Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2021.27829

241 (2021) 183–191
November

Silica-based nano-adsorbent for enhancement of bacterial biodegradation of methylene blue dye

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ABSTRACT

Silica-based nano-adsorbents have attracted much interest for water treatment applications. Herein, spherically-shaped silica-based nano-adsorbents were fabricated using the modified Stöber method, and they were characterized to identify their morphology and structure. The prepared silica-based nano-adsorbent was applied to enhance the biodegradation of methylene blue. Two bacterial sources were investigated for methylene blue (MB) dye biodegradation, including consortia from sand samples and *Azotobacter*. The conditions for biodegradation were adapted twice to confirm the biodegradation activities, which had biodegradation efficiencies of approximately 76.1% and 78.7% for the sand consortium and *Azotobacter*, respectively. Silica-based nano-adsorbents played an important role in enhancing biodegradation efficiency by collecting bacterial cells and dye molecules through adsorption. The biodegradation efficiencies were 99.5% and 99.4% for the sand consortium and *Azotobacter*, respectively, when the silica-based nano-adsorbent was applied. The biodegradation process reached a maximum efficiency at a temperature of 30°C when shaken at 150 rpm. In addition, the rate of the biodegradation of MB was found to follow a first-order kinetic model.

Keywords: Bacterial consortium; Biodegradation; Methylene blue; Silica nano-adsorbent; Water treatment

1. Introduction

The excessive production and use of dyes increase the degree of environmental pollution [1,2]. A large amount of dyes are discharged annually worldwide, from which approximately 10% are discharged to the water system, causing various environmental destabilizations. The most common environmental problems include the slowing of the photosynthetic process, the reduction of the normal growth of aquatic microorganisms due to decreased sunlight penetration and the utilization of dissolved oxygen [3–5]. Industrial activities for textiles and dying extensively consume fresh water and produce wastewater in the same amount. The main environmental issue in the dying processes is the partial fixation of dyes on fabrics; usually, the unfixed dyes are moved during washing and discharged to water surfaces [6,7]. Laing [8] reported that the dye pollution level in wastewater effluent from textile industries was approximately 10–50 mg L^{-1}, while Ghaly et al. [9] reported a range between 10 and 250 mg L^{-1} for dye levels in effluents from textile industries.

Methylene blue (MB) is a cationic dye that has been widely applied in textile industrial activities for various matrices, such as leather, clothes, paper and plastic materials. In addition, it is applied for ink production and printing applications. MB is a heterocyclic organic compound with a molecular weight of 373.9 g that exhibits a blue color during water solvation [10]. MB dye is toxic to humans and

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may result in mutations or cancer, and it is hazardous to the eyes and skin [11,12]. The treatment and removal or degradation of MB is an important applicable research issue that includes oxidation, treatment by ozone, adsorption and reverse osmosis [13–16]. However, the application of these methods is difficult due to high costs and the formation of by-products such as sludge. Biological treatment methods are inexpensive and easy to operate; these benefits can lead to the wide application of biodegradation [17–21].

Biological treatment for decolorization of dyes is widely used to purify industrial effluent from contaminated dyes. The application of bacterial consortia poses a rapid growth rate under various conditions, including aerobic or anaerobic conditions. In addition, the bacterial consortium can be grown in a wide pH range and can resist harsh conditions such as salinity and temperature [22–25]. The biodegradation process can be enhanced by applying adsorbent materials as supports [26]. Activated carbon is most widely used as an adsorbent and solid support matrix for microbial cells in the removal of hazardous chemicals [27].

The combination of the adsorbent with microbial cells may be achieved by the formation of covalent bonds between the adsorbent and the cell in the presence of a binding (crosslinking) agent. Chemical modification of the surface is necessary [28]. In addition, the combination of adsorbent with bacterial cells can also be achieved by adsorption of microorganisms onto porous support materials, which is similar to the adsorption of colloid particles [29]. Bedekar et al. [18] applied ground water hyacinth as an adsorbent to enhance the biodegradation of MB by using two steps for wastewater treatment, including adsorption onto the water hyacinth plant followed by biodegradation under anaerobic and aerobic conditions. This anaerobic treatment showed higher efficiency of dye removal. Spagnoli et al. [30] applied carbon from cashew nut shells as an adsorbent to remove MB and reported that the dipole-dipole interaction between the nitrogen from MB and the oxygen-containing groups in carbon was responsible for the removal efficiency. Ahmed et al. [31] reported the fabrication of pure SnO_2 and SnO_2 modified by silver nanoparticles for MB remediation by simultaneous adsorption with photocatalysis, which enabled high efficiency for the purification of wastewater by the mineralization of organic pollutants. Tong et al. [32] developed carbon modified with montmorillonite for adsorptive MB removal. Nguyet et al. [33] applied coconut fiber for 62 d to support the biodegradation of MB and reported the importance of coconut fiber as a support to keep the degrading bacteria in the reactor, leading to an improvement in the wastewater treatment process. Bharti et al. [21] isolated bacterial strains from dye-polluted water surfaces and applied them for MB dye degradation in a batch process over 5 d at 30°C using a 150 ppm dye concentration, and they reported that 96.2% of the dye was degraded. Biochar was used as a support for bacteria to enhance the MB removal process, and the researchers reported an approximately 86% reduction in dye concentration, or 500 ppm. Elgarahy et al. [34] applied discarded sepia shells as adsorbents to remove MB from water and enhanced the process with microwave irradiation. Wang et al. [35] used a chitosan/cross-linked hydrogel for MB removal.

Thus, this work aimed to fabricate and characterize a silica-based nano-adsorbent to enhance the biodegradation of methylene blue. The prepared silica-based nano-adsorbent was characterized by scanning electron microscope (SEM), the Brunauer–Emmett–Teller (BET)-surface area, Fourier transform infrared (FTIR) and transmittance electron microscope (TEM). The conditions for silica-enhanced bacterial biodegradation were optimized, and the data were analyzed according to the first-order kinetic model.

2. Materials and methods

2.1. Chemicals and reagents

Hexadecyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), ethanol and sodium chloride were purchased from Sigma (St. Louis, MO, USA). Nutrient broth media was purchased from HiMedia Laboratory, India. The sand sample for the bacterial consortium was taken from soil near a petrol station. The *Azotobacter* bacterial isolate was provided by the Department of Botany and Microbiology, Faculty of Science, Beni-Seuf University, Egypt.

2.2. Fabrication and characterization of the silica-based nano-adsorbent

For the synthesis of silica nano-adsorbent, water and ethanol were mixed at a ratio of 2:1. Ammonia solution was added, and the mixture was stirred for 15 min. Then, CTAB was added and stirred for 60 min on a magnetic stirrer. Then, TEOS was added dropwise, and stirring was continued at room temperature for 180 min. The white precipitate was collected through centrifugation, washed with deionized water, and air-dried. The dried silica-based nano-adsorbent sample was calcined in air to remove the surfactant. It was characterized by SEM, TEM, BET surface area analysis and FTIR spectroscopy [36,37].

2.3. Adaptation and optimization of the bacterial biodegradation of methylene blue

Two sources of bacterial cells were investigated to biodegrade MB dye, including consortia from sand and *Azotobacter* strains. One milliliter from the bacterial source samples was used to inoculate 20 mL glass tubes containing 12 mL nutrient broth media where the concentration of dye was 10 mg L^{-1} in this mixture. The tubes were incubated for 5 d under shaking conditions at 150 rpm and 30°C. Blank and control experiments were conducted. The experiments were performed in triplicate. The process was monitored by analyzing the MB concentration by UV-visible spectroscopy with time intervals of 1, 3 and 5 d. The biodegradation efficiency was calculated from Eq. (1):

$$\text{The biodegradation efficiency } \% = \frac{\left(C_0 - C_f\right)}{C_0} \times 100 \quad (1)$$

where C_0 represents the initial concentration of dye in the solution and C_f is the final concentration of dye in the solution.

For adaptation, a portion of the previously screened culture that evidently promoted the biodegradation of methylene blue was transferred to fresh media, tested by incubation under the same conditions and evaluated for MB dye degradation (10 mg L^{-1}). The adaptation tubes were incubated for 5 d under shaking conditions at 150 rpm and 30°C. The efficiency was monitored by analyzing the MB concentration over time intervals of 1, 3 and 5 d. The biodegradation efficiency was calculated from Eq. (1).

The previous steps for the determination of the biodegradation efficiency for MB were repeated to study the effect of temperature at 30°C and 37°C, and the effect of shaking was evaluated by comparing samples that were static and those that were shaken at 150 rpm.

To study the effect of solid support enhancement, silica-based nano-adsorbents were added to the biodegradation mixture (bacterial source, MB dye and nutrient media). The rate of dye biodegradation was studied using a first-order kinetic model.

3. Results and discussions

3.1. Morphology and structure characterization of the fabricated silica nano-adsorbent

The fabricated silica-based nano-adsorbent exhibited a uniform sample distribution with particle sizes between 200 and 250 nm, as shown in Fig. 1A and B, which shows scanning electron microscope images at magnifications of 6,000 (Fig. 1A) and 27,000 (Fig. 1B). The internal structure of the prepared silica-based nano-adsorbent was characterized by TEM, which confirmed the uniform spherical structure around the whole sample, which had a porous nature (Fig. 2A and B). The BET surface area of the fabricated porous silica-based nano-adsorbent is 1,063.2846 $m^2 g^{-1}$.

The surface active sites of the prepared silica-based nano-adsorbent were identified by FTIR spectroscopy (Fig. 3), which showed peaks at 470 and 791 cm^{-1} that were caused by the bending vibrations of Si–O–Si groups, a peak at 1,095 cm^{-1} that was caused by the stretching vibrations of

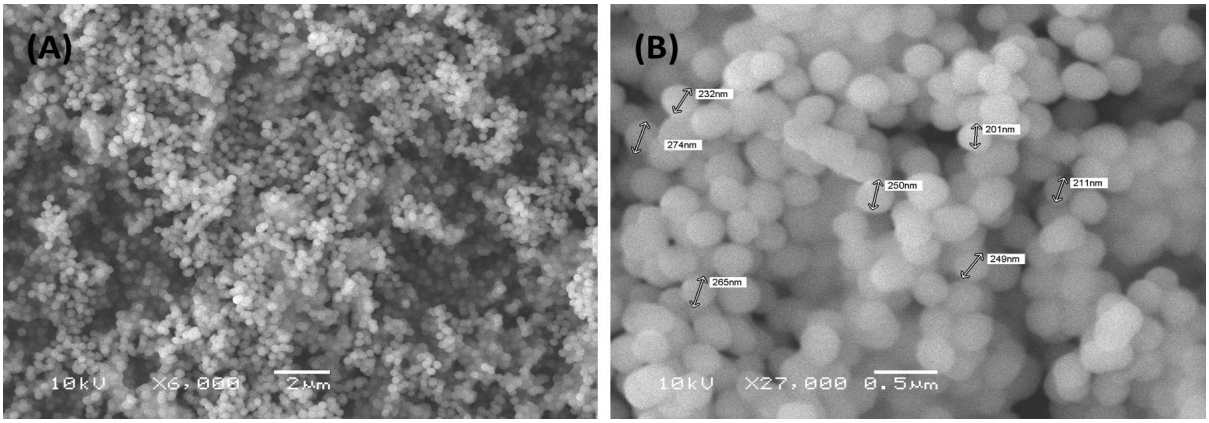

Fig. 1. SEM images of SiO_2-based nano-adsorbents (A) at a magnification of 6,000 and (B) at a magnification of 27,000.

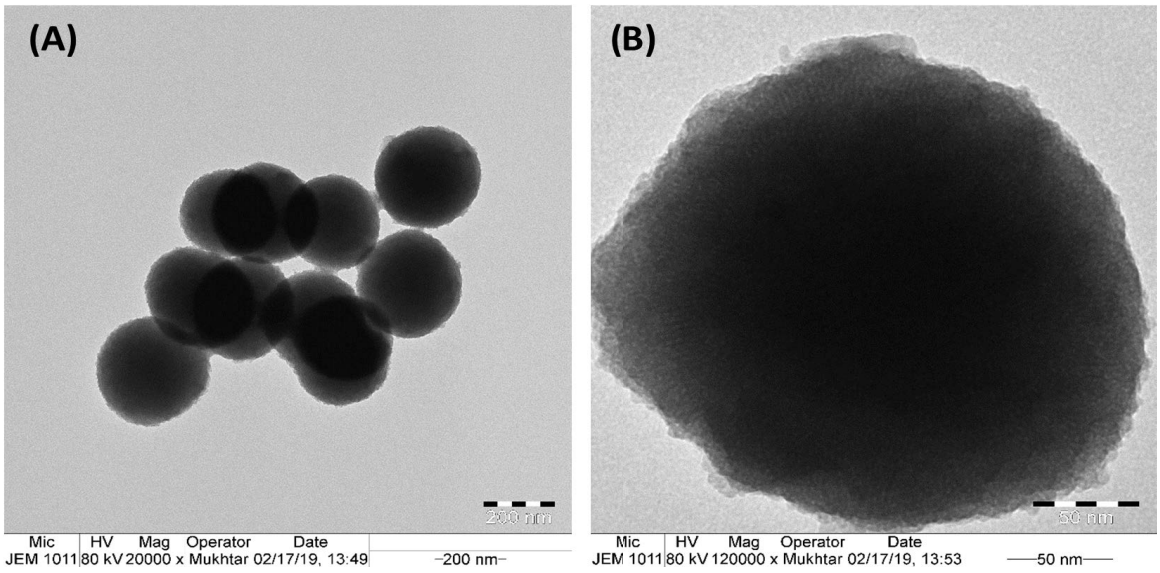

Fig. 2. TEM examination of SiO_2-based nano-adsorbents (A) at a magnification of 20,000 and (B) at a magnification of 120,000.

the Si–O groups, and a peak at 949 cm^{-1} that was caused by the stretching vibrations of the Si–OH groups. The broad peak between 3,400 and 3,600 cm^{-1} is attributed to the OH groups [38,39].

3.2. Primary investigations of MB bacterial biodegradation using *Azotobacter* and consortium from sand samples

Adaptation of the bacterial sources, including the *Azotobacter* and consortium, from sand for MB biodegradation was performed in two steps, each of which was monitored for 5 d, and the biodegradation efficiency was calculated as shown in Table 1. In the first adaptation experiment, the sand consortium showed a biodegradation efficiency of 43.5%, 59.5% and 75.3% for the first, third and fifth days, respectively, while the *Azotobacter* exhibited a biodegradation efficiency of 33.1%, 51.1% and 72.5% for the first, third and fifth days, respectively. In the second adaptation experiments, the sand consortium showed a biodegradation efficiency of 30.4%, 53.3% and 76.1% for the first, third and fifth days, respectively, while the *Azotobacter* strain exhibited a biodegradation efficiency of 66.9%, 71.0% and 78.7% for the first, third and fifth days, respectively. The adaptation experiments were performed using media containing meat extract (1 g L^{-1}), yeast extract (1 g L^{-1}), peptone (5 g L^{-1}) and NaCl (5 g L^{-1}) to allow bacterial growth and enhance the biodegradation activities [40]. The pH of the medium was maintained at 7 because a more strongly acidic medium leads to protonation of the bacterial cell wall and stops the bacterial activities, and basic medium is not suitable for silica-based nano-adsorbent enhancement [41–44].

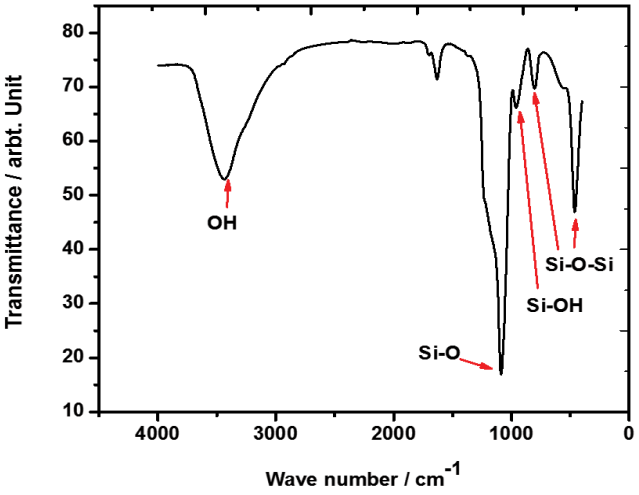

Fig. 3. FTIR examination of the SiO_2-based nano-adsorbent.

3.3. Optimization of the bacterial biodegradation of MB and their enhancement with silica-based nano-adsorbents

The application of a bacterial consortium for dye degradation has received more interest than isolated strains because the isolation process is time-consuming and achieving complete decolonization of dye pollution by pure bacterial culture is difficult in some cases. In addition, the bacterial consortium allows for bacterial cooperation, leading to enhanced biodegradation. However, consortium application is reported to have low reproducibility [45]. Therefore, the application of silica-based nano-adsorbents to support bacterial biodegradation is investigated during this work to overcome the problems associated with bacterial consortia or strains. The application of adsorbents such as carbon derivatives or silica-based materials is suggested to enhance the biodegradation of some organic pollutants. The process includes the adsorption of the dye onto the adsorbent surface together with the bacterial cell through the peptidoglycan in the bacterial cell wall [46–48]. In this work, silica-based nano adsorbents were mixed with the biodegradation system to enhance the uptake and biodegradation of methylene blue, and the most influential factors were studied in the following investigations.

3.3.1. Effect of temperature on MB biodegradation using *Azotobacter* and a bacterial consortium from sand samples

Bacterial biodegradation is a temperature-sensitive process because many functions are dependent on temperature conditions; such processes include bacterial growth and activities to biodegrade dyes to maintain survival. Herein, the bacterial biodegradation of MB was performed at 30°C and 37°C, and the calculated efficiency is presented in Fig. 4. The biodegradation at 30°C enables a high biodegradation efficiency of methylene blue. These results exhibited the opposite trend of those reported by Contreras et al. [49], who mentioned that the biodegradation of MB was approximately 60% at a system temperature of 30°C; however, it reached approximately 72% at a temperature of 35°C. The biodegradation efficiencies in the case of the sand consortium and *Azotobacter* without applied nano-adsorbent at 30°C were 70.3% and 68.8%, respectively, while when the silica nano-adsorbent was

Table 1
Biodegradation efficiency during adaptation of MB biodegradation using *Azotobacter* and consortium from sand samples (n = 3) at 150 rpm and 30°C

Adaptation experiments	Bacterial source	Biodegradation efficiency (%)		
		First day	Third day	Fifth day
First experiment	Sand consortium	43.5 ± 2.0	59.5 ± 1.5	75.3 ± 3.4
	Azotobacter	33.1 ± 1.9	51.1 ± 3.0	72.5 ± 2.7
Second experiment	Sand consortium	30.4 ± 3.2	53.3 ± 1.1	76.1 ± 2.3
	Azotobacter	66.9 ± 1.1	71.0 ± 2.0	78.7 ± 1.8

applied, the efficiencies became 99.5% and 99.4% for the sand consortium and *Azotobacter*, respectively; the addition of the nano-adsorbent resulted in an approximately 30% improvement in the biodegradation efficiency. The mechanism of enhancement may be attributed to the ability of silica-based nano-adsorbents to attract methylene blue molecules by the dipole-dipole interaction between the nitrogen from MB and the oxygen-containing functional groups in silica, such as Si–O–Si or OH, leading to the collection of dye and bacterial cells that facilitate the biodegradation process. Biodegradation wastewater treatment is based on the ability of bacteria to consume dyes instead of nutrients in low-nutrient medium [21,33].

3.3.2. Effect of shaking on MB biodegradation using Azotobacter and a bacterial consortium from sand samples

The mobility of the components of the enhanced biodegradation system, including the bacterial source, silica nano-adsorbent, nutrient media and MB dye, was investigated by comparing the biodegradation rate under static conditions and under 150 rpm shaking conditions. The biodegradation efficiencies of the two bacterial sources in the presence and absence of silica-based nano-adsorbents is shown in Fig. 5. The biodegradation efficiency was improved by shaking the system in all tested cases. These improvements may be attributed to enhanced adsorption of MB

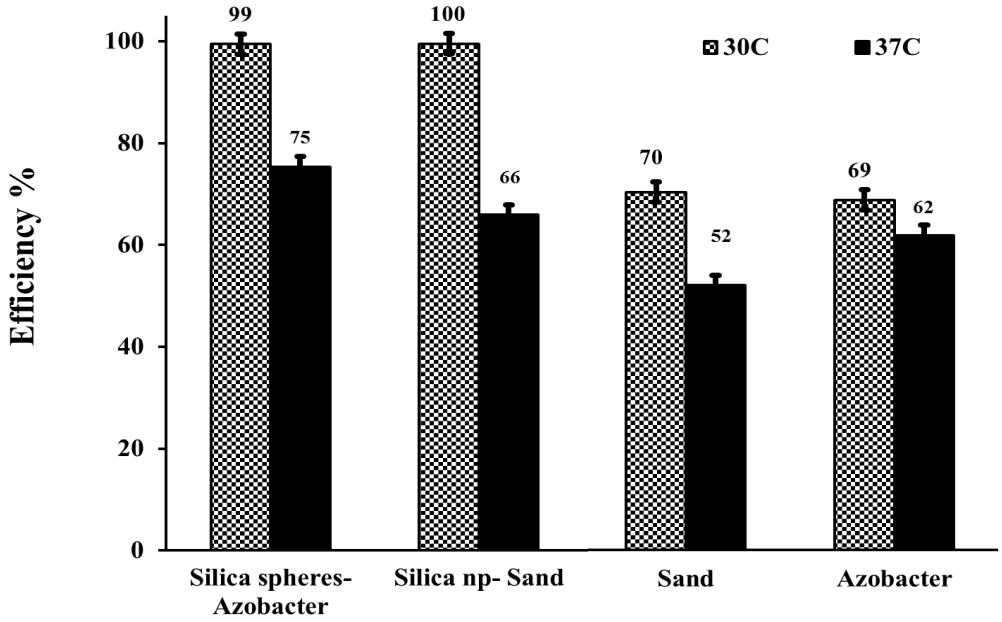

Fig. 4. MB biodegradation efficiency using *Azotobacter* and a bacterial consortium from sand samples at 30°C and 37°C in the presence and absence of silica nano-adsorbents.

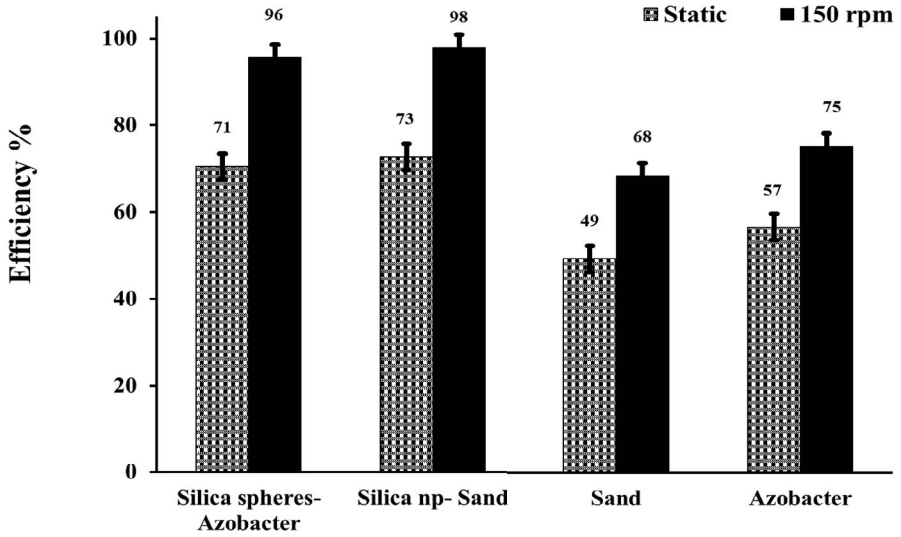

Fig. 5. MB biodegradation efficiency of *Azotobacter* and a bacterial consortium from sand samples without shaking and at 150 rpm in the presence and absence of the silica nano-adsorbent.

dye onto the silica-based nano-adsorbent surface as well as enhanced growth of the bacterial cells, which resulted in an overall enhancement of the biodegradation and removal of MB dye.

3.3.3. Kinetic study of MB biodegradation using Azotobacter and a bacterial consortium from sand samples

In the developed biodegradation system that used *Azotobacter* and a bacterial consortium from sand samples, the rate of dye degradation was assumed to be manipulated by bacterial biodegradation processes; therefore, the Michaelis–Menten kinetic equations [50,51] can be applied:

$$r = -\frac{dC}{dt} \text{ and } -\frac{dC}{dt} = \frac{\lambda(X)C}{\lambda_c + C} \quad (2)$$

where r is the dye degradation rate, mg L^{-1} T^{-1}; C is the dye concentration, mg L^{-1}; X is the biomass concentration, mg L^{-1}; t is the reaction time, T; $\lambda(X)$ is the kinetic constant or Michaelis–Menten constant, mg L^{-1} T^{-1}; λ_c is the half-saturation constant, mg L^{-1}.

Assuming that the biomass concentration is constant, integration of Eq. (2) becomes the following:

$$\lambda_c \ln\frac{C_0}{C} + (C_0 - C) = \lambda(X)(t - t_0) \quad (3)$$

where C_0 is the initial dye concentration, ML~3; t_0 is the initial time, T.

For processes at low dye concentrations, that is, when $\lambda_c >> C$, then Eq. (2), in its simplified form as a first-order kinetics model, is expressed as in Eq. (4):

$$\frac{dC}{dt} = \lambda' C \quad (4)$$

where $\lambda' = \lambda(X)/\lambda_c$.

At the initial conditions, when $t = 0$ and $C = C_0$, the integration gives Eq. (5):

Table 2
The first-order kinetic model constant for MB biodegradation using *Azotobacter* and a consortium from sand in the presence and absence of the silica nano-adsorbent

Biodegradation condition	Kinetic parameters		R^2
	λ′	C0	
Azotobacter	0.38	2.73	0.95
Consortium from sand	0.23	2.61	0.89
Silica nano-adsorbent, Azotobacter	0.88	2.87	0.97
Silica nano-adsorbent, consortium from sand	1.04	3.47	0.94

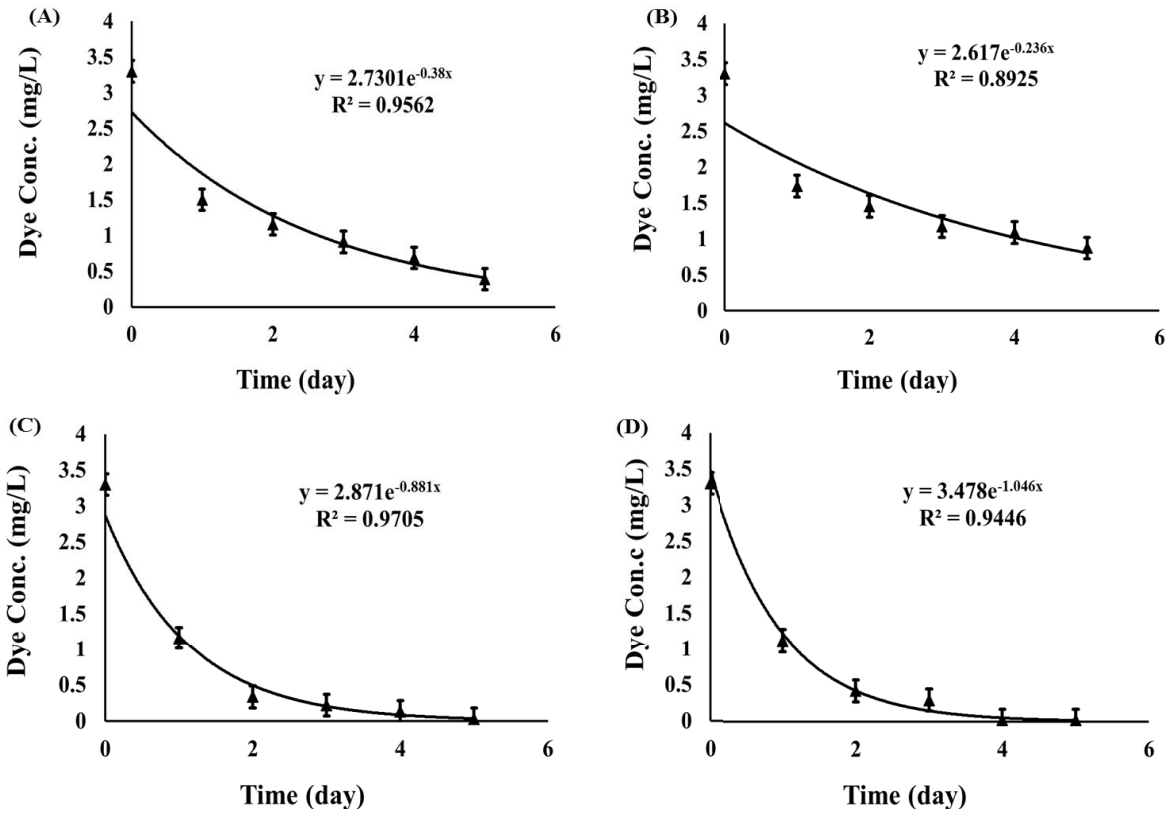

Fig. 6. The first-order kinetic model for MB biodegradation using (A) *Azotobacter*, (B) consortium from sand, (C) silica nano-adsorbent and *Azotobacter* and (D) silica nano-adsorbent and consortium from sand.

Table 3
Comparison of MB removal by biodegradation using silica-based nano-adsorbents and other methods from the literature

Treatment method	Adsorption capacity (mg g^{-1})/Removal efficiency (%)	Reference
Isolated bacterial strains from dye polluted water surfaces applied to MB dye	96.2%	[21]
Cashew nutshell carbons as adsorbents for MB removal	476 mg g^{-1}	[30]
Carbon modified with montmorillonite for MB adsorptive-removal	138.1 mg g^{-1}	[32]
Discarded sepia shells as adsorbents for MB removal from water; enhanced with microwave irradiation	254.05 mg g^{-1}	[34]
Silica based nano-adsorbent for enhancing bacterial biodegradation	99.5%	This work

$$C = C_0 e^{-\lambda'(t-t_0)} \quad (5)$$

By plotting C vs. $(t–t_0)$ as in Fig. 6, C_0 and λ′ can be determined (Table 2).

As shown in Fig. 6, the reduction in the dye concentration occurred more quickly when the biodegradation mixture included silica nano-adsorbents. The values of λ′ (Table 2) were 0.38, 0.23, 0.88 and 1.04 for *Azotobacter*, a consortium from sand, silica-based nano-adsorbent with *Azotobacter* and silica-based nano-adsorbent with the consortium from sand, respectively; these data indicate the enhancement of the biodegradation process. The rate of MB dye biodegradation was found to follow the first-order kinetic model. These results were in agreement with those of Gonçalves et al., who reported the first-order kinetic model for the biodegradation of azo dye by a mixed culture [52]. The efficiency of MB removal using the proposed method of biodegradation enhanced with silica-based nano-adsorbents was compared with the efficiencies of other experiments from the literature (Table 3), which revealed that the efficiency achieved in this work was high.

4. Conclusion

The biodegradation of MB using a bacterial sand consortium and *Azotobacter* can be enhanced by applying a porous silica-based nano-adsorbent. This combination of bacterial biodegradation and the nano-adsorbent led to an improvement in the biodegradation efficiency, which increased from 70.3% and 68.8% for the sand consortium and *Azotobacter*, respectively, to 99.5% and 99.4%. This efficiency enhancement may be attributed to the ability of the porous silica-based nano-adsorbent to collect dyes and bacterial cells and facilitate the feeding of bacterial cells on the MB dye. The biodegradation process was more efficient when the samples were shaken at 150 rpm than under static conditions.

Abbreviations

SEM	—	Scanning electron microscope
BET-surface area	—	Brunauer–Emmett–Teller surface area
FTIR	—	Fourier transformer infrared
TEM	—	Transmittance electron microscope
CTAB	—	Hexadecyltrimethylammonium bromide
TEOS	—	Tetraethylorthosilicate
r	—	Dye degradation rate, mg L^{-1} T^{-1}
C	—	Dye concentration
X	—	Biomass concentration, mg L^{-1}
t	—	Reaction time, T
λ(X)	—	Kinetic constant or Michaelis–Menten constant, mg L^{-1} T^{-1}
λ_c	—	Half-saturation constant, mg L^{-1}
C_0	—	Initial concentration of dye
C_f	—	Final concentration of dye

Acknowledgments

The authors extend their appreciation to the Polymer Research Group, Department of Chemistry, Faculty of Science, Tanta University, Tanta, Egypt, for supporting this project.

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