Synergistic Methodology Based on Ion Exchange and Biodegradation Mechanisms Applied for Metal Complex Dye Removal from Waste Waters

NICOLETA MIRELA MARIN[1,2], OLGA TIRON[2], LUOANA FLORENTINA PASCU[2], MIHAELA COSTACHE[3], MIHAI NITA LAZAR[2], IRINEL ADRIANA BADEA[1]*

[1]University of Bucharest, Faculty of Chemistry, Department of Analytical Chemistry, 2-14 Regina Elisabeta Blvd., 030018, Bucharest, Romania

[2]National Research and Development Institute for Industrial Ecology ECOIND, 71-73 Drumul Podu Dambovitei Str., 060652, Bucharest, Romania

[3]University Bioterra Bucharest, Faculty of Food Engineering Chemistry, Department of Food Technologies, 81 Garlei Str., Bucharest, Romania

This study investigates the synergistic effects of ion exchange and biodegradation methods to remove the Acid Blue 193 also called Gryfalan Navy Blue RL (GNB) dye from wastewater. Ion exchange studies were performed using a strongly basic anion exchange resin Amberlite IRA 400. The equilibrium was characterized by a kinetic and thermodynamic points of view, establishing that the sorption of the GNB dye was subject to the Freundlich isotherm model with $R^2 = 0.8710$. Experimental results showed that the activated resin can removed up to 93.4% when the concentration of dye solution is $5.62 \cdot 10^{-2}$ mM. The biodegradation of the GNB was induced by laccase, an enzyme isolated from white-rot fungus. It was also analyzed the role of pH and dye concentration on GNB biodegradation, so $5 \cdot 10^{-2}$ mM dye had a maximum discoloration efficiency of 82.9% at pH of 4. The laccase showed a very fast and robust activity reaching in a few minutes a Km value of $2.2 \cdot 10^{-1}$ mM. In addition, increasing the GNB concentration up to $8 \cdot 10^{-1}$ mM did not triggered a substrat inhibition effect on the laccase activity. Overall, in this study we proposed a mixt physicochemical and biological approach to enhance the GNB removal and biodegradability from the wastewaters and subsequently the environment.

Keywords: Gryfalan Navy Blue RL, anion-exchange equilibrium, isotherm, kinetics, laccase

Chromate(3-)bis[3-hydroxy-4-[(2-hydroxy-1-naphthalenyl)azo]1naphthalenesulfonato(3-)] sodium hydrogen (1:2:1), also known as Gryfalan Navy Blue RL, Acid Blue 161 or Acid Blue 193 (GNB) is a 1:2 metal complex azo dye obtained in two steps: first through an azo coupling reaction between 4-amino-3-hydroxy-naphthalene-1-sulfonic acid and naphthalen-2-ol and second by a complexation with Cr(III). Its structure reveals a highly stable compound, which is difficult to biodegrade in natural conditions. Therefore, many problems are related to the environmental pollution [1-11] with azo dyes generating human health issues, since most of these dyes are both mutagenic and carcinogenic [12. 13]. This is one of the reasons why the European Parliament had included 22 aromatic amines resulted from some azodyes degradation into the Directive 2006/61/EC [14] and banned from production some azo dyes which could lead by degradation to these aromatic amines. Thus, finding cheaper and easy-to-be employed procedures for the decontamination of azo dyes polluted waters is still a challenging.

Nowadays they are many biological methods [15, 16] as well as chemical and physical, to monitor and remove dyes from waste waters such as coagulation [17], chemical oxidation [18], membrane filtration [19], solvent extraction [20], chemical precipitation [21], flotation [22] and biological degradation [23]. The decontamination process by ion-exchange or polymeric resins remains one of the most efficient ways to remove dyes from waste waters due to several advantages such as high efficiency, simple operation and easy recovery/reuse of the material [24, 25].

Biological methods (biodegradation) in combination with physical and [21] chemical methods for dyes removal could increase the treatment performance and decrease operational costs [26]. White-rot fungi were proved to be the most efficient microorganism for dyes biodegradation [27] due to their non-specific extracellular enzymatic system composed mainly by laccases, lignin peroxidases (LiP) and manganese peroxidases (MnP) [28]. Laccases represent multi-copper oxidases that are able to catalyze the oxidation of a wide diversity of complex substrates [29].

Therefore, in this paper proposed GNB removal of from various polluted waters using a combined ion-exchange (physicochemical) and biological methods. First, the ion-exchange study was carried out on a strongly basic anion exchange resin Amberlite IRA 400. The sorption equilibrium and factors affecting have been investigated. Kinetic and thermodynamic studies have been also performed and the system at equilibrium has been described by a mean of consecrated models. Second, it was investigated the biological removal (biodegradation) efficiency of GNB using laccase from *Trametes versicolor* white-rot fungi. The influence of GNB concentration and the optimal pH value for the laccase enzymatic activity was analyzed by the efficiency of the discoloration process. Biological removal step was proposed as an additional step to increase the dye removal efficiency after the ion exchange process.

* email: irinel.badea@g.unibuc.ro; Phone: (+40)745051286

Experimental part

Materials and methods

Reagents

Analytical grade reagents were used in this study. Amberlite IRA400, a strongly basic anion exchange resin based on styrene/divinylbenzene matrix (particle size 20-25 mesh) modified with quaternary ammonium functional group was purchased from Sigma-Aldrich. 37% hydrochloric acid (ρ= 1.19 g/mL), NaOH pellets and *Trametes versicolor* Laccase (specific activity 0.53 U/mg) were purchased from Sigma-Aldrich. Gryfalan Navy Blue RL (produced in Poland) was kindly donated by The National Research & Development Institute for Textiles and Leather, Bucharest.

UV-Vis measurements

The UV-Vis spectra were recorded using a Jasco V 530 double beam spectrometer controlled by a PC computer with Spectra Manger software. The discoloration was monitored with a UV/Vis spectrophotometer DR/5000™ (Hach Lange, Germany). The UV-VIS spectra of the working solutions were recorded in the range 200-800 nm and the Bouguer-Lamber-Beer law was checked at 578 nm, where GNB has a maximum of absorbance in the UV-Vis spectrum. The calibration graph was constructed and it was linear.

Ion-exchange experiments were carried out using a horizontal plane mechanical shaker (GFL 3006, Germany). Ultrapure water (18 MΩ·cm) was prepared using the Ultra Clear system (Evoqua Water Technologies).

Methodologies

Determination of GNB by UV-VIS spectrometry

An aqueous stock solutions of GNB was prepared by quantitatively transferred of 0.0117 g dye in 50 mL volumetric flask filled up to the mark with demineralized water.

For instance, working solutions were prepared in 25 mL volumetric flasks by dilution of various volumes of stock solution. UV-Vis spectra of these solutions were recorded and the absorbances were determined at 578 nm.

Ion exchange studies

The strongly basic anion exchange resin Amberlite IRA 400 was washed and swelled with ultrapure water and further activated with 4M HCl solution followed by a final washing with ultrapure water up to a negative reaction for the chloride ion (tested with a $Ag_2(NO_3)_2$ 0.02 M solution). At the end, the resin was filtered and left to dry for 48 hours. Ion-exchange studies were carried out using an amount of 0.0100 g strongly basic anion exchange resin Amberlite IRA 400 placed in Erlenmeyer flasks which containing various initial concentrations of GNB dye 23.4, 46.8, 93.6, 187.2 and 234 mg/L respectively . The samples were shaken at 175 rpm for one hour at room temperature then all samples were filtered and the concentration of GNB from filtrate was determined at 578 nm by means of a linear regression equation.

Influence of pH on the stability of the resin loaded with GNB

A quantity of 0.1 g of resin loaded with GNB was stirred for 1h in buffer solutions having the following *p*H values: 1.00, 2.00, 7.00, 13.00 or 14.00. The samples were filtered and the quantity of the dye in filtrate was determined by UV-Vis spectrometry.

GNB discoloration tests

Discoloration experiments were conducted in two steps: i) setting the most efficient experimental conditions; ii) analyzing the role of dye concentration by the enzyme activity. In the first step, the dye biodegradation induced by the laccase enzyme was monitored as a discoloration recorded spectrophotometrically at an absorbance of 578 nm. The assays were carried out at different *p*H values ranging between 2.0 and 9.0 (with a difference of one unit) to analyze the *p*H influence on fungal enzymes activity and implicitly on enzymatic discoloration processes. The following buffer solutions were used to reach specific *p*H values: 0.1 M sodium-citrate buffer for *p*H 2.0; 0.1 M citrate-phosphate buffer for *p*H 3.0, 4.0, 5.0, 6.0 and 7.0; 0.1 M succinate buffer for *p*H 8.0, and 0.1 M sodium-tartrate buffer for *p*H 9.0.

Assays were performed directly in spectrophotometer cuvettes at room temperature, in darkness and in static conditions. Reaction mixture consisted of 1600 µL buffer solution, 200 µL GNB dye (10^{-2} mM, final concentration) and 200 µL laccase solution (0.1 U/mL) prepared in 0.1 M citrate-phosphate buffer (*p*H 4.0). Discoloration efficiency was calculated by monitoring the absorbance read at 578 nm during 340 min compared to control, GNB without enzyme, or one chosen *p*H value, or time 0, or (note: please chose the right set of conditions/controls).

The second step was carried out at the optimum *p*H value set before to give the highest GNB discoloration reaction. The aim of this step was to identify the effect of GNB concentration on discoloration efficiency. As a result, dye concentration was consecutively increased from 10^{-2} mM to 2.5 x10^{-2} mM, 5 x 10^{-2} mM, 1.5 x10^{-1} mM, 4 x 10^{-1} mM and 8 ·x10^{-1} mM. Tested GNB concentrations were selected by taking into consideration the residual concentrations of dye recorded after sorption process on ion-exchange resin. Discoloration efficiency was determined by recording the absorbance of reaction mixture from 400 to 700 nm during 1440 min applying the frequency set up during the first step, excepting the fact that the next reading after 340 min of reaction being performed at 1440 min. The results were used to calculate color removal efficiency by using the following formula proposed by Rodriguez-Couto et al. [27]:

$$D(\%) = \frac{(A_0 - A_t)\cdot 100}{A_0} \quad (1)$$

where: D (%) represents discoloration efficiency determined at the time *t*, A_0 is area of the absorption spectrum from 400 to 700 nm at the initial time and A_t represents area of the absorption spectrum from registered from 400 to 700 nm at the time *t*. The measurements were performed in the same standard conditions as those described in the first step and using the buffer solution whose *p*H value was identified as optimal. Taking into consideration that enzymatic catalysis process transforms substrate's chemical, it was decided to calculate discoloration efficiency based on the absorption spectra obtained from 400 to 700 nm rather than monitoring dye absorbance at 578 nm. Discussed here-by results represent the average of two replicates, experimental error being below 8%.

Results and discussions

Detection of GNB concentrations by UV-Vis spectrometry

The linearity of the method was established on the basis of calibration graph. We have found that our experimental data could be expressed by the equation A = 13.9760xC+ 0.0033, R^2 = 0.9998, absorbance and concentration, respectively. If the Lambert-Beer law was obeyed then the

spectrometric determination could be used for further evaluation of GNB in solution.

Effect of contact time.

The influence of contact time on GNB sorption on a strongly basic anion exchange resin Amberlite IRA 400 was studied by batch method in the time range from 0 to 90 min (fig. 1). Results showed that the sorption changed almost linearly during the first 50 min, and then no significant variations were observed after 65 min. Based

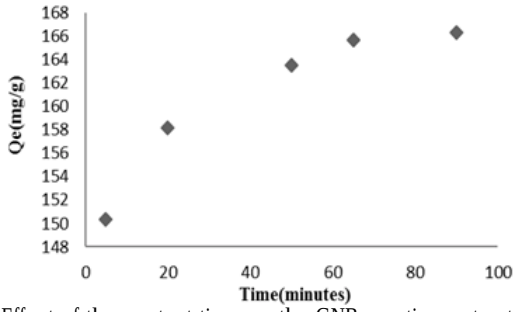

Fig. 1. Effect of the contact time on the GNB sorption onto strongly basic anion exchange resin Amberlite IRA 400

on these results all the next sorption experiments were carried out at a contact time of 65 min.

Thermodynamic studies

Experimental results obtained at equilibrium were analyzed using sorption models described by Langmuir, Freundlich, Temkin and Dubinin-Radushkevich isotherm [30, 31]. The characteristic parameters of each isotherm were obtained using their linear forms. The amount of GNB at equilibrium (Q_e) was calculated with the following formula:

$$Q_e = \frac{(C_0 - C_e)V}{m} \quad (2)$$

where: C_o and C_e are the GNB concentration in the initial and equilibrium solutions (mg/L); m is the mass of dry resin (g), V is the GNB volume initially entered in the study (L).

The sorption capacity at time t, was calculated with the following formula:

$$Q_t = \frac{(C_i - C_t)V}{m} \quad (3)$$

C_i and C_t are the GNB concentration (mg/L) at the beginning and at time t (mg/L); m is the mass of dry resin (g), V is the GNB volume initially used in the study (L).

Langmuir model. Langmuir isotherm describes quantitatively the formation of a saturated monolayer of sorbent molecules on the outside surface of the adsorbent. The linear equation of the Langmuir model is described by the following equation:

$$\frac{C_e}{Q_e} = \frac{1}{bQ_0} + \frac{C_e}{Q_0} \quad (4)$$

where C_e is concentration of GNB at equilibrium in solution (mg/L), Q_o and b are Langmuir constants correlated with the sorption capacity (mg/g) and affinity sorbent (L/mg). An essential characteristic of the Langmuir equation is the R_L, separation factor which was described by the following equation:

$$R_L = \frac{1}{1 + bC_0} \quad (4.1.)$$

where C_o is the highest initial adsorbate concentrations (mg/L).

The values of separation factor show if the sorption process was: favorable $0 < R_L < 1$, un favorable $R_L > 1$, linear sorption $R_L = 1$ and irreversible sorption $R_L = 0$. The parameters obtained for this model are presented in table 1. The low value of the correlation coefficient ($R^2 = 0.2125$) suggests that the GNB sorption on the strongly basic anion exchange resin Amberlite IRA 400 cannot be described with an Langmuir isotherm type. Conversely, the R_L value is 0.28 and being in the range 0-1 at 25 ± 2°C indicates that the sorption of GNB on the strongly basic anion exchange resin Amberlite IRA 400 is thermodynamically favorable.

Freundlich model. The Freundlich isotherm can be used to describe the sorption characteristics on the heterogeneous surface when a multi-layer is formed. The linear logarithmic form of Freundlich isotherm is described by the following equation:

$$\ln Q_e = \ln K_f + \frac{1}{n} \ln C_e \quad (5)$$

where: K_f is the sorption capacity (L/g) and n is the sorption intensity (dimensionless) the values of K_f and n are presented in table 1. The value of the correlation coefficient ($R^2 = 0.8710$) suggests that sorption of GNB on the strongly basic anion exchange resin Amberlite IRA 400 fits better the Freundlich isotherm model.

Temkin model. Temkin and Phyzev model describes principally the interactions sorbent/sorbit and lain on the hypothesis that the heat of sorption (as function of temperature) will decrease linearly with the surface covered. Equation describing this model is:

$$Q_e = \frac{RT}{b} \ln (AC_e) \quad (6)$$

and can be linearized as:

$$Q_e = B \ln A + B \ln C_e \quad (6.1)$$

where: $B = \frac{RT}{b}$, b is the Temkin constant correlated with the sorption heat (J/mol), A is the constant of the Temkin isotherm (L/g), R is the universal constant of gas 8.314 J/(mol · K), while T is the absolute temperature (K).

Applicability of the Temkin isotherm has been studied by plotting of Q_e versus $\ln C_e$ the constants A and b being obtained from the linear regression equation. The experimental values of the constants obtained are shown in table 1.

Dubinin- Radushkevich model

Dubinin- Radushkevich isotherm has been applied in order to get information about the type of sorption whether it is physical, chemical or ion-exchange. The linear form of the isotherm is described by the equation:

$$\ln Qe = \ln qm - \beta \varepsilon^2 \quad (7)$$

qm is the sorption capacity of a theoretical monolayer (mg/g) and ε is the Polanyi potential which ca can be expressed as :

$$\varepsilon = RT \ln\left(1 + \frac{1}{C_e}\right) \quad (7.1)$$

C_e being the equilibrium concentration of GNB in the solution (mg/L), β the constant of the adsorption energy (mol^2/KJ^2), R the universal gas constant (KJ/(mol·K) and T is temperature (K). The value of E (the mean free energy) is used to estimate the type of sorption and is expressed as:

$$E = \frac{1}{\sqrt{2\beta}} \quad (8)$$

if E ranges between 8-16 KJ/mol, the sorption type is ion exchange, if it is ≤ 8 KJ/mol the sorption is a physical process and if it is > 16 KJ/mol the sorption is chemical. The high value of E obtained din this study being greater than16KJ/mol indicated anion exchange process followed by a p-p interaction among the aromatic rings existing in the resin structure and naphatlene rings of GNB.

Langmuir		Temkin-Phyzev	
Qo(mg/g)	435	A(L/mg)	1.71
b(L/mg)	0.01	b(J/mol)	52
R^2	0.2125	B	47
R_L	0.28	R^2	0.6913
Freundlich		**Dubinin – Radushkevich**	
Kf(L/g)	13.7	qm(mg/g)	95
1/n	0.63	β(mol^2/kJ^2)	9.00E-07
n	1.59	E(KJ/mol)	745
R^2	0.8710	R^2	0.5062

Table 1
LANGMUIR, FREUNDLICH, TEMKIN AND DUBININ-RADUSHKEVICK ISOTHERMS PARAMETERS OF THE GNB SORPTION ON THE STRONGLY BASIC ANION EXCHANGE RESIN AMBERLITE IRA 400

Kinetic studies

Four consecrated empirical models [31-33] have been used to evaluate the ion-exchange equilibrium and to understand the factors affecting the kinetic of this process. Usually, three factors are known to control the heterogeneous equilibria and they are mass transfer, film and intraparticle diffusion.

Morris-Weber kinetic model uses the equation:

$$q_t = k_{id}(t)^{0,5} \quad (9)$$

where q_t is the amount of GNB loaded on the ion exchange resin, k_{id} is the intraparticle diffusion rate constant and t is time expressed in minutes. This model shows a linear relation between q_t and $t^{0.5}$. We have found in our study a linear relationship between the two variables with a correlation coefficient value 0.9353. Thus, one can consider that this model describes with accuracy the studied ion-exchange equilibrium, which has the intraparticle diffusion as a rate-limiting step.

Lagergren kinetic model

This model has been used in order to evaluate the rate constant of the reaction between GNB and the strongly basic anion exchange resin. This model is described by the equation:

$$\log(q_e - q_t) = \log q_e - \left(\frac{k}{2,303}\right)t \quad (10)$$

where q_e and q_t represent the amount of GNB (mg/g) loaded on the ion exchange resin at constant rte, and t is the time. The plot of the log (qe-qt) vs t enables the evaluation of the rate constant. The value obtained is presented in table 2

Elovich kinetic model

The Elovich kinetic model equation is:

$$q_t = \ln(\alpha\beta) + \frac{1}{\beta}\ln t \quad (11)$$

where: α expressed in mg/(g·min) is the initial adsorption rate and β (mg/min) is the desorption constant during the experiment. The kinetic constants α and β were determined from the slope and intercept of the plot of qt versus lnt and are presented in table 2.

Pseudo-second order kinetic model

The sorption of GNB on the strongly basic anion exchange resin Amberlite IRA 400 was investigated using the following equations presented in table 2.

Where: qe and qt and are the quantity of GNB (mg/g) adsorbed at equilibrium and at time t respectively, and k_2 (g/mg·min) is the pseudo-second-order rate constant of sorption process. As shown in table 2 the correlation coefficient calculated for the pseudo-second-order kinetic model Type 1was 0.9999 meaning that this model fits better our experimental data

Effect of pH

The stability of the resin loaded with GNB was evaluated at different *p*H values. Buffer solutions of *p*H: 1.00, 2.00, 7.00, 13.00 and 14.00 were used in batch method as described in experimental section. The amount of dye found in solution was determined and it was found to be below the detection limit of this method. Overall the results showed that resin and dye complex was stable regard less of the *p*H values.

GNB dye color analysis

A volume of 15 mL solution of 0.60 mM GNB was added over 1 g of swollen resin. The samples were stirred for 1 h, filtered and the filtrates were analyzed using a CIE $L^*a^*b^*$ colour system. Colour difference ΔEL*a*b* between the sample analyzed before and after sorption process had a value of 74.6. It could be concluded that ion-exchange method removed effectively the GNB form polluted waters and contributes to the esthetical regeneration of water, too.

Lagergren equation	$K(min^{-1})$		q_e(mg/g)		R^2
	0.05		19.9		0.9340
Morris–Weber equation	$K_{id}(min^{-1})$				R^2
	2.12				0.9353
Elovich equation	α(mg/g·min)			β(g/mg)	R^2
	1.95			0.18	0.9912
Pseudo-second order equation	k_2 (g/(mg·min))				
				q_e(mg/g)	R^2
Type 1	$t/q_t = 1/(k_2q_e^2) + (1/q_e)t$	t/q_t vs t	0.007	167	0.9999
Type 2	$1/q_t = 1/q_e + 1/k_2q_e^2(1/t)$	1/qt vs1/t	0.012	164	0.9449
Type 3	$q_t = q_e - 1/k_2q_e(q_t/t)$	q_t vs q_t/t	0.012	165	0.9367
Type 4	$q_t/t = k_2q_e^2 - k_2q_e(q_t)$	q_t/t vs q_t	0.011	166	0.9367

Table 2
KINETIC PARAMETERS DESCRIBING THE SORPTION OF GNB ON AMBERLITE IRA 400

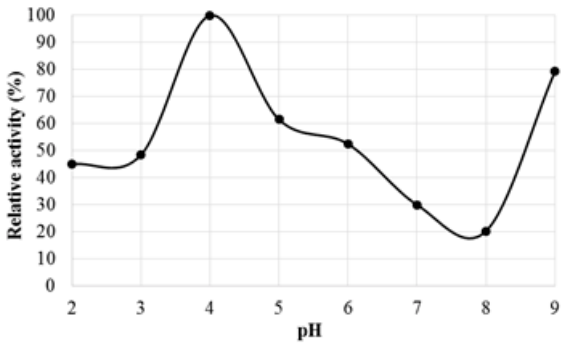

Fig. 2. Influence of *p*H value on GNB discoloration using laccase from *Trametes versicolor* (100% of relative activity is equivalent with maximal discoloration efficiency obtained after testing all experimental variants). The relative laccase activity was measured based on the control sample (GNB without enzyme).

Enzymatic GNB discoloration

Effect of pH on GNB discoloration efficiency

Initially it was established the optimal *p*H value to reach a maximum laccase activity and subsequently the highest GNB discoloration. The most suitable *p*H value for the laccase activity was at *p*H 4.0 (fig. 2) and this result sustained the fact that optimum pH for fungal dyes discoloration was usually found in acidic range [34].

However, it is worth to mention that GNB discoloration was also promoted a new peak of enzyme activity in alkaline condition at *p*H 9.0 (fig.2).

Effect of GNB concentration on discoloration efficiency

The GNB biodegradation conditions by *Trametes versicolor* laccase were set for an optimal *p*H value of $4.0.10^{-2}$mM GNB was incubated up to 1440 min in presence of 0.01U/mL final concentration of laccase (note 0.01U/mL: from mat&met 1600uL + 200uL + 200 uL enzyme of 0.1U/mL) (fig.3). The discoloration was spectrophotometrically monitored at a wavelength range from 400 to 700nm, but the optimal interval for further considerations was from 550 to 650 nm (fig. 3A). The results showed a fast increase in discoloration up to 400 min, then a slower pace up to 75.2% of the discoloration efficiency at 1440 min (fig. 3B). The reaction of the discoloration process followed a first order kinetics ($y = 0.2258x + 14.394$, $R^2 = 0.9912$) with respect to reaction time up to 1440 min (fig. 3C). During investigation time it was also found that highest laccase activity occurred in the first minute of discoloration reaction, at that time rate of laccase catalysis was 13×10^{-4}mM (decolored dye concentration) / min (data not shown). During the incubation time, the rate of enzyme catalysis significantly decreased, so after 10 min of reaction the rate was about 97% lower than the performance achieved in the first minute of discoloration process (fig. 3C).

In the next step, we analyzed the role of GNB concentration in modulating the laccase activity. It was observed an increased trend of GNB discoloration efficiency with an increased substrate concentration from $1 \cdot 10^{-2}$ mM to $5 \cdot 10^{-2}$ mM where the highest reached discoloration efficiency was at 82.9%. A similar degree of efficiency (82.5%) was recorded by testing GNB concentration of 1.5×10^{-1} mM (fig. 4A). However, a decreasing trend of discoloration efficiency continued while the GNB concentration raised from $4 \cdot 10^{-1}$mM to $8 \cdot 10^{-1}$mM reaching the lowest color removal efficiency to 65.3%.

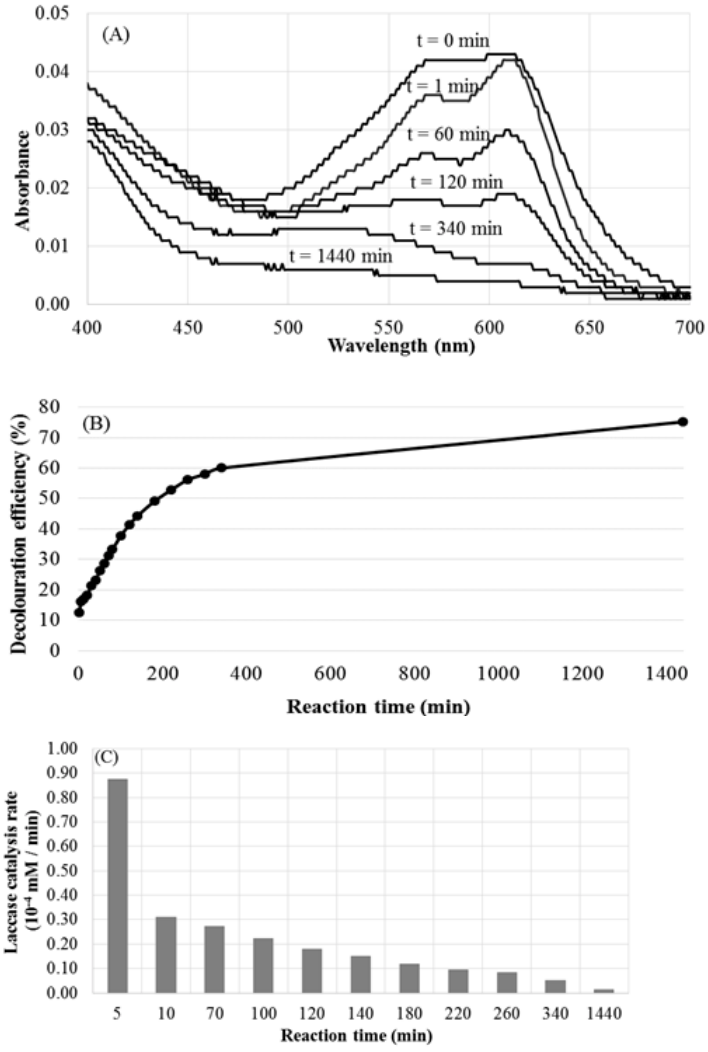

Fig. 3. Dynamics of the spectral curves recorded between 400 and 700 nm during 10^{-2} mM GNB discolorationat *p*H 4.0 (A), the trend of discoloration efficiency (%) with respect to reaction time (min) (B) and variation of the laccase catalysis rate (measured as decolored concentration per minute) during investigation time (C). The laccase catalysis rate was measures based on the absorbance area between 400 and 700nm during the time compared to the control t = 0 min

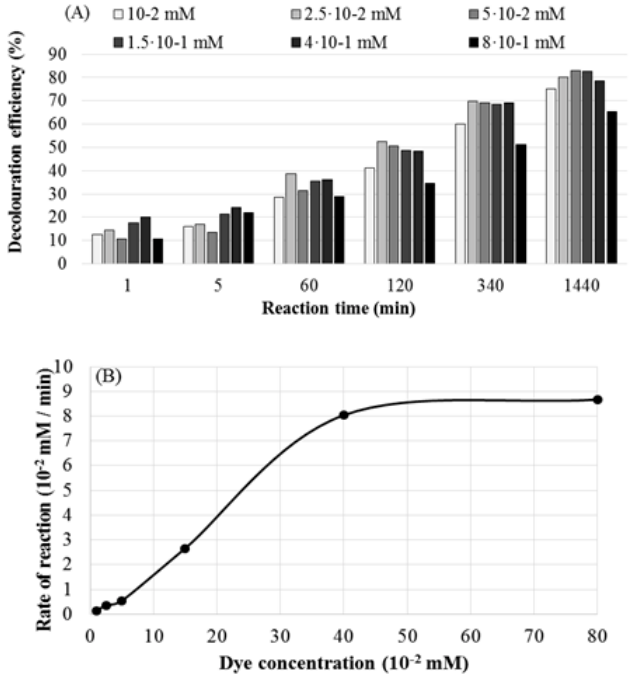

Fig. 4. Variation of GNB discoloration efficiency at different dye concentrations: 10^{-2} mM, 2.5 · 10^{-2} mM, 5 · 10^{-2} mM, 1.5 · 10^{-1} mM, 4 · 10^{-1}mM, 8 · 10^{-1}mM (A) and Michaelis-Menten model obtained by GNB discoloration by *Trametes versicolor* laccase (B)

C_s (mM)	C_e(mM)	Sorption (%)	C_b(mM)	Discoloration (%)
5.62 10^{-2}	0.35 10^{-2}	93.4	10^{-2}	75
1.12 10^{-1}	2.09 10^{-2}	81.4	2.5 10^{-2}	80
2.25 10^{-1}	5.07 10^{-2}	77.5	5 10^{-2}	82.9
4.49 10^{-1}	0.73 10^{-2}	83.7	1.5 10^{-1}	82.5
5.62 10^{-1}	1.16 10^{-1}	79.3	4 10^{-1}	78.6
-	-	-	**8 10^{-1}**	65.3

Table 3
EXPERIMENTAL RESULTS OF SORPTION AND BIOLOGICAL STUDIES

Overall, the 1 ·10^{-2} mM tested GNB concentration induced the highest discoloration efficiency performed per unit of time during the first minute of incubation with the enzyme. The ratio dye concentration (mM) *versus* discoloration rate (mM decolored dye concentration / min) obtained in the first minute of reaction followed Michaelis-Menten kinetics (fig. 4B) and the calculated value of kinetic constant (*Km*) for *Trametes versicolor* laccase was 2.2 · 10^{-1}mM (*Vmax* = 8.7 · 10^{-2}mM/min). Taking in consideration obtained result, it could be concluded that no inhibitory effect occurred on laccase activity up to 8 · 10^{-1}mM GNB concentration.

Comparative results of sorption and biological discoloration

Results of both sorption and biological discoloration experiments are presented in table 3, where: C_s(mM) and C_e(mM) are the GNB concentration of initial and equilibrium solutions when is used in sorption process, C_b is initial concentration in biological experiments.

As one can observe from the results presented in Table 3, a combination of both methods increase the efficiency of removal of GNB. Under these circumstances we propose a sequential procedure having the first step the ion-exchange process and second the biological one.

Conclusions

The strongly basic anion exchange resin Amberlite IRA 400 and laccases constitute an unique methodology having synergistic effect for effective GNB removal. Ion exchange studies showed that GNB was strongly retained on the resin not only by an ion-exchange mechanism but also by a π-π interaction. Furthermore, the GNB retained on the resin subjected to an enzymatic GNB discoloration which was dependent on the reaction *p*H (optimal value was *p*H 4) and on the reaction time. During this enzymatic process the laccase activity reached its peak after 5 min.

Overall, this work showed that the residual GNB concentration obtained after resin sorption process could be further decreased by an efficient degradation through laccase activity.

Acknowledgements: The authors are grateful for the financial support received from the Ministry of Research and Innovation through Program Nucleu, contract no. 38N/2016 (Project code PN 16 25 01 09-2016).

References

1. MARIN, N.M., PASCU, L.F., STANCULESCU, I., IORDACHE, O., JIANU, D., PETRESCU, L., BADEA, I.A., Rev.Chim.(Bucharest), **68**, no. 8, 2017, p. 1726
2. PAUN, I., CHIRIAC, L.F., MARIN, N.M., CRUCERU, L.V., PASCU, L.F., CAROL, B.L., ENE, C., Rev.Chim.(Bucharest), **68**, no. 8, 2017, p. 1732
3. CALINESCU, O., MARIN, N.M., IONITA, D., PASCU, L.F., TUDORACHE, A.,SURUPATEANU, G., BADEA, I.A., ABOUL-ENEINF, Y.H., Environmental Nanotechnology, Monitoring & Management, **6**, 2016, p. 164
4. VASILE, G.G., MARIN, N.M., CRUCERU, L.V., SIMION, M., GALAON, T., LEHR, B. C., Rev. Chim. (Bucharest), **67**, no. 8, 2016, p. 1427
5. VASILE, G.G., MARIN, N.M., PETRE, J., CRUCERU, L.V., J. Environ. Prot. Ecol., **17**, no. 1, 2016, p. 31
6. MARIN, N.M., VASILE, G.G., SIMION, M., PASCU, L.F., LEHR, B.C., Scientific Study and Research-Chemistry and Chemical Engineering Biotechnology Food Industry, **18**, no.1, 2017, p. 43
7. MARIN, N.M., SIMION, M., PASCU, L.F., International Symposium The Environment and the Industry, 2016, p. 127
8. MARIN, N.M., VASILE, G.G., PETRE, J., CRUCERU, L.V., International Symposium The Environment and the Industry SIMI, 2015, p. 215
9. PAUN, I., CRUCERU, L.V., CHIRIAC, L.F., NICULESCU, M., VASILE, G.G., MARIN, N.M., International Symposium The Environment and the Industry, 2016, p. 395
10. PETRE, J., GALAON, T., IANCU, V.I., VASILE, G.-G., STANESCU, E., PASCU, L.F., SIMION, M., CRUCERU, L., Rev. Chim. (Bucharest), **67**, no. 8, 2016, p. 1436

11. GALAON, T., PETRE, J., IANCU, V.I., CRUCERU, L., VASILE, G., PASCU, L.F., LEHR, C.B., Rev. Chim. (Bucharest), **67**, no. 8, 2016, p. 1474
12. NITA-LAZAR, M., GALAON, T., BANCIU, A., PAUN, I., STOICA, C., LUCACIU, I., J. Environ. Prot. Ecol., **17**, no.1 , 2016, p. 237
13. STOICA, C., GHEORGHE, S., PETRE, J., LUCACIU, I., NITA-LAZAR, M., Environ. Engineer. Manag. J., **13,** no. 9, 2014, p. 2243
14. FENG, Y., ZHOU, H., LIU, G., QIAO, J., WANG, J., LU, H., YANG, L., WU, Y., Bioresour. Technol., 125, 2012, p. 138
15. OFOMAJA, A.E., HO, Y.S., Dyes Pigm., 74, 2007, p. 60.
16. ***European Parliament Directive 2002/61/EC of July 2002
17. MORSHEDI, D., MOHAMMADI, Z., BOOJAR, M.M.A., ALIAKBARI, F., Colloid Surface B., **112**, 2013, p. 245
18. TURGAY, O., ERSOZ, G., ATALAY, S., FORSS, J., WELANDER, U., Sep. Purif. Technol., **79**, 2011, p. 26
19. KONSOWA, A., EL-RAHMAN, H.A., MOUSTAFA, M., Alexandria Engineering Journal, 50, 2011, p. 117.
20. LONG, C., MAI, Z., YANG, X., ZHU, B. , XU, X., HUANG, X., ZOU, X., Food Chem., **126,** 2011, p. 1324.
21. CAO, C., XIAO, L., CHEN, C., SHI, X., CAO, Q., GAO, L., Powder Technol., **260**, 2014, p. 90
22. SHAKIR, K., ELKAFRAWY, A.F., GHONEIMY, H.F., BEHEIR, S.G.E., Water Res., **44** , 2010, p. 1449
23. NAVARRO, A.E., CUIZANO, N.A., LAZO, J.C., SUN-KOU, M.R., LLANOS, B.P., J. Hazard. Mater., **164**, 2009, p. 1439
24. OZCAN, A. S., ERDEM, B., OZCAN, A., Colloid Surface A., **266** , 2005, p. 73
25. ÖZCAN, A., ONCU, E.M., OZCAN, A.S., J. Hazard. Mater., **129** , 2006, p. 244
26. SARATALE, R.G., SARATALE, G.D., CHANG, J.S., GOVINDWAR, S.P., J. Taiwan Inst. Chem. Eng., **42** , 2011, p. 138
27. RODRÍGUEZ-COUTO, S., J. Hazard. Mater., **194**, 2011, p. 297
28. SATHIAN, S., RADHA, G., SHANMUGAPRIYA, V., RAJASIMMAN, M., KARTHIKEYAN, C., Water Sci. Appl., **3**, 2013, p. 41
29. YANG, Y., MA, F., YU, H., FAN, F., WAN, X., ZHANG, X., JIANG, M., Biochem Eng. J., **57** , 2011, p. 13
30. ZHENG, H., LIU, D., ZHENG, Y., LIANG, S., LIU, Z., J. Hazard. Mater., **167**, 2009, p. 141
31. ESFANDIAN, H., JAVADIAN, H., PARVINI, M., KHOSHANDAM, B., KATAL, R., Asia-Pac. J. Chem. Eng., **8** , 2013, p. 665
32. OZCAN, A., ONCU, E.M., OZCAN, A.S., Colloid Surface A., **277** , 2006, p. 90
33. SECULA, M., CRETESCU, I., DIACONU, M., J. Environ. Prot. Ecol. **15**, 2014, p. 1583
34. KAUSHIK, P., MALIK, A., Environ. Int., **35**, 2009, p.127

Manuscript received: 11.11.2017