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Nano-Bioremediation of Contaminated Industrial Wastewater Using Biosynthesized AgNPs and Their Nano-Composite

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***Abstract*—**Nanotechnology as multidisciplinary technology is growing rapidly with important applications in several sectors. Also, nanobiotechnology is known for the use of microorganisms for the synthesis of targeted nanoparticles. The present study deals with the green synthesis of silver nanoparticles using aquatic bacteria and the development of a biogenic nanocomposite for environmental applications. 20 morphologically different colonies were isolated from the collected water samples from eight different locations at the Rosetta branch of the Nile Delta, Egypt. The obtained results illustrated that the most effective bacterial isolate (produced the higher amount of AgNPs after 24 h of incubation time) is isolate R3. *Bacillus tequilensis* was the strongest extracellular bio-manufactory of AgNPs. Biosynthesized nanoparticles had a spherical shape with a mean diameter of 2.74 to 28.4 nm. The antimicrobial activity of silver nanoparticles against many pathogenic microbes indicated that the produced AgNPs had high activity against all tested multi-antibiotic resistant pathogens. Also, the stabilized prepared AgNPs-SA nanocomposite has greater catalytic activity for the decolourization of some dyes like Methylene blue (MB) and Crystal violet. Such results represent a promising stage for producing eco-friendly, cost-effective, and easy-to-handle devices for the bioremediation of contaminated industrial wastewater.

***Keywords*—**Bioremediation, AgNPs, AgNPs-SA nanocomposite, *Bacillus tequilensis*, nanobiotechnology.

I. Introduction

NANOTECHNOLOGY (synthesis and applications of nanomaterials) is growing rapidly with significant applications in various areas [1], involving biology, chemistry, medicine, environment and electronics owing to nanomaterials' unique particle size and shape dependent physical, chemical and biological properties [2], [3]. Biosynthesis methods involve the usage of natural organisms or their produced materials in synthesis of nano-structures. They have emerged as a simple, clean and viable alternative to chemical and physical approaches. A wide range of biological resources available in nature, including bacteria, fungi, yeasts, algae and plants, can be used to synthesize nanomaterials. Prokaryotic bacteria have received the most attention in this field. One advantage of using bacteria in the nanoparticles manufacture is the ease of handling and genetic manipulation without much difficulty [4], [5].

To date, nanoparticles are mostly prepared from metals, i.e., silver [6], gold [7], copper [8], zinc [9], iron [10], palladium [11] and titanium [12]. Among those, silver nanoparticles (AgNPs) have received much attention in various fields, such as antimicrobial activity [13], therapeutics [14], water treatment [15], bio-molecular detection [16], coating medical devices [17] and optical receptor [18].

Screening of new microorganisms for AgNPs synthesis is very important, because the size and shape of nanoparticles can be controlled through microbial synthesis [19]. Microbial synthesis of metal nanostructure can happen either inside or outside cell system [20]. Extracellular biosynthesis mechanism is cheap and requires simple downstream procedures than intracellular one (it requires additional steps to release the synthesized nanoparticles like ultrasonic treatment or reactions with appropriate detergents) [21]. Cell-free culture supernatants (extract) of five psychrophilic bacteria, *Ps. antarctica*, *Ps. proteolytica*, *Ps. meridiana*, *Ar. kerguelensis* and *Ar. gangotriensis* and two mesophilic bacteria *B. indicus* and *B. cecembensis* have been used to synthesize AgNPs [22].

AgNPs have received increasing scientific interest because of their unique electronic, catalytic and antibacterial properties leading to applications as nanocatalyst, surface-enhanced spectroscopy, nanosensor and active material in energy devices [23]. The antibacterial properties of AgNPs are attributed to the ability of AgNPs to stabilize and penetrate the bacterial cell wall and modulate cellular signalling [24]. Among all these applications, the use of AgNPs as nanocatalyst in the field of environment remediation is the most noteworthy. It is used as catalyst for degradation of dyes; MB [25], methyl orange [26], reactive red [27], 4-nitrophenol [28], xanthene [27], malachite green oxalate [29], rhodamine B [30] and methyl red [25]. Thus, the objectives of the present study focused on the biosynthesis of AgNPs using the extracellular extract of aquatic bacteria isolated from Rosetta branch of Nile Delta, Egypt, and evaluating its use for bioremediation of wastewater containing various pathogenic microbes and dye residues like methyl blue and crystal violet.

II. Materials and Methods

Isolation of Silver Resistant Bacteria

Water samples were collected from eight different locations at Rosetta branch of Nile Delta, Egypt. Samples were taken in sterile plastic containers and transported to the laboratory in an ice box for bacteria analysis [31]. These samples were tacked the codes from R1 to R8 according to their sites of collection. Isolation of bacterial strains resistant to silver was carried out

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by plating the water samples onto nutrient agar [32]. Obtained colonies were additionally grown on nutrient agar at a concentration of 1 mM sterile filter $AgNO_3$. The plates were also incubated at room temperature for 48 h and bacterial growth in the plates was observed. Isolated colonies were sub-cultured and obtained as pure cultures and used to produce AgNPs [33].

Screening for Extracellular Biosynthesis of AgNPs

In order to screen the efficiency of bacterial isolates for synthesis of AgNPs, bacterial isolates were inoculated into 250 ml Erlenmeyer flasks, each contained 100 ml nutrient broth medium then was incubated at 30 °C for 24 h under shaking conditions at 120 rpm. After incubation, the supernatants of the cultures were obtained by centrifugation at 8000 rpm for 10 min, followed by filtration with 0.24 µm discs. The harvested supernatants were transferred to clean, sterile flasks, mixed with an equal amount of silver nitrate solution (0.5 g/100 ml) and incubated again at 30 °C under continuous agitation at 150 rpm for 72 h. The control containing silver nitrate in the media component was also run. All bacterial strains were examined for their ability to synthesize AgNPs by monitoring visible colour changes and analysing samples by UV-visible spectroscopy (Genway 6800). Only the strains capable for AgNPs synthesizing by showing colour change from yellow to brown and UV spectrum at 430 nm were selected for further screening [34].

Identification of the Most Efficient Isolate

Based on the previous screening, the most potent isolate in nano-silver fabrication was subjected to primary identification according to Bergey's Manual of Systematic Bacteriology [35]. These bacteria were identified on the basis of morphological and biochemical characters. This characterization was confirmed through molecular and phylogenetic methods [13]. The genomic DNA was extracted from the most active isolate and the 16S rRNA gene was amplified by PCR using a Bio-Rad T100 thermal cycler (Bio-Rad Laboratories, CA, USA) as previously described [36], [37]. The PCR products were purified using a QIAquick PCR purification Kit (Qiagen, USA). The purified 16S rRNA fragments were analysed by agarose gel electrophoresis and visualized using UV-transilluminator [38]. Sequencing of the amplified 16S rRNA fragments was performed using a BigDyeR Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) on an Applied Biosystems 3730xl DNA Analyzer. Similarities of the bacterial nucleotide sequences with other known sequences were examined by comparisons with the National Center for Biotechnology Information (NCBI) database for reference and type strains using the BLASTN program. A phylogenetic tree based on partial 16S rRNA sequences was constructed using the neighbour-joining method contained within the Clustal X program and MEGA6 software. The obtained sequences were submitted to GenBank [39], [40].

Optimization of AgNPs Bio-Manufacturing Using Bacillus tequilensis

Microbial synthesis of AgNPs by the most efficient bacterial isolated *Bacillus tequilensis* had been investigated under different conditions. The most efficient strain R3 is used for production of AgNPs at different concentration of silver nitrate (0.025-0.05-0.1-0.2-0.4-0.6%) to determine the best concentration for production of AgNPs [34]. Also, bio-reduction of silver ions was evaluated at initial and final pH values (5 to 9) using 0.2M NaOH and 0.1M HCl. Also, incubation temperature (15-45 °C) was applied twice, the first time is the bacterial growth temperature, another one is the bio-reduction incubation temperature and the pH was 7.0 with incubation period for 72h.

Production and purifica tion of AgNPs by Bacillus tequilensis

In order to find the best cultivation process for AgNPs production, the bacterial strain was cultivated into nutrient broth medium for 24 h. Then supernatant was mixed with 0.2% $AgNO_3$ adjusted at pH 7 at 20 °C for 24 h. Visual observation showed a change of colour from yellow to brown. After centrifugation of brown colour solutions (10 min, 6000 rpm), the produced nanoparticles were washed consequently with absolute ethanol (Merck, Germany). These steps were repeated again in order to gain pure nanoparticles. The AgNPs were dried and stored in lyophilized powdered form until used.

Characterization of the Produced AgNPs

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analysis (EDX)

The morphology and topography of produced AgNPs were visualized using SEM at National Research Center (SEM, Quanta FEG 250, FEI, Republic of Czech). Also, elemental composition of tested AgNPs was analysed by Energy Dispersive X-Ray Spectrometer (EDS) "Phoenix" detector for compositional analysis. Elemental analysis was performed at 20 keV [41].

Transmission Electron Microscopy (TEM)

The dimension and shape of the synthesized AgNPs were analysed using transmission electron microscopy (JEOL, JEM 1400) at accelerating voltage of 80 kv. Samples for TEM analysis were prepared by drop-coating AgNPs solution onto carbon-coated copper grids. The films on the TEM grids were allowed to stand for 2 min. The extra solution was removed using a blotting paper and the grid dried prior for measurement [42].

In vitro Antibacterial Assay of AgNPs by Agar-Well Diffusion Assay

The agar-well diffusion method was used to test the antimicrobial capabilities of the bacterial synthesized AgNPs against multiple pathogenic strains including: Gram positive bacteria, i.e., *Staphylococcus aureus* ATCC-47077, *Bacillus cereus* ATCC-12228, *Listeria monocytogenes* ATCC-35152, *Staphylococcus epidermidis, Enterococcus faecalis* ATCC-29212 and Gram-negative bacteria, i.e., *E. Coli* ATCC-25922, *Pseudomonas aeruginosa* PTCC-1074, *Salmonella typhi*

ATCC-15566, *Aeromonas hydrophila* and yeast i.e. *Candida albicans* ATCC-10231 according to standard methods [43]. Pathogenic microorganisms, used in this study, were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA), and Microbiology Lab, National Institute of Oceanography and Fishers and National Research Centre (NRC), Egypt. The wells contained distilled water were considered as negative control. The sensitivity of the tested pathogens was determined by measuring the diameter of inhibition zone around each well (mm). Experiments were performed in triplicate [44].

Effect of Different AgNPs Concentrations as Antimicrobial Agent

The purified AgNPs dissolved in distilled water at different concentrations (70000, 7000, 5000, 3000, 700, 500, 300, 70 µg/mL) were tested against some pathogenic strains using well-diffusion technique as explained above. The plates were incubated at 37 ºC for 24 h. Antimicrobial activity of AgNPs was determined by measuring the growth inhibition zones around the well in mm [45]. Negative control was applied using water only.

Antibiotic Sensitivity Test of Tested Pathogens

Ten formulated antibiotic discs were purchased from Sigma (Dorset, UK), arranged according to their group (Table I) and used to compare the inhibition of the tested organisms with AgNPs. This test was performed using disk diffusion method, KirbyBauer method.

TABLE I
ANTIBIOTICS USED TO EVALUATE THE ANTIMICROBIAL ACTIVITY OF NANO SILVER

Antibiotic group	Antibiotic	Abbreviation	Concentration
Phenicoles	Chloramphenicol	C	30 mcg
Penicillin	Ampicillin	AM	10 mcg
Aminoglycosides	Amikacin	AK	30 mcg
Rifamycin	Rifampin	RA	5 mcg
Tetracyclines	Doxycycline	DO	30 mcg
Quinolones	Nalidixic acid	NA	30 mcg
Cephems	Cefotaxime	CTX	30 mcg
Glycopeptide	Vancomycin	VA	30 mcg
Nitroimidazole	Metronidazole	MET	5 mcg
Macrolide	Erythromycin	E	5 mcg

Preparation of AgNPs-Alginate Nanocomposites

AgNPs-SA beads were prepared according to the reported method for the AuNPs-SA [46]. Briefly, the as-prepared AgNPs-alginate nanocomposites were added dropwise through a syringe pump to freshly prepared 0.1 M $CaCl_2$ solution. The spherical beads were formed in the presence of Ca^{2+} and left in aqueous $CaCl_2$ solution for further cross-linking to achieve more stability. The beads were filtered out of $CaCl_2$ then washed and stored under purified water for further use. The AgNPs-SA beads were dried at 100 °C in an oven for the evaluation of catalytic activity.

Evaluating the Catalytic Property of AgNPs-SA Beads as Removing Agent for Dyes

At first, 3 ml of the azo-dye solution (30 mg/l) were put in a 10 ml plastic tube, then, 15 AgNPs-SA beads were added. After stirring thoroughly for 10 min, the mixture was transferred into a quartz cell. The change of absorbance was used as criteria to evaluate the reduction efficiency. Also, control of only SA beads was used [47].

III. RESULT AND DISCUSSION

Isolation and Screening of AgNPs Manufacturing Bacteria

A total of 20 morphologically different colonies were isolated from the collected water samples from Rosetta Branch, River Nile. These isolates were re-streaked on new agar plates for purification. The purified isolates were screened for their ability for extracellular production of nano-silver. The change of cell-free filtrate from yellow to brown was the primary assay to determine whether the isolates are capable of synthesizing AgNPs. The supernatant of six bacterial isolates produced brown colour of AgNPs (Fig. 1). The codes of the positive isolates were R1, R2, R3 R5, R6 and R8. The change in colour is due to excitation of surface plasmon resonance (SPR) of AgNPs. The control and blank tubes remained colourless [48], [49]. AgNPs synthesis was validated using UV-visible spectrophotometry. This type of scanning was useful for measuring the SPR adsorption of AgNPs [50]. The typical absorption region for AgNPs falls between 350 and 450 nm. The absorption spectra of AgNPs depend on several factors like shape, size and interaction between particle clusters with the medium [51]. A strong SPR band was observed at 300-460 nm for the, R2, R3.R5, R6 isolates (Fig. 1). UV spectra at 430 nm are used as a property to detect AgNPs [52].

The synthesis of AgNPs in solution was monitored by measuring the time dependent of changes in the onset absorbance at an interval of 2-48 h. The onset absorption was adjusted at 430 nm [53]. A time dependent increase was noticed in the absorption peaks of AgNPs. There is no absorption peak detected corresponding to the control (supernatant without AgNO3). The absorbance of colour formed by different bacterial isolates at 430 nm with different incubation periods was illustrated in Table II. Also, the symmetric plasmon band implies that the solution does not contain much of aggregated particles. Accordingly, isolate R3 was chosen for further studies. The result showed that as the incubation period increased, the AgNPs production was increased gradually until 24 h, then became stable. The colour intensity increased with the incubation period due to the decrease of Ag° [54].

TABLE II
DEVELOPMENT OF BROWN COLOUR FORMATION BY DIFFERENT BACTERIAL ISOLATES MEASURED AT 430 NM

Sample	Incubation period (h)					
	2	4	6	12	24	48
R2	2.2015	2.2102	2.4897	2.6765	2.9367	2.9123
R3	3.7723	3.8298	3.8347	4.5046	4.6164	4.567
R6	2.2128	2.4724	2.5673	2.6531	3.001	3.111

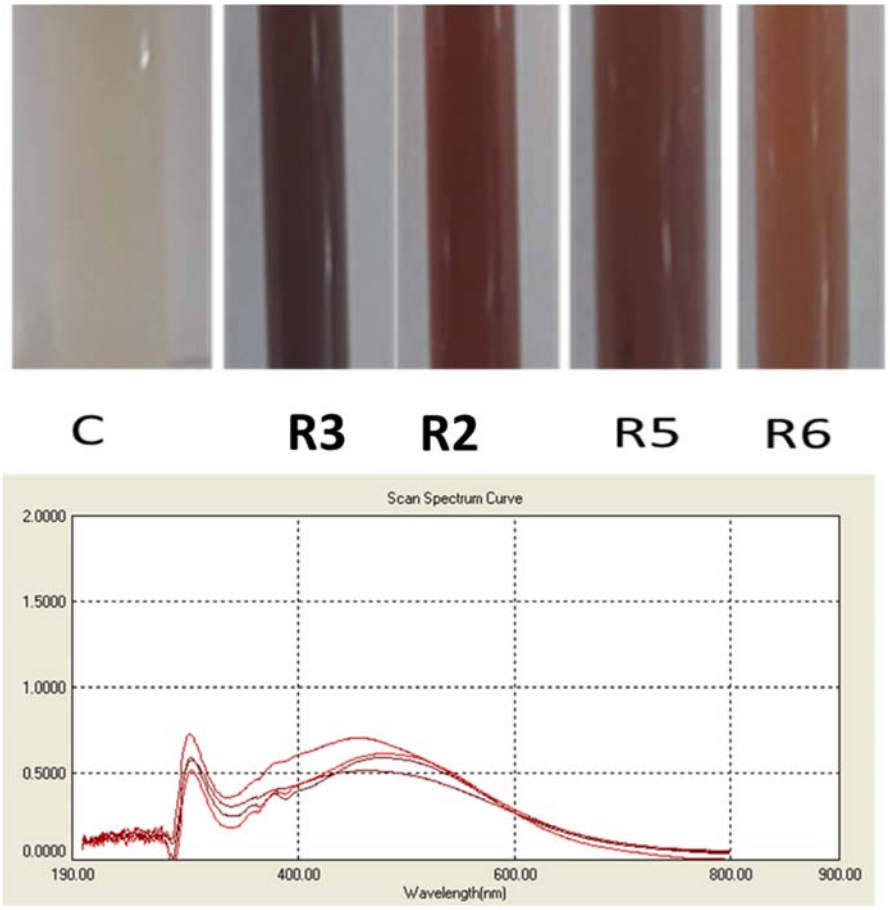

Fig. 1 Visual observation of biosynthesized AgNPs and UV-Vis absorption spectra

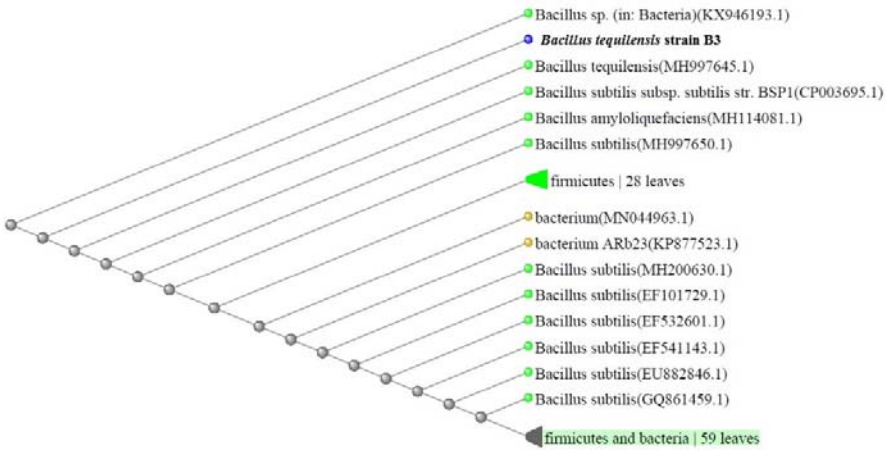

Fig. 2 Phylogenetic tree based on the 16S rRNA gene sequences of (R3*) Bacillus tequilensis* with sequences available in the GenBank

Identification of the Most Efficient Bacterial Isolate (Bacterium # R3)

Many bacterial genera had been recorded for reduction of silver ions to AgNPs [50]. In the present study, the most efficient isolate, R3 had been identified based on morphological and biochemical characteristics, as well as molecular finger prints. In case of classical identifications, R3 was rod in shape, gram positive, oxidase positive, catalase positive, and gelatin hydrolysis positive. For molecular identification of the selected bacterial isolate, the DNA was extracted, purified and 16S rRNA gene amplified. The phylogenetic trees of strain have been displayed in Fig. 2 and the bacterial isolate was identified as *Bacillus tequilensis.*

Optimization of $AgNO_3$ Concentration for AgNPs Bio-Production by Bacillus tequilensis

Six different concentrations of $AgNO_3$ were included in the culture supernatant separately. The purpose of this test was to

determine the effect of various concentrations of $AgNO_3$ on the number of produced silver particles. Minimum and maximum concentrations of $AgNO_3$ were 0.025 and 0.6%, respectively. These experiments were repeated several times. The data illustrated in Fig. 3 represent absorbance of the produced nanoparticles by *Bacillus tequilensis* at different concentrations of $AgNO_3$ after 24 h of incubation at 430 nm. The results showed that with increasing concentrations of $AgNO_3$, the production of AgNPs increased until it reached 0.2% (1.8844), as the concentrations of $AgNO_3$ continued to increase, AgNPs production began to decrease gradually in accordance with [34].

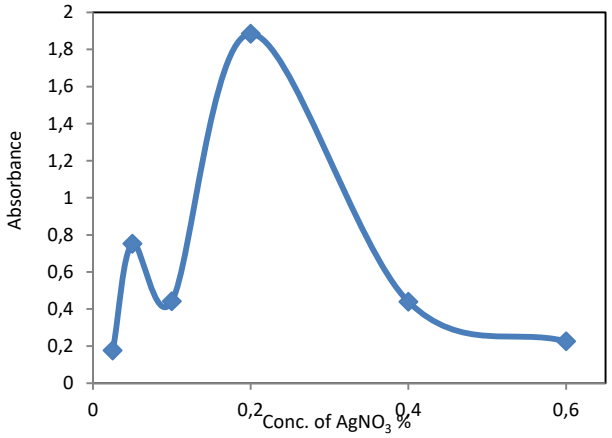

Fig. 3 Absorbance of produced nanoparticles by *Bacillus tequilensis* at different concentrations of $AgNO_3$ after 24 h

Effect of Different Temperatures and pH Values on Production of AgNPs by Bacillus tequilensis

Nanoparticles synthesized microbially by *Bacillus tequilensis* in terms of colour intensity were determined spectrophotometrically at different temperature degrees (15, 20, 25, 30, 35, 40 and 45 °C) and pH values (5-7). The results revealed that maximum AgNPs production was at 20 °C and pH 7 as indicated in Figs. 4, 5. As the temperature of reaction mixture (cell free supernatant + $AgNO_3$) increased, the AgNPs accumulation increased till it reached the maximum production at 20 °C and then began to decrease as temperature continued to increase. In the previous manuscript, Kalimuthu et al. [21] exposed that, when using the cell-free culture supernatant of *P. antarctica*, the stability of the produced AgNPs was affected by the growth temperature. These results indicated that the factors in the cell-free culture supernatants that facilitate AgNPs synthesis and stability vary from bacterial species to others. It was observed that AgNPs formed using cell-free supernatants from *A. kerguelensis* and *P. antarctica* were stable for up to 8 months if stored in the dark.

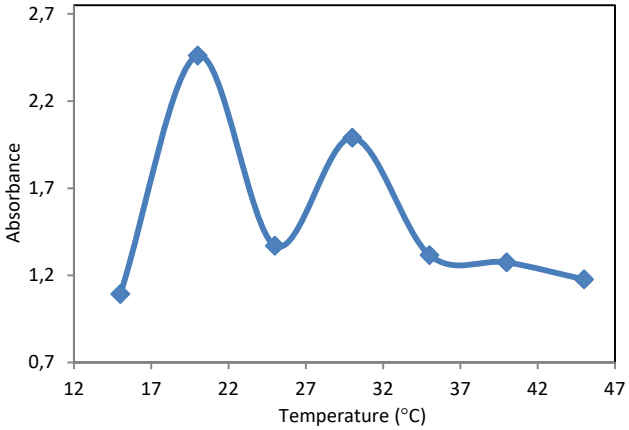

Fig. 4 Different absorbance of the produced nanoparticles by *Bacillus tequilensis* at different temperatures

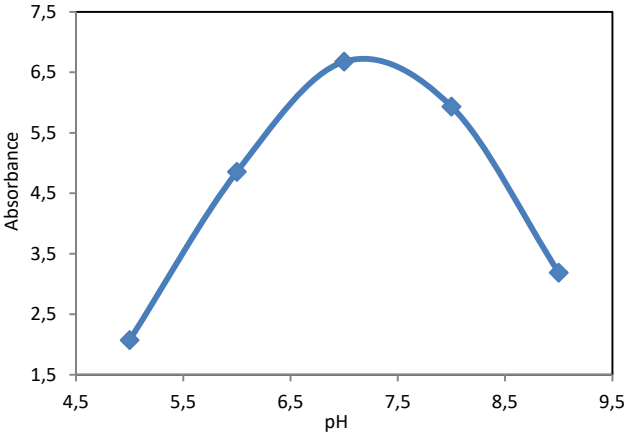

Fig. 5 Different absorbance of the produced nanoparticles by *Bacillus tequilensis* at different pH values

Production and Purification of AgNPs by Bacillus tequilensis

In order to find the best cultivation process for AgNPs production, the bacterial strain was cultivated into nutrient broth medium for 24 h. Then supernatant was mixed with 0.2% $AgNO_3$ adjusted at pH 7 at 20 °C for 24 h. Visual observation showed a change of colour from yellow to brown (Fig. 6). The AgNPs were dried and stored in lyophilized powdered form until used (Fig. 6). The mechanisms for extracellular microbial synthesis of nanoparticles are varied, the microbial agents contained some reduction systems like nitrate reductase, phenols, inactive proteins and other bio-reduction systems [1], [21]. Nangia et al. [55] also suggested that the biosynthesis of nanoparticles and their stabilization via charge capping in *Stenotrophomonas maltophilia* involved NADPH dependent reductase enzyme through electron shuttle enzymatic metal reduction process.

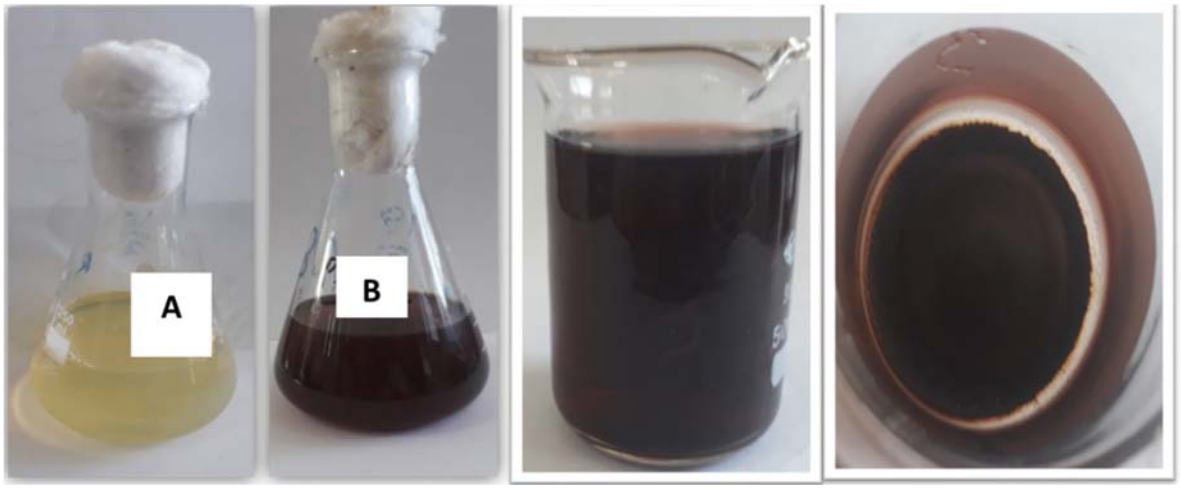

Fig. 6 Conical flasks containing *Bacillus tequilensis* culture supernatant in aqueous $AgNO_3$ solution (A) at the beginning of reaction, (B) After 72 h of reaction and the dried AgNPs

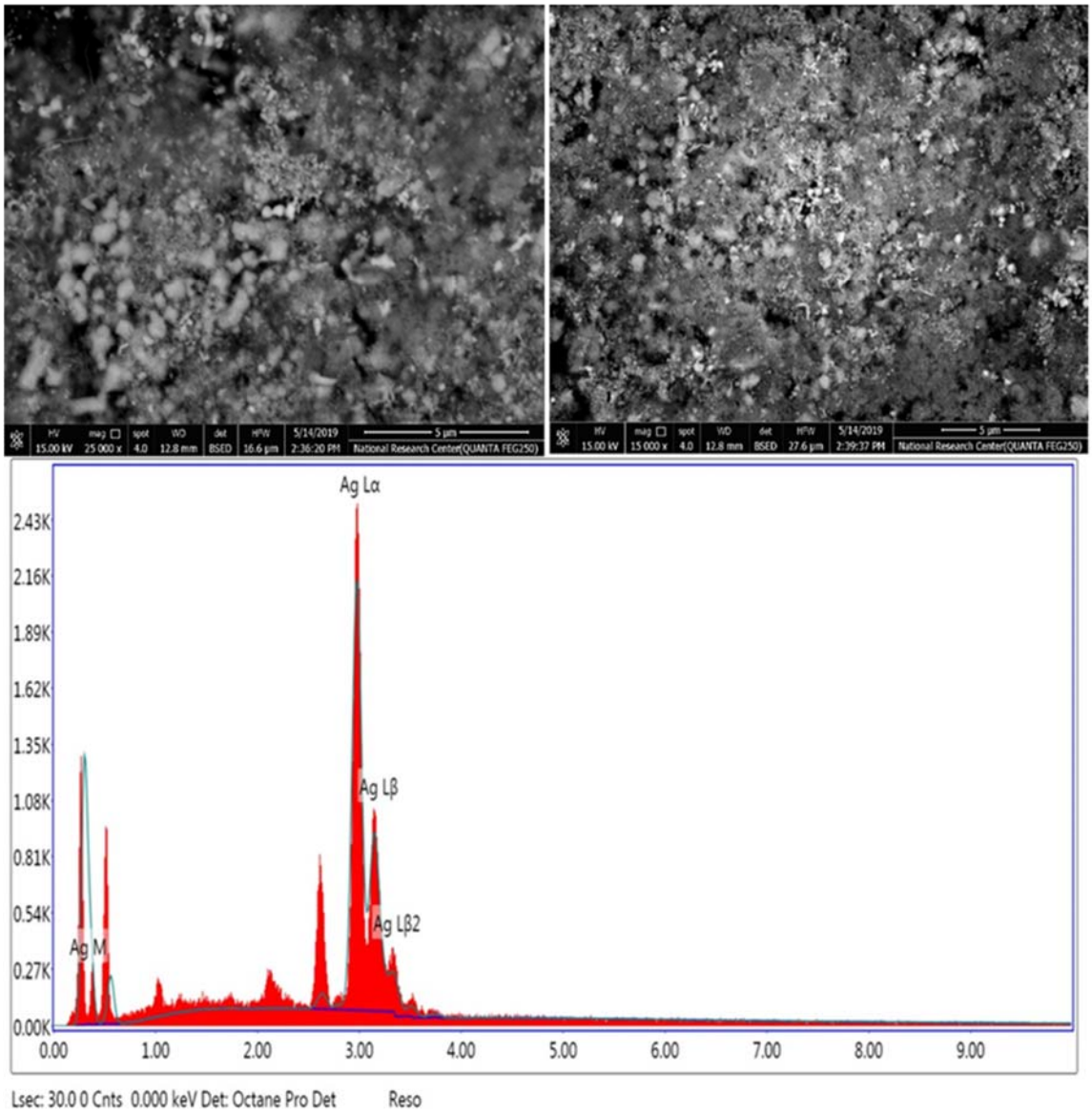

Fig. 7 Electron microscope picture and EDX spectra of AgNPs produced by *Bacillus tequilensis*

Characterization of the Produced AgNPs

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray analysis (EDX)

The formation of AgNPs by *Bacillus tequilensis* was confirmed using SEM. As shown in the electron micrograph, many spherical shaped nanoparticles can be clearly noted (Fig. 7). Energy dispersive spectroscopy was applied for confirming the presence of silver element (Fig. 7). The silver nanostructures were verified by an absorption band peak at 3 KeV [56]. Other EDX signals emitted by C, N and O atoms were also observed. It is may be due to X-ray emissions from proteins and enzymes present in the cell-free filters [57], [58].

Transmission Electron Microscopic (TEM)

Bacterial culture was used to synthesize small, relatively uniform, extracellular AgNPs. The morphology of the AgNPs produced by *Bacillus tequilensis* was analysed using TEM (Fig. 8). The TEM images showed that the mediated nano silver has spherical, hexagonal, triangular, and pseudospherical morphological properties, with traces of agglomerations due to the binding of biological molecules to the nanoparticles [59]. The diameter of the nanoparticles ranged from 2.74 to 28.4 nm. The obtained results are in concurrence with biologically produced AgNPs using bacterial strains like *Bacillus pumilus*, *Pseudomonas aeruginosa* KUPSB12 and *E. coli* [60].

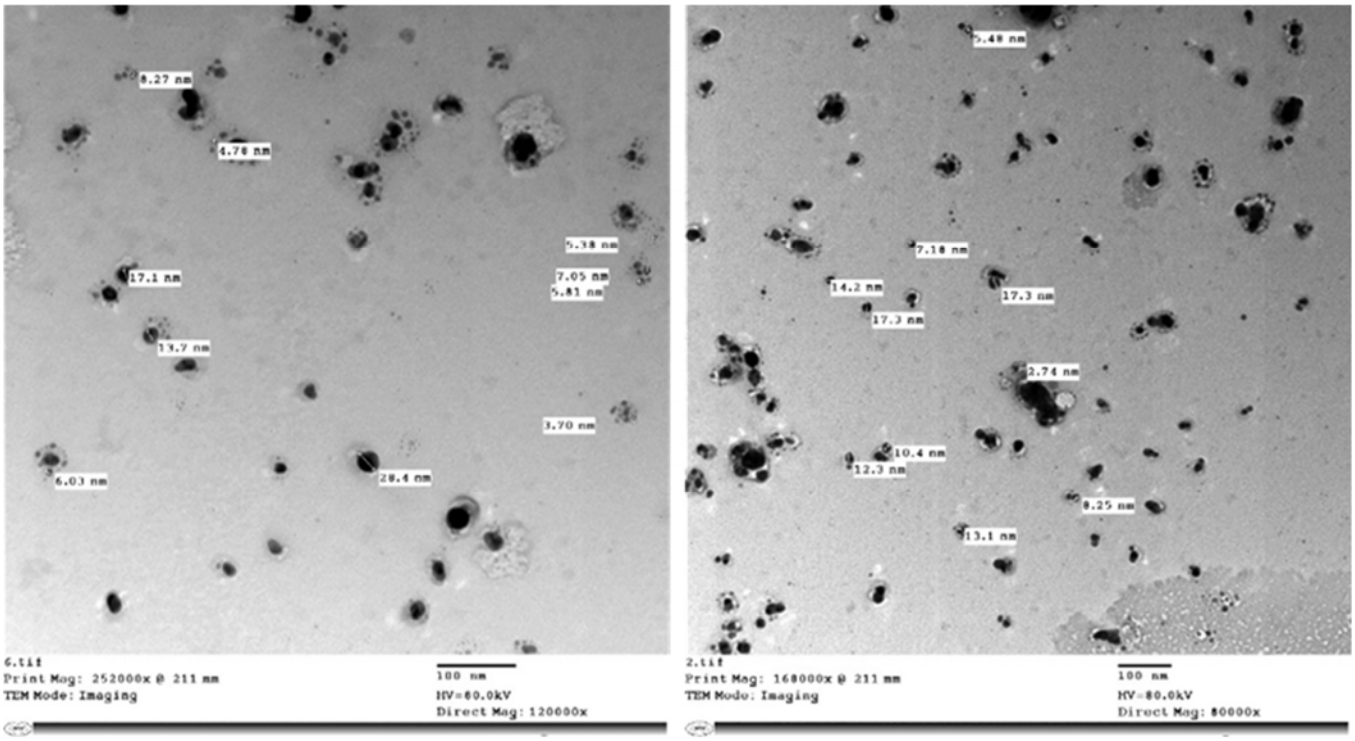

Fig. 8 TEM picture of AgNPs produced by *Bacillus tequilensis*

In vitro Antibacterial Assay of AgNPs by Agar-Well Diffusion Assay

The antibacterial activities of the synthesized AgNPs were examined against some pathogenic bacteria. The AgNPs exhibited antibacterial activity against pathogenic Gram positive & Gram-negative bacteria and yeast. The highest inhibition zone of 21 mm diameter was formed against *L. monocytogenes* ATCC-35152 and the lowest of 9 mm was noted against *Sal. typhi* ATCC-15566 by the synthesized nanoparticles. The mean of inhibition zones diameter (mm) around each well was represented in Fig. 9. The highest antimicrobial activity was observed against *L. monocytogenes* ATCC-35152 followed by *St. aureus* ATCC-47077 and *E. coli* ATCC-25922 (Figs. 9, 10). The results of this study are in agreement with [61] which showed that AgNPs exhibited maximum antimicrobial activity against *Ps. aeruginosa* followed by *St. aureus* and minimum antimicrobial activity against *Escherichia coli*. Shrivastava et al. [62] suggested that Gram-negative bacteria were more sensitive to AgNPs than Gram-positive caused by the interactions of positively charged AgNPs with negatively charged lipopolysaccharides. The effect of AgNPs on bacterial strains may be returned to the release of their ions inside the bacterial cells [63], [64].

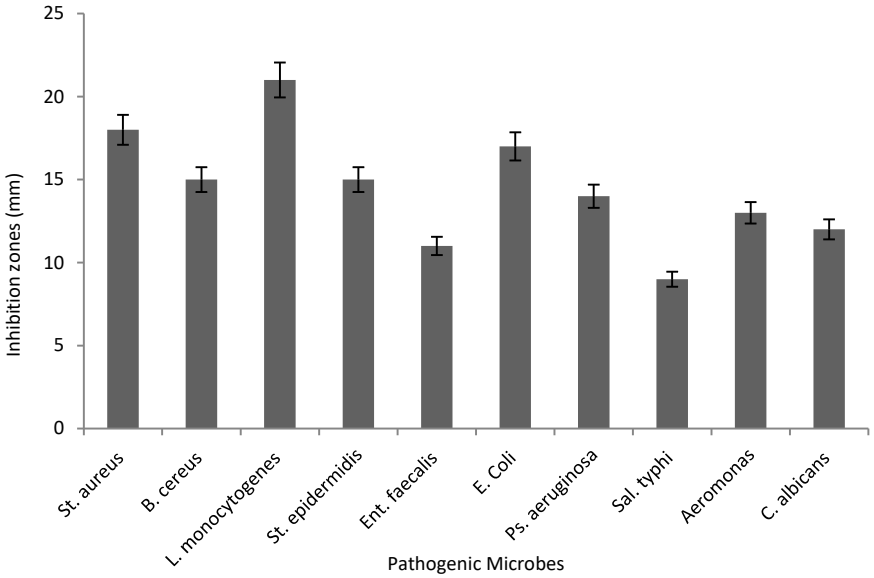

Fig. 9 Antimicrobial activity of AgNPs against ten tested microbes

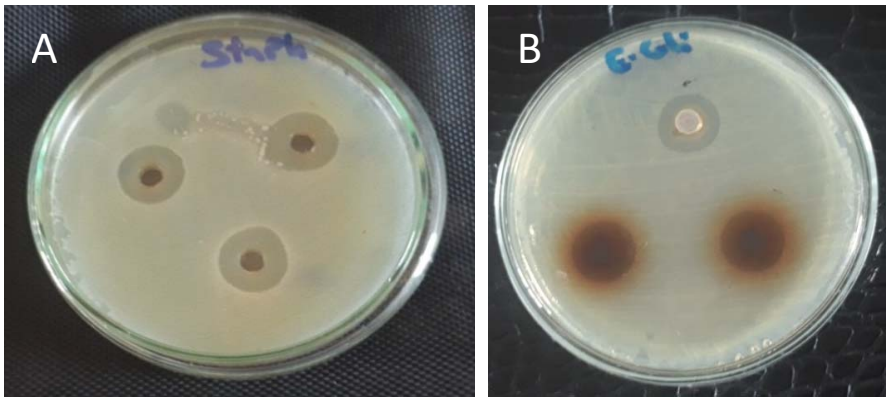

Fig. 10 Inhibitory effect of AgNPs on *St. aureus* (A) and *E. coli* (B)

TABLE III
COMPARISON BETWEEN ANTIBIOTICS SENSITIVITY TEST AND BIOLOGICAL NANO SILVER AGAINST THE PATHOGENIC STRAINS

Antibiotics (AB)	Inhibition zone diameter (mm)									
	Gram negative					Gram positive				Yeast
	Ps. aerginosa	*E. coli* ATCC-25922	*A. hydrophila*	*Salmonella typhi* ATCC 15566	*Bacillus cereus ATCC- 12228*	*St. aureus* ATCC-47077	*St. epidermidis*	*Listeria monocytogenes* ATCC- 35152	*Enterococcus faecalis* ATCC- 29212	*Candida albicans* ATCC- 10231
Ampicillin	22	16	0	19	0	30	21	0	25	9
Erythromycin	0	0	0	0	0	15	8	0	16	11
Cefotaxime	0	15	0	15	0	0	0	0	0	0
Vancomycin	19	7	0	0	15	14	17	0	16	15
Nalidixic acid	0	20	0	17	16	0	0	0	0	16
Rifampin	15	7	0	0	0	21	26	19	12	0
Chloramphenicol	0	29	17	21	10	17	9	25	17	17
Metronidazole	0	0	0	0	0	0	0	0	0	0
Doxycycline	8	19	8	15	13	22	11	22	14	20
Amikacin	16	15	10	16	17	16	23	19	0	19
Nano Silver (70 mg/ml)	18	13	11	9	16	18	16	24	11	17

Ab_{AVG} = Antibiotics Average

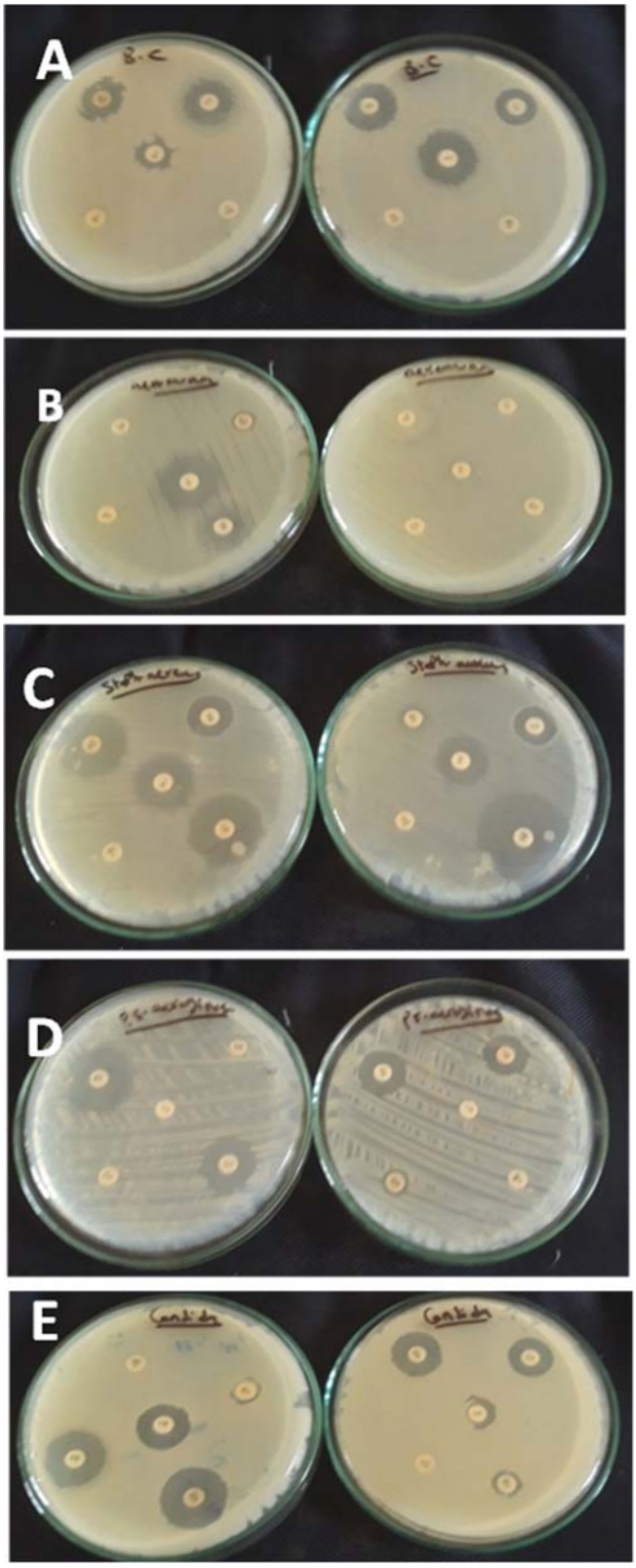

Fig. 11 Antibiotic susceptibility test for (A) *Bacillus cereus*, (B) *A. hydrophila*, (c) *St. aureus*, (D) *Ps. aureginosa*, (E) *C. albicans*

Comparison between the Antimicrobial Activity of AgNPs and Some Antibiotics

The pathogenic bacteria used in this study were subjected to the antibiotic sensitivity test. Ten antibiotics belonging to ten different groups were used. Our results showed that some of these strains were resistant to three or more antibiotics indicating the presence of multiple antibiotic resistances (MAR) strains (Table III and Fig. 11). Also, formulated antibiotics were used to compare with the activity of AgNPs obtained by *B. tequilensis* against the ten pathogens. Our results showed that most of tested pathogens show high sensitivity to produced AgNPs than many used antibiotics. For example, *A. hydrophila* that showed resistance to seven antibiotics, showed sensitivity to the produced AgNPs. Also, *Listeria monocytogenes* ATCC-35152 showed resistance to six antibiotics but showed sensitivity to produced AgNPs.

Catalytic Degradation Activity of AgNPs-SA Nano-Composite Beads for Dangerous Dyes

Metal nanoparticles can exhibit good catalytic activity for some infeasible reactions. AgNPs and their nano-composites have greater catalytic activity for the decolourization of some dyes [65]. In order to investigate the catalytic activity of the green synthesized AgNPs stabilized into SA, the decolourization of MB and crystal violet as models was tested. The progression of the catalytic reduction of dyes can be easily followed by the decrease in absorption at the wavelength of the absorbance maximum of the dye molecules. Also, control of SA beads only was used for degradation of two dyes; the result showed decrease in absorbance of the two dyes, in accordance with results obtained by Thangaraj et al. [51]. MB is a kind of heterocyclic aromatic dye with molecular formula C16H18C1N3S and the usage has increased. MB in water shows a strong absorption band at 615 nm in the visible region [66]. Addition of calculated amount of AgNPs into MB solutions make decrease in the absorbance indicating reduction is taking place in the presence of AgNPs (Fig. 12, Table IV). These results proved that SA based AgNPs act as a highly dynamic catalyst for the reduction of MB. The reduction of MB dye was evident from the gradual decrease of the absorbance value of the dye [67]. Here in our research, addition of AgNPs into MB solutions decreased the absorbance indicating reduction of MB in the presence of AgNPs (Fig. 12, Table IV). These results proved that SA based AgNPs act as a dynamic catalyst for the reduction of crystal violet. The reduction of crystal violet dye was evident from the gradual decrease of the absorbance value of the dye.

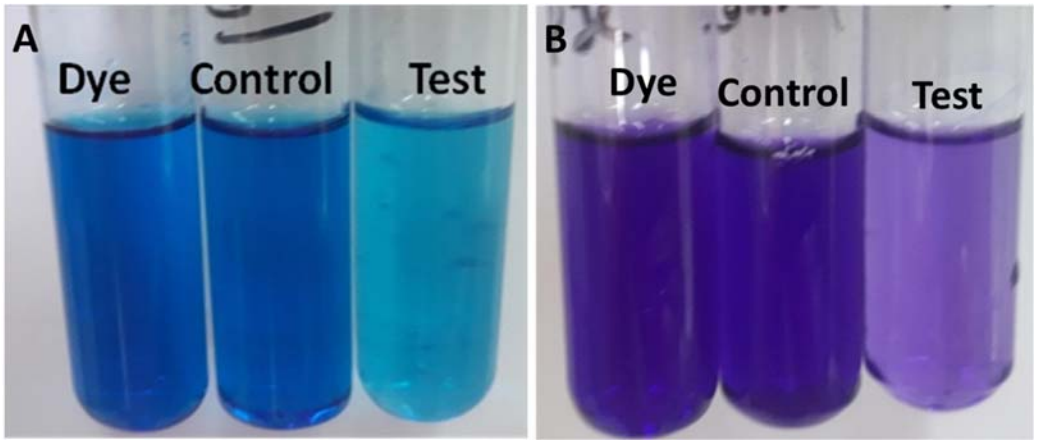

Fig. 12 Decolourization of MB (A) and crystal violet (B) by AgNPs-SA nanocomposite beads

TABLE IV
DECREASE OF ABSORBANCE OF MB AND CRYSTAL VIOLET

Sample	MB (665 nm)	Crystal violet (590 nm)
Dye	3.8960	1.8589
Control	2.9861	1.9122
Test	1.1406	0.9572

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