

Catalytic and antibacterial activity of copper nanoparticle-starch composite

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Abstract

We report the successful synthesis of copper nanoparticle (CuNP)-starch composite employing low cost green protocol without inert gas protection. UV-Vis spectroscopy, X-Ray diffraction (XRD) and Transmission electron microscopy (TEM) were used to characterize the CuNP-starch composite. Mono-disperse almost spherical CuNP having average diameter 4.02 ± 0.076 nm was obtained. The catalytic activity of the as-synthesized CuNP-starch composite during reduction of pollutants, like, 4-nitrophenol (4-NP) and dyes, Eosine Blue (EB), Eriochrome Black T (EBT) and Brilliant Cresyl Blue (BCB) by NaBH_4 has been investigated. High catalytic efficiency of the composite was demonstrated by rapid decrease of the intensity of the UV-Vis absorption peaks at respective λ_{max} of the pollutants with time when reaction mixture contained NaBH_4 and small quantity of CuNP-starch composite. The rate constant of each processes was calculated by considering that the reactions follow pseudo-first-order kinetics with respect to substrate. The obtained rate constants for 4-NP, EB, EBT and BCB are 0.021S^{-1} , 0.011S^{-1} , 0.036S^{-1} and 0.032S^{-1} respectively. The other application, antibacterial activity was tested against Gm positive and Gm negative bacteria. Plate count and minimum inhibitory concentration (MIC) studies show higher susceptibility of Gm positive bacteria towards CuNP-starch composite. The as-synthesized CuNP-starch composite may find potential application in the field of environmental remedies and antibacterial formulations. Copyright©2018 VBRI Press.

Keywords: Green synthesis, CuNP-starch composite, catalytic efficiency, pollutants degradation, antibacterial activity.

Introduction

With the increase of urbanization and industrialization, discharge of many toxic chemicals to the environment has become a practice world-wide. Pesticides and dyes are the most happening examples of such types of toxic chemicals. Complete removal of such toxic chemicals from environment using low-cost and eco-friendly method is one of the major challenging issues before the scientific community. Several methods which include microbial degradation [1, 2], adsorption by different sorbents [3] etc. have been popularly practiced removing various pesticides and dyes from water. However, resistance of such chemicals against microbial degradation limits the application of microbial treatment for environment remedy. Adsorption techniques require further treatment of sorbents to ultimate removal of adsorbed chemicals. On the other hand, nanomaterials based catalytic oxidative and reductive degradation converts the toxic pollutants into harmless components and the process is low-cost, convenient, fast and environment-friendly as well. Oxidative degradation includes the photocatalytic degradation of pollutants by nanoparticle of TiO_2 , ZnO using “advanced oxidation process” [4-6]. Reductive degradation of the pollutants can be achieved by using metal nanoparticle as catalyst in combination with a common reducing agent like NaBH_4 [7]. Recently, Sarkar et al has applied adsorption as well

as photodegradation using TiO_2 -Au nanocomposite for efficient removal of toxic dyes [8]. The success of the degradation of the pollutants using metal nanocrystals like Au, Ag, or Au-Ag, Cu etc has been demonstrated in large number of studies [7-11]. Studies also reveal that the catalytic efficiency may vary as the function of nature, size, shape, matrix of the nanocrystals [4,12].

Besides catalytic applications in chemical transformation, metal nanoparticles have also drawn considerable attention as promising antimicrobial agents. Since microorganisms develop resistance against antibiotics therefore, studies of antimicrobial properties of metal nanoparticles have become more relevant. In recent years a large number of reports have been published on antimicrobial studies of metal nanoparticle against planktonic cell as well as biofilm of various microbial strains.

To meet the above-mentioned issues of environmental pollution and antibiotic resistance, metal nanoparticle based composite synthesized using green as well as low cost protocol may be utilized in different commercial formulations. Recently some of us have successfully developed a low cost CuNP –starch composite employing complete green protocol [13]. The primary objective of the present study was to examine the catalytic and antimicrobial activity of such CuNP-starch composite for its successful utilization in environmental remedy and as an antimicrobial agent.

In the present report, CuNP has been synthesized adopting the procedure described by Dinda *et al.* [13] and as-synthesized nanoparticle was characterized by UV-Vis spectroscopy, TEM and powder XRD analysis. The catalytic activity of such CuNP-starch composite was investigated to study the reductive degradation of several toxic pollutants like 4-NP, EB, EBT, BCB. The other promising application i.e. antibacterial activity of the composite was also investigated against Gm+ve and Gm-ve bacteria.

Experimental

Materials

Copper(II) sulfate pentahydrated (AR, 99.5%, S.D. Fine chemical, India) and starch (Merck, India) were purchased from whereas ascorbic acid (>99%), Peptone, Beef extract, Agar agar were purchased from Merck (India). 4-NP (Loba Chemie, 98%), EBT (65%), BCB(64%, Sodium borohydride (99%) were purchased from Sigma (USA) and EB (85%) was purchased from Loba Chemie (India). All of them were used without further purification. *S. aureus* (MTCC3160) and *P. aeruginosa* (MTCC 424), procured from the Institute of Microbial Technology (Chandigarh, India). Water used throughout the experiment was of double distilled in nature.

Method

Synthesis of copper nanoparticles

CuNP was produced using the method described in our earlier report [13]. In a typical synthesis, a 25ml aqueous reaction mixture containing 10g% starch, 0.4mmol copper sulphate and 1g% ascorbic acid was stirred by magnetic stirrer at 60°C for 3 hrs.

The as-synthesized colloidal solution of starch stabilized CuNP was dried by freeze-drying method after centrifugation and stored in a vacuum desiccator.

Characterization of copper nanoparticles

Sample preparation, conditions during measurement and instrument details for the collection of UV-Vis spectroscopic, TEM and XRD data were similar as described elsewhere [12].

The absolute amount of copper in the CuNP-starch composite was determined by atomic absorption spectroscopy (AAS) using model Chemito AA203.

Catalytic study

3.7 ml aqueous reaction mixture containing 4-NP (0.16mM), NaBH₄ (8mM) and 0.1mg CuNP-starch nanocomposite was taken in a standard quartz cell with a 1 cm path length and reduction of 4-NP was studied by UV-Vis spectrophotometer with time at 25 °C. Similar protocol was used to study the catalytic degradation of dyes. The composition of the aqueous reaction mixture is given in the **Table 1**.

Table 1. The composition of the aqueous reaction mixture.

Total volume of reaction mixture (ml)	Name of the dyes	Concentration of dyes (mM)	Concentration of NaBH ₄ (mM)	Concentration of CuNP - starch composite (mg)
3.7	EB	0.004	3.7	0.1
	EBT	1.7	80	0.1
	BCB	0.05	6	0.1

Microbial test

S. aureus (MTCC3160) and *P. aeruginosa* (MTCC 424) were grown in nutrient media, composed of peptone 5gl⁻¹, and beef extract 3gl⁻¹. For preparing solid media, the nutrient media was supplemented with 2% bacteriological agar as solidifying agent. To investigate the antibacterial effect of CuNP on *S. aureus*, and *P. aeruginosa*, approximately 10⁸ CFU of each strain was cultured on nutrient agar plates supplemented with CuNP-starch composites of 1 to 3 mg/ml concentration. One control plate was also run parallel. The plates were incubated to 24 h at 37°C. The Minimum Inhibitory Concentration (MIC) of the CuNP was examined by batch cultures containing varying concentrations of CuNP-starch composites in suspension (0.1-1.0 mg l⁻¹). Bacterial concentrations were obtained by measuring optical density (OD) at 600 nm.

Result and discussion

Characterization of as-synthesized CuNP-starch composite is depicted in **Fig. 1**. In our earlier report [13], we have already studied the details of the UV-Vis spectra of the aqueous solution of copper sulphate in absence and in presence of starch as well as ascorbic acid. Efficient reduction of Cu²⁺ to Cu⁰ by ascorbic acid was demonstrated and reduction mechanism was discussed there by schematic illustration [13].

In the present study, characteristic Plasmon peak of CuNP was observed at 592 nm (**Panel-A in Fig. 1**). The color of the as-synthesized starch stabilized Cu nanocolloid and freeze-dried Cu-starch composite is red. The XRD data of freeze -dried CuNP-starch composite confirms that copper exists only in metallic form and indexing the peaks according to JCPDS data (card no. No.-04-0836) (**Panel-B in Fig. 1**) reflects the cubic structure of copper. The broadening of the peaks is related to the nanoscale size of the copper crystallites. Mostly spherical shaped particles are seen from TEM micrograph (**panel C in Fig. 1**). Average diameter of the particle is 4.02 ± 0.076 nm which has been measured by fitting the particle size histograms to lognormal distributions (inset of panel C in Fig1). Selected area diffraction pattern (SAED) of particles confirms that those particles are crystalline copper nanoparticle (**panel D in Fig. 1**).

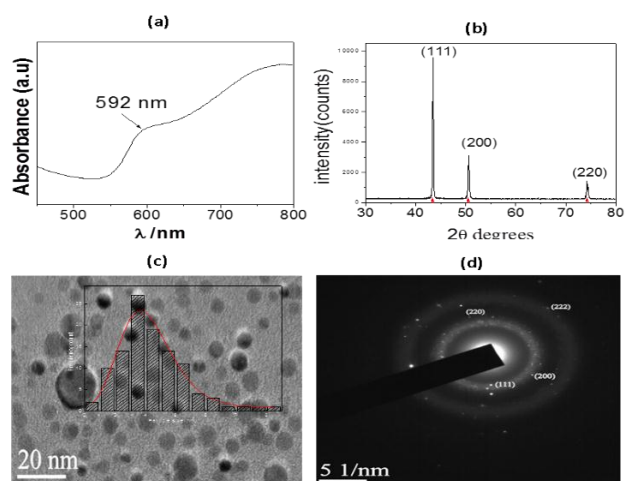


Fig.1. (A) UV-Vis spectrum of copper nanoparticle-starch composite. (B) X-ray diffraction patterns of copper nanoparticle powder samples. (C) HRTEM micrograph of copper nanoparticles, (Inset) Particles size distribution histogram. (D). Selected area diffraction pattern (SAED) of CuNPs.

The catalytic efficiency of the as-synthesized CuNP-starch composite was tested against reduction of aromatic nitro compound and several dyes. Aromatic nitro compound and dyes are the major water pollutants, abundantly found in ground and surface water. Mainly the effluent of paper, textile, leather, pharmaceutical industries is the major source of such pollutants. One of the commonly practiced methods to remove such pollutants from environment is reductive conversion of the pollutants into less harmful chemicals. However, common reducing agent like NaBH_4 alone cannot perform these type reduction processes at a feasible rate. The presence of a catalyst in the reaction mixture may result in a very fast reaction rate, instead. In this study, therefore, all the catalytic reduction processes in presence of CuNP-starch composite as catalyst were monitored by UV-Vis spectroscopy.

The UV-Vis peak for the light yellow colour solution of 4-nitrophenol in aqueous medium appeared at 317nm but with the addition of NaBH_4 , colour of the solution changed to intense yellow and peak shifted to 405nm along with increase of intensity. NaBH_4 makes the solution alkaline in which 4-nitrophenol exists as 4-nitrophenolate anion. Formation of 4-nitrophenolate ion is responsible for such color as well as spectral change (14). No significant decrease of the intensity of the UV-Vis absorption peak at 405nm for solution of phenolate ion was observed in presence of only NaBH_4 even after 60 min reaction time (**Fig 2a**). The intensity of the said peak decreased rapidly along with concomitant appearance of a new peak at 298 nm with progress of time indicating the formation of reduction product, 4-aminophenol when reaction mixture contained NaBH_4 along with catalytic amount of CuNP-starch composite. The underlying chemistry is that kinetic barrier owing to large potential difference between donor (E^0 for $\text{H}_3\text{BO}_3/\text{BH}_4^- = -1.33\text{V}$) and acceptor (E^0 value for 4-NP/4-AP = -0.76V) restricts

this thermodynamically feasible reduction. The catalyst, CuNPs help to overcome such kinetic barrier by facilitating electron relay from BH_4^- to 4-NP.

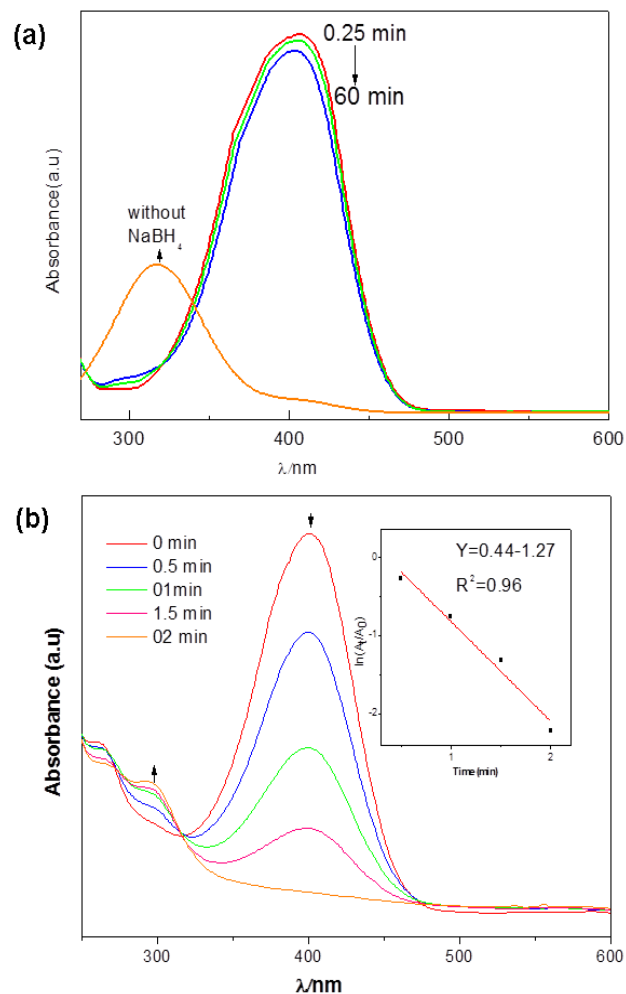


Fig. 2. UV-visible spectra, (a) reduction of 4-nitrophenol mixed with NaBH_4 at different time intervals in absence of CuNP-starch composite. (b) reduction of 4-nitrophenol mixed with NaBH_4 at different time intervals in presence of CuNP-starch composite, (Inset) The determination of apparent rate constants.

Presence of large excess of NaBH_4 in the reaction mixture slows down the decomposition of NaBH_4 by increasing the pH of the solution and also prevents the areal oxidation of 4-AP by liberating hydrogen. Since the concentration of NaBH_4 was large in compare to 4-NP, so the rate constant of the reduction process can be calculated by considering the pseudo-first-order reactions kinetics with respect to substrate (4-NP). A good linear relationship was obtained when $\ln(A_t/A_0)$ was plotted against time. Here A_t and A_0 stands for absorbance at any time, t and absorbance at time 0, respectively. Slope of this straight line represents the rate constant, k and the obtained value is 0.02116 s^{-1} i.e. $21.2 \times 10^{-3} \text{ s}^{-1}$ which is close to previously reported $27.0 \times 10^{-3} \text{ s}^{-1}$ by Ajmal et al (15) when they used Cu nanoparticle containing poly(methylacrylic acid) microgel.

Similar studies were performed to investigate the catalytic degradation of dyes, E B, EBT and BCB by monitoring the UV-Vis absorption spectroscopy. No significant amount decreases of the intensity of the color of these dyes as well as the absorbance of the UV-Vis spectra was observed in the presence of NaBH_4 alone even after 1hr of reaction (Fig. 3a, 4a, 5a).

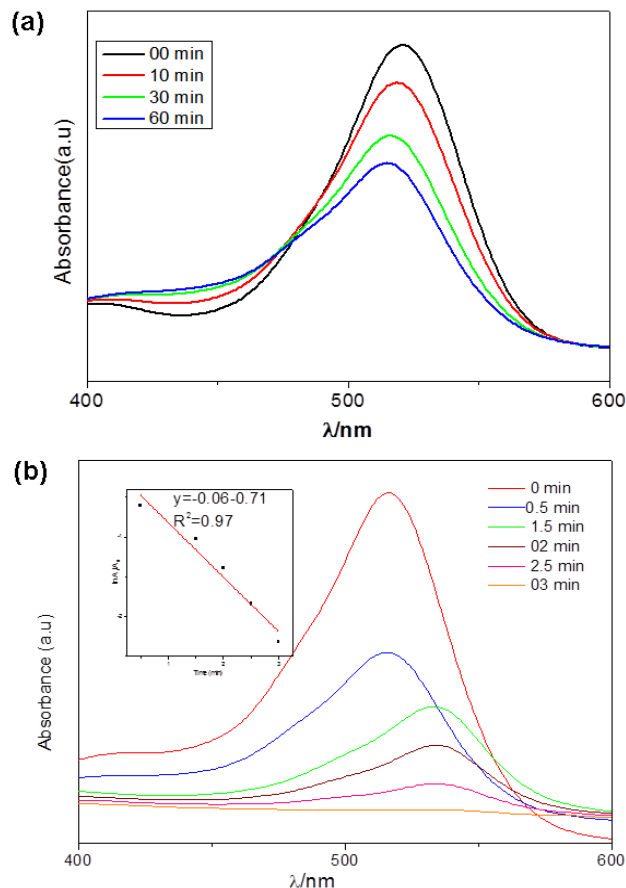


Fig. 3. UV-visible spectra, (a) reduction of Eosine blue mixed with NaBH_4 at different time intervals in absence of CuNP-starch composite. (b) reduction of Eosine blue mixed with NaBH_4 at different time intervals in presence of CuNP-starch composite, (Inset) The determination of apparent rate constants.

Fig. 3b represents the UV-Vis spectra of reaction mixture containing EB and NaBH_4 in presence of CuNP-starch composite. The absorbance of the UV-Vis spectra diminishes to almost zero within 3 min with addition of CuNP-starch composite in the reaction mixture. Simultaneously a shift of λ_{max} value towards higher wavelength during reaction in presence of CuNP may be due to the adsorption of EB dye onto surface of CuNP. The rate constant, k was evaluated in the similar method described for reduction reaction of nitrophenol in earlier section.

The obtained value is $11.4 \times 10^{-3} \text{ s}^{-1}$ taking the A_t and A_0 values at λ_{max} (516nm). The absorbance at 565 nm for EBT decreases to almost zero within 1.25 min in presence of NaBH_4 and CuNP-starch composite in the reaction mixture (Fig. 4b). Degradation of dye, BCB was studied

by monitoring the absorbance at 624nm and reaction was completed within 2mins (Fig. 5b). The calculated rate constants are $36.6 \times 10^{-3} \text{ s}^{-1}$ and $32.8 \times 10^{-3} \text{ s}^{-1}$ for EBT and BCB respectively. To get more appropriate idea about catalytic efficiency of the as-synthesized CuNP-starch composite, the activity parameter (κ) (equal to ratio of rate constant (k) to the total mass of catalyst added) was calculated [14]. The value of k and κ for 4-NP and other three dyes are given in Table. 2. One can interestingly note from Table 2 that CuNP-starch composite most efficiently reduce the EB dye compared to other dyes used in this experiment.

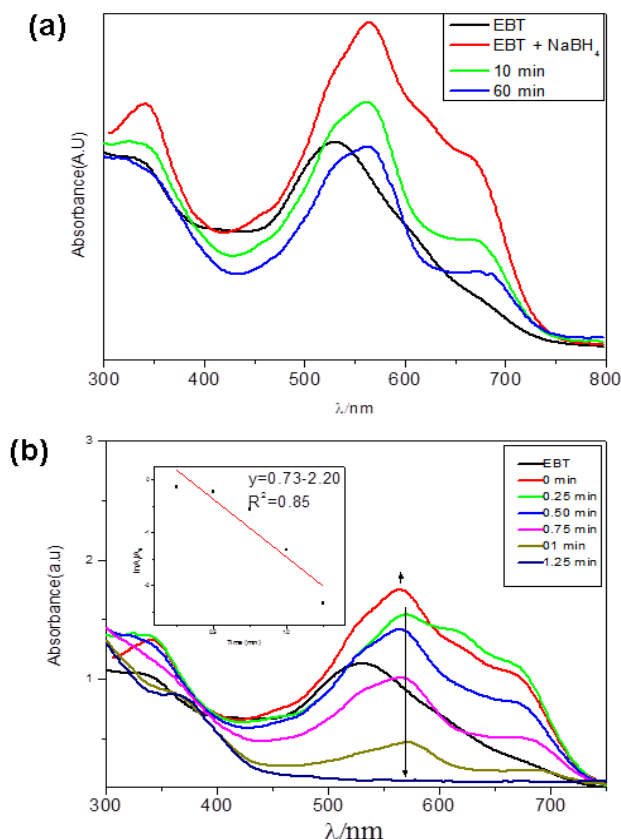


Fig. 4. UV-visible spectra, (a) reduction of Eriochrome black T mixed with NaBH_4 at different time intervals in absence of CuNP-starch composite. (b) reduction of Eriochrome black T mixed with NaBH_4 at different time intervals in presence of CuNP-starch composite, (Inset) the determination of apparent rate constants.

For the reductive degradation of EB, Soomro *et al.* [16] obtained higher k value ($240 \times 10^{-3} \text{ s}^{-1}$) when they used 0.1mg powder of CuNP as a catalyst. However, the AAS result shows that the absolute amount of copper present in each mg of the CuNP-starch composite, reported here was 210 μg . So 0.1mg composite contained only 21 μg of CuNP as active catalyst. Activity parameters (κ) of the reduction of 4-NP in which several nanostructured silvers containing composites were employed have been summarized by Baruah *et al.* [14]. Most of those values are lower than the value $211 \text{ s}^{-1} \text{ g}^{-1}$, obtained for our low cost CuNP-starch composite. However very few of those values are comparable and higher than the $211 \text{ s}^{-1} \text{ g}^{-1}$.

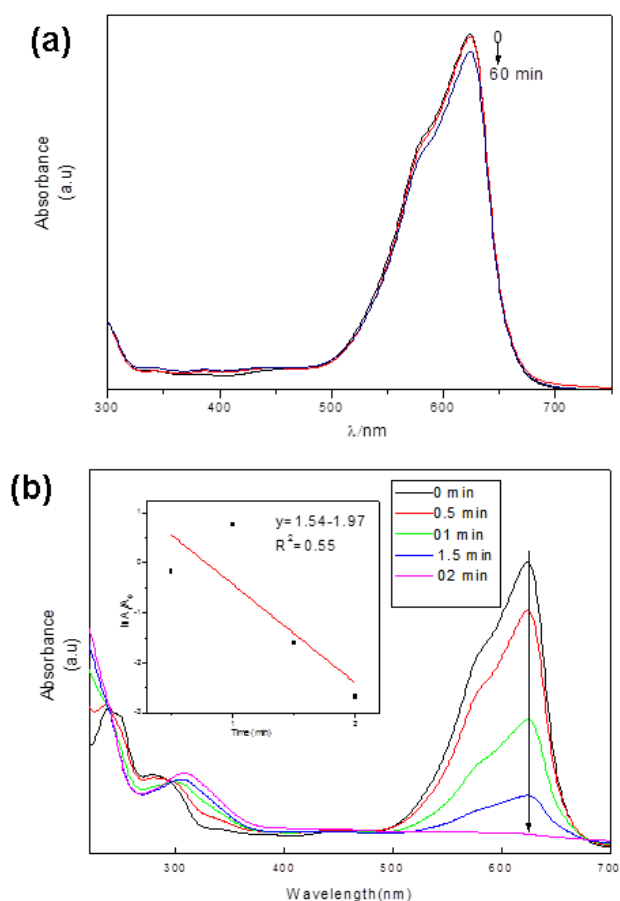


Fig. 5. UV-visible spectra, (a) reduction of Brilliant cresyl blue mixed with NaBH_4 at different time intervals in absence of CuNP-starch composite. (b) reduction of Brilliant cresyl blue mixed with NaBH_4 at different time intervals in presence of CuNP-starch composite, (Inset) The determination of apparent rate constants.

Table 2. Activity parameters, κ , ratio of rate constants of the reaction (k) and the amounts of catalyst used.

Name of substrate	$k \text{ (s}^{-1}) \times 10^3$	$\kappa \text{ (s}^{-1}\text{g}^{-1})$
4-NP	21.1	211
Eosine blue	11.4	114
Eriochrome black T	36.6	366
Brilliant cresyl blue	32.8	328

Antibacterial activity of the CuNP-Starch composite was tested against *S.aureus* (Gm + ve) and *P. aeruginosa* (Gm -ve). As the concentration of CuNP-Starch composite gradually increased, the extent of growth inhibition of both the tested organisms was also increased. At the concentration level of 3mg/ml of the composite no growth was observed for *S.aureus* as well as *P. aeruginosa* (**Fig. 5b**). The MIC values of CuNP-starch composite against *S. aureus*, and *P. aeruginosa* was found to be 0.27mg and 0.34mg / ml, respectively. The AAS

result shows that the amount of copper present in 0.27 and 0.34 mg composite is nearly 59 and 75 μg , respectively. Therefore it is evident that tested Gm positive bacterial strain is more susceptible to the CuNP-starch composite as compared to the strain of Gm negative bacteria. This is perhaps due to their different cell wall composition i.e. greater numbers of binding amine and carboxyl groups are present on the cell surface of Gm Positive *S. aureus* whereas tightly packed Lipo Poly Saccharide (LPS) molecules are present in the outer membrane of *P. aeruginosa* giving an effective resistive barrier against copper nanoparticle [13]. However, Cristena *et al.* reports that casein capped CuNPs exerted an MIC of 0.13mM (almost 8.25mg) against both *S. aureus* and *P. aeruginosa* (17)

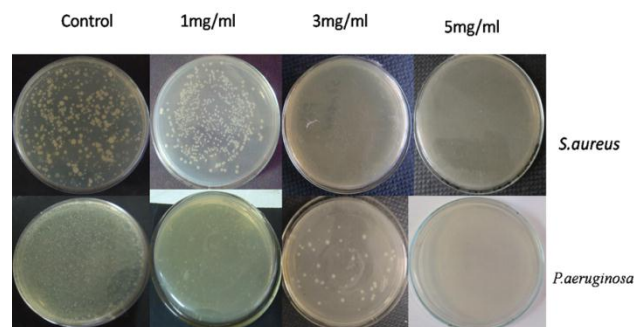


Fig. 6. Growth of bacterial strain (*S. aureus*, and *P. aeruginosa*) on Agar medium loaded with CuNP-starch composite at different concentration level (mg/ml).

Conclusion

Vitamin C may be employed for the synthesis of self-stable CuNP-starch composite which in turn may use as effective catalyst during reduction of toxic pollutants, like, 4-nitrophenol (4-NP) and dyes, Eosine Blue (EB), Eriochrome Black T (EBT) and Brilliant Cresyl Blue (BCB). In addition the composite shows good antimicrobial activity against both gm positive as well as gm negative organisms. Commercial application of this composite may be a low cost and greener approach for environment remediation or packaging field.

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Author contributions

Concieved the plan of work: G. D, A. M, D. H; Performed the experiments: G. D; Data analysis: G. D; Wrote the paper: G.D, A. M, D. H. Authors have no competing financial interests.

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