Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Asterarcys quadricellulare Algae-Mediated Copper Oxide Nanoparticles as a Robust and Recyclable Catalyst for the Degradation of Noxious Dyes from the Wastewater

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1 Experimental section

1.1 Materials and methods

Asterarcys quadricellulare microalgae (NCBI accession no. MW560279.1) were taken from the Department of Botany, Mohanlal Sukhadia University, Udaipur, Rajasthan, India. Bismarck brown Y (BBY), boric acid (H_3BO_3), calcium chloride dihydrate ($CaCl_2.2H_2O$), citric acid, cobalt nitrate hexahydrate ($Co(NO_3)_2.6H_2O$), copper sulfate pentahydrate ($CuSO_4.5H_2O$), dipotassium hydrogen phosphate trihydrate ($K_2HPO_4.3H_2O$), ethylenediamine tetraacetic acid (EDTA), ferric ammonium citrate, magnesium sulfate heptahydrate ($MgSO_4.7H_2O$), manganese chloride tetrahydrate ($MnCl_2.4H_2O$), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), sodium molybdate dihydrate ($Na_2MoO_4.2H_2O$), sodium nitrate ($NaNO_3$), and zinc sulfate heptahydrate ($ZnSO_4.7H_2O$) were purchased from Himedia. Benzoquinone (BQ**),** brilliant green (BG), eriochrome black T (EBT), hydrochloric acid (HCl), isopropyl alcohol (IPA), malachite green (MG), potassium dichromate ($K_2Cr_2O_7$), and sulphuric acid (H_2SO_4) were purchased from LOBA Chemie Pvt. Ltd. Deionized (DI) water was purchased from Merck. Analytical-grade chemicals were used in this study without any additional purification.

1.2 Axenic culture and growth of algae

For the axenic culture of *Asterarcys quadricellulare*, blue green-11 (BG-11) media was utilized in this study. The BG-11 media was prepared by the method described in Rippka et al. literature.[1] 20 mL of BG-11 media and *Asterarcys quadricellulare* algae were poured into the 50 mL test tubes and incubated at 27 ± 1 °C, 14.5 Wm^{-2} light intensity, and a 16 h light and 8 h dark cycle to begin the culture. To obtain a larger amount of biomass, the culture was subcultured in 250 mL Erlenmeyer flasks. Cultures were routinely maintained by replacing the media under the same conditions, and harvesting was carried out during the exponential phase.

1.3 Preparation of aqueous algal extract of *Asterarcys quadricellulare*

After harvesting the algal biomass, it was subjected to centrifugation at 4000 rpm for 20 min at room temperature. Decanted off the supernatant carefully into a beaker. The remaining pellet was washed three times with DI water and dried in an oven at 35 °C for 24 h. The dried algal biomass was then crushed into a fine powder using a grinder. Based on prior research by Banerjee and colleagues, it was analyzed that 5 g/100 mL was the optimal concentration of algal extract for the biosynthesis of CuO NPs.[2] Therefore, in this study, the same concentration of *Asterarcys*

quadricellulare algal extract was utilized to synthesize CuO NPs. To achieve this, 5 g of algal powder was added to 100 mL of DI water in a 250 mL beaker, which was then heated in a water bath at 45 °C for 30 min. After cooling, the aqueous algal extract was centrifuged at 4000 rpm for 20 min, and the supernatant was collected for further use. If required the supernatant should be stored at 4 °C temperature.

1.4 Biosynthesis of CuO NPs using algal extract

For the green synthesis of CuO NPs, 100 mL of 0.2 M copper sulphate solution was taken in a 250 mL round bottom flask and stirred at 60 °C for 5 min. Then, 10 mL of the *Asterarcys quadricellulare* algal extract was added to the above solution at the same temperature (60 °C). Subsequently, 0.1 N NaOH solution was added dropwise to the reaction mixture to reach a pH of 11, and the stirring was continued for 24 h at 60 °C. After 24 h, the obtained CuO NPs were separated from the solution by centrifugation at 10000 rpm for 5 min, dispersed in DI water and centrifuged again to eliminate the impurity. The washing procedure of nanoparticles was repeated three times. The washed CuO NPs were dried in an oven at 80 °C overnight. The obtained CuO NPs were stored in an airtight container for further use.

1.5 Study of the effect of physicochemical factors on the synthesis of CuO NPs

The biosynthesis of CuO NPs using *Asterarcys quadricellulare* algal extract was optimized by varying the contributing parameters sequentially, including the pH of the reaction mixture, reaction time, the volume of algal extract, and the concentration of copper ions, with keeping constant other parameters except one. In our previous study, we discovered that the alkaline pH was found to be appropriate for the biosynthesis of CuO NPs.[3] Therefore, to study the effect of pH, the reaction was carried out by adjusting the pH of the reaction mixture to 10, 11, and 12 using 0.1 N NaOH solution. The effect of reaction time was studied by varying the reaction time to 24 h, 48 h, and 72 h, keeping other parameters constant. The effect of the volume of algal extract was studied by adding the different volumes of algal extract (5 mL, 10 mL, and 15 mL) to 100 mL of 0.2 M copper sulphate solution. To investigate the effect of copper ion concentration on the biosynthesis of CuO NPs, 5 mL of algal extract was added to 100 mL of copper sulphate solution with different concentrations (0.05 M, 0.1 M, and 0.2 M). The influence of these parameters on the synthesis of CuO NPs was confirmed by various techniques below in this literature. Table S1, Table S2, Table

S3 and Table S4 represent the synthesis of CuO NPs at various pH, reaction time, the volume of algal extract, and the concentration of the copper ions, respectively.

Table S1. Conditions of synthesis of CuO NPs samples by variation of pH values.

S. No.	Sample name	pH	Time	Volume of algal extract	Concentration of copper ions	Temperature
1.	**S1**	10	24 h	10 mL	0.2 M	60 °C
2.	**S2**	11	24 h	10 mL	0.2 M	60 °C
3.	**S3**	12	24 h	10 mL	0.2 M	60 °C

Table S2. Conditions of synthesis of CuO NPs samples by variation of reaction time.

S. No.	Sample name	Time	pH	Volume of algal extract	Concentration of copper ions	Temperature
1.	**S2**	24 h	11	10 mL	0.2 M	60 °C
2.	**S4**	48 h	11	10 mL	0.2 M	60 °C
3.	**S5**	72 h	11	10 mL	0.2 M	60 °C

Table S3. Conditions of synthesis of CuO NPs samples by variation of the volume of algal extract.

S. No.	Sample name	Volume of algal extract	pH	Time	Concentration of copper ions	Temperature
1.	**S6**	5 mL	11	24 h	0.2 M	60 °C
2.	**S2**	10 mL	11	24 h	0.2 M	60 °C
3.	**S7**	15 mL	11	24 h	0.2 M	60 °C

Table S4. Conditions of synthesis of CuO NPs samples by variation of the concentrations of copper ions.

S. No.	Sample name	Concentration of copper ions	pH	Time	Volume of algal extract	Temperature
1.	**S8**	0.05 M	11	24 h	5 mL	60 °C
2.	**S9**	0.1 M	11	24 h	5 mL	60 °C
3.	**S10**	0.2 M	11	24 h	5 mL	60 °C

1.6 Chemical synthesis of CuO NPs

The CuO NPs were synthesized by the simple co-precipitation method.[3] The 100 mL of 0.2 M copper sulphate solution was taken in a 250 mL round bottom flask and stirred at 60 °C for 5 min. A solution of 0.1 N NaOH was gradually added to the reaction mixture until a pH of 11 was achieved. Subsequently, the stirring of the mixture was sustained at 60 °C for a period of 24 h. After cooling, the brownish-black precipitate was separated using centrifugation at 10000 rpm for 5 min. The precipitate was rinsed three times with DI water. The washed CuO NPs were dried in the oven at 80 °C overnight. The obtained CuO NPs were stored for further analysis. The chemically synthesized CuO NPs were denoted as S11 in the manuscript.

1.7 Characterizations

Various characterization techniques were employed to investigate the structural, optical, and morphological properties of synthesized nanoparticles. X-ray powder diffraction (XRD) was conducted within the 2θ range from 30° to 70° using a Rigaku Ultima IV X-ray diffractometer with CuKα radiation (λ = 1.5406 Å) to obtain XRD patterns. The obtained XRD patterns were used to determine structural features such as orientation parameters, crystallite size, and dislocation density. From XRD data, Williamson-Hall (W-H) plots were generated to assess the crystallite size and strain in the samples. FTIR, Bruker Alpha, USA, was used to identify functional groups present in the algae and the synthesized nanoparticles. UV-visible absorption spectra were recorded using Shimadzu UV-2600, Japan UV-visible spectrophotometer to determine the nanoparticles' band gap values by Tauc's plots. The FE-SEM coupled with EDS (JEOL-7610F) was utilized to evaluate the structural morphology and elemental composition of the nanoparticles. A digital pH meter (HANNA) was used to measure the pH of the reaction mixture. The absorbance of the dye solution was recorded on a Hitachi UH5300 UV-visible spectrophotometer.

1.8 Photocatalytic degradation of BBY, BG, EBT, and MG dyes

The photocatalytic efficiency of CuO NPs was assessed with photocatalytic degradation of BBY, BG, EBT, and MG dyes using visible light illumination with a tungsten lamp of 60 W (Philips). A water filter was used to block the thermal radiation. A stock solution of 1 x 10^{-3} M concentration was prepared for all four dyes. The CuO NPs (0.05 g) were dispersed in 40 mL of an aqueous solution of dye. The above reaction mixture was placed in the dark on the magnetic stirrer for 30 min to achieve the adsorption-desorption equilibrium. After equilibrium, the solution was

irradiated with a light source to initiate the photocatalytic activity. After a specific interval of time (15 min), 2 mL of dye solution was taken off carefully so that the catalyst particles did not interfere, and the absorbance of this dye solution was measured using the UV-Vis spectrophotometer. The variables such as pH (3–11), catalyst loading (0.01 g–0.09 g), and dye concentration (50 ppm–100 ppm) were optimized individually, keeping the other factors constant.

Equation (S1) was used to determine the percentage of degradation at any given time.

$$\% \text{ degradation} = \frac{A_o - A_t}{A_o} \times 100 = \frac{C_o - C_t}{C_o} \times 100 \quad \text{(S1)}$$

Where A_o and C_o, and A_t and C_t represent the initial absorbance and initial concentration of dye, absorbance and concentration of dye at a specified time t, respectively.

The rate constant (k) was calculated assuming pseudo-first-order kinetics using equation (S2).

$$\ln(A_o/A_t) = \ln(C_o/C_t) = kt \quad \text{(S2)}$$

The pseudo-first-order rate constant, k (min^{-1}), was calculated from the slope of ln (C_o/C_t) versus irradiation time t.

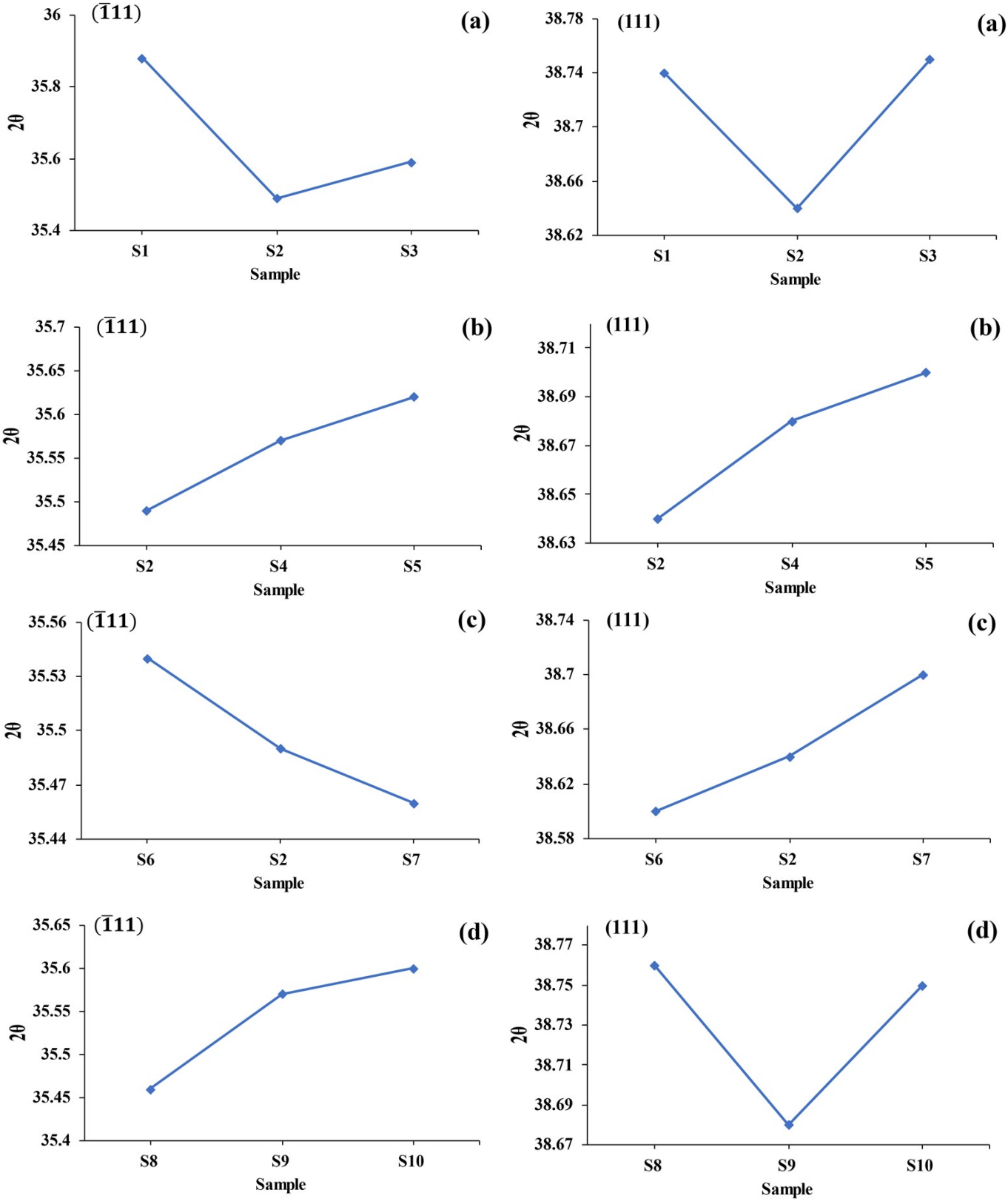
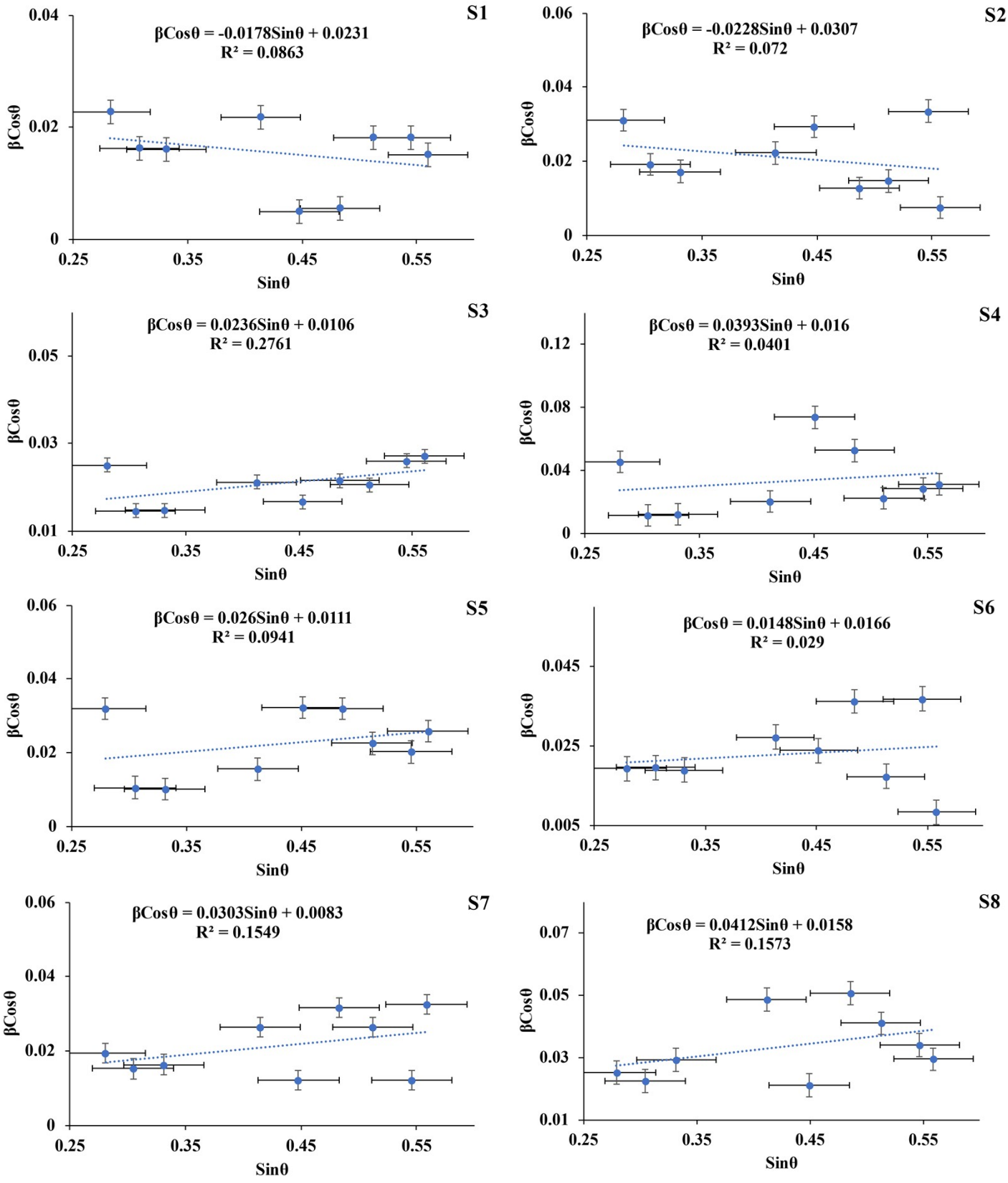
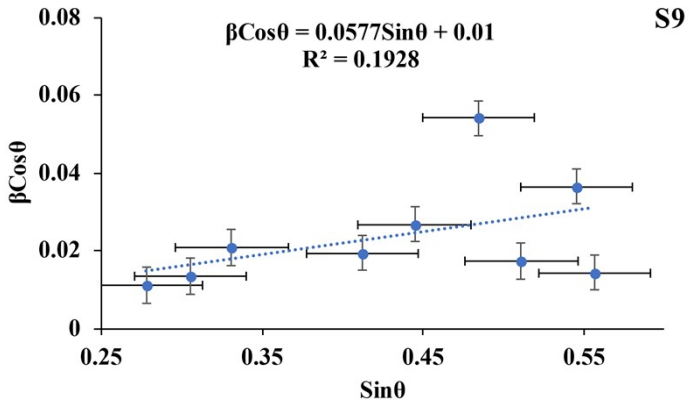
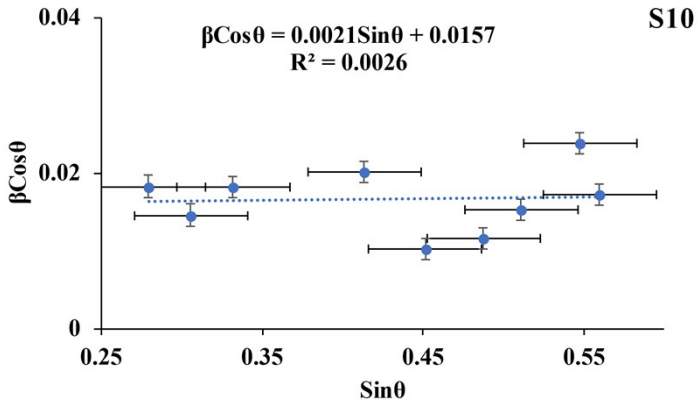

(a)
(111)
36
35.8
35.6
35.4
2θ
S1
S2
S3
Sample
38.78
38.74
38.7
38.66
38.62
(b)
35.7
35.65
35.6
35.55
35.5
35.45
S2
S4
S5
38.71
38.69
38.67
38.65
38.63
(c)
35.56
35.53
35.5
35.47
35.44
S6
S7
38.74
38.7
38.66
38.62
38.58
(d)
35.65
35.6
35.55
35.5
35.45
35.4
S8
S9
S10
38.77
38.75
38.73
38.71
38.69
38.67

Fig. S1. Difference in the angle of diffraction (2θ) associated with two major crystallographic planes, i.e., $(\bar{1}11)$ and (111) for various parameters of biosynthesized CuO NPs. (**a**) pH variation (S1- pH 10, S2- pH 11, S3- pH 12), (**b**) reaction time variation (S2- 24 h, S4- 48 h, S5- 72 h), (**c**) algal extract volume variation (S6- 5 mL, S2- 10 mL, S7- 15 mL), and (**d**) copper ions concentration variation (S8- 0.05 M, S9- 0.1 M, S10- 0.2 M).


S1
βCosθ = -0.0178Sinθ + 0.0231
R² = 0.0863
βCosθ
Sinθ
S2
βCosθ = -0.0228Sinθ + 0.0307
R² = 0.072
βCosθ
Sinθ
S3
βCosθ = 0.0236Sinθ + 0.0106
R² = 0.2761
βCosθ
Sinθ
S4
βCosθ = 0.0393Sinθ + 0.016
R² = 0.0401
βCosθ
Sinθ
S5
βCosθ = 0.026Sinθ + 0.0111
R² = 0.0941
βCosθ
Sinθ
S6
βCosθ = 0.0148Sinθ + 0.0166
R² = 0.029
βCosθ
Sinθ
S7
βCosθ = 0.0303Sinθ + 0.0083
R² = 0.1549
βCosθ
Sinθ
S8
βCosθ = 0.0412Sinθ + 0.0158
R² = 0.1573
βCosθ
Sinθ


S9
βCosθ = 0.0577Sinθ + 0.01
R² = 0.1928
βCosθ
Sinθ
0.08
0.06
0.04
0.02
0
0.25
0.35
0.45
0.55


S10
βCosθ = 0.0021Sinθ + 0.0157
R² = 0.0026
βCosθ
Sinθ
0.04
0.02
0
0.25
0.35
0.45
0.55

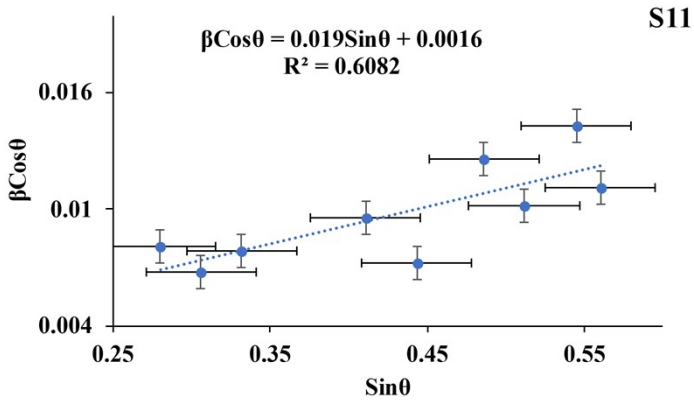
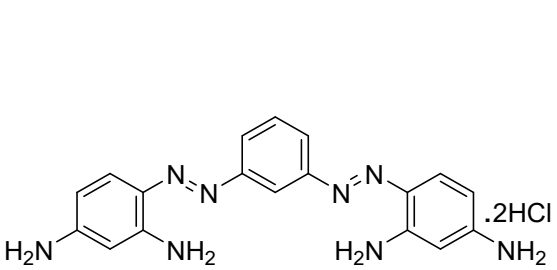
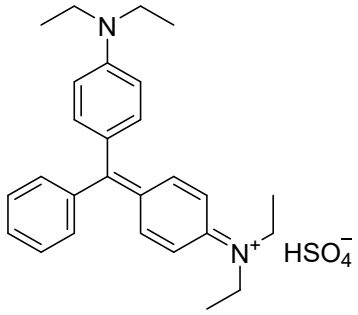

S11
βCosθ = 0.019Sinθ + 0.0016
R² = 0.6082
βCosθ
Sinθ
0.016
0.01
0.004
0.25
0.35
0.45
0.55

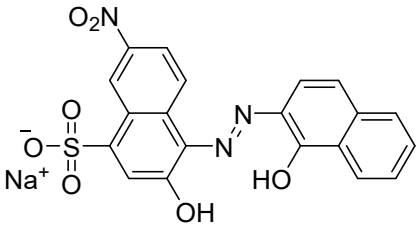
Fig. S2. W-H plots of the biosynthesized CuO NPs (S1-S10) and chemically synthesized CuO NPs (S11) to give a qualitative idea of lattice strain and average crystallite size. The standard error bars demonstrate the uncertainties.



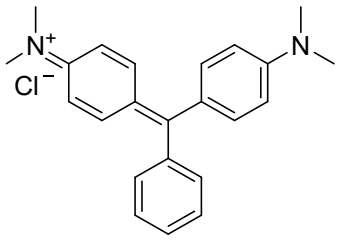
Bismarck brown Y (BBY)



Brilliant green (BG)

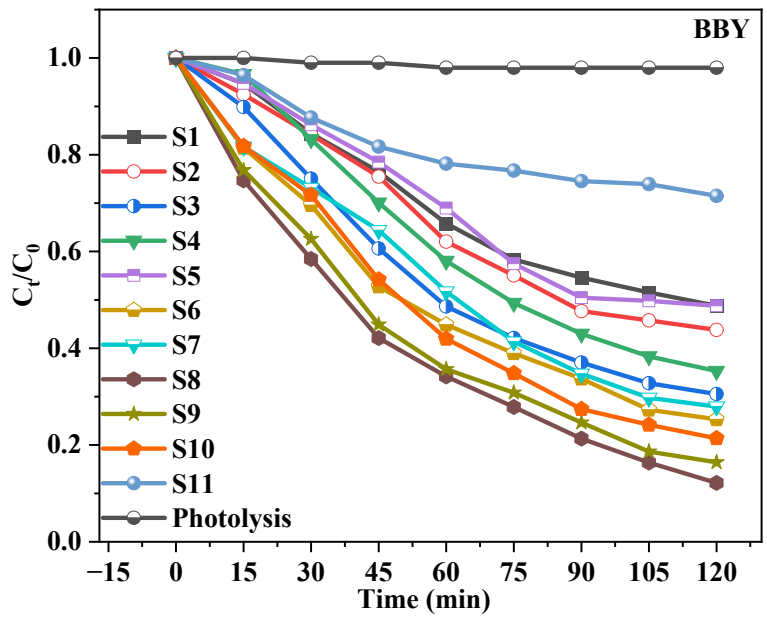


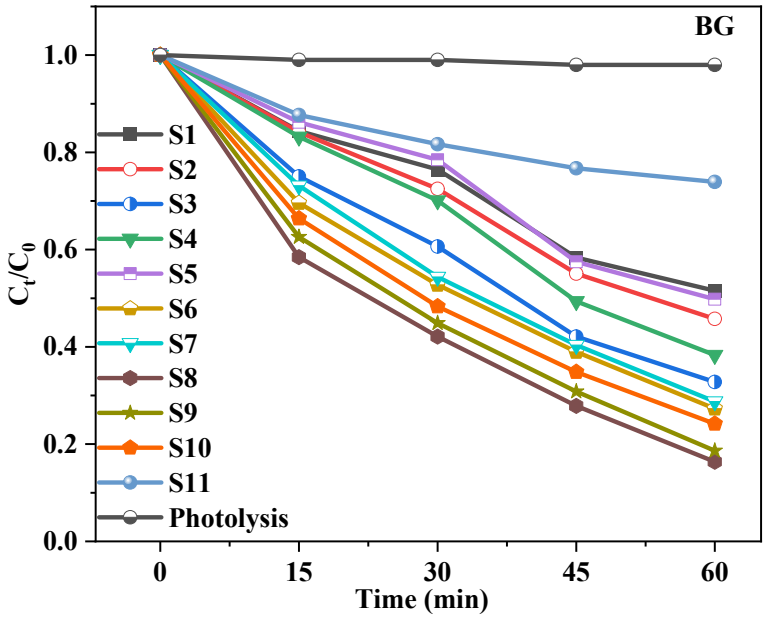
Eriochrome black T (EBT)

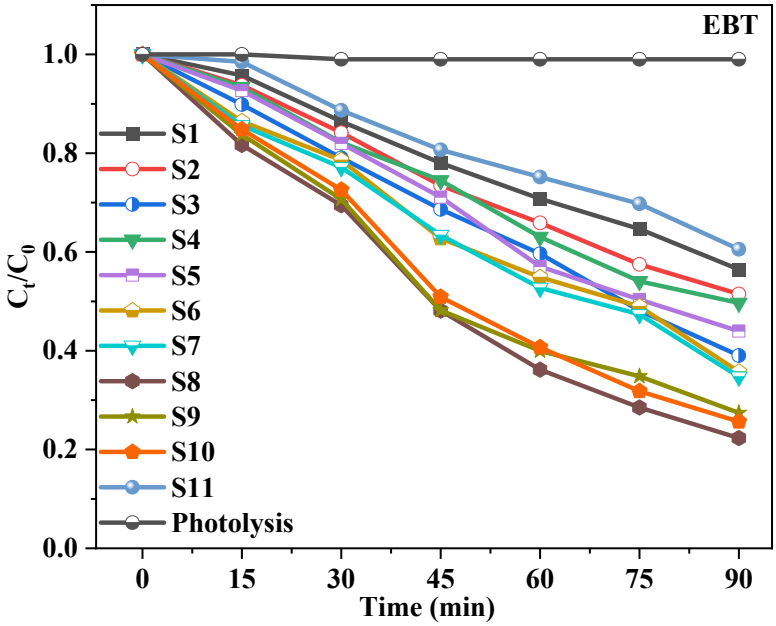


Malachite green (MG)

Fig. S3. Structures of BBY, BG, EBT, and MG dyes.


BBY
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11
Photolysis
C_t/C_0
Time (min)


BG
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11
Photolysis
C_t/C_0
Time (min)


EBT
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11
Photolysis
C_t/C_0
Time (min)

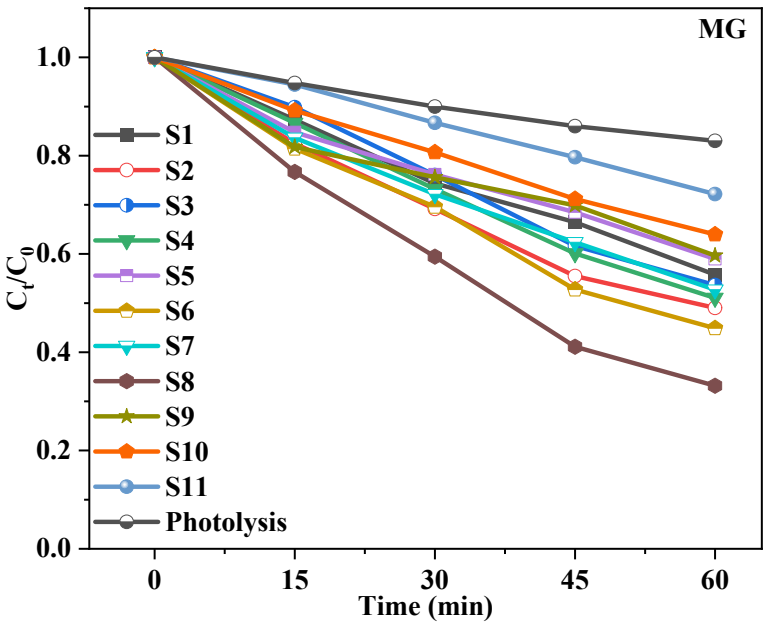

MG
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11
Photolysis
C_t/C_0
Time (min)

Fig. S4. The time dependent C_t/C_0 plots showing the effect of various biosynthesized CuO NPs (S1-S10) and chemically synthesized CuO NPs (S11) on the degradation of the dyes: (**a**) BBY, (**b**) BG, (**c**) EBT, and (**d**) MG, through photolysis and photocatalysis.

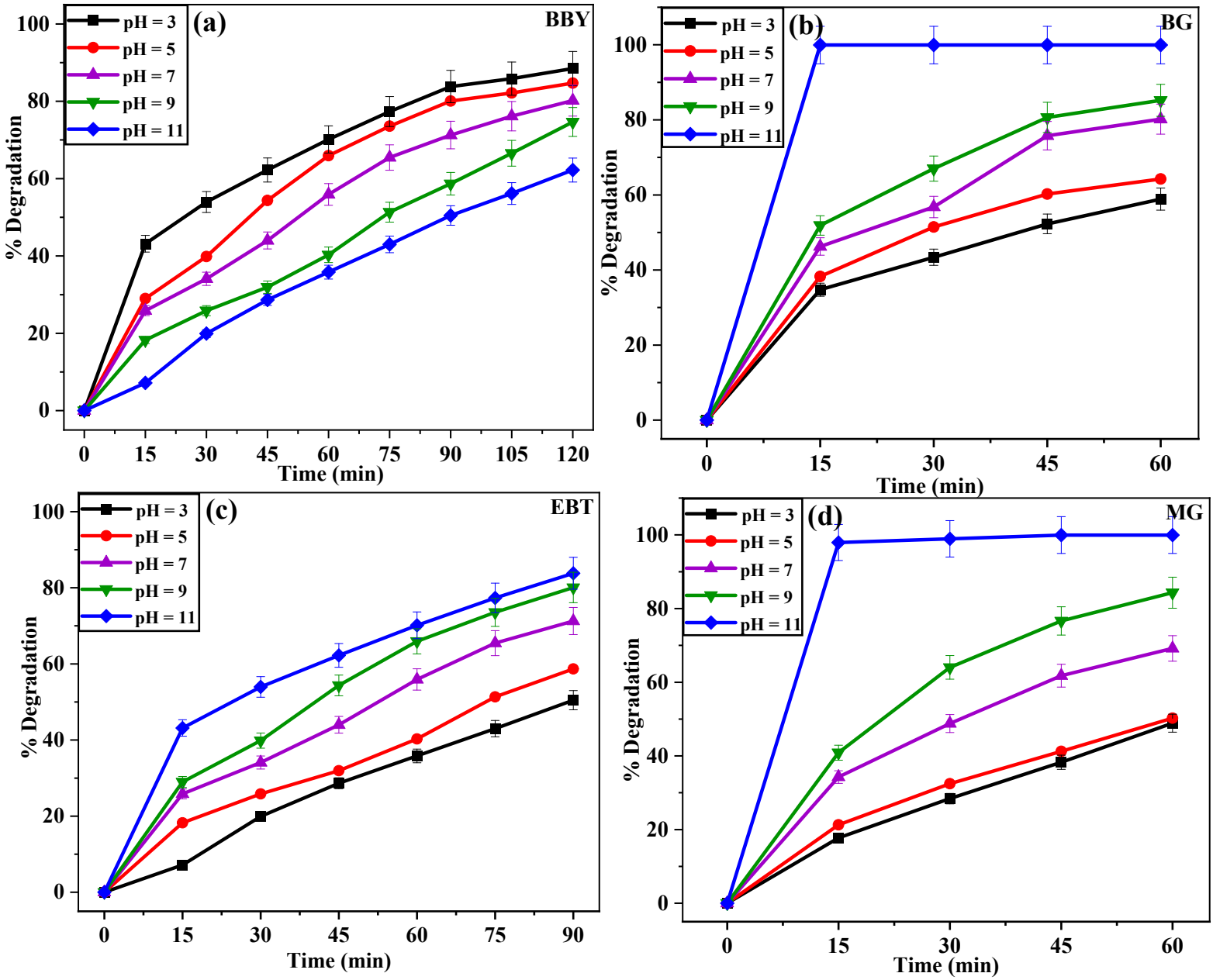


Fig. S5. The effect of pH on the degradation percentage of dyes: (**a**) BBY, (**b**) BG, (**c**) EBT, and (**d**) MG using biosynthesized CuO NPs (S8).

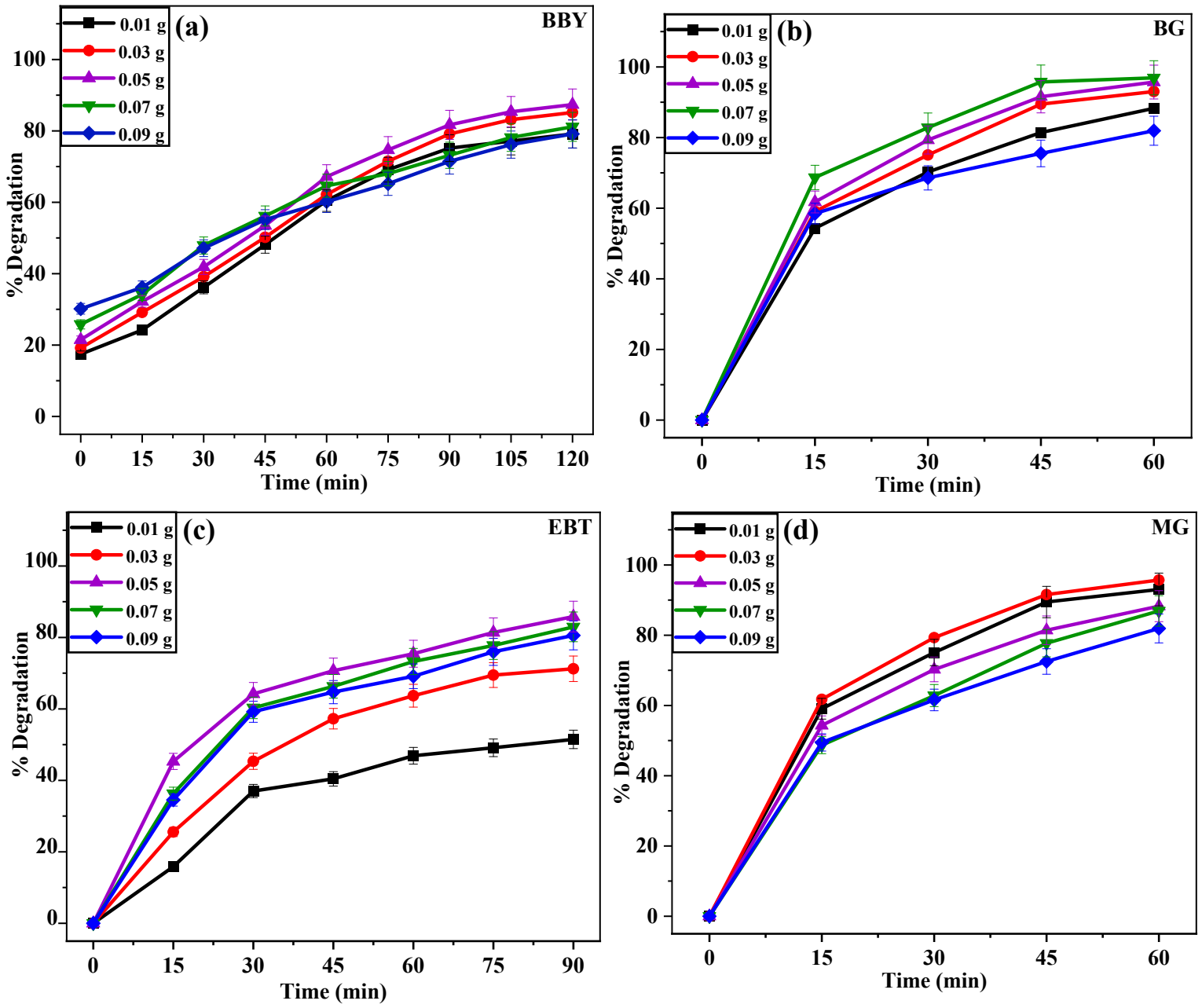


Fig. S6. The effect of catalyst dosage on the degradation percentage of dyes: (**a**) BBY, (**b**) BG, (**c**) EBT, and (**d**) MG using biosynthesized CuO NPs (S8).

References

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