APPLICATIONS OF MICROALGAE IN WASTEWATER TREATMENT. EXPERIMENTAL AND EQUILIBRIUM STUDIES

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Abstract: *The aim of this study was to investigate the capacity of Nannochloropsis oculata (microalgae) and Spirulina (cyanobacteria) biomass for Rhodamine B and Methylene blue removal from aqueous solutions. The results reveal that Nannochloropsis oculata was more efficient for Rhodamine B and Methylene blue removal, than Spirulina. The most favourable biosorption of Rhodamine B onto Nannochloropsis oculata and Spirulina was obtained at pH 4 and pH 1, respectively. The optimum pH for Methylene blue removal by Nannochloropsis oculata and Spirulina was 1. The experimental data were analysed using Langmuir, Freundlich, and Dubinin-Radushkevisch isotherm models. The Freundlich model fitted better the experimental results for the removal of Rhodamine B onto Nannochloropsis oculata and Spirulina biomass. Langmuir isotherm model suggested the monolayer coverage of Methylene blue molecules onto Nannochloropsis oculata. Dubinin-Radushkevich isotherm indicated a chemically process involved in Rhodamine B removal onto Nannochloropsis oculata and Spirulina. In case of Methylene blue removal onto Spirulina, Dubinin-Radushkevich isotherm indicated a physically process.*

Keywords: *Nannochloropsis oculata, Spirulina, Methylene blue, Rhodamine B, biosorption*

1. Introduction

The production and usage of synthetic dyes have increased worldwide due to their high stability and cost-effectiveness in synthesis compared with natural dyes [1]. Thus, the discharge of coloured wastes has become one of the main sources of environmental pollution.

The presence of dyes in water can cause significant changes, including a decrease in the photosynthetic activity and dissolved oxygen, an alteration of the pH, an increase in the biochemical oxygen and chemical oxygen demand, in aquatic life. Also, can exhibit chronic effects towards biota, such as mutagenic damage and carcinogenicity [1, 2, 3]. It has been found that above the concentration of 1 mg/L, the dye is visible in the wastewater and typically the concentration of dyes found in textile wastewater is in the range of 10 to 50 mg/L [3].

The Rhodamine dye is a synthetic dye used as a colorant in textile and food industry. Its presence in drinking water could lead to subcutaneous tissue borne sarcoma [4].

Methylene blue (MB) is a cationic dye used in chemistry, biology, medical science and textile industry. Its long term exposure can cause vomiting, nausea, anemia and hypertension affecting human life [5].

Various physical/chemical methods have been investigated for dyes removal from wastewaters [1]. Among these, the adsorption methods appear to be an efficient alternative for the removal of a wide range of compounds [6].

The application of low cost adsorbents has been extensively applied for colour removal but the results showed that they failed to achieve high adsorption capacity.

Microalgae appears to have an advantage due to the use of wastewater as a low cost nutrient source for its production [7].

Several algae were used for dyes removal, such as dead biomass of *Spirogyra*, living biomass of microalgae *Caulerpa lentillifera* and *Caulerpa scalpelliformis*, Chlorella vulgaris, dry and wet biomass of *Chlorella pyrenoidosa* [7].

The aim of the present study was to evaluate the potential application of *Nannochloropsis oculata* microalgae and *Spirulina* (cyanobacteria) biomass for Rhodamine B and Methylene blue removal from aqueous solutions by taking into account the effect of adsorbent dosage and pH.

2. Experimental protocol

The experiments were performed in batch conditions, contacting different quantities of *Nannochloropsis oculata and Spirulina* (1 – 5 g) with 50 mL dye solutions (Rhodamine B and Methylene blue). All the experiments were carried out at the same initial concentration (50 mg/L) and stirring rate (75 rpm) for 240 min. After the equilibrium was reached, the dye solutions were separated from the biomass by centrifugation at 3600 rpm for 10 min. The concentration of Rhodamine B and Methylene blue in solution was determined using Lambda 25 Perkin-Elmer UV/VIS spectrophotometer at 665 nm and 554 nm, respectively.
The dyes amount in the adsorbent phase, q_e (mg/g), was calculated using equation (1), while dyes removal efficiency, E (%) was calculated using equation (2):

$$q_e=\frac{(C_0-C_e)}{m}\cdot\frac{V}{1000} \tag{1}$$

$$E(\%)=\frac{(C_0-C_e)}{C_0}\cdot 100 \tag{2}$$

where, q_e is the amount of dye adsorbed per gram of adsorbent at equilibrium (mg/g), V is the volume of solution (mL), m is the weight of the adsorbent (g), C_e is the equilibrium dye concentration (mg/L) and C_0 is the initial dye concentration (mg/L) [7].

2.1 Materials

Nannochloropsis oculata and *Spirulina* as powder were used as received without any further purification.

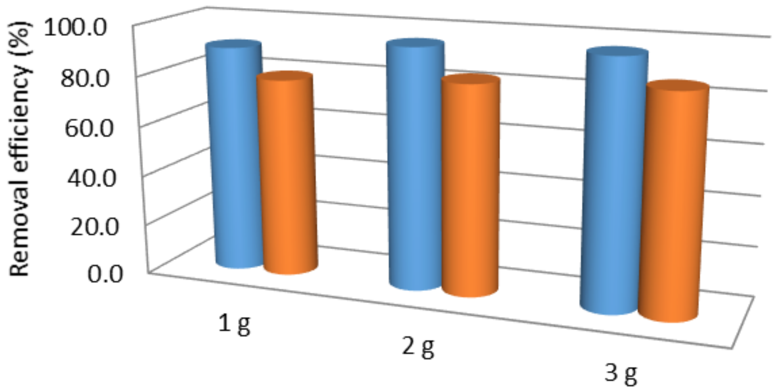
2.2 Chemicals

Rhodamine B and Methylene blue were purchased from Merck, Germany. The calibration standards (2-10 mg/L) and the initial concentration of 50 mg/L were prepared by diluting a stock solution of 100 mg/L Rhodamine B and Methylene blue. All used chemicals were of analytical purity and used as received without any further purification. The pH adjustments were carried out using dilute 1M NaOH and 1N HCl solutions.

3. Results and discussion

3.1 Influence of adsorbent quantity

To determine the influence of adsorbent doses for Rhodamine B and Methylene blue biosorption, the experiments were performed by contacting different quantities of *Nannochloropsis oculata and Spirulina* (1 – 5g) with 50 mL dye solutions (50 mg/L) at room temperature (22 ± 2°C) for 240 min.
The results obtained for Rhodamine B and Methylene blue biosorption onto *Nannochloropsis oculata and Spirulina* are given in Fig. 1 and Fig. 2, respectively.
The percent removal of Rhodamine B (Fig. 1) onto *Nannochloropsis oculata* and *Spirulina* increased as the adsorbent quantity increased from 1 to 3 g at 50 mg/L dye concentration on equilibrium time. This can be due to the number of available biosorption sites. Thus, the surface area increase by increasing the adsorbent quantity reflecting an increase in the amount of adsorbed dye [7].
It was noted that there was 95.1% removal efficiency for Rhodamine B dye removal at 2 g *Nannochloropsis oculata* and 96.4 at 3 g of *Nannochloropsis oculata* (smaller difference in term of removal efficiency can be seen). Therefore, 2g of microalgae biomass was chosen as optimum quantity for further experiments.
In case of Rhodamine B removal onto cyanobacteria *Spirulina* smaller removal efficiency values were obtained.



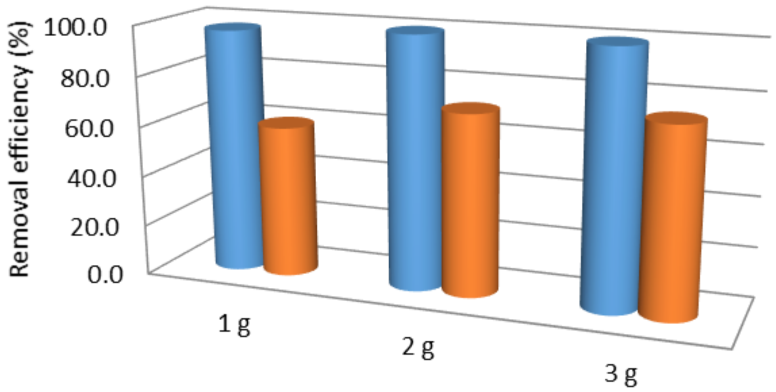
	1 g	2 g	3 g
Nannochloropsis oculata	90.4	95.1	96.4
Spirulina	79.0	82.7	85.4

Fig. 1. Rhodamine B removal onto *Nannochloropsis oculata* and *Spirulina* at different adsorbent quantities. C_0 = 50 mg/L, m = 1 - 5 g, pH = 4, V = 50 mL, t = 240 min

As it can be seen from Fig. 2, the percent removal of Methylene blue onto *Nannochloropsis oculata* and *Spirulina* increased as the adsorbent quantity increased from 1 to 3 g at 50 mg/L dye concentration. The assumption made in case of Rhodamine B removal can be attributed also in this case (the surface area increase by increasing the adsorbent quantity).

It was noted that there was 99.9% removal of Methylene blue dye at 2 g and 3 g of *Nannochloropsis oculata*, respectively.

For Methylene blue removal onto *Spirulina* the removal efficiency values were much smaller than the values obtained for Rhodamine B removal on *Spirulina*.

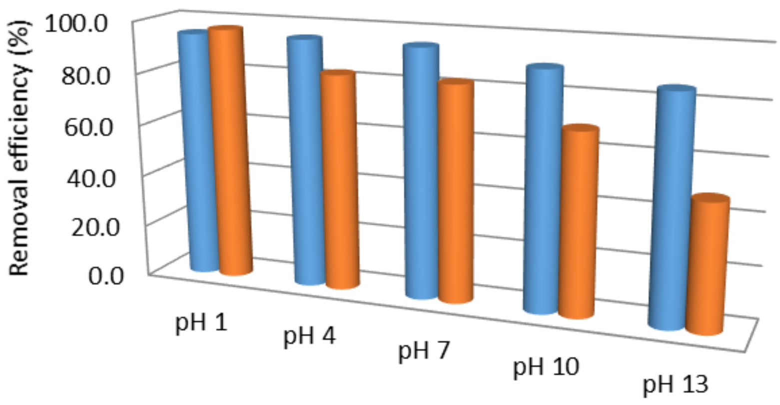


	1 g	2 g	3 g
Nannochloropsis oculata	97.2	99.9	99.9
Spirulina	60.1	71.6	73.6

Fig. 2. Methylene blue removal onto *Nannochloropsis oculata* and *Spirulina* at different adsorbent quantities. C_0 = 50 mg/L, m = 1 - 5 g, pH = 4, V = 50 mL, t = 240 min

3.2 Influence of pH

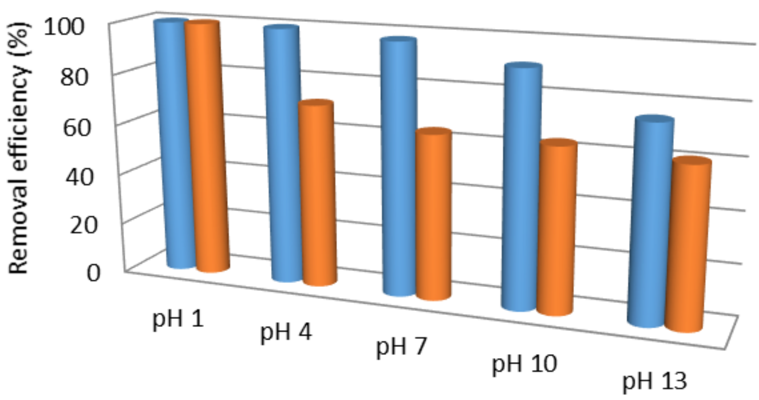
To determine the influence of pH for Rhodamine B and Methylene blue biosorption, the experiments were performed by contacting 2 g of *Nannochloropsis oculata* and *Spirulina* with 50 mL dye solutions (50 mg/L) at different pH values (1 - 13) at room temperature (25 ± 2°C) for 240 min.
The results obtained for Rhodamine B and Methylene blue biosorption onto *Nannochloropsis oculata and Spirulina* are given in Fig. 3 and Fig. 4, respectively.



	pH 1	pH 4	pH 7	pH 10	pH 13
Nannochloropsis oculata	94.8	95.1	94.8	89.9	85.3
Spirulina	97.1	82.7	82.3	68.9	48.0

Fig. 3. Rhodamine B removal onto *Nannochloropsis oculata* and *Spirulina* at different pH values. C_0 = 50 mg/L, m = 2 g, pH = 1 - 13, V = 50 mL, t = 240 min

The most favourable biosorption of Rhodamine B onto *Nannochloropsis oculata* and Spirulina was obtained at pH 4 and pH 1 (Fig.3), respectively.



	pH 1	pH 4	pH 7	pH 10	pH 13
Nannochloropsis oculata	100	99.9	97.7	90.9	75.0
Spirulina	100	71.6	64.2	63.7	61.2

Fig. 4. Methylene blue removal onto *Nannochloropsis oculata* and *Spirulina* at diferent pH values. C_0 = 50 mg/L, m = 2 g, pH = 1 - 13, V = 50 mL, t = 240 min

The optimum pH for Methylene blue removal by *Nannochloropsis oculata* and *Spirulina* was 1 (Fig.4). This can be due to the increase of positively charged adsorbent surface sites at the expense of the number of negatively charged surface sites, at lower pH values. Therefore, with an increase of pH a decrease of adsorption capacity was observed (the electrostatic repulsion between the positively charged surface and the positively charged dye molecule increased) [7].

3.3. Equilibrium studies

In order to investigate the Rhodamine B and Methylene blue removal the experimental results were analysed by taken into account the Langmuir, Freundlich and Dubinin-Radushkevich isotherm models.
The Langmuir isotherm was applied in order to determine if the biosorption process occurred onto a monolayer surface [8]. The linear form of the Langmuir isotherm is given in Table 1, where K_L is the Langmuir adsorption constant (L/mg) and q_{max} is maximum amount of dye adsorbed per gram of adsorbent (mg/g) [9].

Table 1: Linear forms of isotherm models

Isotherm model	Linear form	Plot
Langmuir	$\frac{1}{q_e}=\frac{1}{q_{max}K_LC_e}+\frac{1}{q_{max}}$	$\frac{1}{q_e}$ vs. $\frac{1}{C_e}$
Freundlich	$\log q_e=\log K_F+\frac{1}{n}\log C_e$	$\log q_e$ vs. $\log C_e$
Dubinin-Radushkevich	$\ln q_e= \ln q_{max}-\beta\varepsilon^2$ $\varepsilon = RT\ln(1+\frac{1}{C_e})$ $E_L = \frac{1}{\sqrt{-2\beta}}$	$\ln q_e$ vs. ε^2

Langmuir parameters, q_{max} and K_L, obtained from the slope and intercept of the plot $1/q_e$ *vs.* $1/C_e$ are presented in Table 2.
Freundlich isotherm was applied in order to determine if the biosorption process occurs on a heterogeneous surface [10]. Its linear form is given in Table 1, where, K_F is the adsorption capacity (L/mg), $1/n$ is the adsorption intensity [11]. If n value is below 1 it indicates a normal adsorption, > 1 indicates cooperative adsorption and if 1< n < 10 indicates a favourable adsorption process [12].
Freundlich parameters, K_F and n, obtained from the $\log q_e$ *vs*. $\log C_e$ linear plot are presented in Table 2.
The n value obtained for Rhodamine B onto *Nannochloropsis oculata* was found to be lower than 1, indicating a normal biosorption. Also, Freundlich isotherm parameter (n value) indicated a normal biosorption of Methylene blue removal onto *Nannochloropsis oculata* and *Spirulina.* The n value obtained for Rhodamine B onto *Spirulina* was found to be greater than 1, indicating a favourable biosorption of the considered dye.
Based on the correlation coefficient values (R^2), it can be concluded that Freundlich model fitted better the experimental results for the removal of considered dyes except for Methylene blue removal onto *Nannochloropsis oculata* (in this case Langmuir model fitted better the experimental results).
The Dubinin-Radushkevich model was applied to determine the nature of the biosorption process (physically if E_L < 8 kJ/mol or chemically if E_L value lies between 8 kJ/mol and 16 kJ/mol) [13, 14]. Its equations are given in Table 1, where β is Dubinin Radushkevich constant (mol^2/kJ^2), R is the gas constant (8.314 J/mol·K), T is the absolute temperature (K), ε is the Polanyi potential and E_L is the mean adsorption energy (kJ/mol) (Ayawei et al. 2017).
Dubinin-Radushkevich parameters, q_{max} and β, obtained by plotting $\ln q_e$ *vs.* ε^2 are given in Table 2.

Table 2: Langmuir, Freundlich and Dubinin-Radushkevich isotherm parameters for *Nannochloropsis oculata* and *Spirulina* at different adsorbent quantities.

		Rhodamine B		Methylene blue	
Isotherm model	Parameters	*Nannochloropsis oculata*	*Spirulina*	*Nannochloropsis oculata*	*Spirulina*
Langmuir	q_{max}/(mg/g)	16.23	1.80	142.86	13.87
	K_L/(L/mg)	0.03	0.61	0.13	0.04
	R^2	0.9864	0.9841	0.9483	0.8976
Freundlich	n	0.95	4.65	0.79	0.41
	K_F/(L/mg)	2.26	3.54	21.40	41.73
	R^2	0.9915	0.9961	0.9280	0.9570
Dubinin-Radushkevich	β/(mol^2/ kJ^2)	$7\cdot10^{-9}$	$2\cdot10^{-9}$	$8\cdot10^{-9}$	$2\cdot10^{-8}$
	E_L/(kJ/mol)	8.5	15.8	7.90	5.0
	R^2	0.9931	0.9954	0.9233	0.9582

As it can be seen from Table 2 the mean free energy value indicated a chemically process involved in Rhodamine B removal on *Nannochloropsis oculata* and *Spirulina.* In case of Methylene blue removal onto *Nannochloropsis oculata* and *Spirulina,* the mean free energy value indicated a physically process.

4. Conclusions

In this paper, the use of *Nannochloropsis oculata* microalgae and cyanobacteria *Spirulina* was investigated for Rhodamine B and Methylene blue dyes removal from aqueous solutions.
The results revelled that the percent removal of Rhodamine B and Methylene blue onto *Nannochloropsis oculata* and *Spirulina* increased as the adsorbent quantity increased.
By studying the effect of pH the removal efficiency values decreased as the initial pH increased.
The Freundlich parameter suggested that the removal of Rhodamine B onto *Nannochloropsis oculata* and *Spirulina* biomass occurred on a heterogeneous surface.
Langmuir isotherm model suggested the monolayer coverage of Methylene blue onto *Nannochloropsis oculata.*
Also, was found that a physically process was involved in Rhodamine B removal onto *Nannochloropsis oculata* and *Spirulina.* In case of Methylene blue removal onto *Spirulina* a chemically process was involved and a physically one onto *Nannochloropsis oculata.*
Based on the obtained results, *Nannochloropsis oculata* and *Spirulina* biomass have been proven to be potential dye biosorbents for Rhodamine B and Methylene blue removal.

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