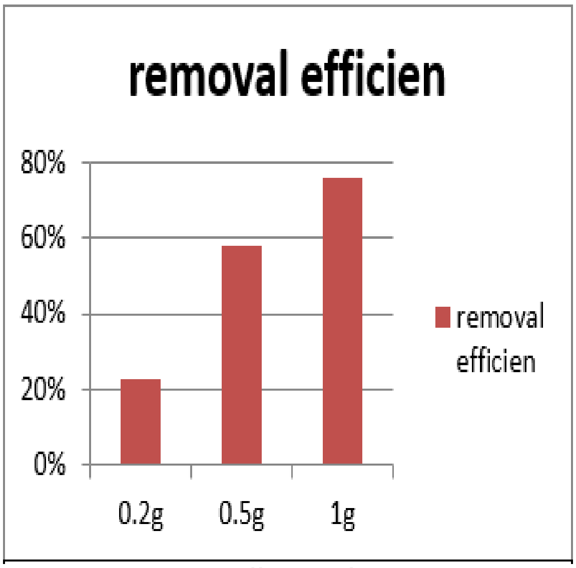


Figure 2: removal efficiency for bentonite –zno nanocomposite beads in 15ml alginate

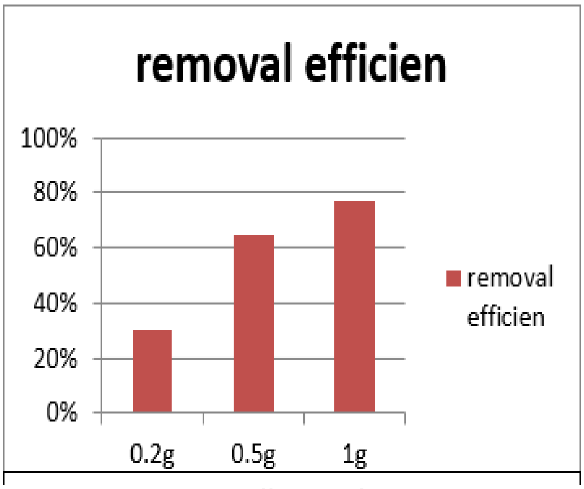


Figure 3: removal efficiency for bentonite –zno nanocomposite beads in 20ml alginate

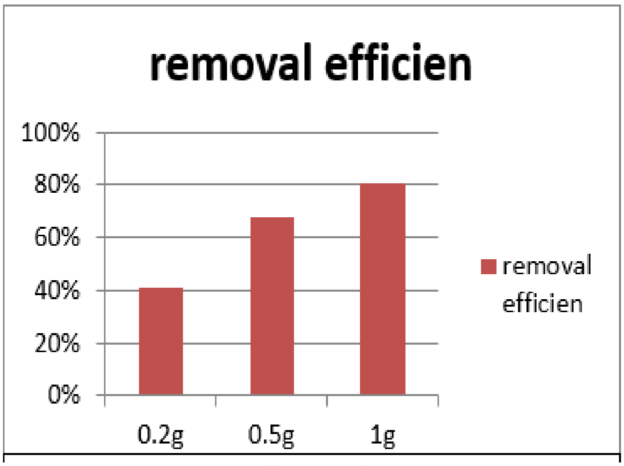


Figure 4: removal efficiency for bentonite –zno nanocomposite beads in 25ml alginate

The number of Enterobacteriaceae before treatment was (2800 cfu/ml), and the removal efficiency of the bentonite ZNO- nanocomposite beads, used different weight (0.2 g,0.5 g, 1 g). the removal efficiency for (0.2g) from beads was(22%, 22%, 24%, 26%) for the concentrations of sodium alginate(10, 15, 20, 25), respectively, and with the same concentrations, the removal efficiency for(0.5 g) from beads of bentonite ZNO- nanocomposite with the same concentration of sodium alginate (33%, 49%, 53%, 67%) and the removal efficiency for(1 g) from beads of bentonite ZNO- nanocomposite (57%,65%, 71%, 75%)

The following figure shows the percentage of removal efficiency of Enterobacteriaceae Count for bentonite ZNO- nanocomposite

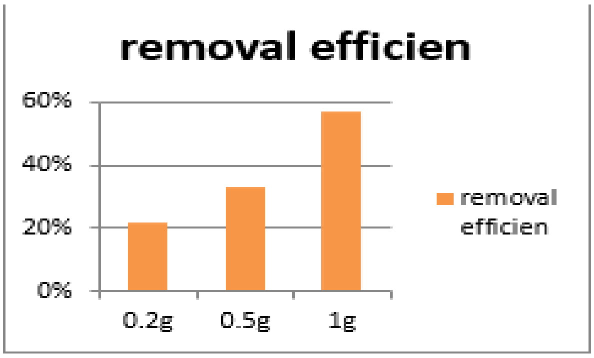


Figure 5: removal efficiency for bentonite –zno nanocomposite beads in 10ml alginate

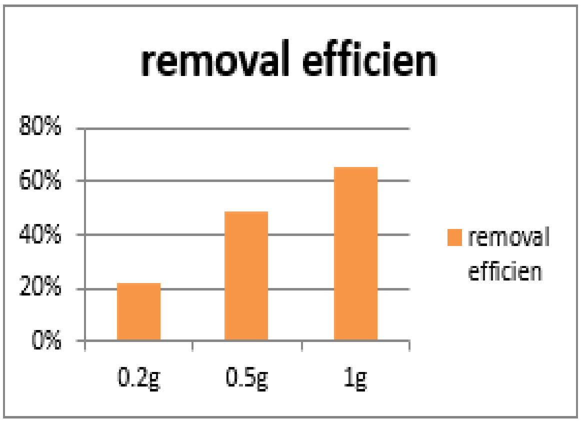


Figure 6: removal efficiency for bentonite –zno nanocomposite beads in 15ml alginate

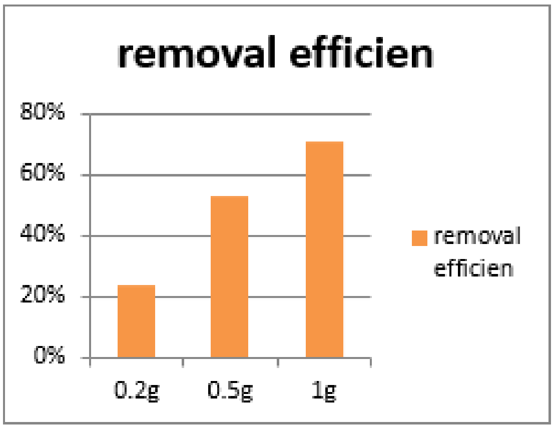


Figure 7: removal efficiency for bentonite –zno nanocomposite beads in 20ml alginate

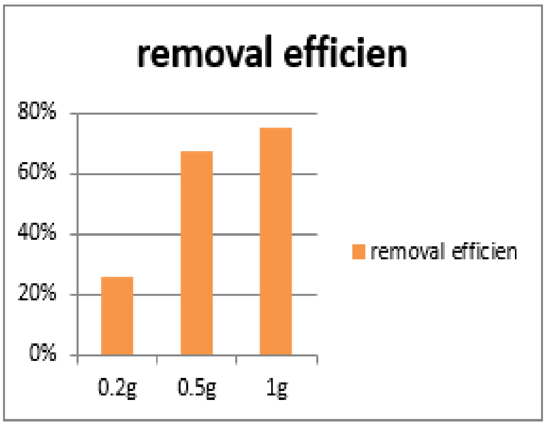


Figure 8: removal efficiency for bentonite –zno nanocomposite beads in 25ml alginate

* The microbiological analyses of wastewater were performed at end of treatment. the results of bacterial profile diagnosis showed that treated water was still polluted with different type of G+ve bacteria included (Staphylococcus pseudintermedius and Staphylococcus heamolyticus and Kocuria varians) and G-ve bacteria included (Pseudomonas aeruginosa, Escherichia coli, Enterobacter aerogenes and, Enterococcus faecalis) all these bacterial species were diagnosis phenotypically in different culture and
chromogenic media like MacConkey, Blood, Nutrient and chromogenic agar, and then confirmed by VITEK- 2 system as follows:
Bionumber: 0405610450402610 (Escherichia coli).
Bionumber: 0405610550506610 (Escherichia coli).
Bionumber: 0405211544500600 (Escherichia coli).
Bionumber: 0405610550506610 (Escherichia coli).
Bionumber: 0405610550524610 (Escherichia coli).
Bionumber: 6627734553577010 (Enterobacter aerogenes).
Bionumber: 156002765773771 (Enterococcus faecalis).
Bionumber: 010006043760231 (Staphylococcus heamolyticus).
Bionumber: 01041217363231 (Staphylococcus pseudintermedius).
Bionumber: 0003453103500252 (Pseudomonas aeruginosa).
Bionumber: 014000140000030 (Kocuria varians).

The obtained results recorded evealed that a significant decrease in the densities of bacterial indicators counts included Total viable counts and Enterobacteriaceae viable counts and increased in bacterial removal rate by using nano composition beads of bentonite in different weight and the decreased in number of bacterial counts have an expulsion relationship with the increased of nano composition beads of bentonite weight as shown in the figures above.

Water pollution is the major problem in the global surrounding. And it is major causes of illness and mortality worldwide (5). Now the awareness for the disposal of industrial wastewater, pretreatment and proper purification of water is the prime responsibility of all the people around the globe. Many laws, policies and a lot of technological advancement have been made related to recycling of industrial water and/or its treatment before it is discharged into the environment.

People are responsible for causing the water pollution on this earth. This water pollution is the results of using different types of chemicals used in agricultural sector (such as herbicides, pesticides, fertilizers), use of detergents and soaps by human being in daily life, and major pollution is cause by industrial sectors (such as textiles, electroplating, mining and other chemical industries) which released highly toxic chemicals (6).

Wastewater treatments are processes in which microorganisms play crucial roles and illustrate well some of the principles of biogeochemistry. Wastewaters are materials derived from domestic sewage or industrial processes, which for reasons of public health and for recreational, economic, and aesthetic considerations cannot be disposed of merely by discarding them untreated into convenient lakes or streams.

Rather, the undesirable and toxic materials in the water must first be either removed or rendered harmless. Inorganic materials such as clay, silt, and other debris are removed by mechanical and chemical methods, and microorganisms participate only casually or not at all. If the material to be removed is organic in nature, however, treatment usually involves the activities of microorganisms, which oxidize and convert the organic matter to CO2. Wastewater treatment usually also results in the elimination of pathogenic microorganisms, thus preventing these organisms from getting into rivers or other supply sources. As a result of the sewage treatment process, the effluent is stabilized and its content of toxic materials is reduced, it can thus be disposed into the environment with less difficulty. (7) recorded that total and faecal coliforms were around 107 in marine water mixed with raw sewage. (8) found that faecal coliform in the raw wastewater was 3.9 x 107 as mean value. (9) reported that faecal streptococci counts in raw wastewater were around 106 to count /100ml either under warm or cold conditions.

X-rays diffraction (XRD)

XRD analysis is a fast and efficient technique that has been widely used to determine the crystalline properties of solid nanomaterials, namely crystalline nature, phase and crystal size. Figure (10) represents the XRD spectrum of bentonite clay and ZnO- bentonite nanocomposite. The spectrum showed the diffraction peaks of the bentonite clay at 2θ = 19.7o, 21.7o, 29.0o, 35.9o, 42.4o, 54.0 o, 56.7 o and 61.8o assigned to (100), (202), (201), (002), (101), (003), (222), and (210) planes, respectively. In contrast, the XRD spectrum of ZnO- bentonite nanocomposite showed the appearance of new characteristic peaks due to the presence of ZnO nanoparticles at 2θ = 31.5o, 34.3o, 35.9o, 47.2o, 56.4o, 62.02o and 67.8o assigned to (100), (002), (101), (110), (103), (200), and (112) planes, respectively. This indicates the wurtzite hexagonal crystalline structure of ZnO. While the diffraction peaks of the bentonite clay have decreased their intensity after their association with ZnO NPs at 2θ = 19.7o, 21.7o, 26.4o, 28.2o, 49.4o, 53.8o, and 58.3o assigned to (110), (210), (124), (144), (112), (113), and (204) planes, respectively. Finally, the appearance of several small peaks of low intensity indicates a loss of crystalline character (10,11).

To find out the values of the average crystal size, the Debye-Scherrer equation was used to calculate it:

$$D = \frac{k\lambda}{\beta\cos\theta} \quad (3\text{-}1)$$

Where D is particle size, λ is the X-ray wavelength, θ is diffraction angle (deg.), k is Scherrer constant (0.9) and FWHM (β) is the full width at half maximum of the peak. It was found that the average crystalline size of bentonite clay and nanocomposite up to 10.2 nm and 14.2 nm respectively. This indicates the success of the synthesis of the nanocomposite as a result of a clear increase in the average crystal size with the appearance of their peaks in the spectrum.

Table .4 XRD data of bentonite and ZnO- bentonite nanocomposite

Sample	2θ	FWHM	Intensity (I/Io) %	d-spacing (Ao)	Crystal size (nm)
Bentonite	19.77	0.432	14.1	0.448	19
	21.77	0.708	100	0.407	11
	29.02	1.073	25.6	0.307	7
	35.95	1.152	47.8	0.249	7
	42.41	0.904	8.8	0.212	9
	54.01	1.059	10.6	0.169	8
	56.74	1.031	11.9	0.162	8
	61.88	0.704	19.3	0.149	13
Sample	2θ	FWHM	Intensity (I/Io) %	d-spacing (Ao)	Crystal size (nm)
ZnO- Bentonite	19.70	0.554	20	0.450	14
	21.72	0.824	100	0.408	9
	26.43	0.846	11.9	0.336	9
	28.22	0.78	11.8	0.315	10
	31.53	0.446	20.2	0.283	19
	34.31	0.758	29.7	0.261	11
	35.98	0.534	52.6	0.249	15
	47.21	0.807	13	0.192	10
	49.46	0.739	10.9	0.184	11
	53.80	0.593	11	0.170	15
	56.45	0.518	24.9	0.162	17
	58.32	0.532	15	0.158	17
	62.02	0.811	26.1	0.149	11
	67.80	0.617	16.7	0.138	15

Field emission scanning electron microscopy (FESEM) analysis

FESEM is an important technique that reveals the morphology, size and crystalline nature of the prepared nanocomposites. Figure (9) shows FESEM images of the ZnO- bentonite nanocomposite before treatment at different magnifications. This indicates the presence of clusters of different shapes deposited on nanoplates with a smooth wavy structure representing the bentonite clay. The shapes confirmed the success of the bonding of ZnO on the surface of bentonite clay. After the treatment process using the nanocomposite, there is a significant change in the symmetric shape with the disappearance of the spherical clusters as a result of the binding of the target bacteria to the surface of the nanocomposite, as shown in Figure (9). In addition, a decrease in the agglomeration rate of the particles and an increase in the size particles and the thickness of the nanoplates (12). It was found that the average particle size of the ZnO- bentonite nanocomposite before and after treatment are 21.45 nm and 65.23 nm.

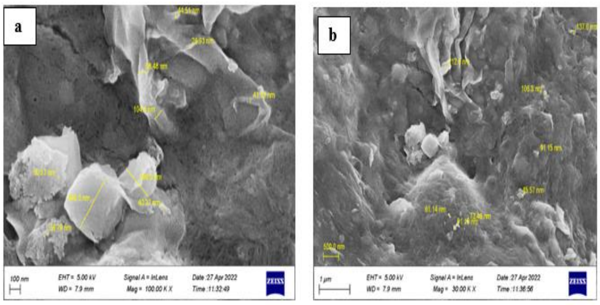


Figure 9. FESEM image of ZnO- bentonite nanocomposite before treatment at different magnifications (a) 100 nm and (b) 1 µm

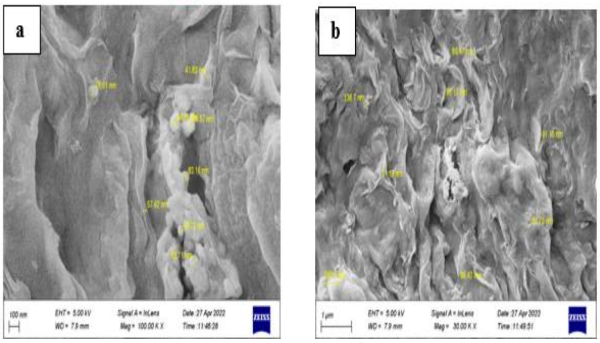
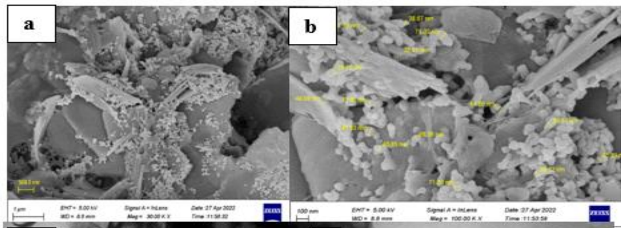


Figure 9. FESEM image of ZnO- bentonite nanocomposite after treatment at different magnifications (a) 100 nm and (b) 1 µm



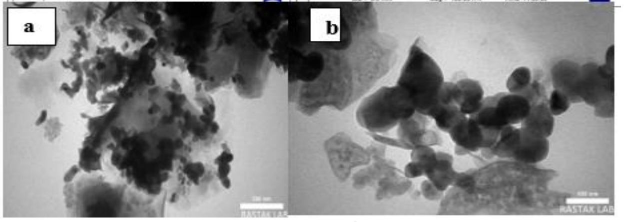


Figure 10 TEM image of ZnO- bentonite nanocomposite at different magnifications (a) 100 nm and (b) 500 nm

TEM images of the ZnO-bentonite nanocomposite at different magnifications. It was noticed that the ZnO nanoparticles appear as black spherical shapes deposited regularly on the surface of transparent sheets representing bentonite clay. It was also noted that bentonite clay has a porous nature that attaches strongly with zinc oxide nanoparticles. This indicates the success of the synthesis of ZnO-bentonite nanocomposites with particulate size up to 90 nm (13, 14).

4. Conclusions

Bentonite alginate nanocomposites were prepared and characterized to develop a new antimicrobial material for the disinfection of water. The alginate nanocomposites encapsulated with antimicrobial ZnO-NPs demonstrated its inhibition of bacteria activity with wastewater outlet. the nanocomposites showed excellent antimicrobial activity within 24 hours by inactivating a large number of the enterobacterobacteriaceae. However, the nanocomposites showed good results after24 hour by inactivating a large number of the total count from bacteria. The inactivation was improved with more nanocomposite amount and contact time. Furthermore, the overall performance of the nanocomposites in the study shows that the nanocomposites are promising as an alternative disinfectant material for contaminated water. In addition, the cost implication for the preparation of the nanocomposites allows for a large-scale production which is good for point-of-use. This treatment is considered environmentally friendly because no reaction by-products were found. It is therefore proposed as an efficient, simple, and economical treatment that can be adopted as an alternative for wastewater treatment.

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