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Using Composite Nanofibers to Remove Microorganisms from Aqueous Solutions

Ahmed K. Suleiman[1*], Omnia I. Aly[1], Elsayed M. Mahdi[1], Alaa Mohamed[2] and Gehad G. Mohamed[3]

[1] Department of Applied Chemistry, Faculty of Sciences, Helwan University, Helwan, Egypt

[2] Department of Mechatronics, Canadian International College, Fifth Settlement, New Cairo, Egypt

[3] Chemistry Department, Faculty of Sciences, Cairo University, Giza, 12613, Egypt

Abstract

Microorganisms present in drinking water sources such as bacteria targeted with the aim of limiting their growth as well as eliminating them, because of their significant negative effects on human health. A chemical used that contains a mixture of chitosan, polyvinyl alcohol, nanomagnetic iron oxide, as well as nanocarbon. This chemical obtained in the form of nanofibers by the electric spinning process, the properties of this substance and its shape studied using the scanning electron microscope (SEM) as well as the transmission electron microscope (TEM). The type and shape of bacteria studied after completing the processes of separation from the sources drinking water according to the approved steps, preparing it for its sensitivity and chemical impact in private microbiology laboratories, where we indeed able to know the type, shape and name of bacteria. The results were clear and distinctive on the ability of this chemical to limit the growth of bacteria and eliminate them. The substance chitosan with a certain concentration had a great ability to clearly influence the bacterial activity in drinking water and eliminate it. This paper aims to show the bacteria effects on the public health, clarify their shape and type, as well as stopping and eliminating the bacterial growth.

Keywords: E. Coli Bacteria, Fecal Coliform, Muller Hunten Dishes, Crystal Pigment, Iodine Dye, Chemical Mixture, nanofiber composite.

1. Introduction

The provision of safe drinking water has been one of humanity's most successful public health interventions and is a defining aspect of a developed country [1]. However, ignorance of the potential risks, inappropriate training of staff and managers working on drinking water systems still results in unnecessary waterborne disease outbreaks in affluent communities Furthermore, re-introduction of once-controlled diseases, such as cholera, may rapidly spread during periods of disasters when sanitation systems are non-functional and drinking water treatment is inadequate

A recent example was the haitian epidemic [2], although a developing region, international aid workers introduced the outbreak strain and then tourists spread infections to more developed regions. Hence, to some degree, differentiating pathogen risks between developed regions and those less developed, particularly rapidly developing regions, is artificial and not very useful [3, 4] .

Therefore, this information is relevant to most regions with functional drinking water treatment provided through a community system [5, 6]. A key realization is the need for ongoing system-wide vigilance, coupled with a preventative rather than just responsive management approach. This approach is best practiced globally using principles from the food industry's hazard analysis critical control point (HACCP) approach, described by the world health organization (WHO) as water safety plans (WSPs).

*Corresponding author e-mail: a4002778@gmail.com

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In addition to existing regulatory framework constraints, this WSP approach may be particularly hard to implement in (developed) regions that have not identified major waterborne outbreaks for decades. Even with well-operated drinking-water treatment systems, there is growing concern that aging drinking water distribution systems (DWDSs) are vulnerable to higher rates of mains breaks/repairs and related pressure losses that may lead to pathogen intrusion scenarios [7, 8].

Traditional end-of-pipe compliance monitoring practices may not identify short periods of DWDS intrusions or short periods of poorer treatment performance, such as is associated with rain-induced dirty-water events that appear to be associated with increased rates of waterborne gastrointestinal disease [9].

Generally, drinking-water gastrointestinal cases are not well quantified, even in developed regions, due to the insensitivities of surveillance and specific epidemiology studies [10, 11]. For the US (with more than 300 million people) estimates of annual drinking-water gastrointestinal cases range from 12–19 million [12]. In addition, beyond the DWDS is a vast network of building or in-premise plumbing that, under certain conditions, allows the growth and release of water-based opportunistic pathogens, many resulting in respiratory or skin diseases, such as from Legionella pneumophila and non-tuberculous mycobacteria (NTM) [13]. At least in the US, these water-based pathogens appear to cause a higher health burden via hospitalization than waterborne enteric pathogens [14].

Due to environmental growth of water-based opportunistic pathogens, quite different, but familiar control strategies (elimination of stagnation zones and related temperature and disinfectant control) are required for in-premise plumbing, particularly in healthcare settings [15]. This review builds on previously conducted reviews [5, 16-18], and is organized around recent findings associated with drinking-water microbial hazards and scenarios that need to be managed as part of a WSP-like system-wide management framework to provide safe drinking water .

However, about 10 percent are harmful known as pathogens, if ingested by humans, they can release toxins causing sickness or even death. Symptoms of waterborne diseases may include gastrointestinal illnesses such as severe diarrhea, nausea, and possibly jaundice as well as headaches and fatigue. It is important to note, however, that these symptoms are not associated only with disease-causing organisms in drinking water. They may also be caused by a number of other factors [19].

This paper investigates the bacteria from the drinking water samples to show their effects on the public health, clarify their shape and type, as well as stopping and eliminating the bacterial growth. A composite contains a mixture of chitosan, polyvinyl alcohol, magnetite nanoparticles, as well as carbon nanotubes were used. This composite obtained in the form of nanofibers by the electric spinning process, where the properties of the prepared composite nanofibers and their shape were studied using the scanning electronic microscope (SEM) and the transmission electronic microscope (TEM).

2. Materials

Polyvinyl alcohol, iron(III) chloride, acetic acid, saffron dye, crystal pigment, iodine dye and distilled water. Iron(III)-acetyl acetone (red crystals) is prepared as previously described [20, 21].

2.1. Preparation of Magnetite Nano Partials by Hydrothermal Process

First, iron (III)-acetyl acetone was dissolved in (70 ml) deionized water taken in a beaker. Then ethylene diamine was added dropwise to the solution under stirring to maintain the pH at 10-11. After that the resulting suspension was transferred into the 100 ml. Teflon lined autoclave and the hydrothermal reaction was carried out at 150 ºC for 12 hr. Later, the obtained precipitate was centrifuged, washed with distilled water repeatedly and finally with alcohol. Then, the obtained precipitate was dried in electric over at 60 °C.

2.2. Preparation of Chitosan (CS)

The extraction of CS from the collected shrimp shellfish was carried out according to Brine's methodology (Brine 1984). This protocol consists of the following tasks: first of all, deproteinization

where proteins related to chitin were removed via continuous stirring of 76.45g of shrimp shellfish in 760 mL of 0.1 N NaOH solution for 2 days. Then, this solution was decanted at room temperature and filtered through a filter paper. The obtained filtrate was afterward washed with distilled water until neutral pH. Then, demineralization was carried out to remove the mineral matter bound to chitin (Fig 1).

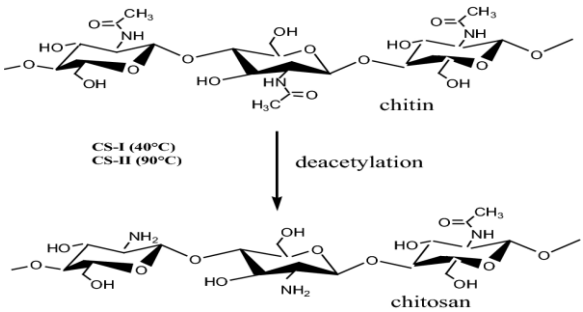


Figure 1. *Deacetylation Mechanism*

This step was performed within 8 days with continuous stirring to rinse the obtained filtrate in 200 mL of 3–5% HCl solution, followed by decantation, filtration and rinsing with distilled water. The obtained product after these two tasks is named chitin. After that the obtained chitin (task 2) is chemically deacetylated in order to remove some or all of its acetyl groups by hydrolysis of chitin in a strongly alkaline medium with 50% NaOH under agitation for 4hrs under controlled temperature. The first type of chitosan (CS-I) was prepared at temperatures of 40 °C, while at 90 °C, the chitosan second type (CS-II) was prepared.

2.3. Preparation of Electrospun Composites Nanofibers

2g of Chitosan was dissolved in 98 ml of acetic acid and the solution was stirred for 24 hr with heating at 40 °C, until the chitosan solution was prepared. A polyvinyl alcohol solution is prepared by dissolving 8g of polyvinyl alcohol in 92 ml distilled water with heating at 90 °C and constant stirring for 2hrs to obtain the polyvinyl alcohol solution .

After several experiments, it was found that the best solution can be used to obtain the nanofibers of chitosan as a mixture consists of 30% chitosan and 70% polyphony alcohol. This can attribute to chitosan solution alone does not give nanofibers for the occurrence of large scattering on the receiver board in the electric spinning device. After the addition of the polyvinyl alcohol solution by weight as mentioned above, there was a significant reduction of the dispersal which gave nanofilaments on the receptor plate. This is due to the mitigation of the positive charge present in chitosan and its equivalent to the negative charge found in the polyvinyl alcohol. After obtaining these nanofibers, different concentrations of MCNTs were used with only one concentration of iron oxide and a solution of glutaraldehyde (GA; 2.5wt.%) wherein kept nanoparticles at room temperature after dipping in solution of GA (2.5 wt.%) for 24 hrs.

2.4. Isolation of Bacteria from the Drinking Water Sample

First of all, water sample of about 3 ml from a source of drinking water was taken and placed in a special glass container for bacterial growth called blood agar. We put the glass container containing the sample of drinking water in a special incubator to help bacterial growth for 24 hrs at a temperature of 36 °C. Then, bacterial growth was observed within the vessel designated for bacterial growth after 24 hrs incubation .

This indicates the presence of bacterial contamination of the water sample. Swab was taken from one of the large and clear colonies using a small plastic spoon and placed on a special dish called Muller Hunten dishes and left in the special incubation for bacterial growth for 24hrs at a temperature of 36 °C.

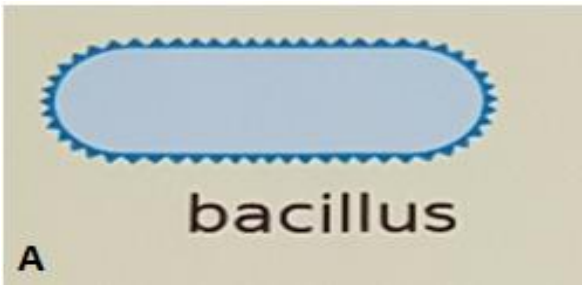
Finally, bacterial cultures were performed under the flame stove to ensure good sterilization and no bacterial air pollution.

2.5. Steps to Check the Type and Shape of Bacteria

First, a small swab was taken with the sterile plastic spoon from the container on which the bacterial growth occurred. This swab was putted on a special glass slide and about 1 ml of the crystal pigment was added to it, and then left for 2 min. After that, the dye was poured on top of the slide, put the iodine dye and left for 2 min. Next, the glass slide was washed with 70% ethanol solution and with distilled water. Then, about 1 ml of saffron dye was added and left for 2 min. After that, the slide was

washed with distilled water and left for 2 min. The slide was transferred to the microscope to find out the shape and type of bacteria.

The indication under the microscope was pink color which means that the bacteria was of the type Gram negative (G^-) and commonly from the *E. coli* family. Thus, it can conclude that the pollution occurred to the drinking water sample is bacterial infection of (G^-) type (*E. coli*), as illustrated in Fig 4, that has bad effects on the general health of humans.



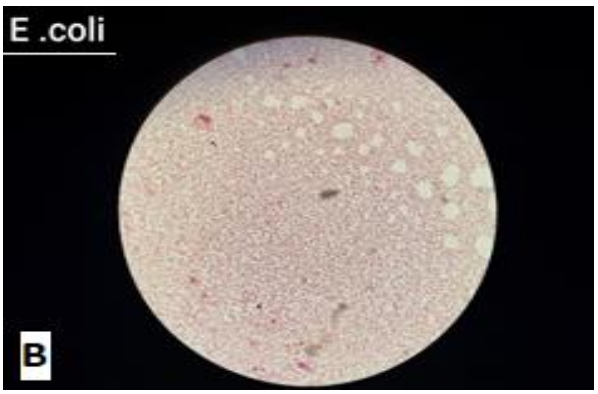
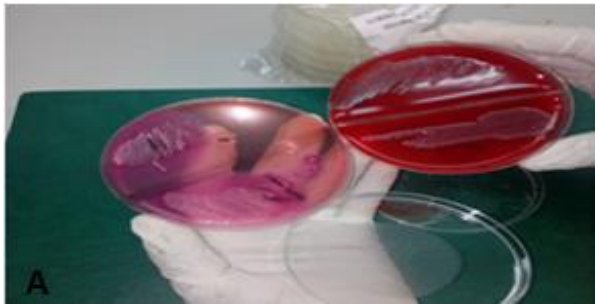


Figure 2. (A) Shape and (*B) Type of the Bacteria*

2.6. Effect of the Fiber Obtained on Bacterial Growth

A sample of the bacteria that separated and prepared was taken from the drinking water. These bacteria were putted on special dishes called Mueller Hunten dishes to test the bacteria's sensitivity to the produced fiber. Different concentrations of fiber samples were distributed inside the dish at which bacterial growth could be followed as shown in Fig. 3 (A and B).



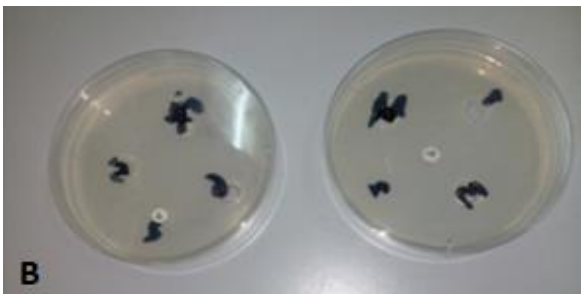


Figure 3. (A) *A special Dish (Blood Agar) for Bacterial Growth* ***and (B).*** *Distribution of Chemical Samples on Muller Hunten Dishes*

These dishes were placed in a private 24hrs nursery at a temperature of 36°C. The target samples were used to study their effect on the bacteria, and they were numbered as follows:

- Sample 1 contains acetic acid that used as a solvent.
- Sample 2 contains a type of Antibiotic called Ampicillin as a comparison sample to know and compare its effect on bacterial growth.
- Sample 3 contains concentrations of 0.5g fiber with magnetite (Fe_3O_4), plus (Chitosan-%2 - PVA 98%).
- Sample 4 contains fiber (P.V.A 98% - Chitosan 2%).
- Sample 5 contains fiber with (P.V.A 98% - Chitosan 2%) with carbon nanotubes 0.5g).
- Sample 6 contains (P.V.A 98% - Chitosan 2% - 1g carbon nanotubes + 0.5g Fe_3O_4).
- Sample 7 contains fiber (P.V.A 70% - Chitosan 30%).

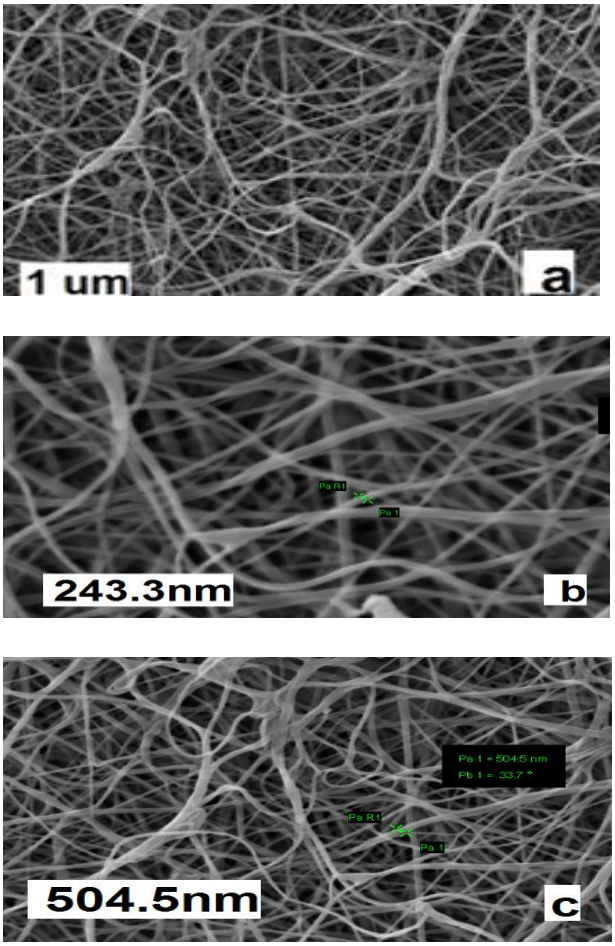
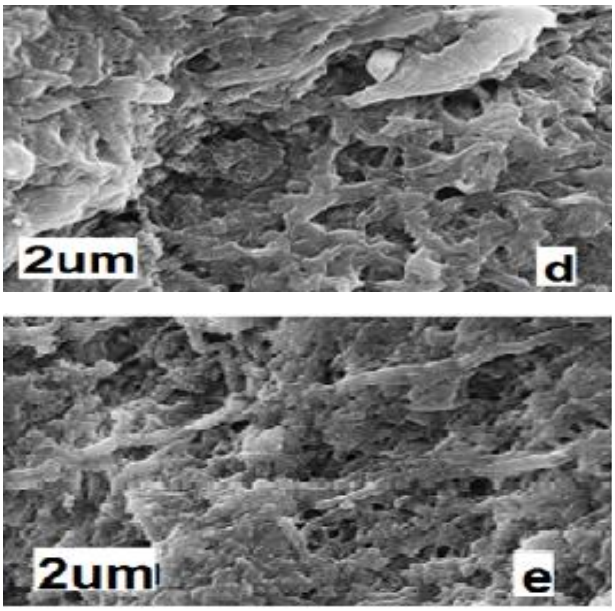


Figure 4. *(a, b, c). SEM Images of (PVA–Chitosan)*



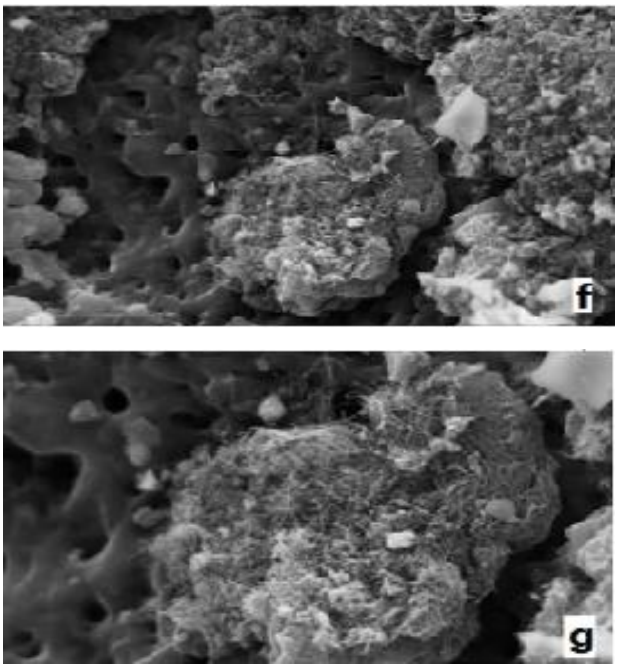


Figure 5 (e, d, f, g). *SEM Images for Fiber with Different Concentrations of Nanoparticles (PVA-Chitosan-Fe_3o_4-MCNTs)*

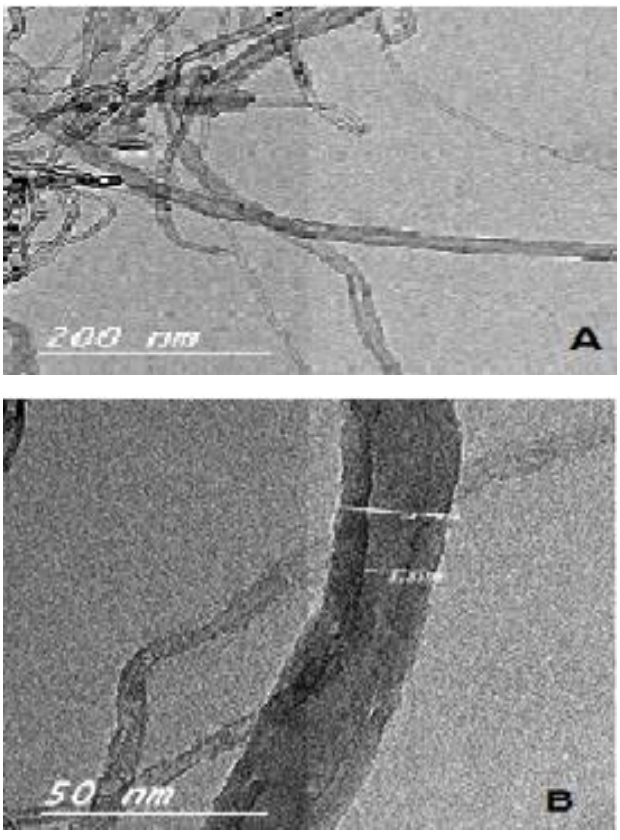


Figure 6 (a, b). *TEM Images of MCNTs*

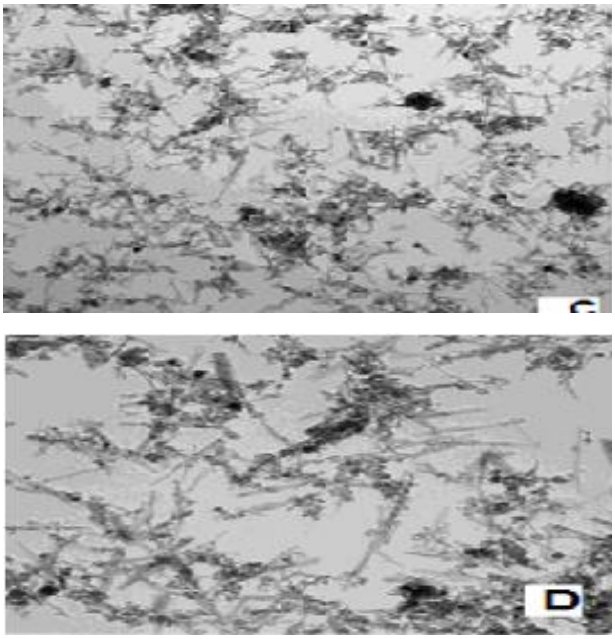


Figure 7 (c, d). *TEM Image of Iron Oxide (Magnetite) nanoparticles.*

2.7. Characterization

The composite nanofiber (Chitosan-PVA-CNTs-Fe_3O_4) was characterized using scanning electron microscopy (SEM, LEO 1430 VP, GRESHAM) and transmission electron microscope (TEM, JOEL JEM-2100). The HR-TEM is operating at 200 kV equipped with Gatan digital camera Erlangshen ES500.

Results and Discussions

3.1. Characterization of Nanocomposites

Figure 4 (a, b and c) shows the nanofibers produced by the electric spinning process without adding other nanomaterials, where the diameter of the fiber was up to 243 nm. The prepared nanofibers were described and studied using SEM. Figure 5 (d, e, f and g) shows the SEM images of the fiber associated with different concentrations of magnetite nanomaterials (0.5g) and carbon nanotube in different masses (0.05g, 0.1g, 0.5g and 1g). Also, nanomaterials, carbonaceous and magnetic iron oxide were described using TEM images as shown in Fig 6 (a, b) and Fig 7 (c, d)

3.2. The Effect of Nanofibers on Bacterial Growth (*E. coli* Bacteria)

According to the experiments conducted in the special microbiological laboratory to study microorganisms, it became clear that the Gram negative (G^-) of bacterium lives in drinking water has bad effects on public health.

This type of bacteria was separated from the sample of drinking water, placed on special dishes called Muller Hunten, distributed over the entire area of the dish, the fiber was distributed as well over each and every numbered sample as illustrated in Fig 8 (a, b). These dishes are placed in a special incubator at 36 °C for 24 hrs according to the following Table 1:

Table 1. *Chemical Content of each Type of Fiber*

Sample no	Chemical content
1	Acetic acid
2	PVA + Chitosan + (0.5g MCNTs)
3	PVA + Chitosan + (MCNTs 0.1g)
4	PVA 80% + Chitosan 20%
5	PVA + Chitosan + 0.5g Fe_3O_4
6	PVA + Chitosan + 1g MCNTs
7	PVA 70% + Chitosan 30%

We note that there is a clear effect on bacterial growth in sample 4 and 7, because both contain high percentage of Chitosan. In comparison, sample 7 contains a higher concentration than sample4 for Chitosan which get a clear and large area in stabilizing bacterial growth and eliminating the bacteria .

These results have been got after measuring the diameter of the circle in which the effect occurred on bacterial growth. It was (2.1 cm) in sample 7 and (1.7 cm) in sample 4.

Chitosan contains the NH_2 groups that are responsible for stopping and eliminating the bacterial growth. Therefore, it is expected that the samples which have the NH_2 groups are interconnected with other nanomaterials get less impact on the bacterial growth as noticed in samples 2, 3, 5 and 6.

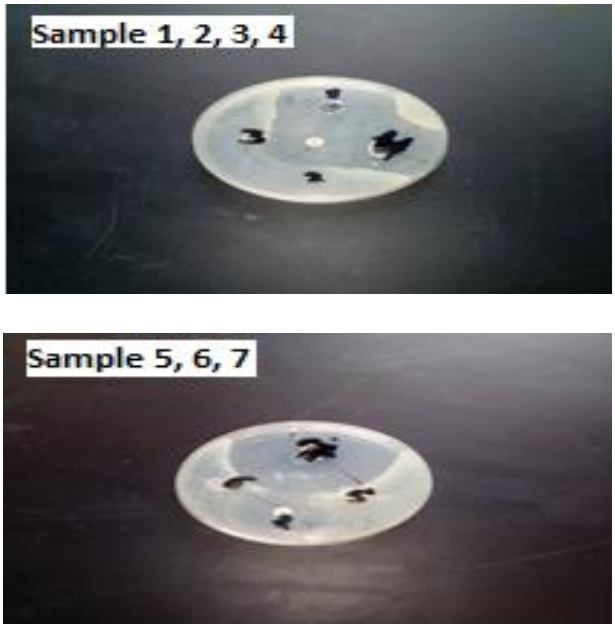


Figure 8. *Effect of Fibers on Bacterial Growth*

4. Conclusion

The process of preparing the fibers was successfully accomplished by using the electrospinning technique, as well as the process of adding nanomaterials to the fibers and its binding by the crosslinking process. Also, the process of separating the bacteria from the drinking water sample, preparing them to study their shape, type and their influence on the prepared fibers are successfully done inside the microbiology labs.

The results clarify that when increasing the concentration of chitosan in the fibers, bacterial growth will be reduced and eliminates it completely.

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