Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (7) Issue (6) (2022), (1-7) **online: ISSN 2356–976x**
http://bjas.journals.ekb.eg

Optimizing the removal process of methyl orange using magnetite nanoparticles synthetized by *Aspergillus tamarii*

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Abstract

Biosynthesis of nanocomposites by plant, fungi and bacteria is considered as one of the promising ecofriendly approach alternative to chemical and physical methods. Nowadays, the application of the biosynthetic nanocomposites in the removal of different recalcitrant dyes has attracted more consideration. Different fungal isolates were examined for their capability for magnetite nanoparticles synthesis. The exo-metabolite of *Aspergillus tamari* was found to be the most suitable for the Fe_3O_4-nps biosynthetic process. The produced nanocomposite was characterized using various devices. Transmission electron microscope (TEM) showed spherical nanoparticles with 9 nm size. SEM micrograph shows an aggregation of spherical nanoparticles. In the batch adsorption process, the removal efficiency of methyl orange (MO) dye was 84.9% within 20 min of incubation period. The adsorbent dosage of 2 g/l displayed the maximum MO removal rate at initial dye concentration of 80 mg/l. The MO removal rate was sharply improved as a response for pH variation, while exhibiting the maximum rate at pH of 5.0. Therefore, the Fe_3O_4-nps synthesized by harnessing the exometabolites of *A.tamarii* can be considered as a promising adsorbent material for removal of toxic/recalcitrant dyes from wastewater.

Keywords: *Aspergillus tamarii*, Biosynthesis, Nanocomposites, Fe_3O_4-nps, Methyl orange, Removal rate.

1. Introduction

Dyes have been considered as chemical substances, which belong to different chromophore groups. They are widely applied in different industrial applications such as rubber, textiles, plastic, trucking, paper, paint, food, coating, and marine industries. Generally, dyes have been eradicated into the water bodies in the developing and developed countries, producing a serious environmental problem and affect the aquatic life through blocking the oxygen they need [**1-4]**.The degraded structure of some dyes has carcinogenic effect on human beings.

Water pollution has remarkable effect on the biodiversity and human health. It is considered as a great worldwide problem, affecting over 40% of the population. The rising application of different synthetic dyes in industry regardless its hazardous effect become a serious issue. There are three ways for removal of dyes including physical, chemical and biological methods. The ways include coagulation, dialysis, Biological oxidation, coagulation and adsorption [**5-7]**. The most preferred way for water treatment is biological way [**8,9**].

The adsorption technique is considered as the important way for removal of toxic material from water like dyes [**10].** The industry of dyes always need to use activated carbon as adsorbent for adsorption of dyes because it has high porosity and surface vicinity almost 500 to 2000 m^2/g [**11]**, but it needs controlled pressure generation and regeneration. Generally, it should be regenerated at regular time, giving it more expensive for industries. In addition, dyes adsorption that use adsorbent coming from crop and agricultural not succeed to attract the researcher for using them [**11].**

Nowadays, adsorbents depended on nanoparticles (NPS) have become more effective for dyes removal from water due to they have chemical and physical properties as nanoscale size, chemical composition, and high surface area [**12]**. Furthermore, the nanoparticles make adjustment for their surfaces by using specific organic ligands and groups that make the adsorption parameters highly **[13]**. The enhancement of the removal process of synthetic dyes using nanoadsorbent materials is highly desirable.

The current study aims to screen the fungal isolate for the biosynthetic process, biosynthesis Fe_3O_4-nanocomposite using the most promising fungal isolate, characterize the developed nanomaterial, optimize and evaluate their potentiality in the removal process of methyl orange dye.

2. Experimetal

Collection of soil samples

Samples of soil were collected from different province in El-Qalyubia without any contaminations. The soil samples were gathered in clean bags.

Isolation of fungi from soil samples

In brief, 1 gm of each soil sample was serially diluted until reach 10^{-5}. One ml of the obtained dilution was aseptically spread onto the surface of plates, containing Czapex'sdox agar medium (Sucrose 30.0; $NaNO_3$ 3.0; KH_2PO_4 1.0; KCl 0.05; $MgSO_4.7H_2O$ 0.05; $FeSO_4.7\ H_2O$ 0.001; agar-agar 2.0; pH 7.0). The plates were incubated at 28°C for 4 days. The growing fungal cultures were purified by subculturing on the same medium. The developed fungi were preserved at 4°C for further work [**3**].

Morphological characterization of the isolated fungi

The fungal cultures were pointly inoculated onto the Czapex'sdox agar medium plates. The colony and microscopical characteristics of the obtained fungal isolates (color, diameter, conidial head, strigmata, and pigmentation) were examined at frequent intervals. The fungal isolates were performed according to the universal identification keys [**14-17**].

Preparation of magnetite nanoparticles using the isolated fungi

The fungal isolates were inoculated into 250 ml Erylenmyer flasks containing 25 ml of the Czapex'sdox

broth. The flasks were incubated at temperature of 30 °C for 5 days with shaking at 150 rpm. After incubation period, the fungal beads were removed by filteration through Whatman no 1. Then, the obtained filtrate was centrifuged at 6000 rpm for 15 min. The developed supernatant was used for the magnetite nanoparticles biosynthesis [**1,3**].

Magnetite nanoparticles (Fe_3O_4-nps) were prepared according to (**3**) with some modifications. Briefly, a metallic precursor was prepared by mixing $FeSO_4 \cdot 7H_2O$ and $FeCl_3 \cdot 6H_2O$. Adequate volume of the fungal filtrate was added to the same volume of the metal solution. The preparation was incubated with continuous stirring for 1 h at 60 °C and pH 12. The initial observation of the black precipitate is an indication for the magnetite nanoparticles formation. The Fe_3O_4-nps were collected by filtration and subsequently thrice washed using distilled water and the final product was dehydrated for 12 hours at 60˚C.

Characterization of the mycosynthesized Fe_3O_4-nps

The surface morphology of the mycosynthesized magnetic nanoparticles (Fe_3O_4-nps) were characterized by using Scanning electron microscope (SEM, Japan, JSM-JEOL). The size of the investigated magnetic nanoparticles was determined via Transmission electron microscope (TEM, Japan, JEOL-JEM).The elemental composition of the Fe_3O_4-nps was determined using Energy dispersive X-ray spectrometer (EDX).

Adsorption studies using batch experiments

The methyl orange (MO) dye has a molecular weight of 327.34 and molecular formula of $C_{14}H_{14}N_3NaO_3S$ (**Figure 1**). Stock solution of the investigated dye was formulated using distilled water at concentration of 1000 mg/l. In order to obtain the required dye concentration, a working solution employed in the current work were prepared throughout the dilution of the previously prepared stock solution.

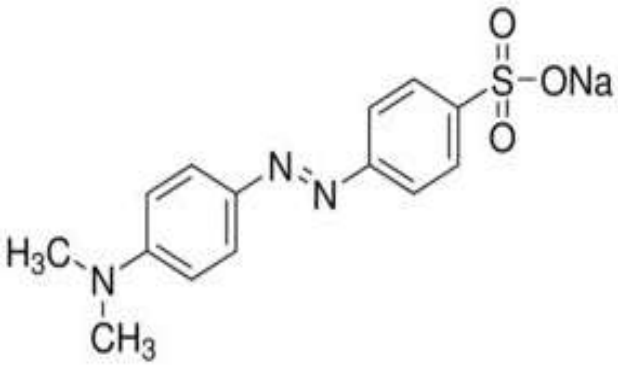

Fig. (1) Methyl orange structural formula.

The adsorption experiments were carried out based on the methods of [**18**] with some modification. The experiments were conducted at various temperatures (20-50˚C) using Erlenmeyer flasks containing various initial concentrations of MO from 10 to 200 mg/l with shaking at 150 rpm for different contact time (5-40 min). The adsorbent doses of the myco-synthesized nanoparticles were examined at various concentration in the range of 0.5 to 2.5 g/l and pH range of 2.0-9.0. Aliquots were withdrawn at frequent intervals, and then centrifuged at 2000 xg for 15 min.

Statistical analysis

The performed experiments were carried out in three replicates and the results are mean of such readings.

3. Results and discussions

Isolation and identification of fungi synthesizing magnetite nanoparticles

Eleven fungal isolates, belonging to four genera, were recovered from different soil samples (**Table 1**). These fungal isolates were characterized to be classified under three classes: Zygomycetes, Ascomycetes, and Deuteromycetes, which represented by two, seven, and two, respectively.

Screening for fungi synthesizing magnetite nanoparticles

All the isolated fungi were examined for their capability for the magnetite nanoparticle synthesis. In brief, the metallic precursor and the fungal filtrate were mixed in a ratio of 1:1. The mixture was heated for 1 h and the pH was kept at pH 8 during the progress of the reaction [**3**]. The color change of the reaction preparations shows the potential of the magnetite nanoparticles synthesis. The formation of a dense black precipitate is an indication for the as-prepared Fe_3O_4-nps production. All the investigated isolates have ability to synthesize the Fe_3O_4-nps. Among the tested fungal isolates, three isolates gave the same quantity of the nanoparticles produced. *A.tamarii* was selected for further study.

Table (1) Fungi isolated from different soil samples.

Genus	Class	Number of isolates	Fungal species
Rhizopus	Zygomycetes	2	*Rhizopus* sp.
Aspergillus		2	*A. niger*
		1	*A. tamarii*
	Ascomycetes	2	*A. flavus*
Penicillium		2	*P. chrysogenum*
Fusarium	Deutromycetes	2	*Fusarium* sp.

Characterization

Transmission electron microscope (TEM) is a tool used in measuring the size and structure of the as-prepared nanomaterial. The TEM image reveals that the as-prepared Fe_3O_4-nps showed a homogenous distribution of spherical and semi-spherical nanoparticles (Figure 2). The size of the as-prepared Fe_3O_4-nps prepared by the 2ry-metabolite of *A. tamari* was determined to be 9 nm. [**19**] recorded the capability of the fungal filtrate for the biosynthesis of nanoparticles.

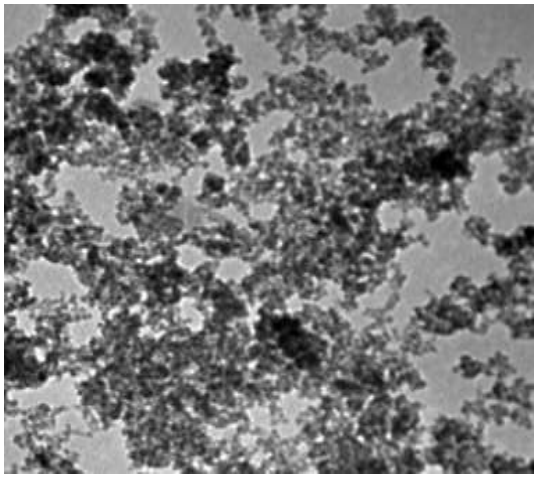

Fig. (2) TEM image for the as-prepared Fe_3O_4-nps prepared by the filtrate of *A. tamarii*.

Scanning electron microscope (SEM) is a significant tool, which is greatly employed for the determination of the morphology and texture of the as-prepared Fe_3O_4-nps prepared by the filtrate of *A. tamarii*. Aggregated semi-spherical and spherical nanoparticles were observed based on SEM micrographs. The EDX result exhibited the existence of peaks for iron, and oxygen (**Figure 3**), hinting the fabrication of Fe_3O_4 nanoparticles. The elemental percentage of Fe and O was represented by 8.87 and 31.5%. Similar results have been reported by other researchers [**19, 20**].

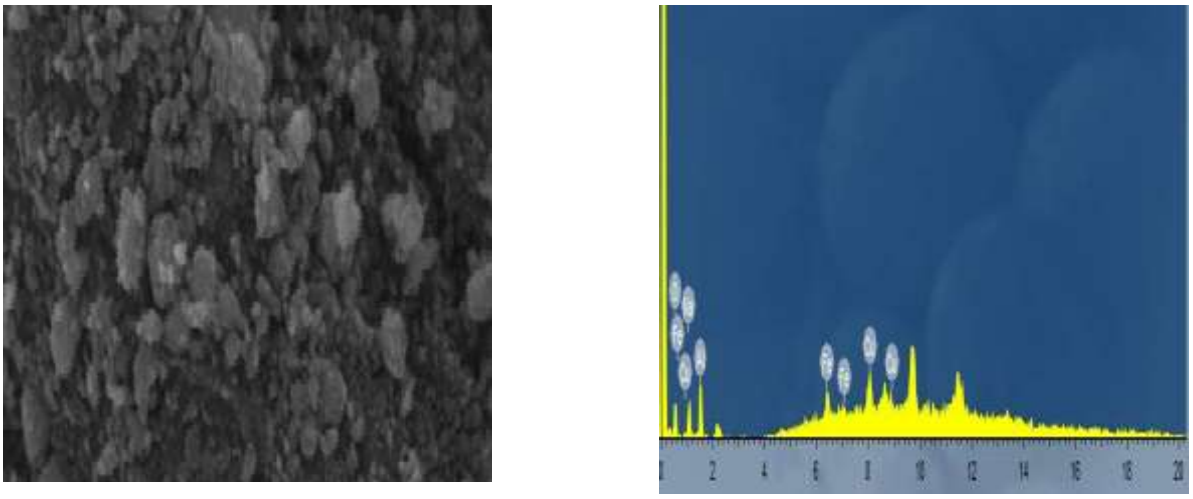

Fig. (3) SEM image for the as- filtrate of *A. tamarii*. EDX pattern of *A. tamarii* -mediated Fe_3O_4-nps synthesis.

Optimization of removal process of methyl orange dye using the prepared Fe_3O_4-nps

Impact of contact time

The influence of contact time on the removal percentage of MO dye is illustrated by **Figure 4**. The removal percent of the investigated dye using the biosynthetic Fe_3O_4-nps was increased during the gradually elevation in the contact time. However, the dye removal efficiency was found to be 84.9% within 20 min of incubation period. The removal efficiency was reached a plateau during the further progress of the reaction. The rising in adsorption rate is attributed to the empty adsorption sites on the biosorbent; however, the progress in adsorption process leads to the saturation of adsorption sites, reach equilibrium. Further, the absence of empty adsorption sites is consequently reduced the adsorption process after getting equilibrium [**7, 20**].

The equilibrium of adsorption rate was detected at different contact time using various adsorptive nanocomposite [**4**]. The contact time was found to be 240, and 140 min for ZnO/activated carbon and Ti-doped layered ZnOH, respectively [**21, 22**]. On contrary, the gelatin/activated carbon showed a contact time of 30 h at equilibrium rate of adsorption process [**23, 24**]. Hence, the biosynthetic nanoparticle performs the least contact time (20 min), compared to the previous nanocomposites.

Impact of adsorbent dose

The influence of adsorbent dosage on the MO dye removal rate is demonstrated by **Figure 5**. Various adsorbent dosage in the range of 0.5-2.5 g/l have been employed in a batch experiments for the MO removal. Results showed that the MO adsorption percentage has severely increased by elevating the adsorbent dose from 0.5 to 2 g/l, reaching the maximum adsorption rate of MO. The MO removal rate displayed no substantial variation during the progress of adsorption process, after the maximum removal at 2 g/l. The rising in the MO adsorption rate at the adsorbent concentration from 0.5 to 2 g/l, indicating a larger accessibility of adsorbent surface at the beginning of the process. However, the continuity of the removal reaction causes a reduction in the adsorption process because of the complete loss of active adsorption sites after achieving the equilibrium state.

Reduction in the removal process of the examined dye indicates the formation of monolayer of MO and the absence of active adsorption sites of the tested nanoparticle. Therefore, the adsorbent dosage of 2 g/l was used for further work as the optimal adsorbent dosage for the maximum MO removal rate. Similar results have been determined for the removal of different synthetic recalcitrant dyes using other various nanocomposites [**7, 21-23**].

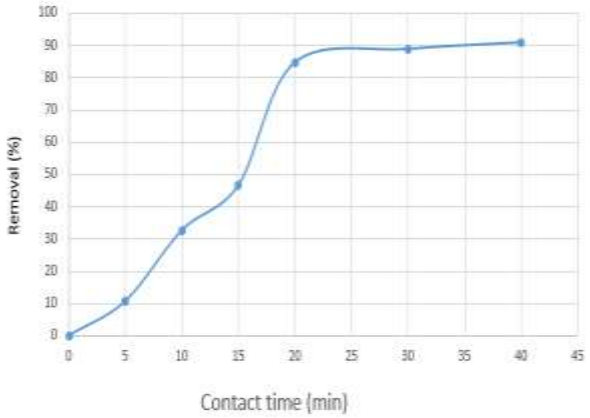

Fig. (4) Effect of contact time on the removal percentage of methyl orange.

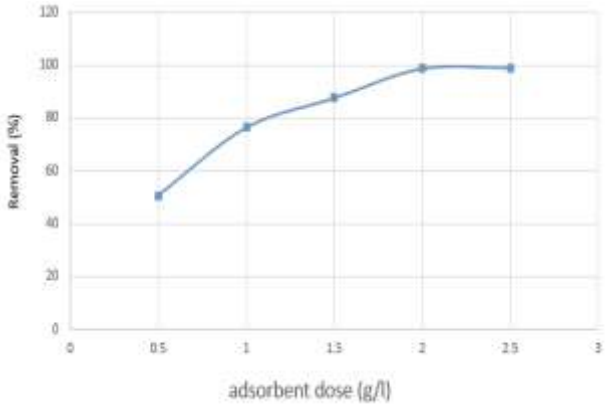

Fig. (5) Effect of adsorbent dosage on the removal percentage of methyl orange using the biosynthetic Fe_3O_4 nps.

Impact of pH on the MO removal rate

The removal process using different adsorbent nanocomposites is mainly affected by the pH value of the dye solution [**4**]. Herein, the removal process of the MO dye was investigated at various pH values in the range from 2.0 to 9.0 as depicted by **Figure 6**. The removal rate was sharply increased during the variation of pH, recording the greatest adsorption rate for MO dye at pH value of 5.0. It is suggested that the adsorbent particles at acidic pH, shows positively charged surface, which in turn are more accessible for electrostatic attraction of a negatively charged dye. On contrary, the adsorbent's surface at basic pH exhibited a negative charge, which repel the anionic dye (adsorbate) [**2-23, 25**]. Hence, pH 5.0 was used in the further study.

Impact of dye concentration on the MO removal rate

The response of the removal rate as a function of the dye concentration was investigated by performing a batch experiments with different concentration in a range of 20-200 mg/l in the presence of nanocomposite (**Figure 7**). The removal percentage of the examined dye decreases by elevating the initial MO dye concentration at pH 5.0, adsorbent dosage 2.0 g/l and contact time 20 min.

The removal rate shows an equilibrium capacity at 80 mg/l while maintaining optimum conditions. At low dye concentration, more active sites are available to the adsorption of dye molecules, which are found in lower concentration. On contrary, the ratio of free active adsorption sites become lower when compared to the available higher adsorbate molecules [**26, 27**].

Impact of temperature on the MO removal rate

Response of the MO removal rate toward the reaction temperature was investigated by incubating the reaction preparations at different temperature from 20°C to 50°C. The removal rate of MO increases with the increasing of reaction temperature. **Figure 8** clearly shows that the optimal temperature for the removal reaction was determined when the reaction was conducted at temperature of 40 °C. The elevation in the temperature of reaction preparations causes a faster diffusion rate of dye molecules into the nanomaterial. Similar results have been detected by [**4, 21, 23, 27**], when they investigated the effect of different temperatures on the dye removal processes. However, the adsorption experiments is generally managed at suitable temperature in order to decrease operation costs. Hence, 40 °C -temperature was selected for conducting the adsorption processes in the current study.

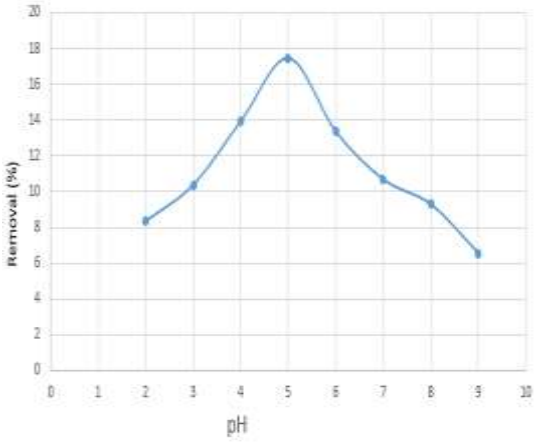

Fig. (6) Effect of pH on the removal percentage of methyl orange using the prepared Fe_3O_4-nps.

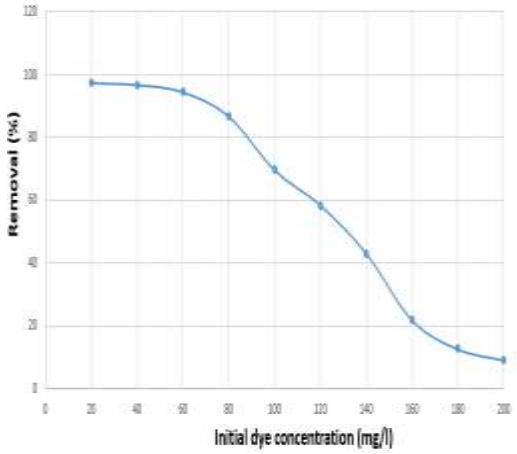

Fig. (7) Effect of initial dye concentration on the removal percentage of methyl orange in the presence of Fe_3O_4-nps.

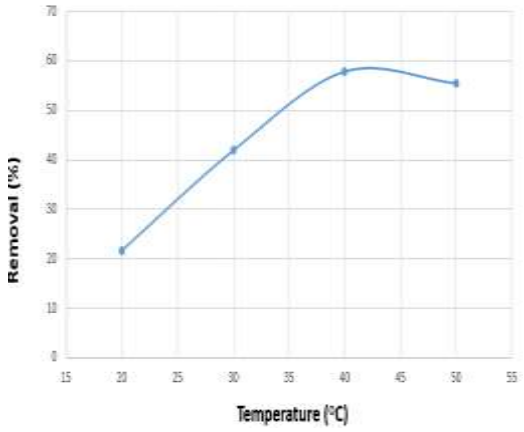

Fig. (8) Effect of reaction temperature on the removal percentage of methyl orange when the experiments conduced with an adsorbent of Fe_3O_4-nps.

4. Conclusions

Performance of suitable ecofriendly nanocomposites for the removal of different toxic/recalcitrant dyes from aqueous solution is of great importance for developing and developed countries. Physical and chemical techniques have been used for the synthesis of different nanocomposite; however, the production of nanomaterials using biological method is preferred due to its cost-effective and environmental impact. The current work conducted to investigate the potentiality of the secondary metabolites produced by fungi in the biosynthesis of magnetic nanomaterial with its application in the removal of methyl orange dye. *Aspergillus tamarii* was selected as the most promising fungal isolate for the biosynthesis of Fe_3O_4-nps. The developed nancomposite was characterized based on the results of TEM, SEM, and EDX. The maximum adsorption process of the MO dye was obtained by optimizing using contact time at 20 min, adsorbent dosage of 2 g/l, pH of 5.0, and initial dye concentration of 80 mg/l. Therefore, the biosynthetic Fe_3O_4-nps is an excellent adsorbent material for removal of toxic/recalcitrant dyes from Wastewater. More analyses require to be conducted for investigating the kinetics and isotherms of the adsorption reaction.

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