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Phyco-biosynthesis of *Chlorella*-CuO-NPs and its Immobilization on Polyester/Cotton Blended Textile Waste Activated by Cellulase Enzymes for Application as Wastewater Disinfection Filter

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Abstract

The accumulation of textile wastes, without use, leads to many environmental and economic problems, so they must be recycled and given "smart" properties to be useful in many applied fields. Immobilization of nanoparticles, particularly biosynthesized one, on textile surfaces may provide additional bioactive capabilities, such as antimicrobial and photoprotection due to its distinct properties. Therefore, in this study, CuO-NPs were biosynthesized by *Chlorella* microalga and immobilized on the surface of Polyester/Cotton (PET/C) blended textile waste modified using cellulase enzymes. Phyco-biosynthesized *Chlorella*-CuO-NPs were characterized using SEM-EDAX, HRTEM, XRD, FTIR, and whose particles were approximately spherical and nano-sized (<10 nm) and capped with organic compounds of biological origin. Both *Chlorella*-CuO-NPs and cellulase-modified PET/C textiles loaded with *Chlorella*-CuO-NPs exhibited antimicrobial activity and UV protection. The modified fabric was applied as a nano-biofilter to reduce the microbial load in wastewater to disinfect it from different pathogens e.g. total coliform bacteria, *E. coli* and *Salmonella* (efficiency exceeding 97, 98 and 99.4%, respectively). Recycling textile waste using microbial nanotechnology is an environmentally friendly and cost-effective way to develop smart fabrics for various industrial and applications in the environment.

Keywords: Polyester/Cotton wastes, *Chlorella*-CuO-NPs, Microbial nanotechnology, Wastewater remediation.

1. Introduction

Nanotechnology research has recently grown more active and interesting due to the promising outcomes and prospects in a wide range of applications. The primary building blocks of the rapidly developing nanotechnology are nanomaterials with particle sizes smaller than 100 nm. The features of a substance utilized, such as its catalytic effectiveness, thermal conductivity, chemical performance, and antibacterial activity, are significantly influenced by the size of the particles. A variety of nanoscale materials, including those made of iron, selenium, cadmium, platinum, titanium, zinc, copper, gold, magnesium, and silver, have been developed **[1,2,3]**. Several synthetic routes are used for the fabrication of nanoparticles of diverse morphology and size. While these methods have resulted in higher-quality nanoparticles, more efficient production methods still need to be developed**[4,5].**There are many ways to create metal nanoparticles, but chemical reduction techniques are the most popular since they are more effective than the other techniques **[6]**. However, such chemical approaches may occasionally be performed by harmful or environmentally hazardous compounds.

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This is why green nanoparticle manufacturing that uses microbes or plant extracts is more acceptable, especially for use in biomedical applications and human meals**[3].**

There have been reports of the use of several microorganisms, including bacteria, fungus, actinomycetes, and microalgae, in the green production of nanoparticles **[7**,**1**,**3]**. These microorganisms develop the capacity to create reducing substances that facilitate the bio-conversion of metal ions into nanoforms both intracellularly and/or extracellularly **[8].** Microalgae are photosynthetic microorganisms that inhabit both terrestrial and aquatic habitats. They have prokaryotic or eukaryotic unicellular or simple multicellular structures. Microalgae can be cultivated in a variety of water resources, such as freshwater, seawater, and even domestic or industrial effluents. As photosynthetic organisms, microalgae can use CO_2 as an inorganic carbon substrate when employing light energy. A variety of bioreactor systems, including open ponds and photobioreactors, can be used to cultivate them on a large scale**[9].**

For the manufacture of metallic nanoparticles, microalgae are regarded as convenient and effective "bionanofactories.". Their capacity to do so depends on the intracellular and extracellular biomolecules they make as they grow (functional groups and enzymes that serve as reducing agents to turn metals into nanoform. There have been reports of both freshwater and marine alga-based nanoparticle synthesis techniques. Furthermore, microalgae offer the advantages of autotrophic growth and faster multiplication, as well as large-scale synthesis possibilities without requiring any safety precautions **[4,10].**

Functional textiles, which have a 30% annual growth rate between 2015 and 2020, have sparked a great deal of interest across several industrial sectors. The automotive, fashion, healthcare, military, and sports industries have mostly supported this rise. Functional fabrics have characteristics such being antimicrobial, ultraviolet (UV) protecting, heat-resistant, insect-repellent, oil/water-repellent, odor-controlling, windproof, sensing, thermoregulating, andself-cleaning**[11]**. Therefore, tremendous attempts have been undertaken to create novel fibers that have the desirable qualities of both natural and synthetic fibers, or to functionalize current fibers by improving their favorable qualities **[12]**. In order to facilitate the addition of nanocomponents to fabrics, the fabric surface must be activated by the addition of charged functional groups, either by chemical or biological means**[13,14]**.

Enzyme biotreatment of textiles is a safe and efficient technique for activating and adding functional groups to the surface of fibers. A wide range of hydrolytic enzymes, such as lipases and cutinases, can activate natural fibre surfaces**[15,16]**. One of the most significant enzymes ideal for textile applications is cellulase, particularly given that many fabrics contain cotton. Cotton textiles are biopolished using cellulases to increase their wettability, softness, and smoothness**[17]**. The success of an enzyme treatment is subject to numerous elements, including the enzymes' ability to access the cellulose substrate, their activity, dosage, pH, temperature, and duration. The coexisting chemicals during activation, mechanical action and history of fabric processing also affect the finishing and activation process **[18,19,20]**.

The advantage of biocatalytic methods is that they can be performed under mild reaction conditions, saving energy and without using a large amount of harmful chemicals.Enzymes are exceptional (bio)catalysts because of their great substrate specificity, regiospecificity and stereospecificity as well as their capacity to boost reaction rates by up to several orders of magnitude **[21]**. New functional performance can be achieved by enzymatic treatment that modifies the treated cloth's surface properties by increasing the amount of free hydroxyl and carboxylic groups**[22,23,24]**. Furthermore, more hydroxyl and carboxyl groups facilitate the nanoparticles' ability to stick firmly to the textile surface**[25]**.It is thought that this technology's use is straightforward and safe for the environment, opening the door to the direct use of fibre waste to create intelligent, functional textiles for a range of cutting-edge uses. The functional fabrics created by this bio-nanotechnology are anticipated to have benefits for the biosensor, packaging, and wastewater treatment industries.

This paper will examine the feasibility of immobilizing CuO-NPs produced by the *Chlorella* microalga's aqueous extract on PET/C blended waste fabrics activated by the enzymatic processes. The study also includes evaluation of expected functional features of the altered fabric, such as enhanced antibacterial activity, ultraviolet protection factor (UPF) and wastewater treatment.

2. Materials and methods

2.1. Microalga and cultivation conditions

The green microalga, *Chlorella* sp., used in this study was previously isolated from wastewater swamp, Gharbia governorate, Egypt, genus identified and stored in the Department of Agricultural Microbiology, National Research Centre, Egypt. Bold

Basal Medium (BBM) was used to maintain and culture the microalga. Per litter, BBM is made up of the following components**[26]**: KH_2PO_4, 175 mg; $CaCl_2$. $2H_2O$, 25 mg; $MgSO_4$. $7H_2O$, 75 mg; $NaNO_3$, 250 mg; K_2HPO_4, 75 mg; NaCl, 25 mg, H_3BO_3, 11.42 mg and the following trace elements: $ZnSO_4$. $7H_2O$, 8.82 mg; $MnCl_2$. $4H_2O$, 1.44 mg; MoO_3, 0.71 mg; $CuSO_4$. $5H_2O$, 1.57 mg; $Co(NO_3)_2$. $6H_2O$, 0.49 mg; Na_2EDTA, 50 mg; KOH, 3.1 mg; $FeSO_4$, 4.98 mg and 1 µl H_2SO_4 (Conc.). The cultivation process took place in 5-L air-bubbled glass flasks with constant lighting (white fluorescent lights) for 28 days. Biomass was harvested (after different cultural ages *i.e.* 14, 21, and 28 days) via centrifugation at 6000 rpm for 10 min.After being washed with DW to remove any remaining salts and contaminants from the culture, the collected biomass was dried at 50°C for 24 h and grounded. The supernatant of the microalgal cultures after centrifugation were used for nanoparticle biosynthesis line by line with the aqueous extracts of microalgal biomass.

2.2. Aqueous extracts of the microalga

The biomass of *Chlorella* sp. was water-extracted using a modification as described in **Somasekharan et al.[27]** and **Saleh et al., [28]**. In brief:one gram of dried and grounded *Chlorella* biomass was suspended in a glass tube containing 20 mL of dH_2O and extracted in a water bath sonicator at 50 ˚C for 30 min. Then, the cells and their solid residues were separated by centrifugation (2000 rpm for 10 min) and re-suspended in a suitable volume of dH_2O for another sonication/extraction cycle which repeated four times at elevated temperatures *i.e.* 60, 70, 75 and 80 °C, respectively. The aqueous fractions were collected after the five extraction cycles, centrifuged and the final volume was adjusted to 100 mL.

2.3. Biosynthesis of CuO-NPs using *Chlorella* sp. aqueous extracts

The biosynthesis of *Chlorella*-CuO-NPs was performed using both the cell-free cultural filtrate and the aqueous cell extract of the freshwater microalga *Chlorella* sp. The effect of cultural age on copper biosynthesis was studied at three different culture ages, *i.e*. two, three and four weeks. An equal volume of supernatant or cell extract was added to 1000 mg/L copper solution (as copper sulphate). The mixtures were incubated for 48 h with shaking (100 rpm). The precipitates were collected by centrifugation (10000 rpm for 15 min) and then washed 3 times by deionized water and two times by absolute ethanol and then dried overnight in an oven at 50°C.The formation of CuO-NPs nanoparticles was checked gravimetrically and by measuring the absorbance values at 570 nm **[29]**.The dried and grounded CuO-NPs of the optimum condition were selected and subjected to further characterization before being applied for immobilization on textile.

2.4. Preparation of activated Fabric Waste

Polyester/Cotton blended fabric waste applied during the present work was in a form of woven fabric peace that was collected from textile factories (Misr Al-Amerya Co., Alex; Misr Al-Mahalla Co., Al-Mahalla El-Kobra and Misr ElBida Co., Kafer El-Dawar). The enzymatic treatment of PET/C fabric was performed using commercial acid cellulases (Cellusoft® L; Novo NordiskA/S, Denmark) using a laboratory dyeing machine with high temperature/high pressure. In brief, the needed concentrations of cellulases enzyme (1 and 3 %) and the fabric samples were mixed into stainless steel bowls with pH 4.5 adjusted by acetic acid, followed by rotating shaking at 45 °C into a closed bath of ethylene glycol. The ratio between fabrics (F) and enzyme (E) solutions (F:E) was 1:15. The temperature of the water bath was increased by 5 °C for each min. After that (40 min), the pH was increased to 10 using Na_2CO_3 for terminating the enzymes, and the samples were removed from the bath, and rinsed with hot and cold distilled water sequentially. And then the treated fabric samples were put onto stainless sheets in the open air to dry. The fabric enzyme-degradation (weight loss, WL) was estimated from the weight loss of the fabric samples based on the following equation:

$$\mathrm{WL}\ (\%) = \frac{\mathrm{W1} - \mathrm{W2}}{\mathrm{W1}} \times 100$$

Where W_1 is the weight of the samples before enzymatic treatments and W_2 is the weight of the sample after enzymatic treatments.

The carboxylic content was used to evaluate the enzymes working. Thus, it was applied on parent and activated fabric waste as mentioned in the previous process described by **Yang and Tsai [30]**.

2.5. Immobilization of *Chlorella*-CuO-NPs on textile waste

PET/C Blend fabrics that had been activated by cellulases as well as parent fabrics were dipped in the *Chlorella*-CuO-NPs dispersion for 1 h. The samples were then squeezed to remove 60% (wt/wt) of the solution, dried in the air for 24 h at 22°C (laboratory temperature), and then cured for 15 min in a 150°C oven. The coated fabrics were washed five times using a conventional procedure in order to assess the Cu-NPs adherence to the fabrics (AATCC Test Protocol, 61-1989)**[31].**

2.6. Analysis

2.6.1. X-Ray Diffraction

The crystalline structure of the *Chlorella*-CuO-NPs was analyzed using X-Ray Diffractometer. X-Ray Diffraction patterns were obtained with the XRD- 6000 series by Shimadzu apparatus using nickel-filter and Cu-Kα X-ray target, PANalytical X'Pert PRO Instruments, Holand, Central lab at Agricultural Research Center, Cairo, Egypt. Under condition of 2θ scan range (10- 80), step side (0.02), scan rate (0.5 sec) and anode source copper.

2.6.2. Carboxylic content

The method described by **Shalaby et al.,[32]**was used to measure the carboxylic content of the parent and activated fabric waste.

2.6.3. Antimicrobial Activity

The *Chlorella*-CuO-NPs were tested as antimicrobial agents against the following pathogens: *Bacillus cereus* ATCC-12228, *Listeria monocytogenes* ATCC-35152, *Enterococcus faecalis* ATCC-29212, *Pseudomonas aeruginosa*, *Salmonella typhi* ATCC-15566, *Escherichia coli* ATCC-25922, *Candida albicans* ATCC-10231, *Aspergillus niger* ATCC-16888, *Aspergillus flavus* ATCC-MYA 4921 and *Fusarium proleferatum* MPVP 328 **[33,34]**. The well diffusion agar method, as previously described by **Darwesh et al., [35]** was used to test the antibacterial activity. Cu-NPs were evaluated against Nystatin, an antifungal reference, and Amoxicillin, an antibacterial reference, at a concentration of 200 mg/mL each. Every sample was run in triplicate and the results were expressed as average values SD±. Additionally, the minimum inhibitory concentration (MIC) was established**[36]**.

The antimicrobial activity of activated PET/C blended fabric waste loaded with biosynthesized *Chlorella*-CuO-NPs was measured utilizing the disc diffusion method.This technique measured the antibacterial efficacy via diffusion by measuring the zone of growth inhibition surrounding the sample (in mm).

2.6.4. SEM and EDX

Photomicrographs of fabric surfaces were taken using a JEOL-Model JSM T20 scanning electron microscope (SEM) operating at 19 kV to evaluate the surface structure and the morphology of the *Chlorella*-CuO-NPs and fabric waste samples.

2.6.5. FTIR

The chemical structure of the biosynthesized *Chlorella*-CuO-NPs and their chemical interaction with the functional groups of loaded fabric waste was determined using the Fourier transformation infrared (FT-IR) spectrometer (model NEXUS 670, NICOLET USA). A spectral range of 4000 to 500 cm^{-1} was used for the measurements. All of the samples under investigation underwent reflection percentage measurement (R%).

2.6.6. Ultraviolet protection factor (UPF)

The amount of ultraviolet protection provided by the finished samples was evaluated by measuring UVR transmission using UV-Shimadzu 3101-PC-Spectrophotometer. The UPF values were calculated according to the Australian/New Zealand Standard (AS/NZS-4399-1996). UPF values were calculated, and the protection category is classified as follows: (0–10) non-ratable, (15–20) good, (25–35) very good, and (40–50, +50) excellent protection categories**[37].**

2.7. Wastewater treatment

Municipal wastewater sample was collected from municipal treatment station and analyzed its content of microbial communities before remediation it by syringe filter containing PET/C blended fabric waste loaded with biosynthesized *Chlorella*-CuO-NPs. Municipal wastewater sample was passed through the syringe filter containing modified PET/C wastes with contact time 10 min. The collected sample after treating was subjected to microbiological analyses. Total bacterial count was determined using the plate count agar medium. Total coliform counted using Violet Red Bile (VRB) Agar; Pink colonies surrounded by bile precipitation were counted as coliforms. For *E. coli* counting, Eosin Methylene Blue (EMB) Agar medium was used as selective medium. In case of *Staphylococci* and *S. aureus* counting, bairde parker medium supplemented with egg yolk tellurite was used. Bairde Parker agar was used to count *Staphylococci* where representative gram positive clustered cocci, typical black appearance colonies and surrounded by clear zone were picked up, and tested for catalase. Colonies showed egg yolk lysis and positive catalase were confirmed as *S. aureus*. Mold and yeast species were counted using potatoes dextrose agar (PDA). Plates were incubated for 72 h at 30 ºC.

3. Results and discussion

3.1. Extracellular biosynthesis and characterization of *Chlorella*-CuO-NPs

The age of microalgae cultures is very important to produce bioactive compounds and metabolites**[3]**. In this regard, three ages of the tested microalga were studied and evaluated for its impact on biosynthesis of copper nanocomposities. Moreover, both cultural filtrate and cell extract of *Chlorella* were examined in the terms of quantitative

CuO-NPs productivity. According to obtained results, the aqueous cell extract of *Chlorella* biomass was more efficient than the cultural supernatant in the biosynthesis of CuO-NPs (**Fig. 1**). The productivity of biosynthesized nanoparticles was higher in case of three-week old cells. As a biological reduction system, the aqueous extract of the microalgal biomass was employed to transform copper ions into its nanoform. The microalgal extract led to the production of a greenish precipitate after overnight of incubation with copper sulphate solution. A spectrophotometric analysis of the precipitate absorbance, which peaked at 570 nm, indicated the production of *Chlorella* based Cu-NPs. The CuO-NPs that were biosynthesized utilizing the fresh water microalga *Chlorella* sp. aqueous extract were considered using SEM-EDX, TEM, XRD, and FT-IR methods. The findings demonstrated that the biomass extract of microalgae that were three weeks' old included higher CuO-NPs than microalgae that were two or four weeks old. For the production of bioactive compounds utilized as reducing, capping, and protecting agents, this era was comparatively significant.

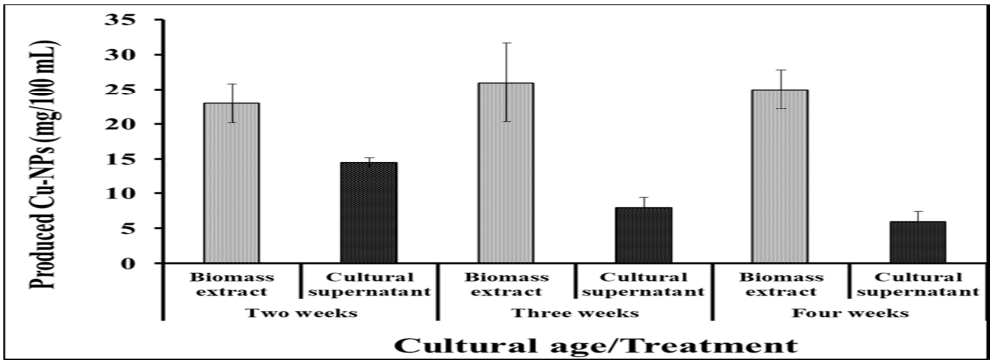


Figure 1: Gravimetrically evaluation of Cu-nanoparticles production using cultural filtrates or biomass extracts of *Chlorella* sp

The size, morphology and topography of the *Chlorella*-CuO-NPs were examined using a scanning electron microscopeas illustrated in **Fig. (2a**).The image taken with an electron microscope demonstrates the production of almost spherical Cu nanoparticles. The scanning map (**Fig. 2b**) shows 2 elements (Cu and O) detected in the *Chlorella*-CuO-NPs.Furthermore, the elemental analysis technique (EDX) proved that the generated copper nanoparticles were primarily CuO-NPs, as shown in **Fig. (2c),** and confirmed results detected in scanning map. In case of the size of *Chlorella*-CuO-NPs, the picture obtained by HRTEM illustrated that *Chlorella*-CuO-NPs were less than 10 nm in size (**Fig. 2d**). Our results are in line with other studies that have shown how microalgae, which are photosynthesis microorganisms, function as a factory to produce bioactive components that are useful as dipping agents in the production of nanomaterials, such as proteins, polyphenols, polysaccharides, and flavonoids**[38,39]**.

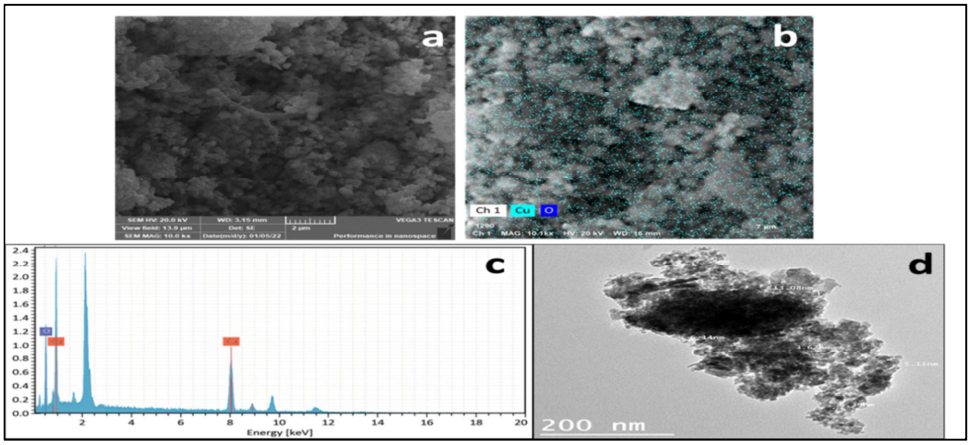
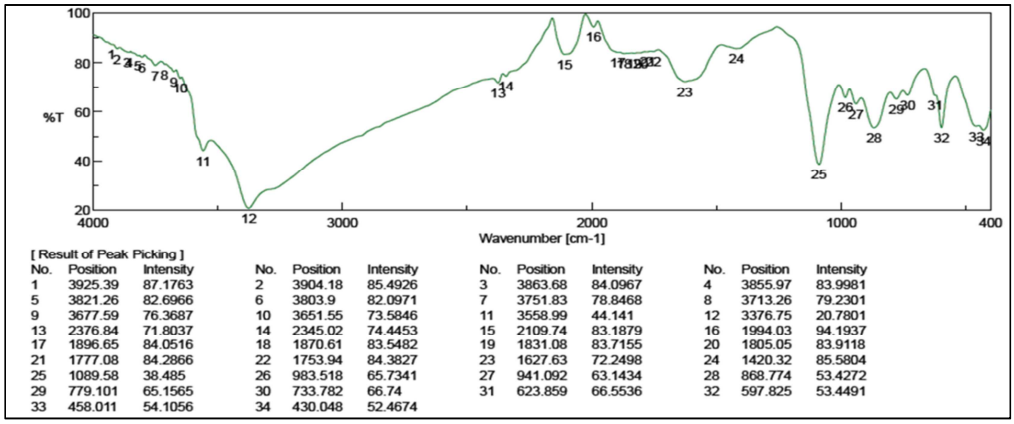


Figure 2: Some characteristics of *Chlorella*-CuO-NPs: Scanning electron microscopic micrographs (a & b), elemental analyses (c) and HRTEM micrographs (d)

The probable biomolecules involved for the reduction, capping, and efficient stabilization of *Chlorella*-CuO-NPs were identified using Fourier Transform Infrared Spectroscopy (FT-IR) **(Fig. 3)**. An analysis of the FT-IR spectrum of the biosynthesized CuO-NPs made from microalgal extract revealed the presence of functional groups,that could help in understand how distinct photochemicals, which may work simultaneously as a reducing, stabilizing, and capping agent, cause NPs to convert from straightforward inorganic copper salts to elemental one. The FT-IR spectra of the biosynthesized CuO-NPs clearly demonstrates that the *Chlorella*-CuO-NPs were biofabricated using a microalgal extract array with absorbance bands ranging from 400 to 4000 cm^{-1}. The IR spectrum of copper nanoparticles was shown in **Fig. (3)** with certain vibration bands at 3558, 3376, 2109, 1089, 868, 597 and 430 cm^{-1}. Furthermore, it is evident that the peaks at 597 and 430 cm^{-1} represent copper oxide nanoparticles. In addition, the broad absorption peak at around 3400 cm^{-1} is caused by the adsorbed water molecules since the nano crystalline materials display a high surface to volume ratio and thus absorb moisture **[40]**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3925.39	87.1763	2	3904.18	85.4926	3	3863.68	84.0967	4	3855.97	83.9981
5	3821.26	82.6966	6	3803.9	82.0971	7	3751.83	78.8468	8	3713.26	79.2301
9	3677.59	76.3687	10	3651.55	73.5846	11	3558.99	44.141	12	3376.75	20.7801
13	2376.84	71.8037	14	2345.02	74.4453	15	2109.74	83.1879	16	1994.03	94.1937
17	1896.65	84.0516	18	1870.61	83.5482	19	1831.08	83.7155	20	1805.05	83.9118
21	1777.08	84.2866	22	1753.94	84.3827	23	1627.63	72.2498	24	1420.32	85.5804
25	1089.58	38.485	26	983.518	65.7341	27	941.092	63.1434	28	868.774	53.4272
29	779.101	65.1565	30	733.782	66.74	31	623.859	66.5536	32	597.825	53.4491
33	458.011	54.1056	34	430.048	52.4674						

Figure 3: FT-IR spectrum of microalgal synthesized CuO-NPs

X-ray diffraction (XRD) was used to determine the crystalline structure of the CuO-NPs produced by microalga, and the resulting graph is shown in **Figure (4)**. The XRD spectrum of the biosynthesized CuO-NPs with the JCPDS card (card no: 89-2531) revealed the unique peaks for metallic crystalline copper. CuO-NPs have distinctive diffraction peaks at 2θ of 32.37, 35.19, 37.5, 48.14, 58.01, 65.01, and 67.66, which were ascribed to the (100), (002), (101), (102), (103), (200), and (201) planes, respectively (**Fig. 4**).

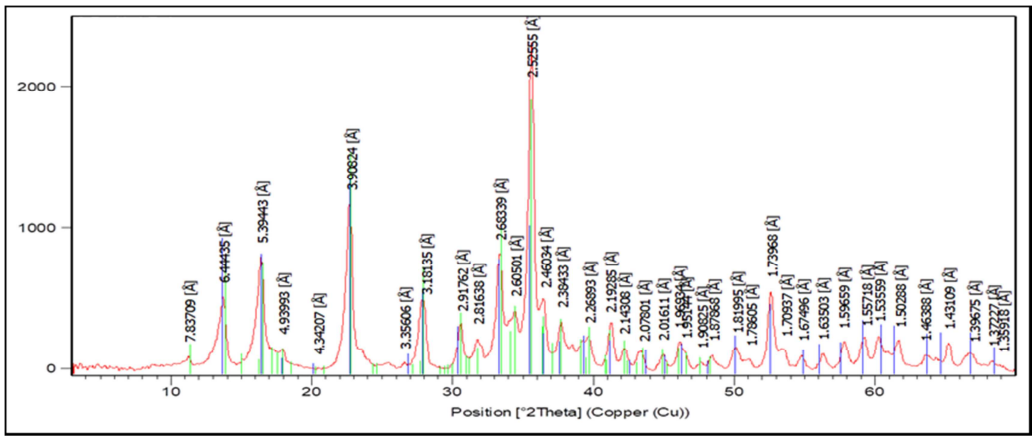


Figure 4: XRD pattern of microalgal synthesized CuO-NPs

3.2. Polyester fabrics treated by cellulases and biosynthesized CuO-NPs

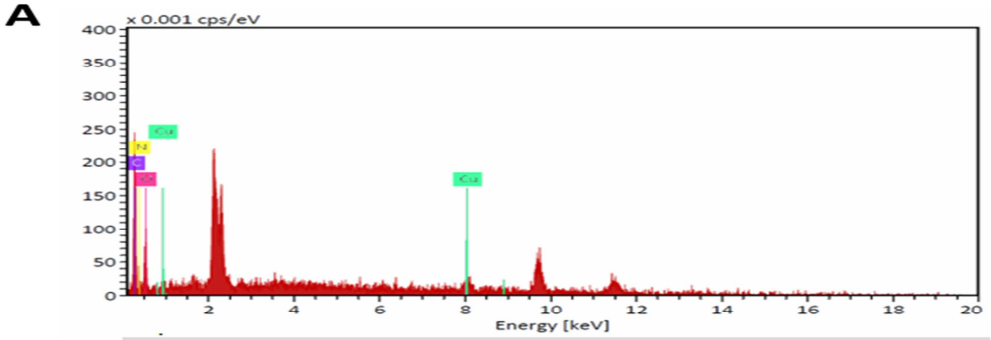
The primary goal of transferring chosen nanoparticles and ascribing them to textile materials is to provide new necessary features to enhance the conditions of the finished invention. Adding more active cites and functional groups to the fabric's surface may be necessary or advantageous for loading nanomaterials onto fabrics. Cellulases treatment of the cellulosic material may result in such alteration**[31]**. Therefore, in this study, fungal cellulases (3%) were used to biologically treat PET/C blended materials. The partial destruction of cellulose fibres caused by the action of cellulases enzymes led to the release of several soluble substances, including glucose and short chain oligomers, and a three percentage loss in fiber weight**[41]**. Additionally, the surface of the biotinylated tissues alteration increased the carboxylic content, improving the capacity of the nanoparticle to be adsorbed on its surfaces. The results show that the enzymatic treatment raised the carboxylic content of the fabric, which went from 8.10 to 22.6 meq/100 g. The results showed that the enzymatic treatment caused the carboxylic content to increase from 8.10 to 22.6 meq/100 g of fabric. Thus, The EDX study demonstrated that as the quantity of carboxylic groups increased, so did the amount of CuO-NPs loaded on the surfaces of activated textile waste.

3.3. Characterization of Fabrics Waste Loaded with CuO-NPs

Selective nanoparticles are transferred to textile materials with the primary goal of adding new desirable features to raise the product's specifications. The loading of nanoparticles onto fabrics may be improved by increasing the number of active sites and functional groups on the surface of the fabric material itself. Scanning electron microscopy, EDX, and FTIR were used to visualize the surface characteristics of the waste from activated textiles with respect to shape, surface coating, and distribution of the biosynthesized Cu-NPs. Additionally; the textile loaded with Cu-NPs underwent an antibacterial study.

3.3.1. Surface Topography using EDX

By using EDX analysis, it was verified that the surface of PET/C blended fabrics waste included biosynthesized CuO-NPs. EDX spectra of the fabrics loaded with Cu-NPs after five washing cycles are shown in **Figure (5A)**. On the basis of these spectra, it is significant to arrive at the conclusion that the deposited substance consisted of copper and oxygen based on these spectra. This demonstrates that metal oxide NPs are still present on the waste surfaces of the fabrics even after five washing cycles (25 household washings) **(Fig. 5B)**. Additionally, EDX measurements show that cellulases-activated textiles have increased NPs content. This indicates that the CuO-NPs produced by biosynthesis have adequate adhesion to the cellulases-activated PET/C fabrics.



B

Element	At. No.	Netto	Mass [%]	Mass Norm. [%]	Atom [%]	abs. error [%] (1 sigma)	rel. error [%] (1 sigma)
Carbon	6	637	46.76	46.76	56.62	12.11	25.90
Oxygen	8	264	32.29	32.29	29.35	11.20	34.68
Nitrogen	7	39	11.41	11.41	11.85	8.43	73.88
Copper	29	149	9.53	9.53	2.18	0.89	9.37
		Sum	100.00	100.00	100.00		

Figure 5: EDX micrographs of activated PET/C blended fabrics and loaded with biosynthesized CuO-NPs*(1000x). (B) PET/C+E+CuO. *After five washing cycles according to AATCC Test Method (61-1989). E=Cellulases

3.3.2. Scanning Electron Microscope (SEM)

The form, surface coating, and CuO-NP distribution of the activated fabric waste were all characterized using scanning electron microscopy. Comparing the parent untreated fabric (control) to activated fabric waste that was either loaded with or not with biosynthesized NPs, SEM images of the activated fabric waste were taken **(Fig. 6)**. The parent and activated PET/C blended textiles with cellulases have spotless and smooth surfaces, as shown in **Fig. (6A and B)**. Furthermore, partial hydrolysis by cellulases gave the materials a smooth surface with increased resilience and a soft handle. This is because the amount of weight lost together with the removal of hairiness from the fabric's surface reduced stiffness and thickness and gave the surface a smooth appearance. When it comes to activated fabric surfaces, deposits occur after the biosynthesized NPs are applied to the fabrics. Additionally, the size and shape of these deposits change according on the NPs employed when loading fabrics that have been treated with the cellulase enzyme**[31]**.

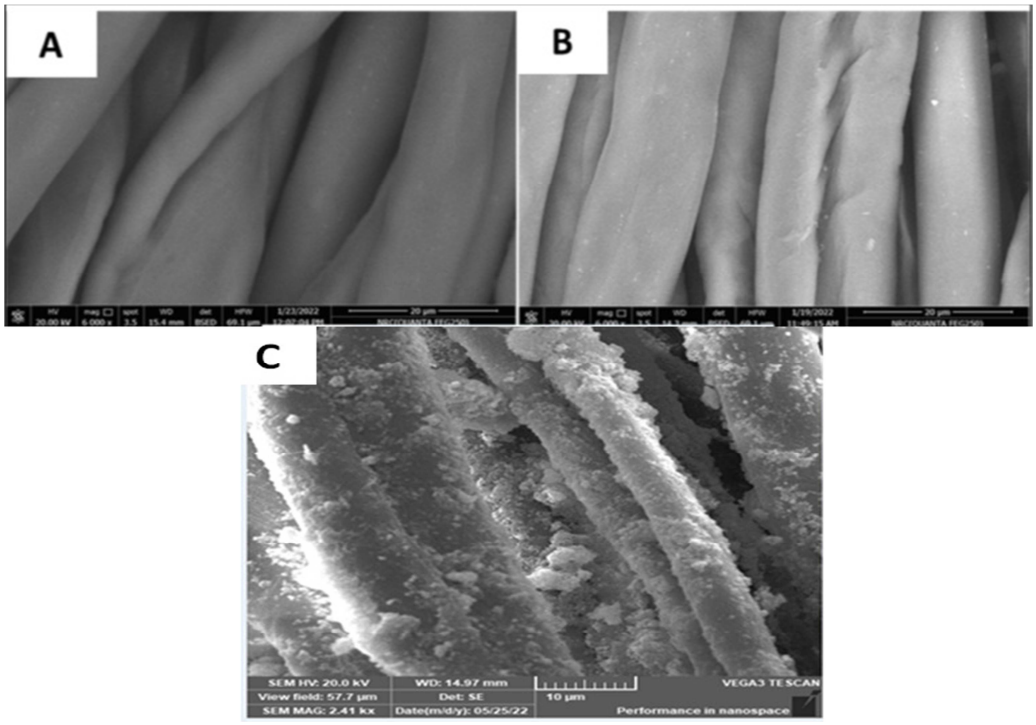


Figure 6: SEM Micrographs of Activated PET/C Blended Fabrics waste and Loaded with Biosynthesized NPs* (1000x). (A) PET/C; (B) PET/C+E; (C) PET/C+E+ CuO. *After Five Washing Cycles According to AATCC Test Method (61-1989). E=Cellulases

3.3.3. FTIR

The creation of the molecules' covalent bonds was examined using the FTIR spectrum in order to examine changes in the biomolecular composition of the created cloth. Evidently, the chemical composition of the fabric surfaces underwent a major alteration as a result of enzyme activity. **Figure (7A)** of the parent fabric's FTIR spectrum displays absorptions at 1649–1712, 3408–3388, and 2317 cm^{-1}, which are indicative of the stretching caused by C=O, OH, and CH, respectively. The FT-IR spectra of activated PET/C blended textiles waste that activated with enzyme and loaded by NPs **(Fig.7)** reveals that new distinctive peaks are emerging. These peaks correlate to CuO bonds, and a similar result was published by **Hong et al [42]**. The investigation's conclusions explained why NPs from dispersion solutions could only be fastened on surfaces that were active.

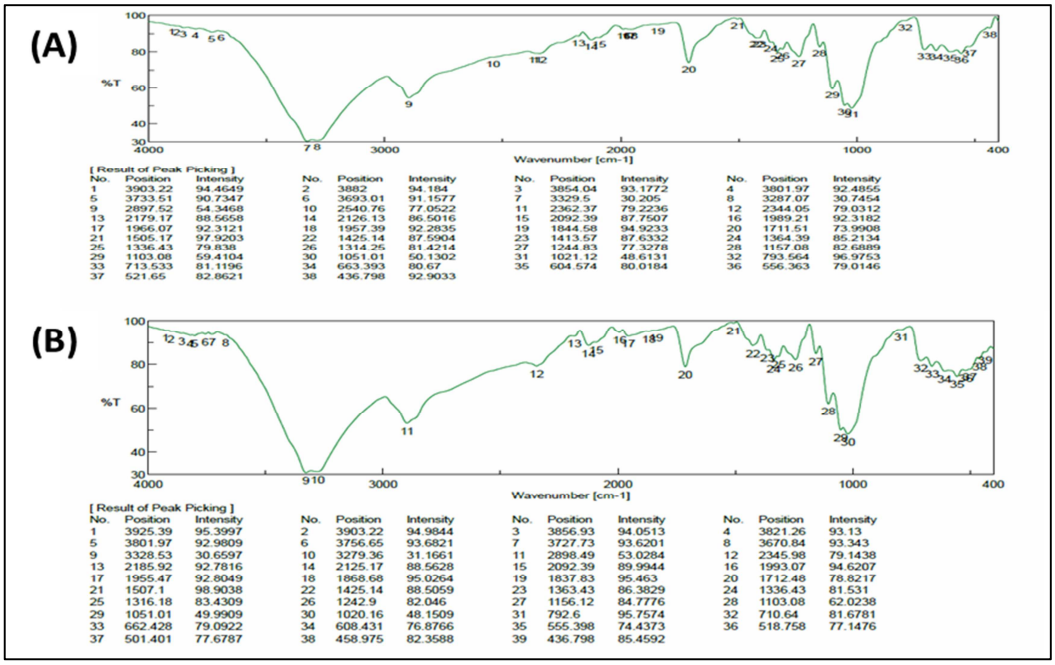


Figure 7: FT-IR Spectra of PET/C Blended Fabrics Waste Activated and Loaded with Biosynthesized NPs*. (A)PET/C+E; (B)PET/C+E+CuO after Five Washing Cycles According to AATCC Test Method (61-1989) E=Cellulases

3.4. Antimicrobial Activity

Investigated was the effect of cellulase enzyme-activated PET/C blended fabric waste loaded with CuO-NPs against pathogenic microorganisms. Gram-positive *B. mycoides*, gram-negative *E. coli*, and non-filamentous fungus *C. albicans* were the microorganisms addressed. The task involved calculating the clear zone (inhibition zone) surrounding the CuO-NPs/loaded textile samples on agar plates. According to the data in **Table (1)**, all fabrics displayed high antibacterial activity against the three pathogens indicated earlier after five washing cycles. Evidently, all of the analyzed fabric samples had considerable inhibitory zones, but all of the untreated fabric samples have no such zones. Given that the samples were repeatedly washed in the launder-Ometer, it appears that the effect of activating PET/C blended textiles waste with cellulases before loading with CuO-NPs on the antibacterial activity is more significant. This demonstrates the viability of utilizing biosynthesis technology for creating NPs and using them as finishing agents with waste PET/C mixed fabrics.

Table 1: Impact of PET/C Blend Fabric Activation on Antimicrobial Activity

Fabrics	Inhibition zone diameter (mm) in case of loaded Polyester Fabrics with Biosynthesized NPs:					
	Staph. aureaus		*E. c*		*C. a*	
	1*	5*	1*	5*	1*	5*
PET/C	-ve		-ve		-ve	
PET/C+E	-ve		-ve		-ve	
PET/C+E+CuO NPs	66.1	55.9	99.3	95.6	43.9	31.4

Where Conditions for Enzymatic Treatment: [Cellulases], 3%, pH = 4.5, Temperature: 45°C; time: 40 minutes; M: L: 1:15. Treatment parameters include: [CuO NPs], 1.0 g/l; 150˚C for the temperature; and 15 minutes for the cure. * Washing cycles 1 and 5 in accordance with the AATCC Test Method (61-1989). E is for celulases

3.5. Ultraviolet Protection Properties

The effectiveness of UV protection was examined after PET/C mixed textiles waste were activated with cellulases and before being loaded with biosynthesized NPs. UPF values are provided in **Table (2)** as a way of quantifying and expressing the rate of UV protection. It was found that the average UPF factors for parent PET/C blended fabrics were 9.6. These materials' superior UV protection efficacy even after five washing cycles demonstrates the exceptional endurance of its laundry. This further establishes the viability of using biosynthetic technology to create NPs that can improve the characteristics of textile materials. Comparable outcomes were noted by **Ran et al., [43]**, who noted that even after five washing cycles of textile material laden with chemically synthesized CuO nanoparticles, the UVF reached 157.8. Similarly, our results may differ from this one because of the biomolecules that are added as enhancing agents to biosynthesized CuO-NPs. CuO-NPs ultrafine nanoparticles are widely applicable in smart textiles to improve their characteristics since they are well-documented UV blocking materials.

Table 2: Impact of PET/C Blend Fabric Waste Activation on its UPF Values

Fabrics	UPF Values After No of Washing Cycles:			
	1*		5*	
	UPF Value	UPF** Rating	UPF Value	UPF** Rating
PET/C	10.5	Good	-	-
PETC+E	18.3	Good	-	-
PET/C+E+CuO NPs	165.0	Excellent	145.7	Excellent

Where Conditions for Enzymatic Treatment: [Cellulases], 3%, pH = 4.5, Temperature: 45°C; time: 40 minutes; M: L: 1:15. Treatment parameters: [1.0 g/l of CuO NPs; 150˚C for the temperature; 15 min for the duration of the cure]. **In accordance with Australia (AS) / New Zealand (NAS) Standard No. 4399 (1996). *In accordance with AATCC Test Method (61-1989). E is for celulases

3.6. Application of the modified CuO-NPs based fabric as filter for wastewater disinfection

The modified fabric based on CuO-NPs was applied as a filter to evaluate its ability to control pathogens in municipal wastewater (**Fig. 8**). The original microbial load of the applied wastewater was represented as CFU as direct counts on agar plates. After 10 min retention time for disinfection traits, the microbial counts of the treated wastewater were analyzed and the "disinfection efficiency" of the applied modified CuO-NPs based fabric filter was recorded (**Table 3**). Obtained results reflect the capability of the applied filter to disinfect total bacterial contaminants with the asperity towards total coliform, *E. coli*, and *salmonella* bacteria (>97, 98 and 99.4%, respectively). However, the efficiency of the modified CuO-NPs based fabric filter against molds (90.7%) was lower comparing to total bacterial load (94%). From the documented results, the filter containing CuO-NPs based fabric was success as a one part of advanced treatment step in domestic station to remove pathogenic microbes.

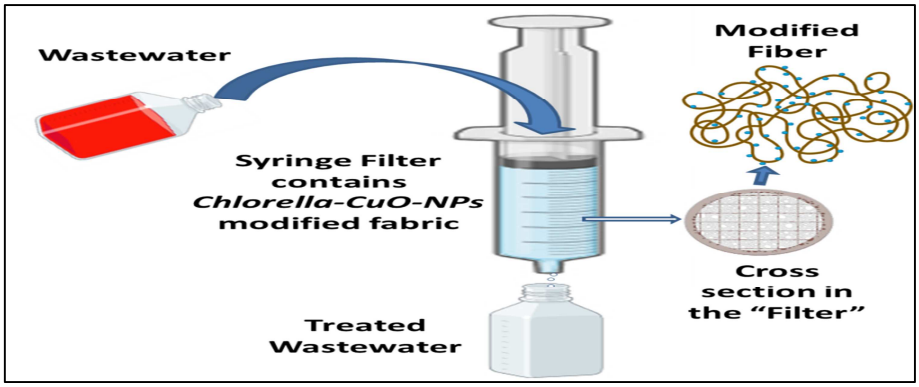


Figure 8: Flowchart of municipal wastewater treatment process against pathogens by syringe filter containing *Chlorella*-CuO-NPs

Table 3: Efficiency ofsyringe filter containing *Chlorella*-CuO-NPs for remediation of municipal wastewater from pathogens

Parameters	Counts of Microbesbefore treatment (CFU/mL)	Efficiency of decreasing after remediation (%)
Total bacteria	**6.5×10^8**	**94**
Total molds	**3.2×10^6**	**90.7**
Total coliform	**1.7×10^8**	**97.2**
Total *E. Coli*	**4.2×10^4**	**98**
Total *Staph.*	**1.2×10^2**	**95.8**
Total *Salmonella* spp.	**2.1×10^1**	**99.4**

4. Conclusion

It has been demonstrated that the freshwater microalga, *Chlorella* sp. has the capability to biologically reduce and transform copper ions into their nanoform. The phyco-synthesized *Chlorella*-CuO-NPs could be immobilized on to the textile fabric (PET/C) blended fabric waste, giving them photoprotective and antibacterial properties. Partial hydrolysis of cellulosic fibers due to cellulase treatment results in an increase in free hydroxyl and carboxyl groups, which promotes the immobilization of phyco-synthesized *Chlorella*-CuO-NPs on fabrics. The antimicrobial properties of *Chlorella*-CuO-NPs can be used as a core component in a "disinfection biofilter" against wastewater pathogens for environmental applications.

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6. Conflict of Interest: The authors declare no conflict of interest.

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