GREEN AND COST EFFECTIVE PREPARATION OF COPPER OXIDE NANOPARTICLES USING WITHANIA COAGULANS FOR THE PHOTOCATALYTIC REMOVAL OF ORGANIC EFFLUENTS FROM WASTEWATER

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ABSTRACT: The metal oxide nanoparticles (NPs) play an important role in heterogeneous photodegradation and removal of organic dyes from water sources. This method is more preferable for being ecofriendly as compared to the other methods having low cost and no use of high temperature, pressure or other parameters. Previous studies have demonstrated that CuO exhibits significant photocatalytic activity. Subsequently, in the present study the CuO NPs were synthesized using aqueous extract of *Withania Coagulans* berries with copper sulphate pentahydrate ($CuSO_4.5H_2O$) served as precursor. The characterization of the synthesized NPs was carried out using UV-Visible spectroscopy to determine the characteristic absorption peak, which was observed at 296nm. Fourier transform infrared spectroscopy (FT-IR) was used to study the functional groups and to detect any impurities present in the NPs due to plant extract. The X-ray diffraction (XRD) study was carried out to examine the crystalline nature of synthesized NPs while the surface morphology was assessed by scanning electron microscopy (SEM). The photocatalytic degradation efficiency of biologically fabricated NPs was tested against the potentially harmful dye methylene blue (MB) under sunlight as the light source. Factors affecting degradation efficiency like photocatalyst dosage, initial dye concentration and pH of the solution were optimized over a time range of 5 to 115 minutes. Under the optimized parameters the CuO NPs showed high degradation efficiency of around ≈96% after 115 minutes which is significantly higher as compared to the previously reported works.

Key words: Withania coagulans berry, Copper oxide, organic dyes, photocatalysis.

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INTRODUCTION

Nanotechnology is a fast developing multidisciplinary area that allows the maneuvering of material at the nanosized (1–100 nm), where matter reveal distinctive physical, chemical, catalytic, and optical, properties unique that are different from their bulk counterpart [1]. At this scale, nanoparticles own a high surface-to-volume ratio, improved surface reactivity, and modifiable electronic properties, which markedly enhance their functional performance. These properties have assists in the vast utilization in catalysis, environmental remediation, medicine, and sensing technologies [2]. Amongst the miscellaneous nanomaterials, metal oxide nanoparticles are particularly remarkable due to their stability, low cost, and superior catalytic effectiveness in chemical and photochemical processes [3]. Copper oxide nanoparticles (CuO NPs) have earned notable focus outstanding due to their narrow band gap, powerful redox properties, antimicrobial activity, and excellent photocatalytic behavior [4]. Compared to noble metal catalysts, CuO NPs are chemically stable, cost-effective, and environmentally compatible, making them suitable for large-scale wastewater treatment applications [5]. The release of dye-containing industrial sewage specifically from textile industries as well as other sectors that poses severe environmental and health troubles [6]. Organic dyes are highly persistent, non-biodegradable, and toxic in nature [7]. Their complicated aromatic structures resist bio-degradation and decrease water transparency and negatively affect the aquatic environment [8]. Hence, developing effective, durable, and green methods for dye degradation is a crucial research objective [9].

Conventional physical and chemical nanoparticle fabrication methods often involved toxic chemicals, high energy consumption, and environmentally harmful by-products. In response, green synthesis routes using biological resources as reducing and stabilizing agents have emerged as safer alternatives. Plant-based synthesis is simple, cost-effective, and environmentally friendly. Naturally occurring phytochemicals in plants act as a reducing and capping agents, enhancing nanoparticle stability and biocompatibility [10]. In this context, *Withania coagulans*, which is rich in bioactive components like flavonoids, phenolics, and alkaloids, has shown potential for eco-friendly synthesis of stable metal oxide nanoparticles with improved catalytic and biological properties [11]. The current research focuses on the bio-

synthesis, characterization, and application of CuO NPs for the photocatalytic degradation of methylene blue (MB) dye which has not been reported earlier. Spectroscopic and microscopic techniques are used to analyze structural, optical, and morphological properties, which directly affect photocatalytic effectiveness. This study aims to synthesize a low-cost, environmentally friendly nanocatalyst for wastewater remediation, contributing to durable pollution control and safer environmental practices.

MATERIALS

The chemicals employed in the present study were of analytical grade and did not require further purification. Copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$) was bought from Sigma-Aldrich, sodium hydroxide (NaOH) pellets were obtained from Merck Millipore, methylene blue (MB) and methanol was purchased from Merk (Germany). Deionized water that was used during the course of the experiment was bought from the Al Beruni Scientific Store located at Hyderabad, Sindh, Pakistan.

Preparation of Extract: *Withania coagulans* berries were bought from, Shah medicinal and Herbal store Hyderabad Pakistan. Berries were washed with deionized water and shaded dried. Dried berries were ground to make powder form. The Powder was then kept for two hours in an oven at 40°C to ensure complete dryness. Finally, the obtained dried powder of *Withania coagulans* kept in an air tight container for further use. In this method 15g of finely powdered berries of *Withania coagulans* were dissolved in 100mL of deionized water in 250mL beaker and kept for heating and stirring at hot plate at 37°C for 4 hours. Then extract was kept for overnight and filtered with whatman filter paper grade 1. After that extract was kept at 4°C for further use.

Synthesis of CuO NPs: Green synthesis of CuO NPs was carried out by using 0.5g of $CuSO_4.5H_2O$ which was dissolved in 100mL of deionized water in a 250mL beaker. Then, 5mL of *Withania coagulans* extract was added in it and pH was stabilized up to 12 using 2M NaOH. A sudden color change was observed indicating the formation of copper hydroxide $Cu(OH)_2$ NPs. The mixture was then kept in an oven for 4 hours at 78°C to promote further growth of $Cu(OH)_2$ NPs. Afterward NPs were filtered using ash less filter paper and calcinated at 400°C in a muffle furnace for purification from moisture content and convert $Cu(OH)_2$ NPs into CuO NPs. The synthesized CuO NPs we then stored in an air tight container for further study [12].

Instrumentation: The UV-Visible spectrometer was used to confirm the formation of NPs. The FTIR (Fourier transform infrared) spectroscopy was performed to examine the fundamental functionalities and linkages involved in plant mediated synthesized CuO NPs. The XRD analysis was carried out for biologically synthesized samples to evaluate the crystallinity, phase identification, structure and particle size using scherrer's equation. The surface morphology of the NPs was assessed by using Scanning electron microscopy (SEM). Furthermore, a pH meter was used during the experiment to maintain the required pH throughout synthesis process.

Photocatalytic Degradation of Methylene Blue Dye: The biologically synthesized CuO NPs were used as a catalyst for the photocatalytic degradation of MB dye. 10mg of synthesized NPs were added to 20mL of 8μg/mL MB dye and placed in the direct sunlight for photo catalytic degradation. After that the absorbance of the sample was measured every 10 to 15 minutes up to 115 minutes. The gradual disappearance of the MB dye color indicated degradation of dye [13].

RESULTS and DISCUSSION

UV-Visible Analysis: The presence of characteristic UV Visible absorption band confirmed the successful preparation of CuO NPs synthesized with the help of *Withania coagulans* extract which is in line with other existing literature on green-synthesized CuO NPs. The peak absorption was recorded in the UV region (around 280−300 nm), which is typical to the CuO NPs and it is because of core electronic transitions in CuO (Fig.1). This range of UV absorption has been extensively reported in plant-mediated CuO NPs, implying the effective reduction of copper ions and the formation of nanoparticles. The well-defined and sharp absorption peak indicates the uniform particle size distribution and excellent stability. However the absence of additional peaks indicates minimal aggregation of CuO NPs. These optical characteristics confirm that the *Withania coagulans* extract acted as an effective reducing and stabilizing agent, providing an environmentally friendly approach to the synthesis of green CuO NPs [14].

Fourier Transformation Infrared (FTIR) Spectroscopy Analysis: Fourier Transform Infrared (FTIR) spectroscopy was employed to analyze the functional groups and chemical bonds present in CuO NPs synthesized using *Withania coagulans* extract. The FTIR spectra of biologically synthesized CuO NPs (Fig. 2) recorded in the wavenumber range of 4000–500 cm^{-1} revealed distinct absorption bands corresponding to specific functional groups. A broad absorption band at approximately 3465 cm^{-1} was attributed to O–H stretching vibrations, indicating the presence of hydroxyl groups, likely due to adsorbed water molecules on the surface of NPs or due to O–H stretching vibrations of

plant phytochemicals [15]. Weak absorption peaks in the region of 2900–3000 cm^{-1} were identified as C–H stretching vibrations of aliphatic group or aromatic hydrocarbon, suggesting the presence of organic residues or biomolecules, which often acts as capping or stabilizing agents in biological synthesis [16]. A significant absorption band around 1632 cm^{-1} was linked to C=O stretching vibrations, indicating carbonyl groups from polyphenols, protein or other biomolecules from plant extract that play a role in reducing and stabilizing the CuO NPs. Additionally, the peak observed at 1410 cm^{-1}, particularly due to amide group bending vibrations [17]. The peak at 1045 cm^{-1} was observed due to C–O and C–O–C stretching vibrations of alcohol and ester. However, the peak at 834 and 620 cm^{-1}, were assigned to specifically metal-oxygen (Cu–O) stretching vibrations [18]. The FTIR analysis confirmed the presence of hydroxyl groups, carbonyl groups, and organic residues on the surface of biologically synthesized CuO NPs, highlighting the successful integration of biomolecules during the synthesis process.

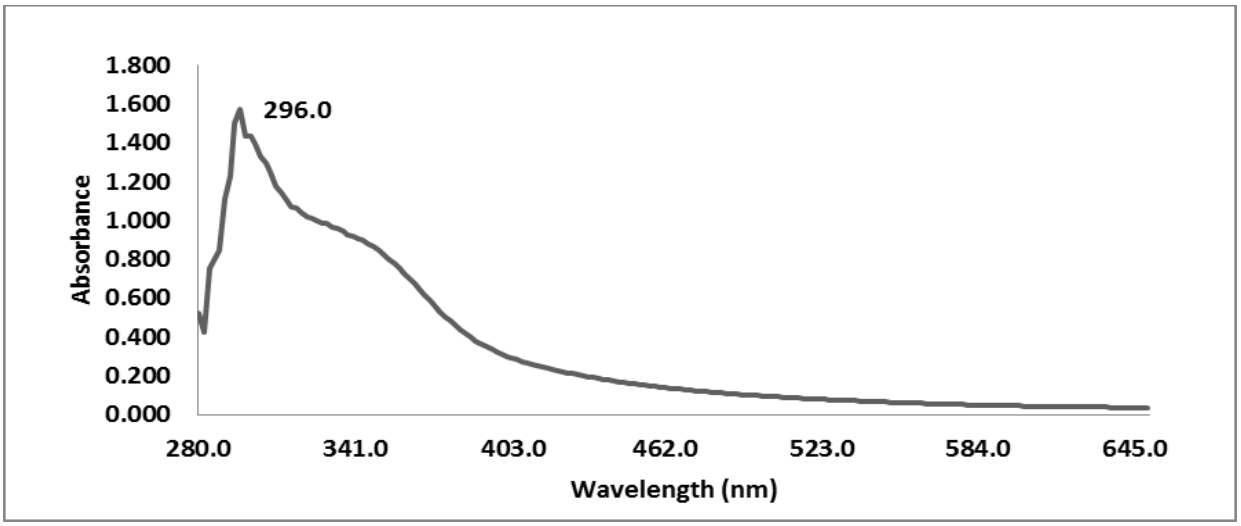

Figure. 1 UV-Visible spectrum of biologically fabricated CuO NPs

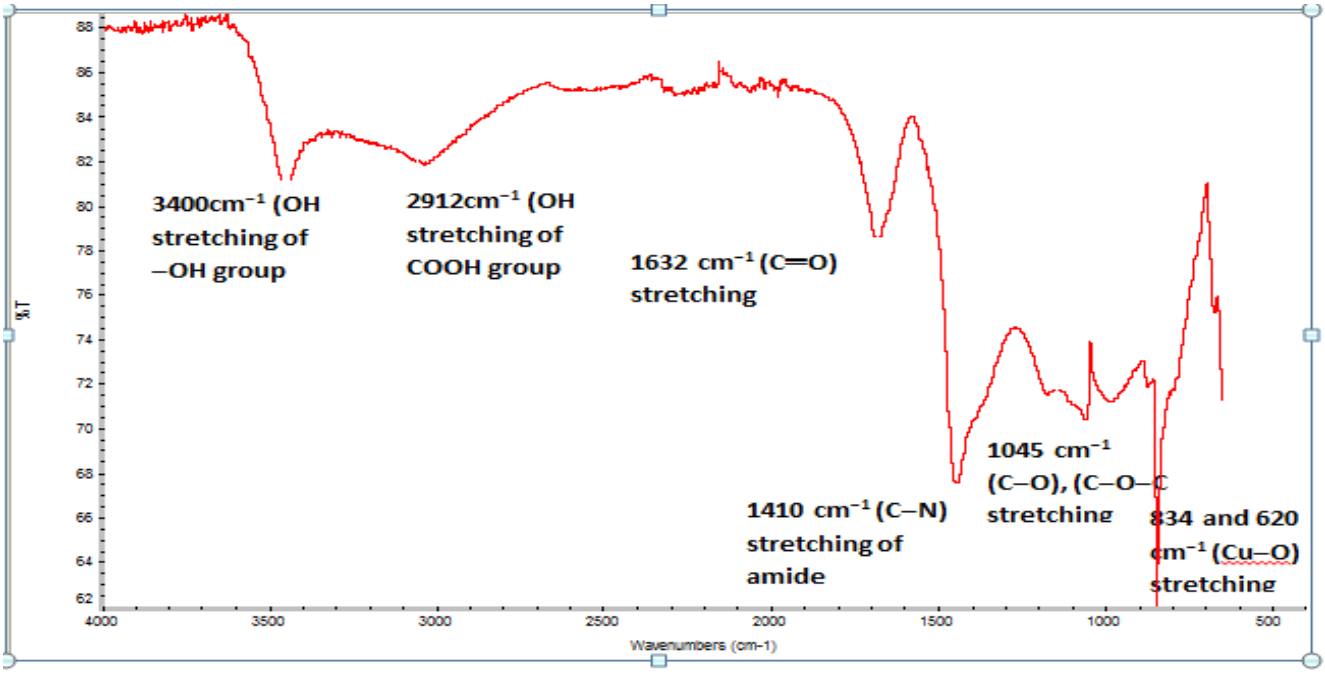

Figure. .2 FT-IR Spectrum of biologically synthesized CuO NPs

Scanning Electron Microscopy (SEM): The particle size and morphology of biologically synthesized CuO NPs derived from *''Withania coagulans''* were extensively characterized using advanced SEM techniques, which provide high-resolution imaging. The CuO NPs, were studied initially in the range of 500nm. The average sizes of synthesized NPs were found to be 54.69 to 67.75nm. Fig. 3 clearly showed the SEM morphology of CuO NPs which were spherical in shape and exhibited slight aggregation of small particles [19]. Similar results have been reported by Ruataba A. *et al*., for the Green Synthesis of Copper NPs by means of *Fortunella margarita* Leaves which yielded NPs of spherical morphology with a an average size of 51.26 to 56.66 nm [20]

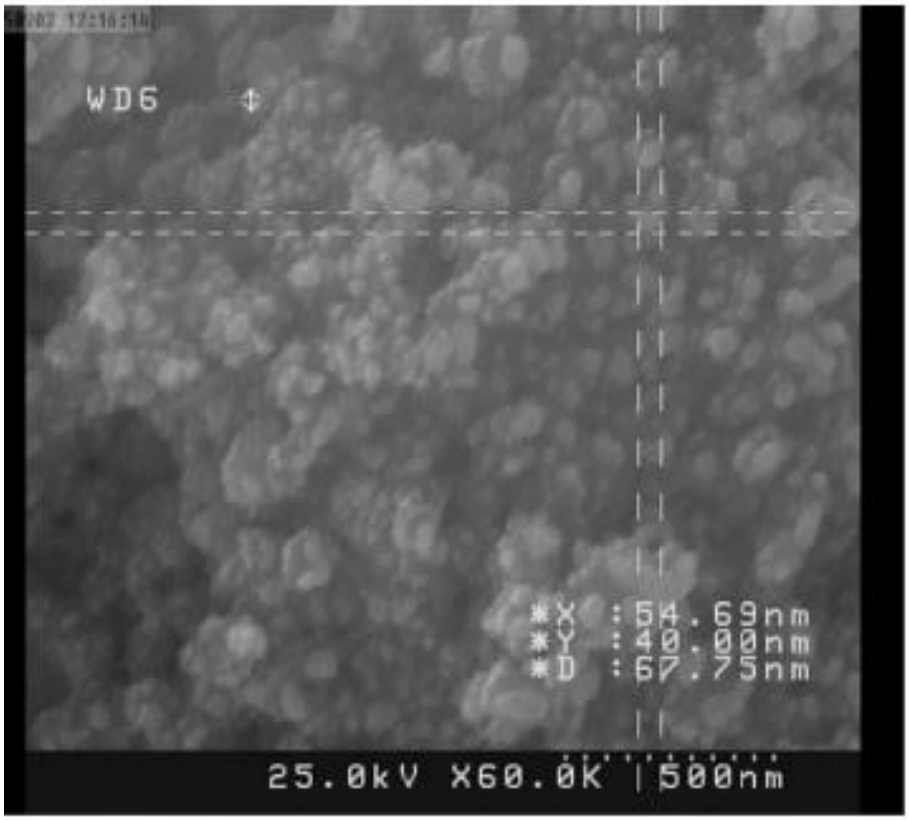

Figure 3. SEM images of biologically fabricated CuO NPs

X-Ray Diffraction Analysis (XRD): The crystalline structure and phase purity of CuO NPs synthesized through biological methods were investigated using X-ray diffraction (XRD) analysis as represented in Fig. 4. The peaks observed in X-ray diffraction arise from the constructive interference of a monochromatic X-ray beam scattered at precise angles by the lattice planes in the sample. The intensity of these peaks is influenced by the arrangement of atoms within the lattice [21]. The XRD patterns of synthesized CuO NPs revealed distinct peaks corresponding to the monoclinic lattice structure. The characteristic pattern showed distinct peaks at specific 2θ values, typically around 34.4°, 36.5°, 38.8°, 48.7°, 54.6°, 58.5°, 62.4°, 67.7°, 72.5°, 75.4° and 83.6° which were indexed to the crystallographic planes (110), (002), (200), (202), (020), (202), (113), (311), (311), (220), and (222) respectively. These results confirm the formation of monoclinic CuO structures. The observed diffraction peaks are consistent with the standard monoclinic phase of CuO and align well with the reported literature (JCPDS card no. 45-0937) [22-23]. The average crystallite size of the CuO NPs was calculated using the Debye-Scherrer's equation: $d = 0.9\lambda/\beta \cos\theta$ where d is the average crystallite size, λ is the X-ray wavelength (1.5406 Å), β is the full width at half maximum (FWHM) of the peak, and θ is the Bragg angle. The most intense peak at $2\theta = 38.8°$ used to calculate the FWHM giving an estimated crystallite sized of 16.9 nm. The absence of additional peaks in the XRD patterns confirmed the phase purity of CuO NPs, with no detectable impurities or secondary phases. The synthesis route significantly influenced the crystallographic properties of the CuO NPs.

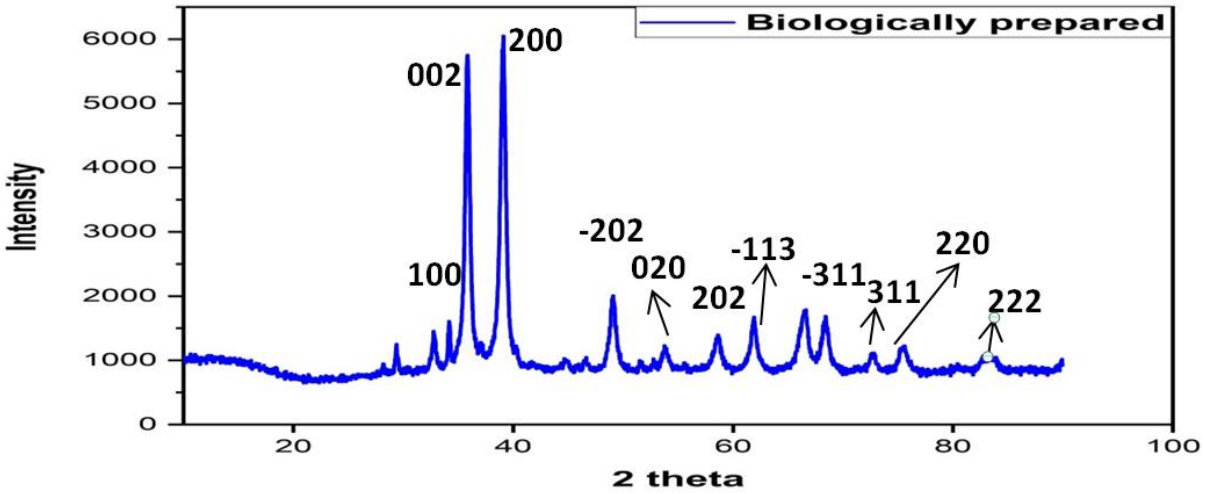

Figure:4 XRD spectrum of biologically synthesized CuO NPs

Effect of pH on Dye Degradation: The pH is considered as an important factor that affects the surface charge of the catalyst, dye stability and the generation of reactive radicals. The degradation efficiency was analyzed at range of pH from 4 to 10 by adjusting the pH of MB solution using 0.1N sodium hydroxide solution (NaOH) and 0.1N hydrochloric acid solution (HCl) respectively. The efficiency of CuO NPs photodegradation was examined under various pH conditions of the solution (between 4 to 10). The degradation efficiency increased with an increase in the pH values and slightly decreased at higher pH values as shown in Fig.5. This may be explained by the fact that at pH values greater than 8, the nanoparticles are negatively charged due to the adsorbed OH^- group on the surface of nanoparticle which occupies the active sites on the catalyst surface and reduces the interaction between dye molecules and CuO NPs, thereby decreasing the degradation efficiency. Conversely, the positively charged methylene blue is readily repelled by the positively charged CuO NPs at acidic pH 4-6, leading to a lower photodegradation rate. However, the maximum degradation efficiency was observed at optimum pH value of 8 that comparatively similar with the literature [23-24]. At pH=8 better adsorption of dye on the surface of catalyst occur which enhances the photocatalytic degradation.

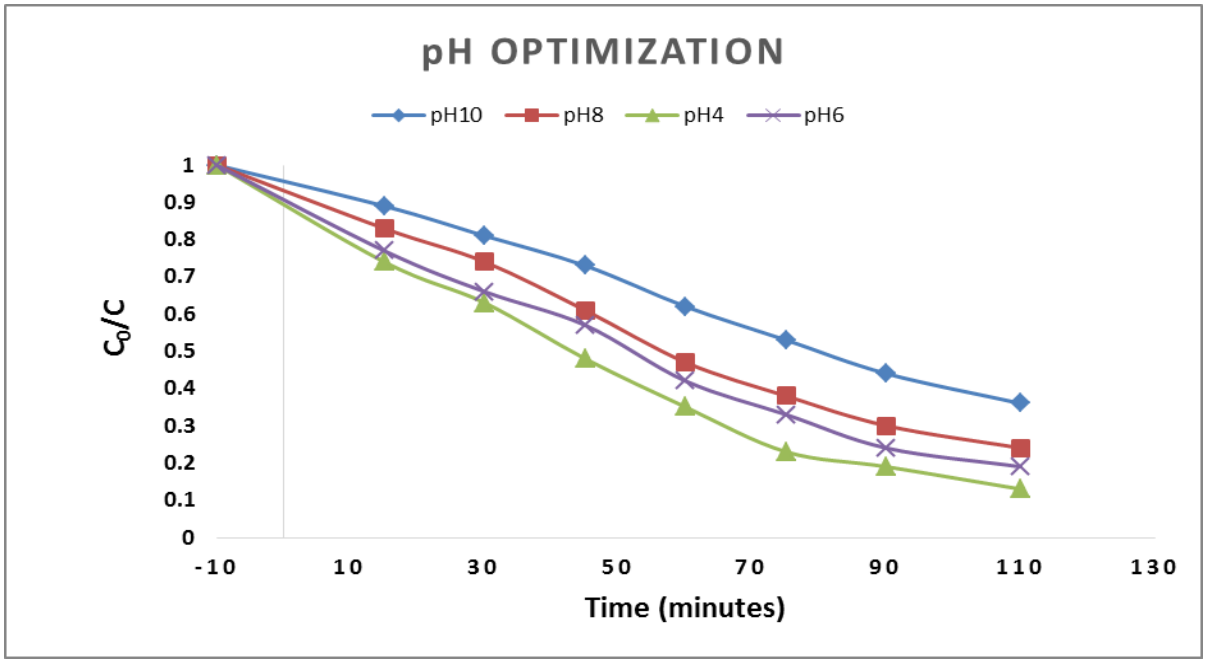

Figure 5. Photocatalytic degradation efficiency of organic dye (MB) at different pH levels

Effect of Initial Dye Concentration: The optimization study of initial dye concentration in the range of 10µg/mL to 70µg/mL of MB dye was performed to evaluate the percent degradation efficiency using 10mg of CuO NPs (Fig. 6). A continuous decline in degradation percentage was observed as the concentration increased from 10µg/mL to 70µg/mL respectively. The concentration directly relates to degradation time; as the concentration of MB dye is increased a longer time is required to achieve maximum degradation. The degradation efficiency was highest around 87.67% at an initial concentration of 10µg/mL and then decreased to 42.30% at 70 µg /mL concentration. This decreased can be attributed to the maximum number of dye molecules which adsorb on the surface of catalyst causing shield for lesser light penetration hence the degradation proficiency decreased with increasing the dye molecule concentration. A similar trend was reported by Khedr, A. I. *et al* using CuO NPs where the MB dye degraded from 99% to 87% as concentration increased from 10 to 100µg/mL [25].

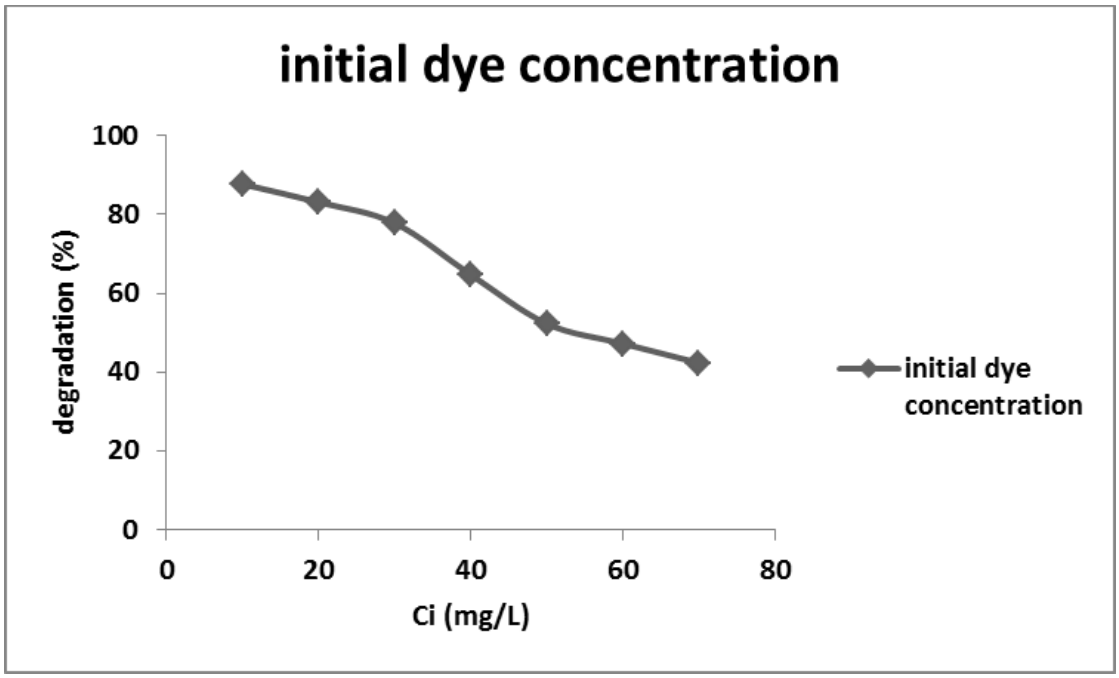

Figure 6. Effect of initial dye concentration on degradation efficiency of MB dye solution.

Effect of Photocatalyst Dosage: The dosage of photocatalyst is significantly influence the degradation efficiency of organic dyes. To investigate these effects the degradation study was carried out using different dosages 8mg, 10mg and 12mg of CuO NPs in 20mL of MB dye solution as shown in Fig 7. The results revealed that the lowest degradation percentage was obtained with 8 mg of CuO NPs after 120 minutes of irradiation. In comparison increasing the dosage to 12 mg resulted in maximum degradation efficiency for the MB dye solution. The enhancement in degradation performance at higher catalyst loading could be due to increase number of active available site and larger effective surface area which promote better catalyst interaction by adsorption and generation of reactive species, consequently greater catalyst and dye molecule interaction resulting in a larger percentage of dye removal. A similar study on different dosage of photocatalyst was reported by Parethe G. T. *et al*., using CuO NPs for the MB dye degradation [26].

Photocatalytic Degradation under Sunlight Irradiation: The process of photocatalytic degradation of MB dye in an aqueous solution was studied using CuO NPs under the sunlight irradiation (Fig. 8). The UV-Visible spectrum indicated a definite reduction pattern in the characteristic absorption peak of the MB dye at 664 nm with increasing irradiation time, which confirms the successful degradation of the dye solution. The maximum absorption was continuously decreased over the reaction time of 5 to 115 minutes which showed that the dye molecules were progressively being degraded (~96%) as upon exposure to sunlight. Such decrease in the intensity of absorbance is related to an increase in degradation efficiency since the photocatalytic reaction progresses, which indicates the formation of reactive species and the breakdown of the dye chromophore. The same observation has been documented in the recent literature on semiconductor photocatalysts. Wang *et al*. have demonstrated that CuO NPs were capable of reacting with MB dye to produce alterations in the UV-Vis

absorption characteristics during photocatalytic tests, however full degradation in their system needed to be conducted under advanced oxidative conditions [27]. Similarly, Shazia N. *et al*., developed the biogenic CuO NPs using plant extract which showed high photocatalytic degradation of MB (almost 97 %) under the influence of the visible light within 210 minutes. This highlighting the significance of green synthesis methods for the effective removal of organic toxic dyes using plant mediated nanocatalyst in waste water treatment [28].

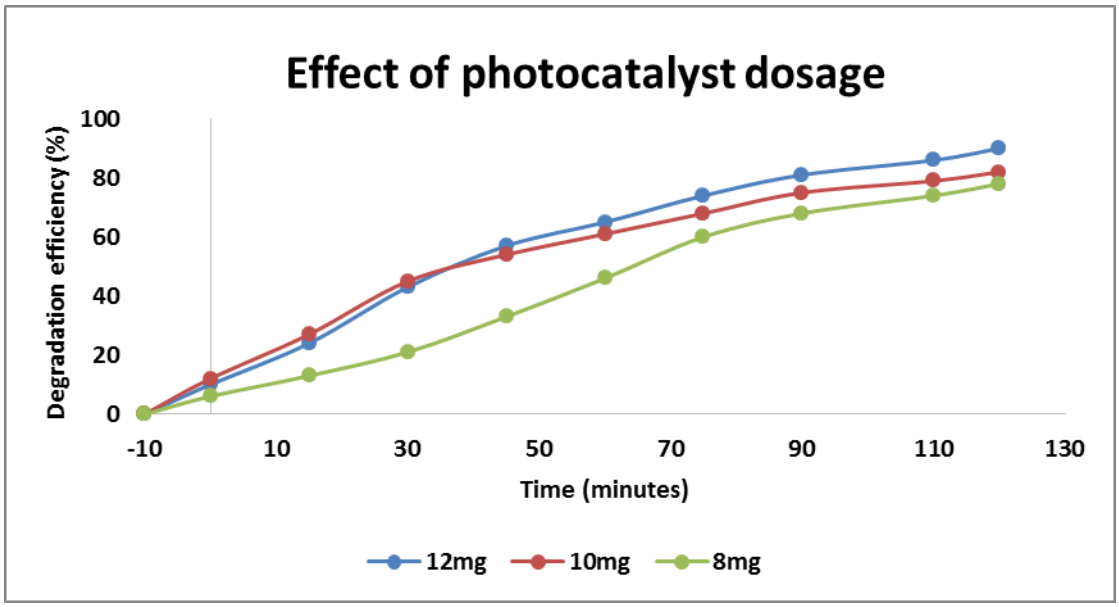

Figure 7. Effect of CuO NPs dosages on degradation efficiency of MB dye.

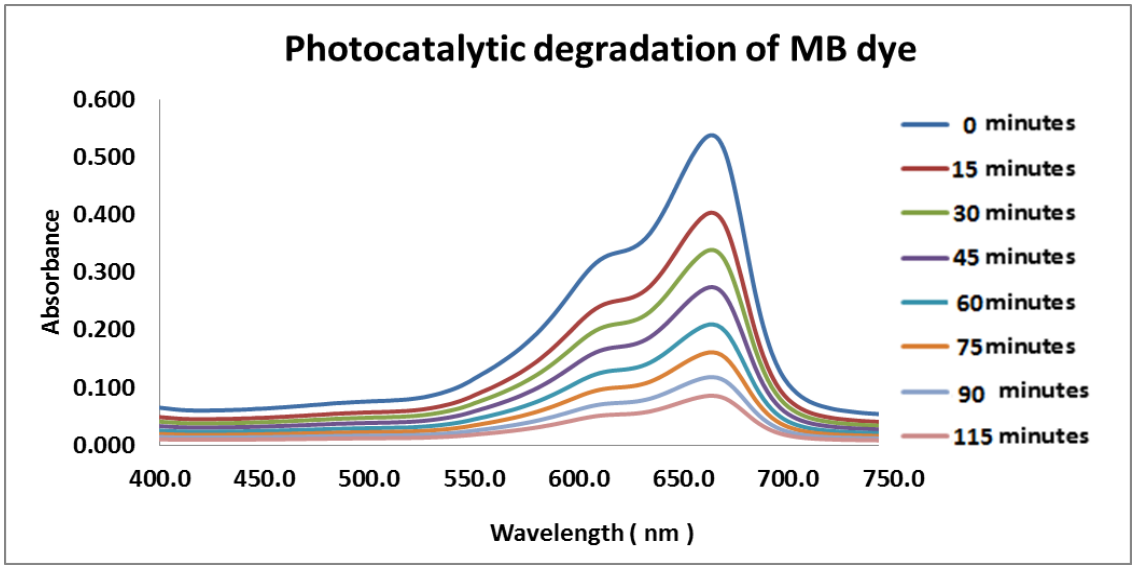

Figure 8. Photocatalytic degradation of MB using CuO NPs solution with different time intervals

Mechanism of Photocatalytic Degradation: Photocatalytic degradation is a reaction in which photons with an adequate amount of energy reacts with a semiconductor photocatalyst that has particular band gap energy. When the incident photons have energy comparable to or exceeding the band gap energy, the electrons in the valence band (VB) of CuO NPs are boosted to the conduction band (CB), leaving behind positively charged holes (h^+) in the valence band (Fig 9). The process generates electron-hole pairs in the photocatalyst. The photogenerated electrons in the conduction band have the ability to react with a molecular oxygen (O_2) that is adsorbed onto the catalyst surface to produce an anionic superoxide radical ($O_2^{\bullet -}$).

Such superoxide anions can further react with hydrogen ions (H^+) in a sequence of intermediates to form hydroperoxyl radicals ($HO_2^{\bullet}$), hydrogen peroxide (H_2O_2), and ultimately very reactive hydroxyl radicals ($OH^{\bullet}$). In the meantime, the holes in the valence band may directly participate in oxidation reactions or assist in the formation of hydroxyl radical. These reactive oxygen species (ROS), especially hydroxyl radicals, are a powerful oxidizing agent that reacts and splits methylene blue (MB) dye molecules into non-toxic and simple end product (carbon dioxide, CO_2, water, H_2O, and other mineralization products)[29].

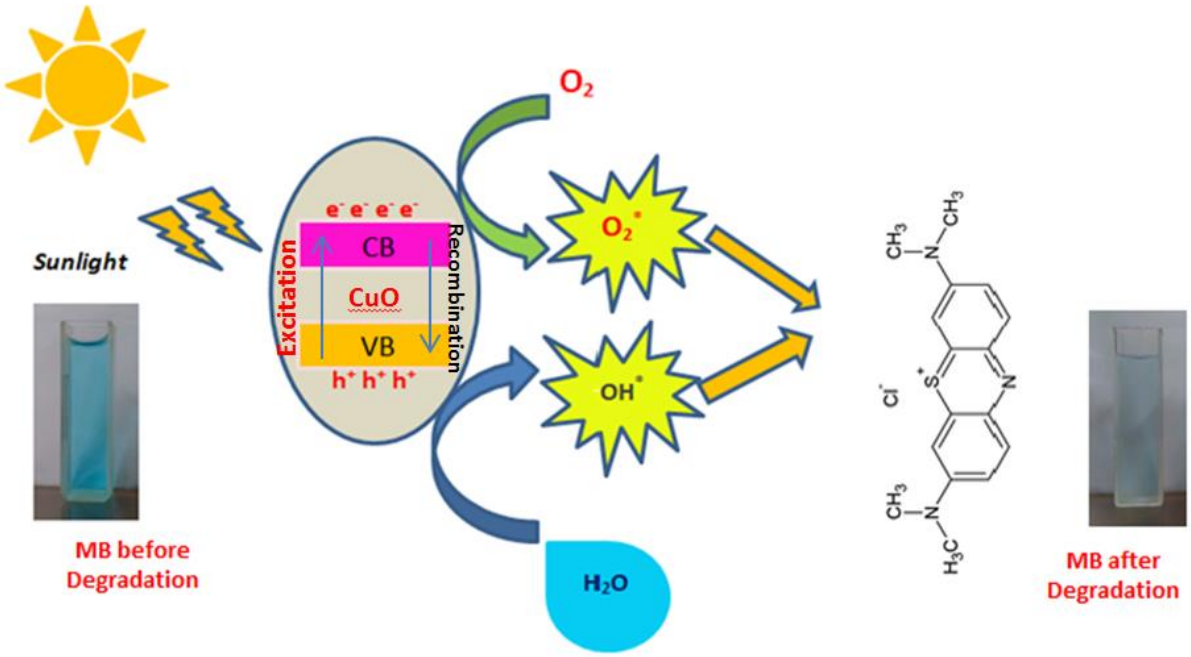

Figure 9. Mechanism of photocatalytic degradation of MB dye

Conclusion: The CuO nanoparticles produced by the biological synthesis method using *Withania coagulans* berries extract exhibited high efficiency in terms of photocatalysis of MB dye degradation under sunlight. Maximum nanoparticle was formed through the synthesis mechanism in an alkaline environment (pH of 12), with nanocrystals stabilized by the bio-reducing and capping agents presents in the extract. UV-Visible and FTIR studies confirm the formation of CuO NPs. XRD analysis of the strongest peak at 2θ= 38.8° showed that the crystallite size was approximately 16.9nm. Whereas the SEM images indicated that the aggregated size of the particles is 54 to 67nm in range, which reflects the formation of smaller crystallites into slight aggregated particles. The CuO NPs could degrade around 96 % of the dye molecule under optimized condition (pH=8, 115 min) without an extra reducing agent. The nanoscale crystallinity, hierarchical morphology, and efficient electron transfer can be attributed as the reasons of the high photocatalytic performance, which features these green-synthesized CuO NPs as promising, low-cost candidate for practical applications in wastewater treatment.

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