Materials Letters 325 (2022) 132853

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

Fabrication of myoglobin hybrid nanoflowers for decolorization process of evans blue and congo red

Merve Turk [a,*], Cevahir Altinkaynak [b,*], Nazlican Yangin [b], Nalan Özdemir [a]

[a] Department of Chemistry, Faculty of Science, Erciyes University, 38039 Kayseri, Turkey
[b] Department of Plant and Animal Production, Avanos Vocational School, Nevsehir Haci Bektas Veli University, 50500 Nevsehir, Turkey

ARTICLE INFO

Keywords:
Myoglobin hybrid nanoflowers
Biomaterials
Microstructure
Decolorization

ABSTRACT

Here, myoglobin hybrid nanoflowers (MbNFs@Cu) were synthesized in various pH values and protein concentrations considering the organic–inorganic hybrid nanoflower method for the first time. Synthesized material was characterized by using EDX, XRD, and FTIR analyses. After determining the optimum morphology, the MbNFs@Cu exhibited great stability and peroxidase-mimic activity towards various pH values and temperature, and provided excellent dye decolorization efficiency for evans blue (EB) and congo red (CR) dyes with more than 90 % within 20 min. These results prove that this material has a potential in the removal of some organic dyes and in catalytic applications.

1. Introduction

With the rapid development of industry, the decrease in usable water resources all over the world and the increase in water pollution are seriously threaten humanity as a critical problem on global scale [1–3]. Specially, organic dyes' used in industries are one of the main causes of water pollution due to the discharge into rivers and lakes [4]. These aromatic compounds have harmful effects such as toxic, teratogenic, carcinogenic, mutagenic on the human, animals and plants [5]. Therefore, removal of dyes from wastewater is an important step to prevent environmental pollution. Many approaches such as adsorption, filtration, oxidation, etc. have been used for decolorization process, but these conventional methods have disadvantages like high cost, high energy and time consumption, low productivity [6,7]. In order to overcome these disadvantages, new approaches based on the immobilization of biomolecules are needed that can be synthesized easily, are inexpensive, have high efficiency, have high stability and show high activity [8,9].

In recent years, an organic–inorganic hybrid nanoflowers synthesis method has attracted attention [10]. The large surface area in structure of nanoflowers are cause high catalytic activity and stability [11]. Myoglobins (Mb), which are members of the hemoprotein family, are played an active role in important biological functions such as oxygen transport, oxygen reduction, electron transfer and oxidative reactions [12]. Mb protein shows a very low level of peroxidase-like activity due to the heme groups it contains, and it has the ability to detoxify many environmental pollutants due to this bio electrocatalytic feature.

In this work, MbNFs@Cu was synthesized under different experimental conditions by using commercially available, inexpensive Mb protein, had low peroxidase activity, as the organic part and Cu^{2+} metal ion as the inorganic part in the first time. The effect of synthesis conditions was investigated on the morphology of the hybrid material. The peroxidase-like activity of the synthesized structures under optimum conditions was compared with natural Mb, and the reusability cycle number was determined. After determining the optimum activity conditions of the material, it has been shown that they can be used in the removal of EB and CR textile dyes.

2. Material and methods

2.1. Materials

Myoglobin, CR, EB, BSA, copper sulphate pentahydrate, disodium hydrogen phosphate, NaOH, ABTS, ethanol, HCl and guaiacol were purchased from Sigma-Aldrich(USA). KH_2PO_4, Na_2HPO_4, H_2O_2 was obtained from Merck. Bradford assay reagent was purchased from Thermo Scientific, USA.

2.2. Synthesis and characterization of MbNFs@Cu

MbNFs@Cu was synthesized according to previously reported

* Corresponding authors.
E-mail addresses: mervedurmus2910@gmail.com (M. Turk), caltinkaynak@nevsehir.edu.tr (C. Altinkaynak).

https://doi.org/10.1016/j.matlet.2022.132853
Received 3 June 2022; Received in revised form 28 June 2022; Accepted 14 July 2022
Available online 18 July 2022
0167-577X/

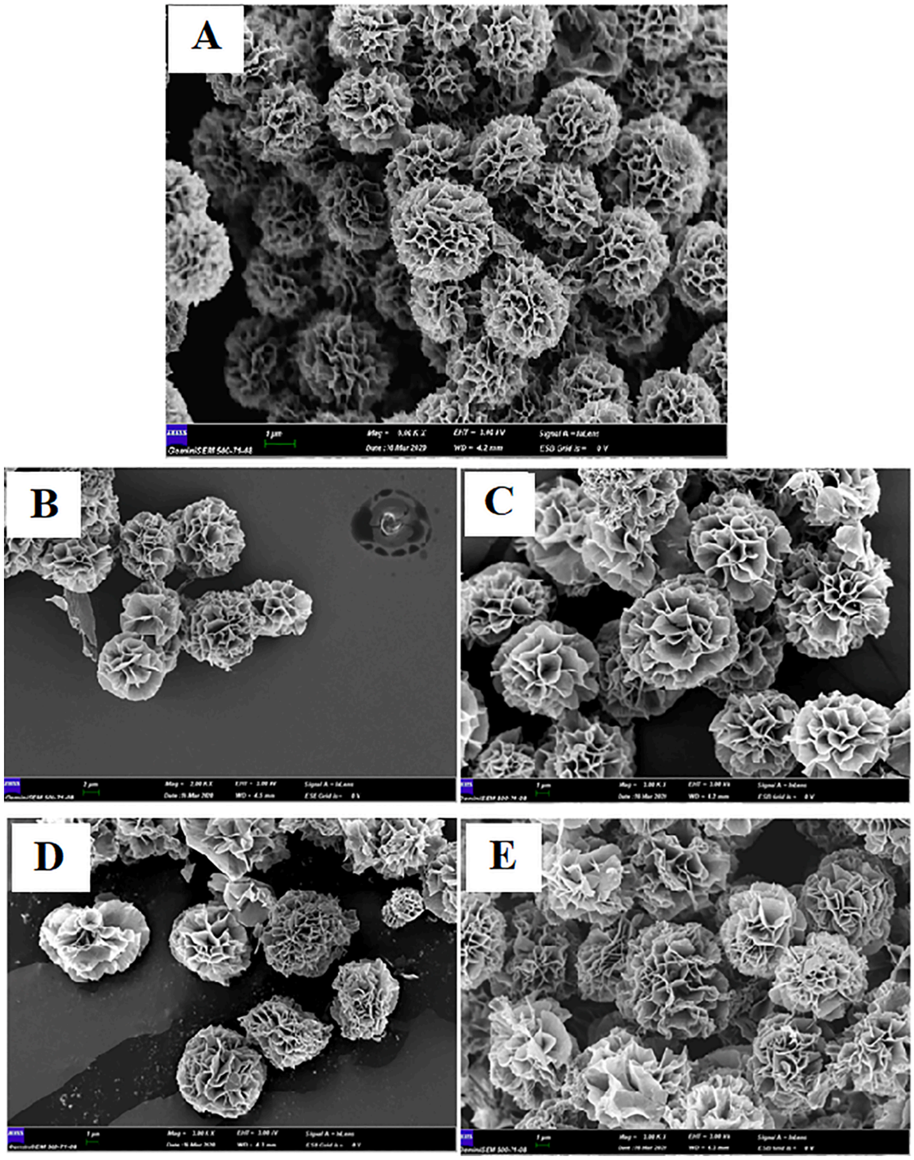

Fig. 1. SEM images of synthesized MbNFs@Cu at different pH levels A) pH 6, B) pH 7, C) pH 7.4, D) pH 8, E) pH 9 (Mb: 0.02 mg mL^{-1}, $[Cu^{2+}]$ = 0.8 mM, Temperature: 25 °C, Incubation time: 72 h).

methods [10,13]. This process was carried out at different myoglobin concentration (0.01–0.1 mg/mL) in phosphate buffered saline (PBS) solution with different pH (pH 6–9). Bradford method was used for determination to encapsulation yield of MbNFs@Cu using UV–vis (HITACCHI UH5300). The morphologies of MbNFs@Cu were visualized by SEM (Scanning Electron Microscopy; ZEISS EVO LS10). Atomic and weight percentage of elemental compositions in the MbNFs@Cu were determined using by EDX (Energy dispersive X-ray; ZEISS EVO LS10). The crystal and chemical structure of MbNFs@Cu were characterized using XRD (X-ray Diffraction; BRUKER AXS D8) and FTIR (Fourier transform infrared spectroscopy; PerkinElmer Spectrum 400) analysis, respectively.

2.3. Peroxidase like activity and reusability of MbNFs@Cu

Peroxidase-like activity of Mb and MbNFs@Cu were determined spectrophotometrically with oxidation of ABTS substrate [14]. 25 mM H_2O_2, 1 mM ABTS solution and MbNFs@Cu was added to PBS in different pH (10 mM, pH 4–9). After 25 min incubation, centrifugation was done at 6000 rpm. The supernatant absorbance was monitored at 420 nm. According to the peroxidase-like activity results of MbNFs@Cu, optimum conditions were determinate. Reusability of MbNFs@Cu was evaluated for eight cycles the same procedure.

2.4. Dye decolorization studies

Dye decolorization of MbNFs@Cu was performed by using CR (λ_{max} = 498 nm) and EB (λ_{max} = 611 nm). For this, H_2O_2 (25 mM), CR (16 µg mL^{-1}), EB (8.3 µg mL^{-1}) and KH_2PO_4 buffer were used. The measurement was made by adding MbNFs@Cu to the solution containing H_2O_2, KH_2PO_4 buffer and dye using UV–vis [15]. The effect of pH (pH 4–9), temperature (25–50 °C) and MbNFs@Cu concentration (0.5–2.5 mg mL^{-1}) on dye decolorization were also investigated. First measured decolorization before adding MbNFs@Cu was accepted as 100 % and other residual activity results of each catalytic cycle were calculated based on this percentage value. The changes in absorbance were determined by spectrophotometrically. Decolorization % of dye was calculated with the formula: [Dye Decolorization % = ($A_{Initial\ Abs.}$-$A_{final\ Abs}$) × 100 / $A_{Initial\ Abs.}$].

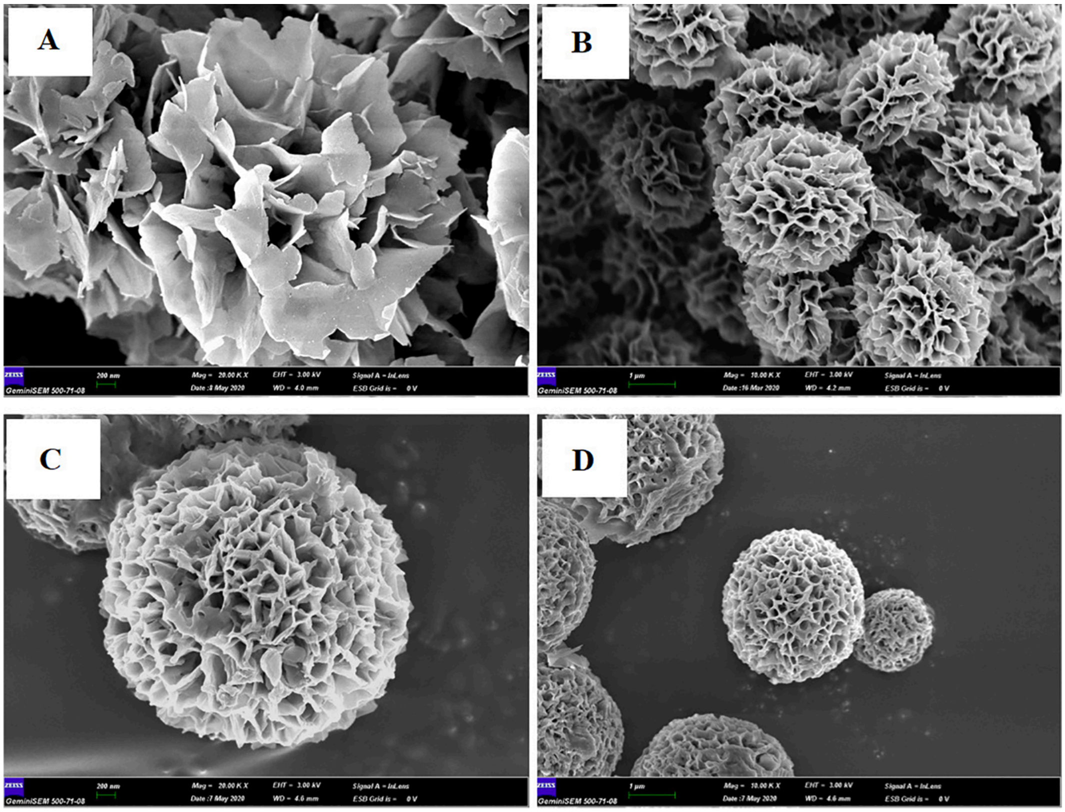

Fig. 2. SEM images of synthesized MbNFs@Cu at different protein concentrations Mb: A) 0.01 mg mL^{-1}, B) 0.02 mg mL^{-1}, C) 0.05 mg mL^{-1}, D) 0.1 mg mL^{-1}, (pH:6, $[Cu^{2+}]$ = 0,8 mM, Temperature: 25 °C, Incubation time: 72 h).

3. Results and discussion

3.1. *Effect of synthesis conditions on morphology of MbNFs@Cu*

Mb and copper ions were used as an organic part and inorganic part, respectively in the synthesis of organic–inorganic hybrid nanoflowers, for the first time. The pH condition (pH 6–9) was evaluated as the primarily investigated parameter to synthesis of MbNFs@Cu. Fig. 1 showed the SEM images of synthesized hybrid nanoflowers. The most uniform and compact flower-like morphology was seen at pH 6. It was detected as the optimum synthesis condition due to having the high surface area and porosity. The average size of MbNFs@Cu was 2.5 μm at pH 6. When the pH increased, the homogeneity between the petals were deteriorated and the size of the structure also increased due to the negatively charged of the Mb molecule.

When the amount of Mb was increased from 0.01 to 0.1 mg mL^{-1}, the

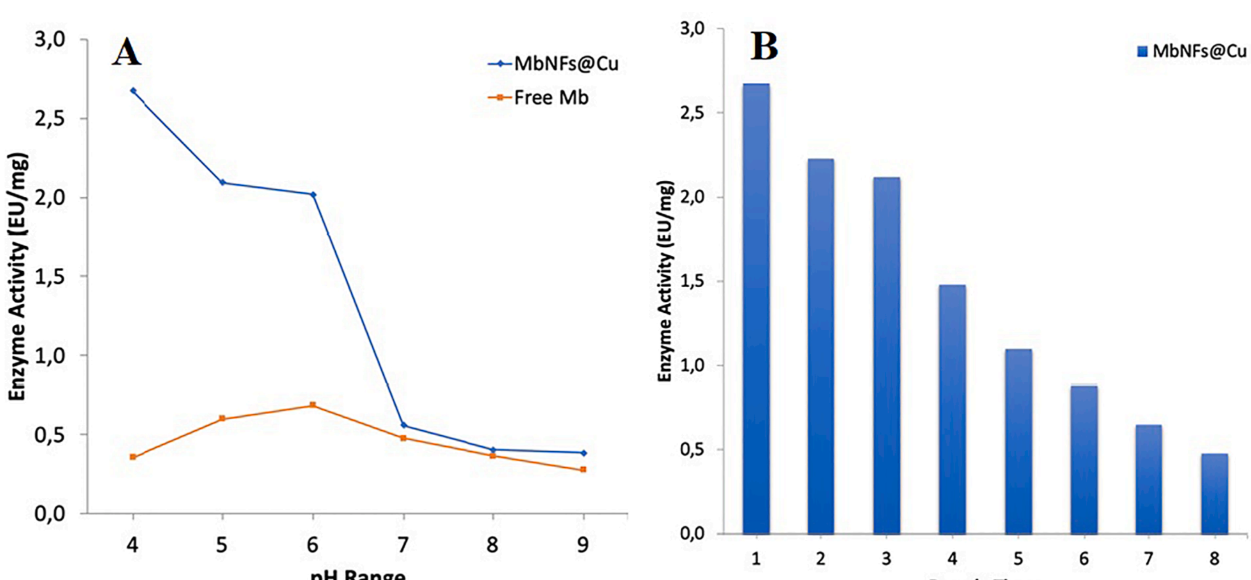

Fig. 3. A) The effects of pH on peroxidase like activity of MbNFs@Cu **B)** Reusability of MbNFs@Cu.

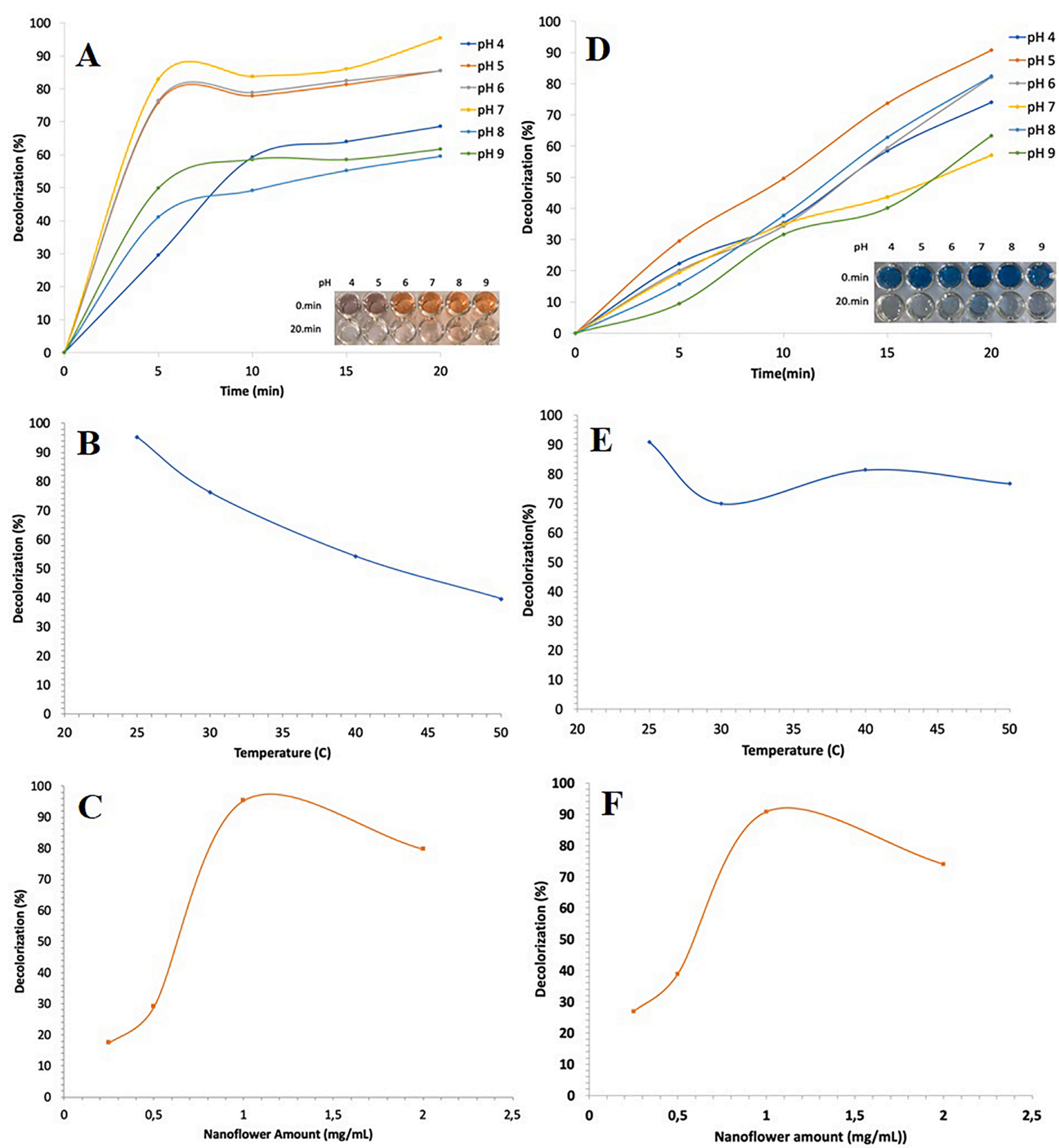

Fig. 4. **A)** Effect of different pH conditions for decolorization of CR **B)** Effect of different temperature for decolorization of CR **C)** Effect of different amount of NFs for decolorization of CR **D)** Effect of different pH conditions for decolorization of EB **E)** Effect of different temperature for decolorization of EB **F)** Effect of different amount of NFs for decolorization of EB.

morphology of the nanoflowers completely changed. As seen in Fig. 2, although the morphology was smooth at 0.05 and 0.1 mg mL^{-1} Mb concentrations, more compact structure was formed and pore size is also reduced. In the light of the data obtained, the optimum synthesis condition for MbNFs@Cu was pH 6, 0.02 mg mL^{-1} of myoglobin, $[Cu^{2+}]$ = 0.8 mM, temperature: 25 °C, and incubation time 72 h. The size of MbNFs@Cu structures obtained under optimum conditions was an average size of 3–4 µm. The encapsulation efficiency was found 90 %, and the weight efficiency is 8.7 %. To investigate the chemical structure and elemental composition of MbNFs@Cu; EDX, XRD and FTIR analysis were performed. EDX analysis indicated the presence of Cu, O, N and P elements (Fig S1). XRD pattern of the MbNFs@Cu was well-matched with that of $Cu_3(PO_4)_2{\cdot}3H_2O$ (JCPDS card 00–022-0548) (Fig S2). In FTIR spectra (Fig S3), the characteristic peaks at ~ 3282 cm^{-1} and ~ 1644 cm^{-1} were associated with specific absorption peaks of proteins. These results confirmed successful immobilization of protein in the synthesized hybrid nanoflowers.

3.2. *Peroxidase-like activity and reusability of MbNFs@Cu*

To find maximum peroxidase-like activity of MbNFs@Cu, the peroxidase-like activities of MbNFs@Cu and free Mb were investigated in the range of 4–9 (Fig. 3A). It was found that the MbNFs@Cu exhibited higher activity than free Mb in broad pH range. As seen in Fig. 3A, while the highest peroxidase activity of MbNFs@Cu was 2.672 EU/mg at pH 4, free Mb was 0.6844 EU/mg at pH 6. The increased (Fig. 4) peroxidase-like activity may be due to the nanoflower's acting as Fenton reagent and the synergistic effects between copper ion and Mb. The materials stability plays a crucial role in industrial applications. After eight cycles, the MbNFs@Cu was exhibited good peroxidase-like activity (Fig. 3B). Reusability is one of the most important superiorities of immobilized enzymes compared to free Mb.

3.3. Dye decolorization studies

In this study, two organic dyes CR and EB were used as model dyes for decolorization studies. After 20 min, MbNFs@Cu exhibited 95.4 % at pH 7 and 90.8 % at pH 5 decolorization for CR and EB, respectively. This experiment was realized at RT. Interestingly, the best decolorization rates were obtained at RT for both dyes. Considering the possible effect for decolorization for MbNFs@Cu amount, the best results were found as 1 mg mL^{-1} for both dyes. MbNFs@Cu can be acted like redox mediator in the decolorization pathway.

4. Conclusion

In summary, in our study, easily, cheaply, efficiently, eco-friendly, and reusable myoglobin incorporated hybrid nanoflowers (MbNFs@Cu) were fabricated and characterized, for the first time. Optimum conditions were also determined for dye decolorization. Synthesized NFs indicated both superior peroxidase mimic activity and excellent dye decolorization. Especially, decolorization of MbNFs@Cu for CR and EB was found 95,4% and 90,8% in room temperature after 20 min, respectively. Yang and coworkers used photocatalytic activity of the g-C_3N_4 for degradation of rhodamine B. They decolorated all dyes in 20 min similar to our work [16]. Moreover, NFs have shown remarkable reusability due to its structural and mechanical strength even after eight uses. The novel material which has excellent peroxidase-like activity could be successfully used in wastewater treatment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by 2209/A University Students Domestic Research Projects Support Program of TUBITAK (Project Numbers: 1919B011904313).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.matlet.2022.132853.

References

[1] K.T. Kubra, M.S. Salman, M.N. Hasan, J. Mol. Liq. 328 (2021), 115468.
[2] M.J. Uddin, R.E. Ampiaw, W. Lee, Chemosphere. 284 (2021), 131314.
[3] S.A. Ansari, R. Kumar, M.A. Barakat, M.H. Cho, J. Mater. Sci.: Mater. Electron. 29 (2018) 7792–7800.
[4] J. Rong, T. Zhang, F. Qiu, Y. Zhu, ACS Sustain Chem. Eng. 5 (2017) 4468–4477.
[5] M.A. Ahmad, N.B. Ahmed, K.A. Adegoke, O.S. Bello, Chem. Data Collect. 31 (2021), 100578.
[6] M. Luo, M. Li, S. Jiang, H. Shao, J. Razal, D. Wang, J. Fang, J. Hazard. Mater. 381 (2020), 120947.
[7] M.O. Ansari, R. Kumar, S.A. Ansari, S.P. Ansari, M.A. Barakat, A. Alshahrie, M. H. Cho, J. Colloid Interface Sci. 496 (2017) 407–415.
[8] S. Dube, D. Rawtani, Adv. Colloid Interface Sci. 295 (2021), 102484.
[9] R. Kumar, S.A. Ansari, M.A. Barakat, A. Aljaafari, M.H. Cho, New J. Chem. 42 (2018) 18802–18809.
[10] J. Ge, J. Lei, R.N. Zare, Nat. Nanotechnol. 7 (2012) 428–432.
[11] C. Altinkaynak, New J. Chem. 3 (2021).
[12] H.F. Xiang, J.K. Xu, J. Liu, X.Z. Yang, S.Q. Gao, G.B. Wen, vd. Y.W. Lin, RSC Adv. 11 (2021)16090-16095.
[13] C. Altinkaynak, S. Tavlasoglu, R. Kalin, N. Sadeghian, H. Ozdemir, I. Ocsoy, N. Ozdemir, Chemosphere. 182 (2017) 122–128.
[14] Z.F. Wu, Z. Wang, Y. Zhang, Y.L. Ma, C.Y. He, H. Li, C. Lei, Q.S. Huo, L. Wang, Z. Q. Li, Sci. Rep. 6 (2016) 22412.
[15] F. Jamal, P.K. Pandey, T. Qidwai, J. Mol. Catal. B Enzym. 66 (2010) 177–181.
[16] X. Yang, L. Zhang, D. Wang, Q. Zhang, J. Zeng, R. Zhang, R. Soc, Chem. 11 (2021) 30503–30509.