



Bioremoval of Azo Dye derivatives from aqueous solution by application of natural flora; free and immobilized bed reactor

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Abstract

Bioadsorption is a promising technique for the removal of textile dyes at relatively low cost and with satisfactory efficiency. This study focuses on the evaluation of bioadsorption capacity of a free and immobilized bed reactor for the removal of Azo Dye derivatives. The selected natural flora from industrial pollutants was used to improve the removal of contemplated pollutants with high performance. Immobilized bed reactor with high surface area at 37°C had the best modified protocol for the microbial community formation with surface adsorption to be ready for. After preparation of immobilized beds, the obtained structure was characterized by SEM, and TEM. The influences of adsorbent mass, pH, initial concentration of dye and contact time on the bioadsorption process were characterized by GC-MS. The bioadsorption capacity of the Azo dyes derivatives was controlled for the most part by pH value. TEM analysis for obtained beds showed particles size in the range of 50 to 138 nm. Also, the results showed that several adsorptive improvements that took place in the surface morphology and topology of the immobilized beds. Additionally, the optimum conditions were determined as follows, pH of 8.5, the initial dose of 5mg/L Azo dye and contact time of 60 min, that in these circumstances the removal of derivative was around 85.4%.

Keywords: Bioadsorption; Natural; free and immobilized bed; Azo dyes; derivatives

1. Introduction

Contaminated water, as a result of industrial activities, exists ubiquitously in the environment, which release a large amount of hazardous materials and contaminants with carcinogenic, mutagenic, and toxic properties (Davis, 1997). Government and international organizations have already recognized the potential dangers of these contaminants to human health and natural environment. For sustainable development and in response to an increasing need to address environmental problems, many remediation technologies such as chemical and bioremediation were developed to remediate that contaminated water (Svab et al., 2008; Paria 2008).

Azo dyes and pigments are colorants that give a color to a material, making it more attractive. The major difference between dyes and pigments is the particle diameter. Dyes are much smaller than pigments and solubility in liquids. While, dyes are soluble and penetrate into the textile or a material, the pigments are insoluble and are suspended in liquid forming a thin layer and painting the surface of the material (Ledakowicz and Pazdzior, 2021). Nowadays, there are more than 8000 kinds of synthetic dyes which listed in the Color Index (C.I.) with 40,000 producers and trade names (DPMS, 2020).

Azo dyes are the most powerful synthetic chemical compounds for

textile and printing, paper manufacturing and other industries (Benkhaya et al., 2020). Annually, around 70 million tons of synthetic dyes are produced specific for the textile industry all over the world, of which nearly 10% of the dyestuff and its derivatives are discharged to the environment as wastewater influent (Benkhaya et al., 2020), because even up to 50% of the dyes used are not fixed to the textile fibers, but persist as pollutants for environment in the liquid phase (Yuan et al., 2020).

Various biological, chemical, and physical technologies such as adsorption, photolysis, chemical precipitation, chemical oxidation and reduction, electrochemical precipitation, and membrane processes as well as their combinations have been employed for the removal of dyes from highly polluted industrial textile effluents (Pal, 2017). However, many of these methods have considerable drawbacks, including high cost, or harsh reacting conditions or, as with adsorption techniques, are nothing more than the transfer of pollutants from the liquid phase to the solid ones (Fast et al., 2017; Pazdzior et al., 2019).

In contrast to these techniques, biological methods of treating textile wastewater are much cheaper, efficient, and green. Specifically, two biodegradation methods, microbial and enzymatic systems have been shown to be useful approaches in industrial textile effluent treatment

(Abiri et al., 2017; Chao et al., 2018). However, the conventional biological processes do not always provide satisfactory results, especially for industrial textile wastewater, since many of the organic substances emitted from dye houses are toxic or resistant to biological treatment. Therefore, the only feasible option for such biologically persistent wastewater is the use of a combination of biodegradation and advanced oxidation processes (AOPs), widely recognized as highly efficient treatments for recalcitrant wastewater (Oller et al., 2011; Miklos et al., 2018).

The aim of this work was to highlight the local new biological treatment of Azo dyes and its derivatives. Free and immobilized bed reactor that could be used in the textile industry wastewater treatment. It is characterized by cheap and available in abundance in our local. The obtained free and immobilized bed reactor were characterized using different techniques such as SEM, and TEM. Bye products from the treatment of contaminated wastewater with Azo dyes were measured for minimizing.

2. Materials and methods

All chemical materials purchased from Merck Company and used without any further purification, the Azo dye was purchased commercially from local market, Egypt. All the analyses were done with three replications using 18.2 Ω water (Elga PURELAB, Veolia system, UK). After every ten sample readings, the standards were run to assure the working of instrument with more than 95% accuracy.

2.1. Free and immobilized microflora treatment

To study the influence removal of the dye, experiments were designed to find optimum pH, optimum contact time, and optimum free cell count with 400 mL included 5 mg/L Azo dye solutions shaken at (200rpm) as separated ones. The solids and solutions were separated, and the filtrate was collected for analysis at 600 nm with visible light by spectrophotometer.

2.2. Isolation of selected Microflora from contaminated textile industry west water

Waste water from ELROBAIA Textile Company, Sadat city, Menofia, Egypt was collected to isolate the flora that able to biodegrade the Azo-dyes in waste water. Ten ml of waste water sample was mixed with 90 mL of 0.9% NaCl solution, tenfolds serially diluted and spread onto the selected media, then incubated at 30 °C for 48–96 h. The medium composition (g/L), 5 g peptone, 3 g beef extract, 0.42 g NaHCO₃, and 0.53 g Na₂CO₃, the pH was adjusted to 9.5 using 1 M NaOH. The selection was followed by picking the colonies and sub-culturing to get pure colonies of the isolates on Luria–Bertani (LB) agar medium containing: g/L (10 g yeast extract, 10 g peptone, and 5 g sodium chloride). The pH of the medium was adjusted to 9.5 with the high pH medium (Ahmed et al., 2021)

2.3. Bacterial Count

Samples from textile industrial water are collected for bacterial counts at deferent pH (6, 7, 8, 9, 10, 11 and 12) as 10 ml of each sample is added to 90 mL of sterile 0.9% NaCl solution and left over night to release the bacterial cells and spores, then serial dilutions of each sample were done, afterwards 1 mL of the final dilution is added to petri dish containing LB medium dispensed

over each plate in triplicates for each dilution. Finally, the plates are incubated at 30°C for 3 days and the CFU/mL in each plate is counted during the time course (Ahmed et al., 2021)

2.4. Preparation of bed immobilized reactor

The microbial inoculum used in this study was obtained from the sewage treatment plant (STP) from ELROBAIA Textile Company, Sadat city, Menofia, Egypt. STP was operated under ambient temperature conditions (30±5oC). The collected active sludge was collected as a part of return activated sludge to keep it in a good status prior to immobilization process on gravel (1 to 1.5 cm) with the following conditions: pH = 7.8 for 96 h at 37 °C. The raw textile wastewater was used as circulating substrate. The dissolved oxygen (DO) of the solution was kept at more than 3 mg/L (Georgiou et al., 2005).

2.5. Analytical methods

Colour of dyestuff wastewater, TDS, COD concentration, and pH were measured in supernatants from samples centrifuged at 8500 rpm for 10 min at 25 ± 1 oC. Wastewater blue concentration was measured using the absorbance peak at 690 nm measured with a UV–Vis spectrophotometer (PJ Instrument, model T80+, UK). Absorbance was translated into concentrations (mg/L) using a standard curve (R₂ = 0.994), thus allowing for determination of initial and Final concentrations as *ci*(0) and *ci*(*t*) of the dye at time 0 and *t*, respectively. pH measurements were taken with a pH meter (Adwa, Romania). COD analyses were performed by the APHA (2017) method with colorimetric determination. The COD and colour removal efficiency of the process was calculated according to Mousa (2016) following the equation (1).

$$\text{Recovery\%} = \frac{ci(0) - ci(t)}{ci(0)} * 100 \quad (1)$$

where *ci*(0) and *ci*(*t*) are the initial concentration value via optical density at 600nm (at the process start) and after contact time (*t*), respectively.

2.6. Azo dye derivative detection

The GC/MS analysis has been carried out using a Thermo Scientific™ ISQ™ Series single quadrupole GC/MS system. The analytical conditions for GC and MS following method of APHA (2017) which included Injector: split/splitless, Injection mode: splitless, 1 min splitless time, Injection volume: 1 μ L, Injector temp. 200 °C, Column dimensions: 30 m length×0.25 mm ID×0.25 μ m film thickness (Cat. No. 26094-1420), Carrier gas: Helium, 1.0 mL/min, constant flow, Oven program: 60 °C, 1 min, 15 °C/min to 200 °C, 25 °C/min to 310 °C, 310 °C, 5 min and finally transfer line temp.: 295 °C.

2.7. Effect of free and immobilized microbes

To study the contact time influence on the dye bioremediation, at optimum pH value, and optimum contact time, free and immobilized microbes were shaken at (200rpm) with 400 mL (5 mg/L) azo dye solutions as separated tests. The solids and solutions were separated, and the filtrate was collected for analysis at 600 nm visible light by spectrophotometer (PJ +80, UK).

2.8. Electron microscope characterization

Scanning Electron Microscope (SEM) and transmission electron microscopy (TEM) analyses was done using a scanning electron

probe micro analyzer (JXA-840A, Japan). The specimen in the form of films were mounted on the specimen stabs and coated with thin film of gold by the sputtering method. The micrograph was taken at magnification of 500, 1000, and 2000 using (KV) accelerating voltage. The TEM observation was carried out at 200kV acceleration voltages (Jeol-Jem 100cx Electron Microscope, Japan.). The Nano composite samples were carefully grounded dispersed in acetone followed by sonication to get a solution of metal nanoparticles and placed on a micro grid using a drop of the dispersion. The sample chamber for the observation was kept cold using a liquid nitrogen bath to prevent the destruction of the sample by the high-voltage electron beam.

2.9. Statistical Analysis

The collected data on the wastewater physic-chemical parameters, optical density were appropriately subjected to statistical analysis to find their corresponding mean variations. Statistical Analysis of the obtained data were analyzed using Microsoft excel software.

3. Results and Discussion

Azo dye is a compound characterized by their clear colors and provides an excellent coloring property. They are widely used as blue coloring agents in the textile industries. In addition, the use of certain azo dyes as textile coloring agents causes a serious health concern. Table (1) shows Physico-chemical characteristics for textile wastewaters that use azo dyes as coloring agent. The risk of its wastewater arises mainly from the carcinogenicity, toxicity, and potential mutagenicity of thus formed aromatic amines (European Commission, 1999). The results reveal that the textile wastewater has low biodegradability features. The biological treatment faces a limiting factor in these waters due to low biodegradability and high saline condition and need more time to get quantity and quality biodecomposers.

Effect of the contact time on removing efficiency

The addition of free and immobilized consortium for time contact of 1, 24, 48 and 72h with solution were used for azo dye biodegradation. The removal % was calculated according to spectrophotometric measurement at OD₆₀₀ that are reported in Table 2. The removal% increased with the time contact with free and immobilized consortium, and then stabilizes after 48h in case of free cell treatment. The obtained results were 66.6% and 76.5% of color removal for free and immobilized consortium, respectively. Free cell was less efficiency compared with those results obtained using immobilized consortium (76.53%) as shown in Figure (1).

Effect of the pH

The pH has a great influence on the growth of microorganisms. Normally, the textile wastewaters are alkaline features that need pH correction to neutralization point to allow microbe growth. The treatment with free and immobilized biofilm on gravels with neutralized textile wastewater was the best solution to get suitable condition in case of low biodegradability ratio of tested wastewater as

shown in Figure (2). The results show that there are good correlation ($r=0.7736$) between number of microorganisms and water pH. The highest total counts were detected in pH 7 and the lowest count in water pH 10-12.

Transmission (TEM) and scanning (SEM) Electron Microscope

The transmission electron microscope (TEM) and the scanning electron microscope (SEM) were used a high voltage electron beam to create an image and provided more information about the size and distribution of the immobilized consortium formed on gravel after 72 h contact time. The TEM image shown in Figure (5a) was found to exhibit a particles size in the range of 26 – 150 nm using 10,000 magnification orders which confirms the successful incorporation of microbial clusters on the surface of gravel (El-Masry et al., 1994). The SEM image shows the distribution of immobilized microbes on gravel that approve the stability of adhesion after contact with synthetic chemicals as shown in Figure (5b).

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Table (1) Physical /Chemical characteristics for textile wastewaters:

Parameter	Unit	Value		
		mean	Min	Max
pH		8.23	7.76	11.98
Conductivity	mS/cm	11016	9375	18125
Total dissolved solids	ppm	7050	6000	11600
Color density	OD ₆₀₀	4.80	2.2	13.2
COD	ppm	2500	2200	3700
BOD	ppm	320	93	780
BOD/COD	%	12.8	4.2	21.1

Table (2) Biodegradation and color removal of wastewater containing azo dye measured by optical densities:

Time (h)	Immobilized consortium				Free cell			
	Mean	Min	Max	Removal%	mean	Min	Max	Removal%
0	5.11	3.30	10.92		5.00	3.90	11.32	
1	4.89	3.28	8.30	4.44	4.80	3.20	8.60	4.00
24	2.95	1.73	4.31	42.24	3.89	1.67	6.54	22.24
48	2.04	1.32	4.79	60.01	2.39	1.27	4.97	52.10
72	1.20	0.90	2.64	76.53	1.67	0.97	3.78	66.64

Removal% were calculated according to Equation (1) on the mean values

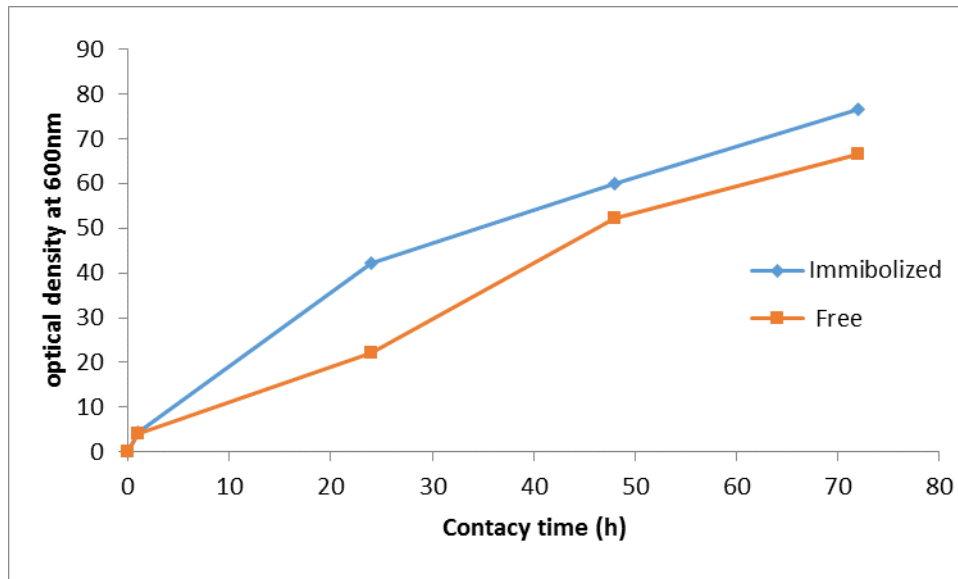


Figure 1. Effect of the contact time of free and immobilized microbes' on the color intensity of wastewater containing azo dyes at wavelength 600 nm.

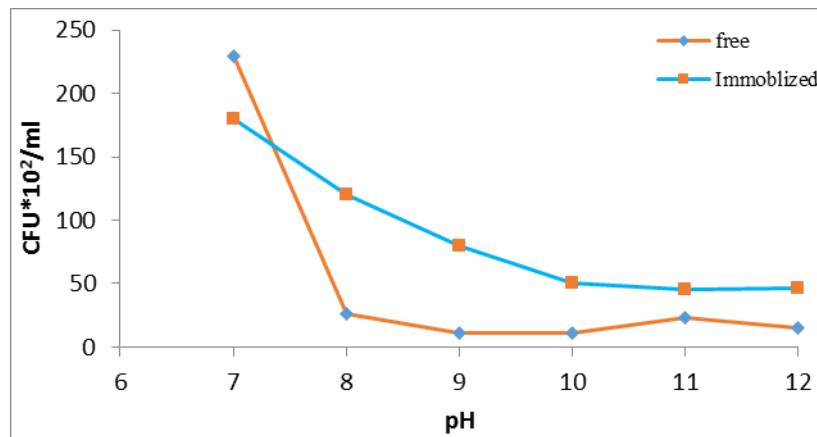


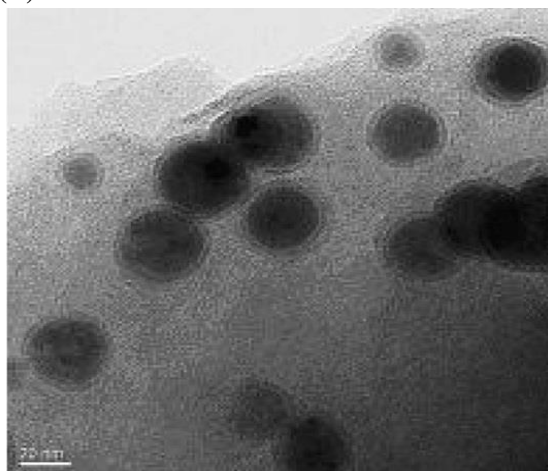
Figure 2. Effect of water pH on the count of free and immobilized microbes of raw wastewater containing azo dyes.

Table (3) Azo dyes derivatives analysis of influent and effluent textile wastewater

Azo dye derivatives	Case #	RT	Unit	Method*	Influent	Effluent	LOD
Aniline	62-53-3	5.42	ppb	GC-MS, APHA 2017	ND	ND	0.1
o-Toluidine	95-53-4	6.43	Ppb		ND	ND	0.1
2,4-Xylidine	95-68-1	7.35	Ppb		ND	ND	0.1
2,6-Xylidine	87-62-7	7.39	Ppb		ND	ND	0.1
Naphthalene-d8	1146-65-2	7.50	Ppb		ND	ND	0.1
2-Methoxyaniline	90-04-0	7.61	Ppb		ND	ND	0.1
p-Chloroaniline	106-47-8	7.97	Ppb		ND	ND	0.1
m-Anisidine	536-90-3	8.40	Ppb		ND	ND	0.1
p-Cresidine	120-71-8	8.50	Ppb		ND	ND	0.1
2,4,5-Trimethylaniline	137-17-7	8.57	Ppb		ND	ND	0.1
4-Chloro-o-toluidine	95-69-2	8.90	Ppb		ND	ND	0.1
1,4-Phenylenediamine (1,4-Benzendiamine)	106-50-3	8.91	Ppb		ND	ND	0.1
2,4-Toluenediamine	95-80-7	10.09	Ppb		ND	ND	0.1
2,4-Diaminoanisole	615-05-4	10.91	Ppb		ND	ND	0.1
2,4,5-Trichloroaniline	636-30-6	11.08	Ppb		ND	ND	0.1
2-Naphthylamine	91-59-8	11.44	Ppb		ND	ND	0.1
5-Nitro-o-toluidine	99-55-8	11.85	Ppb		ND	ND	0.1
4-Aminodiphenyl	92-67-1	12.66	Ppb		ND	ND	0.1
p-Aminoazobenzene	60-09-3	14.36	Ppb		ND	ND	0.1
4,4-Oxydianiline	101-80-4	14.62	Ppb		ND	ND	0.1
4,4-Diaminodiphenylmethane	101-77-9	14.66	Ppb		1.9	ND	0.1
Benzidine	92-87-5	14.71	Ppb		ND	ND	0.1
o-Aminoazobenzene	2835-58-7	15.01	Ppb		ND	ND	0.1
2,3-dihydro-3-oxoindolyiden	64784-13-0	15.15	Ppb		4.3	0.9	0.1
3,3-Dimethyl-4,4-diaminodiphenylmethane	838-88-0	15.31	Ppb		ND	ND	0.1
3,3'-Dimethylbenzidine	119-93-7	15.47	Ppb		ND	ND	0.1
4,4'-Thiodianiline	139-65-1	16.04	Ppb		ND	ND	0.1
4,4-Methylene-bis-2-chloroaniline	101-14-4	16.23	Ppb		ND	ND	0.1
3,3'-Dimethoxybenzidine	119-90-4	16.24	Ppb		ND	ND	0.1
3,3-Dichlorobenzidine	91-94-1	16.26	Ppb		ND	ND	0.1

*With reference to APHA 2017, by Gas Chromatographic-Mass Spectrometric (GC-MS).

(A)



(B)

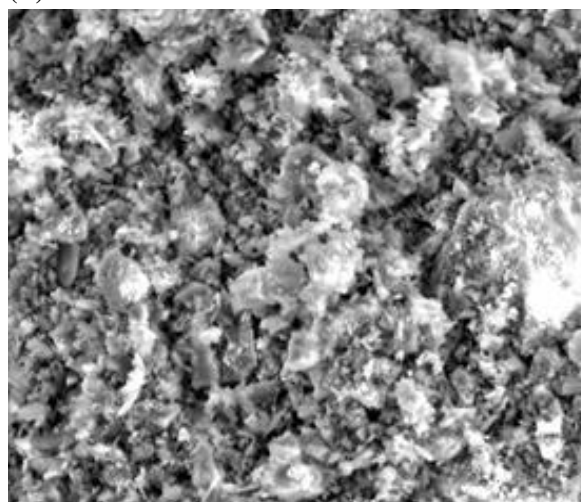


Figure 5: Transmission electron microscope (TEM) (A) and scan electron microscope (SEM) (B) images of immobilized biofilm at 10000X after incubation with azo dyes for 72 hours

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