JOURNAL OF PURE & APPLIED MICROBIOLOGY, Nov. 2014. Vol. **8**(Spl. Edn. 2), p. 343-348

Utilization of Algal *Spirogyra* sp in Environmental Cleanup: A Malachite Green Dye Biosorption Study

S.M. Yakout[1,2]*, A.A. Abdeltawab and Ibrahim M. Aziz[1]

[1]Biochemistry Department, College of Science, King Saud University,
P.O. Box 2455, Riyadh 11451, Kingdom of Saudi Arabia.
[2]Hot Laboratories Center, Atomic Energy Authority, P.O. Box 13759, Cairo, Egypt.
[3]Chemistry Department, College of Science, King Saud University,
P.O. Box, 2455, Riyadh 11451, Kingdom of Saudi Arabia.
[4]Chemical Engineering Department, Tabbin Institute for Metallurgical Studies, Cairo 11413, Egypt.

(Received: 20 July 2014; accepted: 02 September 2014)

***Spirogyra* sp biomass used for bioremediation of malachite green dye from aqueous solution. Biosorption process has been investigated as a function of pH, contact time and equilibrium. The adsorption is strongly dependent on pH of the medium where the removal efficiency increases as the pH turns to alkaline range. This reveals the basic nature of the Malachite green and acidic nature of the *Spirogyra* sp biomass. Langmuir model gave the best fitting to the biosorption data. Bioremediation results shows that *Spirogyra* sp has accumulated varying amount of malachite green dye intracellularly and could be employed for the removal of dyes from spent industrial effluents before discharging it into the environment.**

Key words: Malachite green, Biosorption isotherm, Equilibrium Kinetic.

Malachite green (MG), a synthetic basic cationic dye imparts violet color in aqueous solution. It is a member of the triphenylmethane group, which is widely used in textile dyeing industries, as a biological stain, dermatological agent, veterinary medicine, temporary hair colorant, additive to poultry feed to inhibit propagation of harmful bacteria[1]. The cationic dyes are more toxic than the anionic dyes[2], as these can easily interact with negatively charged cells membrane surfaces, and can enter into cells and concentrate in cytoplasm[3]. MG is toxic to mammalian cells and also a mutagen, mitotic poison [4] and a proven potent carcinogen [5]. It is responsible for moderate eye irritation, causing painful sensitization to light. It can cause permanent injury to the cornea and conjunctiva[6]. Inhalation of MG may cause irritation to the respiratory tracks, vomiting, diarrhea, pain, headache, and dizziness. Long term exposure may cause damage to the mucous membrane and gastrointestinal tract[7].

The color in water bodies reduces penetration of sunlight to the lower layers and hence, affects the aquatic life. Water soluble dyes are characterized by their poor biodegradability and it is estimated that about 20% of the total dye remains in the effluent during the production process[7]. With the legislations becoming more stringent, considerable importance has been given to the treatment of the dye containing effluents. Therefore, it is highly desirable to remove the dyes in general and MG in particular from water/ wastewater not only to protect water resources but also for the protection of human health. Conventional methods for the removal of malachite green from waste water include physical–chemical and biological treatment technologies. Among them, biosorption has been found to be superior

* To whom all correspondence should be addressed.
Tel.: +966558448693; Fax: +96614675931;
email:sobhy.yakout@gmail.com

to other techniques for the treatment of wastewaters containing dyes, pigments and other colourants [8] in terms of cost, simplicity of design, ease of operation and insensitivity to toxic substances[3].

Various low-cost biomasses, including peel of fruit, potato plant wastes and dried algae and ferns, have been used as the potential biomass materials to remove dyes from aqueous solutions containing dyes [9-13]. Algae have been found to be potentially proper biosorbents because functional groups on the cell wall and their high binding affinity, fast growth and natural abundance around the world. Many studies have shown that *Spirogyra* spp. can effectively remove nutrient and heavy metals from aqueous solutions [14]. However, there are few reports on the biosorption of dyes from contaminated waters using *Spirogyra* spp.[14,15] The main objective of this study was to investigate the potentiality of using *Spirogyra* sptreatment for the biosorption of malachite green MG dye from aqueous solutions. The effects of contact time and pH on the selective cationic dyes were studied. Biosorption isotherms and kinetics parameters were also calculated and discussed.

MATERIALS AND METHODS

All chemicals were of analytical grade and were used without any further purification. Double distilled water was used throughout this study. Malachite green (MG) was obtained from Merck. A stock solution of the dye was prepared by dissolving the dye in twice distilled water. Working solutions of the desired concentrations were obtained by successive dilutions. The solution pH was adjusted using 0.1N HCl or 0.1N NaOH obtained from Merck, India. Concentrations of dye solutions were measured by monitoring the maximum absorbance at $\lambda_{max.\ abs}$.= 586 nm using Schimatzu UV–Vis spectrophotometer (Shimadzu Model: UV 1601). *Spirogyra* species algal biomass sorbent was thoroughly washed with distilled water to remove filth and unwanted macro/ microorganisms. After drying in sunlight, the biomass was dried in oven at 75 ^{o}C for 24 h and then ground and sieved to obtain constant particle sizes (178 mm). Prior to use.

In 250mL Erlenmeyer flasks, 1.5 g/L of sorbent were dispersed in water solutions containing malachite green (MG) concentrations in the range 25–300mg/l. The flaks were agitated in an isothermal water bath shaker at 120rpm and 30æ% until equilibrium was reached. Each experiment was duplicated under identical conditions.

The amount of biosorption at equilibrium, qe (mg/g), was calculated by:

$$q = \frac{V(C_i - C_e)}{m}$$

Where C_i and C_f are the initial and final concentrations of dye (mg/L) in aqueous solution, respectively, V is the volume of the solution (ml) and m represents the weight of the sorbent (mg). Dye removal percentage (E%) was calculated as

$$E(\%) = \frac{C_i - C_e}{C_i} \times 100$$

Where C_t (mg/L) is the liquid-phase concentrations of dye at time t.

RESULTS AND DISCUSSION

Effect of contact time

The effect of contact time on malachite green biosorption onto *Spirogyra* sp biomass, at initial concentration of 100 mg/L, was studied in Fig. 2. It was observed that the curve is smooth, and continuous, leading to saturation. This suggesting possible formation of dye monolayer coverage on the surface of *Spirogyra* sp biomass[16]. Malachite green uptake was rapid via first 200 min, and then proceeded at a slower rate and finally attains saturation. The next experiments were done overnight to be sure that full equilibrium was attained.

Effect pH

Fig. 3, show the effect of solution pH (2-10) on *Spirogyra* sp biomass biosorption capacity for malachite green dye (100 mg/L) was studied. It was found that malachite green dye biosorption increase with pH increase from 2 to 5, but further increase in pH from was no affected on the sorption. *Spirogyra* sp biomass surface has positive charge at lower pH, thus making (H+) ions compete effectively with malachite green dyes causing a decrease in the amount of dye adsorbed. At higher pH the surface of on *Spirogyra* sp biomass, may be negatively charged which

enhance the biosorption positively charged cationic dyes through electrostatic force attraction[17].

Biosorption isotherms

As shown in Fig. 4, the isotherm rose rapidly over the initial stage of biosorption where low Ce and qe values existed. This behavior indicates that there were plenty of readily accessible sites available on the sorbents. Eventually a slow approach to equilibrium at high concentrations occurred. As more sites are filled, it becomes difficult for the solute molecules to find a site for biosorption and/or the difficulty of molecules in penetrating the layer of adsorbed molecules already covering the surface sites. Two common isotherm models were tested: the Langmuir and Freundlich models. The linear form of Freundlich isotherm can be described by following equation (1):

$$\log q_e = \log K_F + \left(\frac{1}{n}\right) \log C_e \qquad ..(1)$$

Where K_F (mg/g), is roughly an indicator of the biosorption capacity and 1/n is the biosorption intensity. Values of K_F and n are calculated from the intercept and slope of the plot and are listed in Table 1. The R^2 value is lower than Langmuir isotherm. The value of Freundlich exponent n is the range of n > 1, indicating a favorable biosorption [18].

The linear form of the Langmuir equation can be described by following equation (2).

$$\frac{C_e}{q_e} = \left(\frac{1}{Q_0 b}\right) + \left(\frac{1}{Q_0}\right) C_e \qquad ...(2)$$

Where C_e (mg/L) is the equilibrium concentration of the adsorbate, q_e (mg/g) is the amount of biosorption per unit mass, Q^0 and b are Langmuir constants related to biosorption capacity and rate of biosorption, respectively. The linear plot of specific biosorption (C_e/q_e) against the equilibrium concentration C_e shows that the biosorption obeys the Langmuir model. The Langmuir constants Q^0 and b were determined from the slope and intercept of the plot and are presented in Table 1. The R^2 values (0.998) suggest that the Langmuir isotherm provides a good fit to the isotherm data. This confirms the monolayer coverage process of malachite green dyes onto *Spirogyra* sp biomass.

The essential characteristics of the Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor R_L [19] given by Eq. (3)

$$R_L = \frac{1}{1 + bC_0} \qquad ...(3)$$

Where C_0 (mg/L) is the highest initial concentration of sorbent, and b (L/mg) is Langmuir constant. The parameter R_L indicates the nature of

Table 1. Langmuir and Freundlich isotherm Parameters for biosorption of malachite green dyes onto Spirogyra sp

Isotherm	Parameters	Value
Freundlich	K (mg/ g)	89.13
	n	2.6
	R^2	0.668
Langmuir	qo (mg/g)	320.5
	b (1/mg)	0.48
	R2	0.995

Table 2. Comparison between pseudo-first, second-order biosorption rate constant calculated and experimental q_e values

Model	Parameter	
First-order model	qe,exp (mg/g)	399.1
	K1 (h-1)	0.0074
	qe,cal (mg/g)	233.1
	R2	0.88
Second-order model	K2 (g/mg h)	$5.66*10^{-5}$
	h (mg/g h)	9.66
	qe,cal (mg/g)	413.2
	R2	0.998

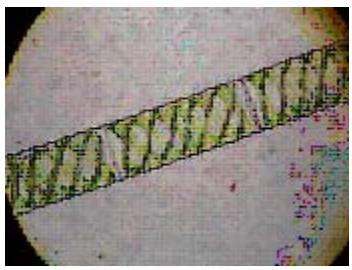

Fig 1. Microscopic picture of *Spirogyra* sp. used in this study.

shape of the isotherm accordingly: $R_L > 1$; Unfavorable biosorption, $0<R_L<1$; Favorable biosorption, $R_L = 0$; Irreversible biosorption, $R_L = 1$; Linear biosorption. The value of R_L in the present investigation has been found to be 0.9737at 30æ%C showing that the biosorption of malachite green dyes on *Spirogyra* sp biomass is favorable at the temperature studied.

Biosorption kinetics

To measure the rate-controlling and mass transfer mechanism of malachite green biosorption on *Spirogyra* sp biomass, kinetic data were calculated by the pseudo-first-order and pseudo-second-order kinetic models [20].

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303}t \quad ..(4)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \left(\frac{1}{q_e}\right)t \quad ...(5)$$

Where k_1 (1/min), is pseudo-first-order rate constant, k_2 (g/mg min) is the second-order rate constant. q_e (mg/g) is the amount of solute adsorbed on the surface at equilibrium and q_t (mg/g) is the amount of solute adsorbed at time t (min). Table 2 showed the rate constants and correlation coefficients (R^2), which were calculated by the pseudo-first-order and pseudo-second-order kinetic models. The R^2 of the pseudo-second-order biosorption kinetic model presented higher values than those of the pseudo-first-order one, and their calculated equilibrium biosorption capacities (q_e calc) fitted the experimental q_e values well. The results indicated that the pseudo-second-order biosorption kinetic model was more appropriate for dye biosorption process than the pseudo-first-order one.

The kinetic results were further analyzed

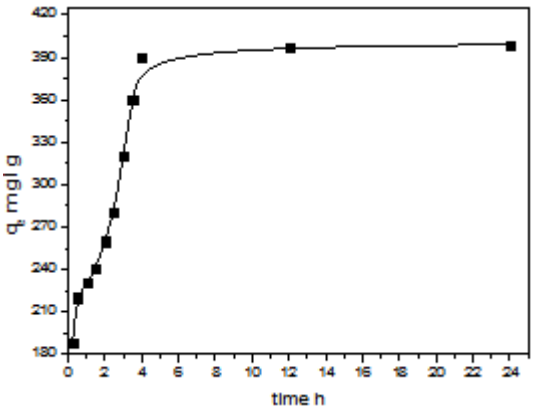

Fig 2. Effect of time on malachite green biosorption unto Spirogyra sp

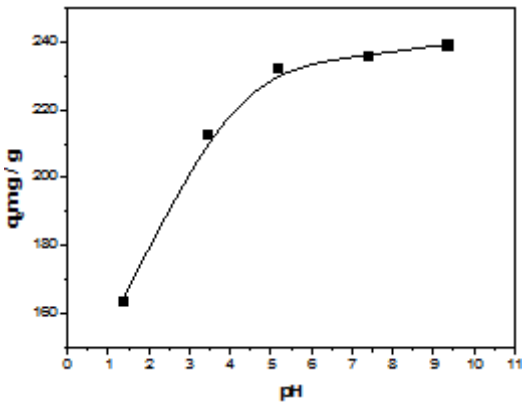

Fig 3. Effect of pH on the biosorption of malachite green by Spirogyra sp

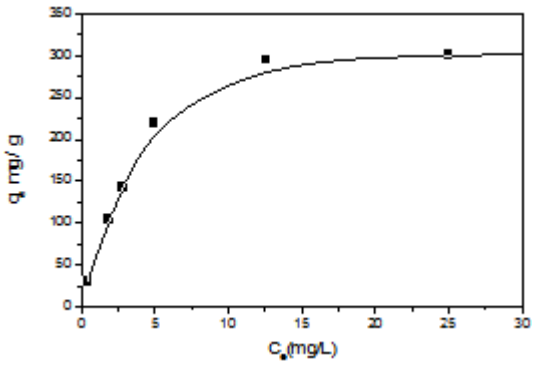

Fig 4. Biosorption isotherm of malachite green by Spirogyra sp.

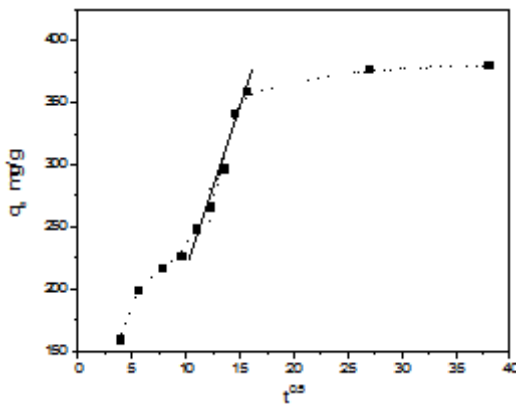

Fig 5. Intraparticle diffusion of malachite green by Spirogyra sp

by the intraparticle diffusion model to elucidate the diffusion mechanism [21].

$$q_t = k_{id} t^{1/2} + C \quad \ldots (6)$$

where q_t is the amount adsorbed at time t, and kid (mg/g min 0.5)) is the rate constant for intra-particle diffusion. The intercept, C, in a plot of q_t versus $t^{1/2}$ represents the thickness of the boundary layer. If C is zero then intra-particle diffusion is the sole rate-limiting step, and the larger the value of I the greater the contribution of surface biosorption. The plot of q_t (mg/g) versus $t^{1/2}$ is shown in Fig. 5. The plots were not linear over the whole time range, but could be separated into three linear regions, which suggests that there are multiple stages to the biosorption process. The R^2 value >0.9, which indicates that biosorption of malachite green can be described by intra-particle diffusion, but since these lines do not pass through the origin, we can conclude that intra-particle diffusion is not the sole rate controlling step.

CONCLUSIONS

Equilibrium and kinetic studies were carried out for the removal of malachite green from aqueous solution by sorption onto *Spirogyra* sp biomass. The biosorption kinetics followed pseudo-second-order model indicating chemisorption. Intraparticle diffusion was not the sole rate controlling step. Malachite green biosorption followed Langmuir isotherm models. The values of the Langmuir separation factor, R_L, indicated that the dye/*Spirogyra* sp biomass-system was a favorable biosorption

ACKNOWLEDGMENTS

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group project No RGP-VPP-184.

REFERENCES

1. Eiichi, I.; Ogawa, T.; Yatome, T. C.; Horisu, H.: Behavior of activated sludge with dyes. *Bulletin of Environmental Contamination and Toxicology* 1985, **35**; 729–734.
2. Hao, O. J.; Kim, H.; Chiang, P. C.: Decolorization of wastewater. *Environmental Science & Technology* 2000, **30**; 449–505.
3. Li, S.: Removal of crystal violet from aqueous solution by sorption into semi-interpenetrated networks hydrogels constituted of poly(acrylic acid-acrylamide-methacrylate) and amylose. *Bioresource technology* 2010, **101**; 2197-202.
4. He, H.; Yang, S.; Yu, K.; Ju, Y.; Sun, C.; Wang, L.: Microwave induced catalytic degradation of crystal violet in nano-nickel dioxide suspensions. *Journal of hazardous materials* 2010, **173**; 393-400.
5. Senthilkumaar, S.; Kalaamani, P.; Subburaam, C. V.: Liquid phase adsorption of Crystal violet onto activated carbons derived from male flowers of coconut tree. *Journal of hazardous materials* 2006, **136**; 800-8.
6. Jones, J. J.; Falkinham, J. O., 3rd: Decolorization of malachite green and crystal violet by waterborne pathogenic mycobacteria. *Antimicrob Agents Chemother* 2003, **47**; 2323-6.
7. Ghosh, D.; Bhattacharyya, K. G.: Adsorption of methylene blue on kaolinite. *Applied Clay Science* 2002, **20**; 295–300.
8. Akbal, F.: Adsorption of basic dyes from aqueous solution onto pumice powder. *Journal of colloid and interface science* 2005; **286**; 455-8.
9. Hameed, B. H.; El-Khaiary, M. I.: Sorption kinetics and isotherm studies of a cationic dye using agricultural waste: broad bean peels. *Journal of hazardous materials* 2008, **154**; 639-48.
10. Pengthamkeerati, P.; Satapanajaru, T.; Singchan, O.: Sorption of reactive dye from aqueous solution on biomass fly ash. *Journal of hazardous materials* 2008, **153**; 1149-56.
11. Hameed, B. H.; El-Khaiary, M. I.: Kinetics and equilibrium studies of malachite green adsorption on rice straw-derived char. *Journal of hazardous materials* 2008, **153**; 701-8.
12. Hameed, B. H.; Hakimi, H.: Utilization of durian (Durio zibethinus Murray) peel as low cost sorbent for the removal of acid dye from aqueous solutions, Biochem.. *Engineering Journal* 2008, **39;** 338–343.
13. Han, R.; Ding, D.; Xu, Y.; Zou, W.; Wang, Y.; Li, Y.; Zou, L.: Use of rice husk for the adsorption of congo red from aqueous solution in column mode. *Bioresource technology* 2008, **99**; 2938-46.
14. Khataee, A. R.; Vafaei, F.; Jannatkhah, M.: Biosorption of three textile dyes from contaminated water by filamentous green algal *Spirogyra* sp.: Kinetic, isotherm and

thermodynamic studies. *International Biodeterioration & Biodegradation* 2013, **83**; 33-40.

15. Mohan, S. V.; Ramanaiah, S. V.; Sarma, P. N.: Biosorption of direct azo dye from aqueous phase onto *Spirogyra* sp. I02: evaluation of kinetics and mechanistic aspects. . *Biochemical Engineering Journal* 2008, **38**; 61-69.
16. Senthilkumaar, S.; Varadarajan, P. R.; Porkodi, K.; Subbhuraam, C. V.: Adsorption of methylene blue onto jute fiber carbon: kinetics and equilibrium studies. *Journal Colloid Interface Science* 2005, **284**; 78-82.
17. Ncibi, M. C.; Mahjoub, B.; Seffen, M.: Kinetic and equilibrium studies of methylene blue biosorption by *Posidonia oceanica* (L.) fibres. *J Hazardous materials* 2007, **139**; 280-5.
18. Ho, Y. S.; McKay, G.: Sorption of dye fromaqueous solution by peat. *Chemical Engineering Journal* 1998, **70;** 115–124.
19. Hall, H. R.; Eagleton, L. C.; Acrivos, A.; Vermeulen, T.: Pore- and solid-diffusion kinetics in fixed-bed adsorption under constant-pattern conditions. *Industrial & Engineering Chemistry Fundamentals* 1966, **5**; 212–223.
20. Rosa, S.; Laranjeira, M. C.; Riela, H. G.; Favere, V. T.: Cross-linked quaternary chitosan as an adsorbent for the removal of the reactive dye from aqueous solutions. *Journal of hazardous materials* 2008, **155**; 253-60.
21. Ho, Y. S.; McKay, G.: Kinetic model for lead(II) sorption on to peat. *Adsorption Science & Technology* 1998, **16**; 243–255.