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In Compared to Traditional Agents, Use of Biosynthesised Silver Nanoparticles as Disinfection Agent for Domestic Wastewater Treatment

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Abstract

Domestic wastewater, originating from household activities, commonly contains numerous pathogens that can lead to waterborne diseases. Therefore, ensuring effective disinfection during wastewater treatment is essential for protecting public health. However, conventional disinfection approaches such as chlorination, UV treatment, and ozone application have many drawbacks. Silver nanoparticles (AgNPs) are a promising alternative disinfectant for wastewater treatment. In general, AgNPs can be synthesized by physical, chemical, and biological methods. The use of biosynthetic AgNPs is eco-friendly and cost-effective. This study investigates the synthesis of AgNPs using the cell-free extract of three actinomycete strains, including Streptomyces sp. (βB1) and two Actinomycetales bacterium (βC1 and βA15). The confirmation of AgNPs formation was conducted through UV-visible spectroscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM). Domestic wastewater samples from various sewage treatment plants were treated with minimum inhibitory concentration (MIC) of AgNPs and then analyzed for various physicochemical variables and microbial groups. Compared to control the treated samples characteristics complied with standards for discharging liquid wastes and the reduction in microbial groups exceeded 99%. Different AgNPs concentrations and exposure times were investigated to minimize both time and concentration of AgNPs while maintaining disinfection activity. Treatment with 15 μg/ml concentration of AgNPs biosynthesized by βB1 was found efficient to disinfect domestic wastewater. The efficiency of the designated concentration versus UV and chlorination was compared. Although chlorination and UV eliminate all the targeted groups, a comparable disinfection capability of the tested AgNPs was proved. In conclusion, the investigated AgNPs hold significant promise as a cost-effective and environmentally friendly disinfection solution, particularly in domestic wastewater treatment. Additionally, biosynthesized AgNPs can be comparable to common disinfection techniques such as chlorination or UV irradiation with less drawbacks.

Keywords: Silver nanoparticles, Biosynthesis, Wastewater treatment, Antimicrobial, Disinfection

1. Introduction:

Household activities generate domestic wastewater, which is typically highly concentrated in nutrients and organic materials, along with the majority of pathogens [1, 2]. Wastewater treatment technologies help safeguard the environment and public health [3]. Pollution and eutrophication result from the release of wastewater that has been improperly treated. Furthermore, water-borne illnesses may spread as a result of untreated wastewater [4, 5]. The three main stages of wastewater treatment are primary, secondary, and tertiary treatment. Providing high-quality water for human consumption and other applications is the main goal of tertiary treatment [6, 7].

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Disinfection is a vital step in wastewater treatment for safeguarding human health by eliminating pathogenic and harmful microorganisms. Ultraviolet (UV), chlorine, chlorine dioxide, chloramine and ozone are common disinfection techniques [8, 9]. Chlorination is the most widely used method for disinfecting effluents from wastewater and/or drinking water treatment plants [10]. There are more than 7,000 municipal UV disinfection installations in the world, and small household UV disinfection systems are also available [11]. However, there are drawbacks associated with these techniques: chlorination leads to the production of carcinogenic disinfection by-products (DBPs), UV lacks residual disinfection, and ozone is costly with high energy requirements [10, 11]. Searching for new disinfection agents to overcome these drawbacks is an issue of several research. AgNPs is a promising alternative disinfectant for wastewater treatment.

With benefits over bulk materials due to the special features of materials at the nanoscale, nanotechnology is a fast-developing field [14]. Nanoparticles' unique chemical, physical, and biological properties make them essential in a variety of fields, including water, health, and energy [13, 14]. Silver NPs are unique among them because of their remarkable physicochemical characteristics which make it recommended in wastewater treatment [17]. Since ancient Babylonian and Greek times, silver has been used for its antibacterial properties, which improve the effectiveness of drinking-water filtering systems [16, 17].

The production of AgNPs typically falls into two main categories: bottom-up and top-down approaches [18, 19]. Generally, these nanoparticles can be synthesized using physical, chemical, or biological methods [20, 21]. Biosynthesized nanoparticles offer advantages such as environmentally friendly manufacturing processes and cost-effectiveness. Additionally, the components of plants and microbes serve as capping and stabilizing agents, eliminating the need for extra stabilizers [22, 23]. Mohanpuria et al,. [24], offer a comprehensive examination of biosynthesized nanoparticles, emphasizing the benefits of affordability, scalability, and environmental sustainability, their work highlights the potential uses of biosynthesized nanoparticles in medicine, energy, and electronics, including solar cells, drug delivery, sensors, displays, and cancer treatment.

Actinomycetes have the natural capacity for growth in a variety of conditions, which is advantageous for large-scale manufacturing, making their use in biosynthesis of AgNPS is cost-effective and sustainable. Actinomycetes are a valuable resource for the extracellular or intracellular synthesis of nanomaterials, making them an environmentally acceptable method of producing nanomaterials. Actinomycete-facilitated synthesis has the advantage of having protein, which significantly increases the synthesis of nanoparticles and makes scaling up the process easier. Another advantage is that, unlike other procedures that need meticulous instruments for thorough extraction from their sources, actinomycetes are easily extracted by filtration. Since actinomycetes don't need expensive equipment, they are consequently significantly more economical [27].

Incorporating these environmentally friendly and cost-effective approaches into wastewater treatment aligns with the Sustainable Development Goals (SDGs), specifically SDG 6, which aims to ensure the availability and sustainable management of water and sanitation for all. By advancing these green technologies, we are moving towards more sustainable and responsible consumption and production patterns, supporting SDG 12. This integration will address the current environmental challenges and ensure healthier ecosystems and communities. This study aims to biosynthesize AgNPs using actinomycetes, apply them for disinfecting domestic wastewater, and compare their effectiveness with other disinfection techniques in wastewater treatment.

2. Materials and method:

2.1. Biosynthesis of AgNPs:

Silver NPs were biosynthesized using cell free extract (CFE) of three different actinomycete strains, Actinomycetales bacterium MZ067956, MZ067957 (βC1, βA15), and Streptomyces sp. MZ067955

(βB1). The strains were obtained from Actinomycetes Laboratory, Botany and Microbiology Department, Faculty of Science, Suez Canal, where their ability to synthesize AgNPs was proved [28]. The strains were grown in starch casein broth, their biomass was harvested by centrifugation and washed twice with distilled water. One-gram wet weight of the harvested biomass was soaked in 50 ml distilled water overnight with shaking at 120 rpm, then the mixture was centrifuged to get rid of cell remnants and obtain the CFE. Silver nitrate (Merck – Germany) solution was mixed with the CFE in a 1:1 ratio for final concentration 1 mM at room temperature in light with shaking at 120 rpm for 24 hours. Biosynthesis of AgNPs was monitored by developing a brown color. The biosynthesized AgNPs were purified by cooling centrifugation at 15000 rpm for 90 min to get rid of Ag^+ and then re-suspended in deionized water.

2.2. Characterization of the biosynthesized AgNPs:

The change in color from colorless to dark brown gives a visual confirmation of the formation of AgNPs. UV–visible spectroscopy, X-ray diffraction and High-Resolution Transmission Electron Microscopy are the most common techniques used in the confirmation and characterization of AgNPs [25, 26]. UV-Vis spectroscopy measurement was carried out on T60 spectrophotometer (PG Instruments-China) in Actinomycetes Laboratory, Botany and Microbiology Department, Faculty of Science, Suez Canal University. The XRD analysis was carried out using X-ray Diffractometer D8 Discovery (Bruker-Germany) in Egyptian Nanotechnology Center, Cairo University, while HR-TEM (Jeol JEM 2100, Japan) was in Electron microscope center (EMC), Faculty of agriculture, Mansura University.

2.3. Collection of Domestic Wastewater from Sewage Treatment Plants

Wastewater samples were collected from four different sewage treatment plants along Suez Canal region (Figure 1), namely Abu Khalifah, El-Kasasin, Sarabium, and Fayed. The outlet wastewater samples were collected before the chlorination stage.

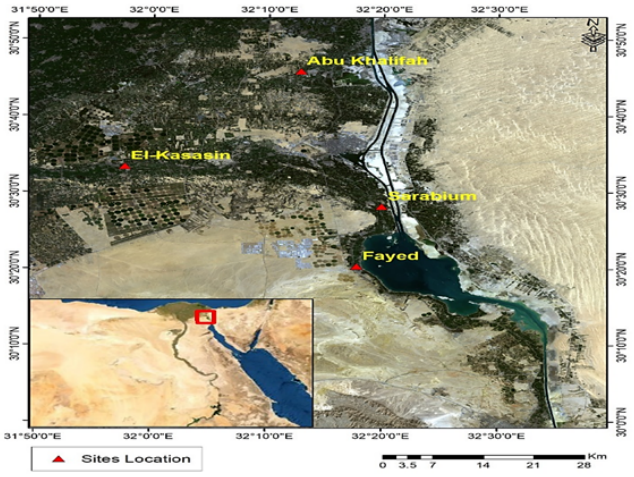

Figure 1: Location map of the selected sewage treatment plants.

2.4. Treatment of Wastewater Using the Biosynthesized AgNPs

The MIC (minimum inhibitory concentration) and the MBC (minimal bactericidal concentration) of AgNPs synthesized using the three actinomycete strains against Escherichia coli (ATCC25922) were provided from Actinomycetes Lab (Table 1). Since the lowest MBC was recorded for AgNPs biosynthesized using Streptomyces βB1, it was selected for disinfection of wastewater samples collected from the four sewage treatment plants. The disinfection was performed in 250 ml sterile conical flask, 100 ml of each water sample was treated by AgNPs and were kept on shaker overnight. Another 100 ml of water samples were accomplished as a control. The experiment was conducted in duplicate.

Table 1: MIC and MBC of AgNPs biosynthesized using the three actinomycete strains.

	MIC (μg/ml)	**MBC (μg/ml)**
Streptomyces βB1	15	30
Actinomycetales βA15	25	40
Actinomycetales βC1	15	40

2.5. Physicochemical analyses and microbial analyses of the treated wastewater samples

2.5.1. Physicochemical analyses:

Physicochemical parameters for control and treated water samples included color using a colorimetric device (HANNA– HI93727 – Hungary), Electric Conductivity (EC), pH and Redox Potential using (Adwa – AD800 - Hungary), Turbidity using digital turbidimeter device (Orbeco-Hellige – 965-10 – USA), Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). All methods were according to the Standard methods of APHA 23^{rd} Ed. [29].

2.5.2. Microbial analysis:

Total viable bacteria (TVB), total yeast, fecal streptococci (FS), total coliform (TC) and fecal coliform (FC) were the microbial group which were isolated from the control and the treated wastewater samples. The different microbial groups were isolated in the most suitable medium and most suitable incubation conditions for each according to the Standard methods of APHA 23^{rd} Ed. [29]. TVB medium was Nutrient agar (Dehydrated Himedia, India), incubation was at 37^{o}C. Medium used for Yeast isolation was Sabouraud Agar medium (Lab M, England) and was supplemented with chloramphenicol (0.125 g/l, prepared in ethanol), incubation was at 28^{o}C. Total and Faecal Coliform Medium was Endobase agar (Lab M, England). For 1000 ml medium 4 ml of 10% Basic Fuchsin were added. Incubation was at 37^{o}C for total coliform and 44.5^{o}C for fecal coliform. While for fecal streptococci, Membrane Enterococcus Agar (Lab M, England) was used, incubation was at 44.5^{o}C. All media were sterilized by autoclaving at 15 psi for 15 minutes. Ten ml of each sample were mixed with 90 ml sterilized saline solution (0.9% NaCl) as standard diluents and shaked for 5 min. The diluents used with different dilution factors. Diluted samples were cultured by using pour plate technique on the appropriate medium. For developing colonies, the plates were incubated inversely at desired temperature for each group. Each set of experiments was carried out in duplicate and reduction percentage of each microbial group was calculated as follow:

$$Reduction\,\% = \frac{count\,after\,experiment}{Initial\,count} \times 100$$

2.6. Disinfection of Wastewater Using AgNPs Synthesized by the Three Strains:

The outlet wastewater samples from Sarabium sewage treatment plant were collected and were immediately transferred to the laboratory in an ice box. 50 ml of wastewater sample were added to flasks then the MBC of AgNPs biosynthesized by each strain as shown in (Table 1) were introduced into the flasks with shaking overnight along with control water sample without AgNPs. In this experiment total and faecal coliform were targeted, the bactericidal effect was designated as the absence of colonies on agar plate. The experiments were conducted in duplicate. The most efficient AgNPs was used in the following investigations.

2.7. Disinfection of Wastewater Using Different Concentrations of the Most Efficient AgNPs:

Lower concentrations and shortening the exposure time may reduce the cost on a larger scale. Consequently, another experiment was performed to test various concentrations of the most efficient AgNPs. Five concentrations around the MIC of each strain were tested; the highest concentration tested was the MBC. The designed concentrations were introduced into flasks containing the samples and were kept on shaker, other flasks containing the samples without AgNPs were prepared for control. A subsample was withdrawn from the flasks after 1, 3, 5 hours and after overnight shaking then plated on endobase agar to count total and fecal coliform as mentioned before, the experiments were conducted in duplicate.

2.8. Disinfection Capabilities of The Biosynthesized AgNPs in Compared to Other Techniques (UV and Chlorination :

In this comparison experiment, disinfection was performed using the most efficient AgNPs while concentration and exposure time were determined in the previous experiment. For chlorination, wastewater samples were collected from the treatment plant after chlorination stage (dose:

5 mg/l). UV-treatment was carried out using UV-transilluminator by filling the wastewater sample in a transparent plastic bag and then was exposed to UV radiation for one hour (Figure 2). In this experiment TVB, yeast, TC, FC and FS were targeted.

Figure 2: UV Treatment

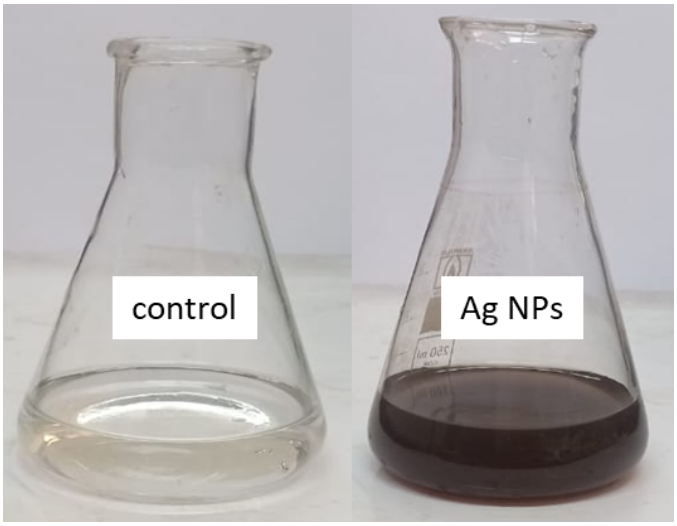

Figure 3: Biosynthesized AgNPs showing color change of silver solution into brown.

2.9. Statistical analysis:

The obtained data were collected and handled using Microsoft Excel. Differences between control and treatment groups were performed by ANOVA test using Minitab 17 program.

3. Results and discussion:

3.1. Biosynthesis of AgNPs:

After adding CFE of each strain separately to $AgNO_3$ (1 mM) for 24 hours, the color of the solution changed to dark brown. The change in color from colourless to dark brown give a visual confirmation of AgNPs formation (Figure 3). The change in color was due to the formation of elemental silver (Ag^0) from the reduction of Ag^+ ions which cause excitation of surface Plasmon vibration typical for AgNPs [28, 29, 32]. Many researchers have examined the ability of actinomycetes, particularly Streptomyces, to synthesize AgNPs [26, 28]. The manufacture of AgNPs utilizing Streptomyces using cell biomass [33], supernatant of cell culture [25, 30, 32, 33] or cell free extract [26], as adopted in the current study.

3.2. Characterization of the formation of AgNPs:

3.2.1. Ultraviolet-Visible (UV/Vis Spectroscopy:

UV–visible spectroscopy is most commonly used technique for characterization of noble nanoparticles which confirm the formation of nanoparticles and their stability. The wavelength in the range of 200– 700 nm is generally used for characterization of nanoparticles in size range of 2–100 nm. The strong surface plasma absorption band in the range of 390–470 nm is used in characterization of AgNPs [26, 36]. Silver NPs biosynthesized by the 3 strains showed peaks between 400 – 460 nm which confirm the formation of AgNPs (Figure 4). Several studies used UV- Vis scanning to monitor AgNPs and showed peaks in the same visible region [32–35]. The highest absorbance ($\mathbf{l}_{max}$) was 420, 426 nm and 461 nm of AgNPs biosynthesized βB1, βA15 and βC1, respectively. Taher et al. [33] biosynthesized AgNPs with surface plasmon absorption band with a maximum of 434 nm in the visible region, while Sambangi and Gopalakrishnan [32] biosynthesized AgNPs with $\mathbf{l}_{max}$ at 420 nm.

3.2.2. X-ray diffraction (XRD :

The XRD pattern of the biosynthesized AgNPs samples were illustrated in (Figure 5). The XRD pattern showed the Bragg's diffraction peaks of AgNPs at 38.16°, 45.04°, and 64.75°, respectively, corresponding to (111), (200), and (220) planes of the face-centred cubic lattice, similar to AgNPs in other studies [28, 30, 37].

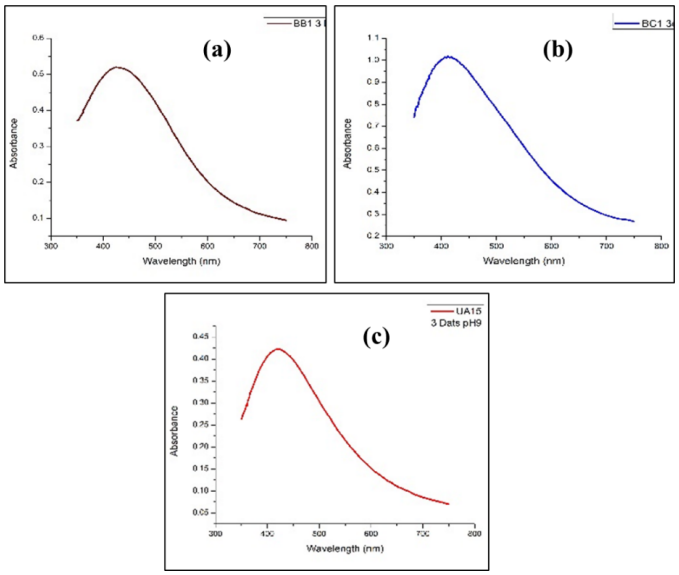

Figure 4: UV-Vis scan of biosynthesized AgNPs by (a) βB1, (b) βC1 and (c) βA15.

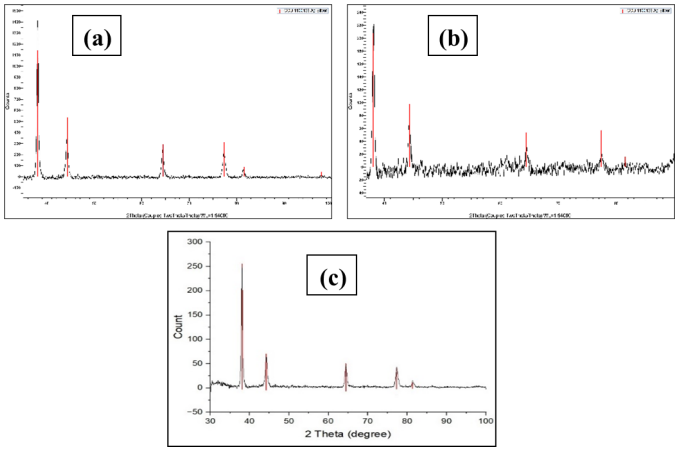

Figure 5: XRD patterns of biosynthesized AgNPs by (a) βB1, (b) βC1 and (c) βA15.

3.2.3. *High-Resolution Transmission Electron Microscopy (HR-TEM*

The size of AgNPs is determined by measuring the diameter of the particles on TEM images. The TEM images had shown in (Figure 6) that the biosynthesized AgNPs biosynthesized by βB1 are well dispersed, spherical in shape and range in size from 6 to 25 nm, which have the same shape but a different size when compared to experiments conducted by researchers [26, 28, 35]. While the biosynthesized AgNPs biosynthesized by βC1 and βA15 are not well dispersed and have spherical to irregular shape and their sizes ranged from 7 to 21 nm and from 5 to 24 nm, respectively. The nucleation properties of nanoparticles are significantly influenced by the degree of dispersion. The higher size and the poorer the dispersion the more aggregation occurs in the nanoparticles [38]. Based on their characteristics, the AgNPs biosynthesized by βB1 are thought to be better to those produced by βC1 and βA15 because they were the smallest and well dispersed.

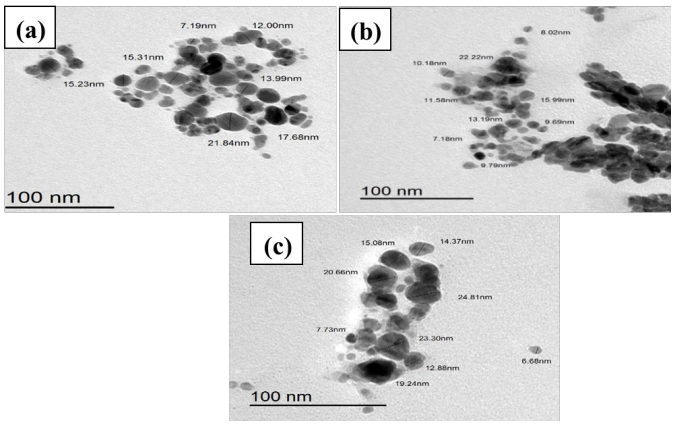

Figure 6: TEM images of AgNPs biosynthesized by (a) βB1, (b) βC1 and (c) βA15.

3.3. *Treatment of Wastewater Using βB1-Biosynthesized AgNPs*

3.3.1. *Physicochemical analysis:*

In order to verify the treatment efficiency of AgNPs for differently characterized wastewater samples, AgNPs with the lowest MBC were selected and treatment was performed with MBC. Wastewater samples were collected from different sewage treatment plants along Suez Canal region. Physicochemical parameters included pH, Redox potential, Conductivity, Color, Turbidity, BOD, and COD were measured to the control and samples treated with 30 μg/ ml of AgNPs synthesized by βB1. The result shown in (Table 2), after treatment with AgNPs, redox potential was reduced while pH, colour and turbidity were increased. All the changes in the physicochemical parameters complied with to standards and requirements for discharging liquid waste into aquatic environments based on Prime Minister's Decree No. 964 of 2015, except of the color and turbidity due to AgNPs suspension in

wastewater samples. p value was < 0.05 which indicate the significant effect of AgNPs in wastewater samples in compare to the control.

The discharge of AgNPs into the environment can indeed pose various risks [39]. The degrees of AgNPs toxicity vary depending on the overall amount of exposure, and the biological community that exists in the environment determines the highest levels of toxicity in each taxon [40]. Direct exposure to AgNPs can have biological impacts on microorganisms, plants, and soil invertebrates as well as decrease activity, abundance, and variety of soil microorganisms. It can also cause silver accumulation in plants and animals [41].

In addition to the risks of discharging, the direct application of AgNPs in water has some limitations. They frequently form aggregating clusters which reduce their effectiveness, and it is difficult to separate them from treated water [42–44]. To overcome direct application problems, AgNPs should be fixed on support materials either porous or nonporous materials such as sand, ceramic filter, polyurethane foam, zeolite, paper, fiberglass, activated carbon, polyurethane, hydrogels and chitosan [42–44].

Differences were assessed by ANOVA test with p value < 0.05 which indicate the significant effect of AgNPs in wastewater samples in compared to the control

Biological oxygen demand and chemical oxygen demand are important parameters, are measured to indicate the organic pollution in the wastewater, COD is a dominant parameter in comparison to BOD [45]. The increasing BOD and COD levels are harmful to aquatic life because they cause a drop in DO, which makes it impossible for living things to survive [46]. Figure 7 showed that treatment with AgNPs caused a significant (p value < 0.05) reduction in the BOD and COD levels which was consistent with other studies done by Qian et al,. [45], Thamilselvi and Radha 2018 [47] and Najafpoor et al,. [48].

3.3.2. *Microbiological analysis:*

Microbiological analyses of TC, FC, FS, TVB and yeasts were also conducted after the AgNPs treatment, the reduction% in their count is shown in

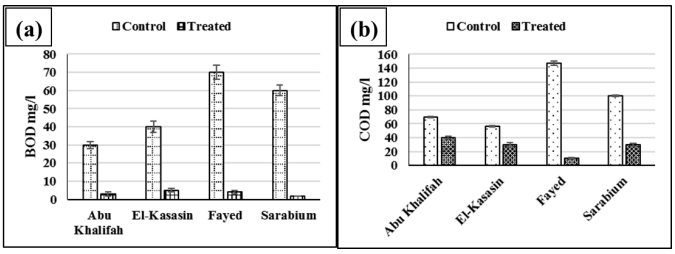

Figure 7: (a) Biological Oxygen Demand (BOD) and (a) Chemical Oxygen Demand (COD) of wastewater samples collected from four sewage treatment plants after treatment withAgNPs synthesized by βB1 compared to their control. Bar is the standard deviation and the differences were assessed by ANOVA test with p value <0.05.

(Figure 8). The reduction% of all tested microbiological group was more than 99 %. The representative isolation plates in (Figure 9) show the distinguished reduction in all groups after treatment. The biosynthesized AgNPs which produced by βB1 are more efficient in the disinfection of wastewater than AgNPs studied by Qian et al., [45], who treated raw household wastewater with 80 mg/l of AgNPs biosynthesized by leaf extract of Phyllanthus niruri and found that the AgNPs only cause about 67% reduction in coliforms.

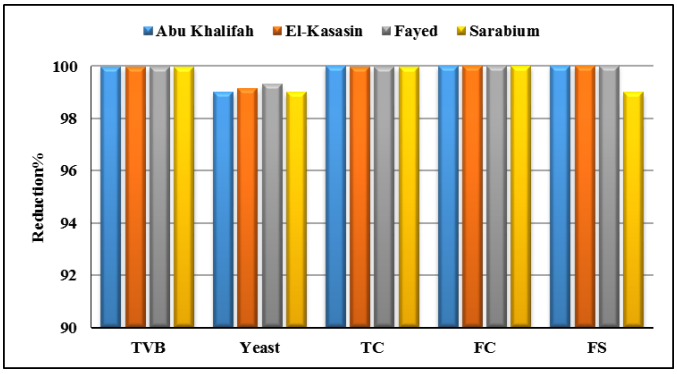

Figure 8: Reduction% in different microbial groups, total viable bacteria (TVB), yeast, fecal streptococci (FS), total (TC) and fecal (FC) coliforms in wastewater samples afterAgNPs treament.

These results confirmed the antimicrobial properties of AgNPs as it exhibited a broad antibacterial action against wide range Gram-negative and Gram-positive bacteria [49]. The mechanism of antimicrobial effect of AgNPs is due to three main mechanisms: first, AgNPs can directly damage the bacterial membrane by altering respiration and

Table 2: Physicochemical characteristics of wastewater samples collected from four sewage treatment plants after treatment with AgNPs synthesized by βB1 compared to their control. Data are presented as mean ± standard deviation.

	Abu Khalifah		**El-Kasasin**		**Fayed**		**Sarabium**	
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**	**Control**	**Treated**
pH	7.6±0.1	6.9±0.1	7.7±0.1	7.2±0.1	7.9±0.1	6.7±0.1	7.5±0.1	6.8±0.1
Redox Potential (mV)	- 19.3±0.1	9.7±0.1	- 40±0.1	- 9.7±0.1	- 48.6±0.1	7.3±0.1	- 13.6±0.1	6.5±0.1
Conductivity (μS/cm)	363±3	181±4	417±4	299±1	1070±6	1368±2	1050±5	1706±4
Color (PCU)	250±1	110±1	350±1	150±1	200±1	170±1	270±1	130±1
Turbidity (NTU)	11±0.4	2.1±0.2	8.2±0.3	23.5±0.5	15.7±0.6	4±0.2	13±0.5	10±0.3

Differenceswere assessed by ANOVA test with p value < 0.05 which indicate the significanteffect of AgNPs in wastewater samples in compared to the control

permeability, leading to leakage of cytoplasmic content such as lipopolysaccharides, membrane proteins and intracellular biomolecules. Second mechanism is AgNPs may be responsible for the generation of reactive oxygen species (ROS) which cause oxidative stress. Third mechanism is AgNPs become source of free form of silver ions can cause interference with proper generation of ATP (Adenosine triphosphate) and DNA (Deoxyribonucleic acid) molecules [50].

3.4. Disinfection of Wastewater Using AgNPs Synthesized by the Three Strains:

In order to minimize the concentration of AgNPs while maintaining disinfection activity, the antimicrobial activities against total coliform of the three AgNPs with concentration 30 μg/ml for βB1 and 40 μg/ml for both βA15 and βC1 were tested. It was found that AgNPs by βC1 was the less active as shown in (Table 3), (Figure 10), the reduction% of AgNPs by βC1 is 98% in TC and 97.5% in FC while the reduction% of the two other AgNPs by βB1 and βA15 is 99.5% in TC and 100% FC.

3.5. Disinfection of Wastewater Using Different Concentrations of the Most Efficient AgNPs

Disinfection activity of different concentrations of AgNPs from βB1 and βA15 was tested in order to test disinfection capabilities various concentrations of AgNPs synthesized by βB1 and βA15 with

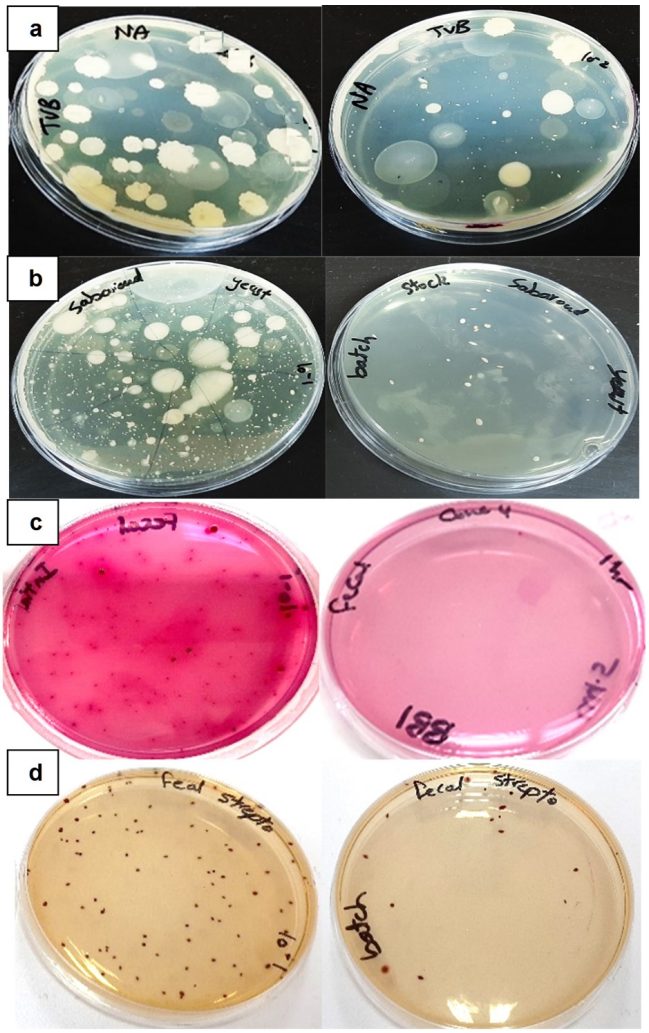

Figure 9: Representative isolation plates of total vaible bacteria (a), yeasts (b), coliforms (c) and fecal streptococci (d) from wastewater samples control (left) and after AgNPs treament (right).

Table 3: Total (TC) and fecal (FC) coliform counts measured in CFU/ml (mean ± standard deviation) in wastewater samples before and after treatment with MBC concentration of AgNPs synthesized by βB1, βC1 and βA15.

AgNPs	MBC (μg/ml)	TC		FC	
		Before	After	Before	After
		CFU/ml			
βB1	30		2		0
βA15	40	5300±250	2	2000±190	0
βC1	40		100		50

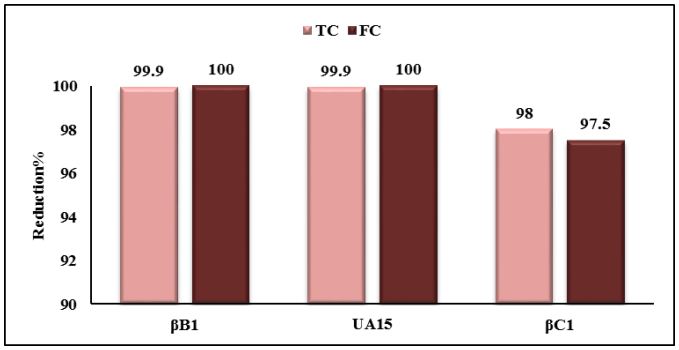

Figure 10: Reduction% in total (TC) and fecal (FC) coliforms in wastewater samples treated with MBC concentration of AgNPs synthesized by βB1, βC1 and βA15.

different contact time. The lowest efficient concentration of AgNPs to disinfect wastewater at the shortest time was targeted. All the concentrations tested reduced TC and FC counts in the first hour of treatment (Table 4 and 5). Most of concentrations tested of AgNPs synthesized by βB1 completely eliminate FC in the first hour of treatment. Only the highest concentrations of AgNPs synthesized by βA15 eliminated FC and after 3 hours of treatment (Table 5). Hence, using 15 μg/ml of AgNPs synthesized by βB1 for one hour was considered sufficient to disinfect the tested wastewater.

Total coliform bacteria or in particular E. coli is considered indicator microorganism have used to assessment the quality of wastewater [51] [52]. In this study the lowest dose can kill coliform bacteria was 15 μg/ml of AgNPs produced by βB1, while Buszewski et al., [30] found that the lower dose AgNPs synthesized by Streptacidiphilus durhamensis can kill E. coli was 50 μg/ml, and Pallavi et al., [34] reported that 128 μg/ml of AgNPs biosynthesized by Streptomyces hirsutus is the MBC of E. coli. One hour treatment with 15 μg/ml AgNPs biosynthesized by βB1 was sufficient to eliminate coliforms. Raja et al., [53], needed 6 h to get zero count in sewage water after treatment with 100 μg/ml of AgNPs produced by the leaf extract of Prosopis juliflor.

3.6. *Disinfection Capabilities of The Biosynthesized AgNPs in Compared to Other Techniques (UV and Chlorination :*

Figure (11) shows reduction% of different microbial groups in wastewater samples treated with 15 μg/ml of AgNPs synthesized by βB1 after one hour exposure compared to chlorination and UV at the same exposure time. Although chlorination and UV eliminate all the targeted groups, a comparable disinfection capability of the tested AgNPs is shown. Using AgNPs, FC and FS were eliminated and the reduction% of remaining groups exceed 99%. There were non-significant differences between AgNPs and the other disinfection techniques whereas the p value was 0.203.

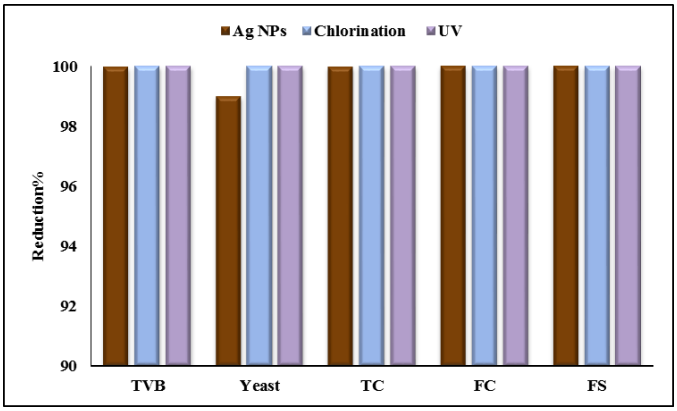

Figure 11: Reduction% in different microbial groups representing the disinfection efficiency of AgNPs compared to chlorination and UV.

The exposure of the wastewater sample for an hour to the UV light cause 100 % reduction in all the target microbial group. Due to the absorption of UV irradiation prompts the formation of DNA photoproducts like cyclobutane pyrimidine dimers and pyrimidine 6–4 pyrimidone photoproducts, which obstruct transcription and replication leading to mutagenesis and cell death [54]. Although UV light have high disinfection ability, but the use of UV light has some drawback such as: low dosage

Table 4: Total coliform counts measured in CFU/ml (mean ± standard deviation) in control and samples treated with various concentrations (μg/ml) of AgNPs synthesized by βB1 and βA15 at different time intervals.

Time (hrs)	Control CFU/ml	βB1					βA15				
		μg/ml									
		5	10	15	22.5	30	15	20	25	32.5	40
		CFU/ml									
1	6500±250	90	19	1	4	2	24	21	14	14	9
3	6900±300	11	22	1	7	2	26	35	12	9	5
5	7500±190	5	9	0	1	0	2	2	14	3	4

Table 5: Fecal coliform counts measured in CFU/ml (mean ± standard deviation) in control and samples treated with various concentrations (μg/ml) of AgNPs synthesized by βB1 and βA15 at different time intervals.

Time (hrs)	Control CFU/ml	βB1					βA15				
		μg/ml									
		5	10	15	22.5	30	15	20	25	32.5	40
		CFU/ml									
1	1400±150	2	0	0	1	0	2	1	1	1	0
3	1600±120	0	1	0	0	0	1	1	1	0	0
5	2000±210	3	0	0	0	0	2	0	0	1	0

may not effectively inactivate some viruses, spores, and cysts. Organisms can sometimes repair and reverse the destructive effects of UV through repair mechanism, turbidity, and total suspended solids (TSS) in the wastewater can render UV disinfection ineffective [55].

Chlorine has strong bactericidal effects such as penetrating cell wall and alters specific functions of proteins. Chlorine's main mechanism is to change the biological structure of bacterial enzymes that play important roles in bacterial nutrition, thereby inhibiting their growth and development [10]. Certain extremely resistant waterborne organisms have shown resistance to chlorination, making higher disinfectant dosages necessary which increases the formation of DBPs [8]. Egypt is currently discouraging the use of chlorine in wastewater management, with the growing awareness of the effects of chlorinated organics in sewage effluent on receiving waters and the tendency toward switching wastewater disinfection to the most promising disinfectant alternatives [11]. The AgNPs consider a suitable alternative disinfectant as it has good disinfection ability in comparison to UV and chlorination as well as it is cost-effective and ecofriendly.

4. Conclusion:

Utilizing the cellular free extract (CFE) of actinomycetes to biosynthesize AgNPs presents an environmentally friendly and economically viable synthesis method. Owing to the antimicrobial activity of AgNPs, these nanoparticles can effectively disinfect wastewater at low concentrations, offering comparable efficacy to conventional techniques such as UV and chlorination which have numerous drawbacks.

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