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Statistical design of experiments as a tool for optimizing the batch conditions to methylene blue biosorption on yellow passion fruit and mandarin peels

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Abstract

In order to reduce the total number of experiments for achieving the highest removal of methylene blue (MB) from aqueous solutions using yellow passion fruit peel (*Passiflora edullis, F. flavicarpa*) and mandarin peel (*Citrus reticulata*) as biosorbents two independent sets of full 2^3 factorial designs with two central points (10 experiments) were experimented. In order to continue the optimization of the system a new full 2^2 factorial design with two central points (six experiments) and a central composite surface analysis (13 experiments, divided into four cube points, five centre points, and four axial points) were employed for yellow passion fruit peel (PFP) and mandarin peel (MP), respectively. Using these statistical tools, the best conditions for MB removal from aqueous solution were initial methylene blue (C_0) of 3.20 mg l^{-1}, pH 9.0 for PFP and 11.0 for MP and time of contact higher than 48 h for PFP and 42.9 h for MP.

Keywords: Yellow passion fruit peel; Mandarin peel; Methylene blue removal; Biosorption; Factorial design; Central composite surface analysis

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1. Introduction

Industrial activity is responsible for generating a large volume of hazardous effluents. Colour is one of the most important hazard in industrial effluents, which needs to be treated [1–6], because the presence of dyes in water reduces light penetration, precluding the photosynthesis of aqueous flora [7]. Besides that, some dyes may cause allergy, dermatitis, skin irritation, and cancer to humans [8] in addition to being mutagenic [9]. Synthetic dyes are extensively employed in textile [3], paper [4], photoelectrochemical cells [4], printing [1], leather [1,4], food [4], cosmetics [1], etc. industries, which employ these substances to colour their final products.

A wide range of procedures for removal of synthetic dyes from industrial effluents are based on the decolorization by activated sludge containing some additives such as powdered activated carbon, bentonite, clays [5]; oxidation processes with ozone [4] and hydrogen peroxide [4]; photodecomposition catalysed by titanium dioxide [4,6] and other metallic complexes [4]; microbiological decomposition using mixtures of aerobic and anaerobic microorganisms [2–4]; electrochemical decomposition of dye containing effluents [3,10]; and adsorption [1,4,7–9,11–20]. Of all these procedures for the removal of synthetic dyes from industrial effluents, the adsorption procedure is the most efficient, because the hazardous species is transferred from the water effluent to a solid phase, diminishing the effluent volume to a minimum. The adsorbent can be regenerated afterwards or kept in a dry place without direct contact with the environment. Besides that, the

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microbiological, photocatalytic and electrochemical decomposition procedures are not efficient because several dyes are very difficult to be decomposed, and also these procedures are prone to give rise to colourless hazardous species in the water effluents [4,6,10].

Activated carbon is the most employed adsorbent for dye removal from aqueous solution because of its excellent adsorption properties [4,11,21]. However, the extensive use of activated carbon for dye removal from industrial effluents is very expensive [17], limiting its large scale application in wastewater treatment. Therefore, there is a growing interest in finding alternative low cost adsorbents for dye removal from aqueous solution. Amongst these the following can be cited: bagasse pitch [17,20]; carbonized bark [17]; carbonized sewage sludge [17]; tree-fern [22]; wood sawdust [1]; tree and eucalyptus bark [17]; peat [17]; biogas waste slurry [4]; carbon slurry waste [8]; recycled alum sludge [12]; diatomaceous earth [15]; fuller's earth [16]; apple pomace [7]; wheat straw [7]; fly-ash [17,23]; moss [24]; orange peel [4,19]; banana pitch [4,24]; water hyacinth roots [24]; peanut hull [9]; microorganisms such as fungus [4,17], bacteria [17], algae [25]; chitin [17]; chitosan [26]; coffee wastes [27]; rice husk [17] and rice hull ash [28].

The *Passiflora edullis, F. flavicarpa*, commonly known as yellow passion fruit, is cultivated in large scale in Brazil and it presents an agronomic importance because its fruits are used *in nature* and in the juice industry. Mandarins (*Citrus reticulata*) are cultivated and well adapted in several countries, mainly in those where a high temperature predominates in summer, followed by a mild one in winter. This fruit is very commonly cultivated in Brazil, and it is consumed *in nature* and also used in the juice industry. As the yellow passion fruit (PFP) and mandarin (MP) peels are very abundant in Brazil, there is a need to find a use for these by-products of industrial activities. Due to the need to find new and inexpensive adsorbents for synthetic dye removal, and also to diminish the large amounts of PFP and MP wastes, in this work is presented the first application of the use of PFP and MP wastes as a biosorbent for successful removal of methylene blue from aqueous samples.

2. Experimental

2.1. Biosorbent preparation

The yellow passion fruit (PFP) was obtained from a local market in Brazil. Yellow passion fruit peel is composed basically of cellulose, pectin, other associated elements such calcium, iron and principally phosphorous [29]. These constituents can bind to ionisable organic compound. The yellow passion fruit peel was washed with deionised water, dried overnight at 60 °C and ground in a knife-mill. The dry material was sieved to the 600 μm size. The powder resulting was placed in a flask bottle for further use.

The mandarins were purchased from a local market. The waste white peel was removed from the coat rejecting the yellow part of the cover. The obtained material was dried at room temperature and ground in a knife-mill. The resulting powder was washed with doubly distilled water and then dried in an oven at 100 °C for 24 h. After drying, this material was sieved to 600 μm size.

2.2. Reagents and solutions

Doubly distilled water was employed throughout.

The basic dye, methylene blue (MB, chemical formula $C_{16}H_{18}N_3SCl$; FW 319.86 g mol^{-1}; λ_{max} 665 nm, see Fig. 1) was obtained from Sigma Chemical Co., USA. A stock solution of 832 mg l^{-1} MB was prepared in water. Working solutions from 3.20 to 32.0 mg l^{-1} were prepared by suitable serial dilution of the stock solution using doubly distilled water.

The pH adjustments of the solutions were made with aliquots of 1.0 mol l^{-1} of HCl and NaOH, utilizing a pH meter Digimed provided with combined glass electrode Model DM 31.

2.3. Methylene blue batch biosorption procedure

Amounts of 200.0 mg of PFP and MP were accurately weighed, with the precision of one tenth of a milligram, on 100 ml glass Erlenmeyer flasks, containing 50.00 ml of 3.20–32.0 mg l^{-1} MB at pH ranging from 4.0 to 11.0. The glass Erlenmeyer flasks were capped and agitated mechanically at 60 rpm using a rotary orbital shaker at 25 °C for 1–48 h. The sorbent was separated from the solution by centrifugation at 3000 rpm for 20 min. The solid phase was then separated by sedimentation/centrifugation, and then the amount of dye in the sample solution was determined by spectrophotometry using a Perkin Elmer UV–vis spectrophotometer model 554 with silica cell of length 1.00 cm. Absorbances were determined at wavelength of 665 nm, which corresponds to the maximum absorption peak of MB. In order to avoid construction of calibration curves of MB at each pH value, the removal of MB from aqueous solution is given by Eq. (1)

$$\%\ \text{Removal} = \left(\frac{A_0 - A_f}{A_0}\right) \times 100\% \qquad (1)$$

where A_0 and A_f are absorbance values, before and after, respectively, the batch adsorption procedure. The A_0 and A_f are related with the initial and final dye concentrations, respectively, at a given pH value.

2.4. Statistical design of experiments

2.4.1. Full factorial design

Factorial design is employed to reduce the total number of experiments in order to achieve the best overall optimization

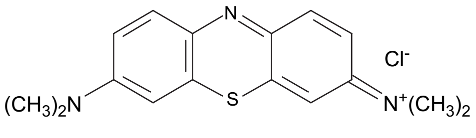

Fig. 1. Chemical structure of methylene blue dye.

of the system [30–32]. Factorial designs allow the simultaneous study of the effects that several factors may have on an optimization of a particular process. The factorial design determines which factors have important effects on a response as well as how the effect of one factor varies with the level of the other factors. Effects are differential quantities expressing how a response changes as the levels of one or more factors are changed [30,31]. Also, factorial designs allow measuring the interaction between each different group of factors. Interactions are the driving force in many optimizations of processes. Without the use of factorial experiments, important interactions may remain undetected, and the overall optimization may not be attained [30,31].

One of the simplest types of factorial designs used in experimental work is one having two levels (2^k) [30,31]. In a full factorial experiment, responses are measured at all combinations of the experimental factor levels. The combinations of factor levels represent the conditions at which responses will be measured. Each experimental condition is called a run and the response measurement an observation. The entire set of runs is the design.

In this investigation of MB removal using PFP and MP, the percentage of removal of MB from aqueous solution could depend on initial dye concentration (C_0), the acidity of the medium (pH), and the time of contact (t). Others variables such as mass of biosorbent was fixed at 200.0 mg, speed of shaking was kept at 60 rpm, and temperature was kept at 25 °C. The effects of these factors and their interactions were measured by performing two sets of 10 experiments (each one was carried out for each biosorbent) containing two central points, in order to evaluate the standard deviation of each factor and to detect if there is any inflection point [30,31] forming the 2^3 full factorial designs given in Table 1. The factor levels were coded as −1 (low), 0 (central point) and 1 (high) [30,31]. Each factor presents four high values, four low values and two medium values. The response was expressed as the percent of dye removal by each biosorbent. For treatment of data, the Minitab Statistical software [33] release 14.1 was employed throughout.

Table 1
Optimization of MB removal by PFP and MP biosorbents

Experiments	C_0	pH	t	% Removal	
				PFP	MP
1	−1	−1	−1	7.05	3.68
2	1	−1	−1	2.83	1.99
3	−1	1	−1	14.00	7.57
4	1	1	−1	9.82	2.98
5	−1	−1	1	23.34	5.56
6	1	−1	1	16.00	3.27
7	−1	1	1	48.43	22.90
8	1	1	1	24.23	14.90
9	0	0	0	19.23	4.68
10	0	0	0	18.87	4.98

Factors	Levels		
	−	0	+
C_0 (mg l^{-1})	3.20	17.6	32.0
pH	4.0	6.5	9.0
t (h)	1.0	5.5	10.0

Full 2^3 factorial designs containing two central points for each biosorbent. Mass of biosorbent fixed at 200.0 mg, speed of shaking 60 rpm, and temperature 25 °C.

After performing the first factorial design for PFP biosorbent, a continuation of this statistical design was carried out fixing the MB initial concentration at 3.20 mg l^{-1} and performing a new 2^2 full factorial design with two central points (six experiments) using different levels of pH and time of contact, as described in Table 2.

Table 2
Continuation of the optimization of MB removal by PFP biosorbent

Experiments	pH	t	% Removal PFP
1	−1	−1	25.79
2	1	−1	27.01
3	−1	1	97.10
4	1	1	95.15
5	0	0	85.37
6	0	0	86.01

Factors	Levels		
	−	0	+
pH	9.00	10.0	11.0
t (h)	12.0	30.0	48.0

Full 2^2 factorial design containing two central points. Initial concentration of MB fixed at 3.20 mg l^{-1}, mass of biosorbent fixed at 200.0 mg, speed of shaking 60 rpm, and temperature 25 °C.

2.4.2. Central composite response surface design

Response surface methods are used to examine the relationship between one or more response variables and a set of quantitative experimental factors. These methods are often employed after a screening of important factors, usually by performing a previous factorial design. After that, it is necessary to find the factor settings that optimize the response. Designs of this type are usually chosen when the previous factorial design indicated a curvature in the response surface [30]. Response surface methods may be employed to [30]

- find factor settings (operating conditions) that produce the best response;
- find factor settings that satisfy operating or process specifications;
- model a relationship between the quantitative factors and the response.

In this work, after performing a screening of the factors with the factorial design for the MP biosorbent a response surface analysis statistical procedure [30] was employed in order to achieve the highest dye removal from aqueous solution. The experimental set was carried out according to Table 3.

Table 3
Optimization of MB removal using MP as biosorbent

Experiments	pH	t	% Removal
1	−1	−1	19.79
2	1	−1	21.99
3	−1	1	92.91
4	1	1	94.79
5	−1	0	78.99
6	1	0	82.53
7	0	−1	17.90
8	0	1	90.90
9	0	0	81.80
10	0	0	81.78
11	0	0	81.73
12	0	0	81.78
13	0	0	81.72

Factors	Levels		
	−	0	+
pH	9.0	10.0	11.0
t	12.0	30.0	48.0

Central composite surface analysis containing 13 experiments, divided into four cube points, five centre points in cube, and four axial points. Initial concentration of MB fixed at 3.20 mg l^{-1}, mass of biosorbent 200.0 mg, speed of shaking 60 rpm, and temperature 25 °C.

3. Results and discussion

3.1. Screening of factors for methylene blue removal using yellow passion fruit and mandarin peels as biosorbents

Synthetic dye removal by biosorbents in a batch system usually depends on several factors, such as initial metallic ion concentration (C_0), acidity of medium (pH), time of contact between the dye and the biosorbent (t), mass of biosorbent (m), speed of shaking, temperature, etc. The optimization of all those variables using the univariate procedure is very tedious, because any variable (factor) is optimized, by varying just one factor by the time and fixing the others. Then, the best value achieved by this procedure is fixed and other factors will be varied by the time. The disadvantage of this univariate procedure is that the best condition could not be attained, because the interactions among all the factors are disregarded, and also it is not known if the set of other fixed variables was fixed at other levels, the results would lead to the same optimization. In order to overcome these disadvantages, statistical design of experiments can be carried out to achieve the best optimization of any possible system [32]. Obviously not all factors could be studied, because it would lead to the need of performing so many experiments that would invalidate the advantage of using the statistical design of experiments [32].

In this work, the factors screened were initial MB concentration (C_0), pH, and time of contact between the biosorbents and MB (t), for best dye removal from aqueous solution using a batch adsorption system. The experiments given in Table 1 were carried out, obtaining the dye removal expressed in percentage (%). The Minitab Statistical Software release 14.1 was employed throughout in order to obtain the effects, coefficients, standard deviation of coefficients, and other statistical parameters of the model. The definitions of the factors and levels used in the complete design are presented in Table 1. Main effects, interaction effects, coefficients of the model, and standard deviation of each coefficient, and probability for the full 2^3 factorial designs are presented in Table 4.

For the yellow passion fruit peel biosorbent it can be verified that all main factors and their interactions were significant at a 5% probability level ($p < 0.05$). All the effects and interactions that present probability lower than 0.05 were significant. The central point carried out in duplicate was useful to obtain the standard error of the coefficients. The probability results showed that the central point is not significant in the chosen model ($P = 0.149$) at 5% probability level. It means that it was not detected at a curvature of the factors when the levels were changed from the lower level (−) to the higher level (+), passing through the central point (0). In addition, the fit model presents an adjusted square correlation coefficient (R^2 adjusted) of 99.96%, fitting very well with the statistical model.

For mandarin peel biosorbent it was observed that all main factors and almost all interactions were significant at a 5% probability level ($p < 0.05$). The interactions $C_0.t$ and $C_0.\mathrm{pH}.t$ were not significant, since they presented probability higher than 0.05. The fit model presents an adjusted square correlation coefficient (R^2 adjusted) of 99.89%, which is very well fitting with the statistical model.

In order to better evaluate each factor and its interaction, in Fig. 2A and C are presented the normal probability plot of standardised effects for PFP and MP biosorbents, respectively. These graphs could be divided into two regions, the region below 50%, where the factors and their interactions present negative coefficients (C_0, $C_0.t$, $C_0.\mathrm{pH}$, and $C_0.\mathrm{pH}.t$ for PFP biosorbent and C_0 and $C_0.\mathrm{pH}$ for MP biosorbent) and the region above 50%, where the factors present positive coefficients (pH, t, pH.t for PFP biosorbent and pH, t and pH.t for MP biosorbent). All these factors and interactions which are represented as a square are significant figures, and they are out of the central line that crosses the zero value at the abscissa at the 50% probability. The effects positioned in this line are represented by a circle and correspond to the estimate of errors of the effects, being not significant ($C_0.t$ and $C_0.\mathrm{pH}.t$ for MP biosorbent). The positive values of effects mean that an increase in their levels leads to an increase in the dye removal promoted by the biosorbent, on the other hand, the negative values of the effects, lead to a diminution of the response (% removal), when their levels are increased.

In Fig. 2B and D are presented the Pareto Chart of standardised effects at $p = 0.05$, for PFP and MP biosorbents, respectively. All the standardised effects are in absolute values (to verify which are positives and negatives, see Fig. 2A and C, respectively). All the values presenting an absolute value higher than 12.7 and 12.71 for PFP and MP ($p = 0.05$), respectively, which are located right of the dashed line, are significant. The absolute standardised value of the effect of each factor and its interaction appear at the right of each bar.

Table 4
Factorial fit: percentage of removal of MB versus C_0, pH, and t

Term	Effect	Coefficient	SE of coefficient	P
Yellow passion fruit peel (PFP)				
Constant		18.215	0.08889	0.003
Main factors				
C_0	−9.986	−4.993	0.08889	0.011
pH	11.815	5.908	0.08889	0.010
t	19.574	9.787	0.08889	0.006
Interaction of two factors				
C_0.pH	−4.204	−2.102	0.08889	0.027
$C_0.t$	−5.784	−2.892	0.08889	0.020
pH.t	4.846	2.423	0.08889	0.023
Interaction of three factors				
C_0.pH.t	−4.224	−2.112	0.08889	0.027
Central point (cp)		0.833	0.19876	0.149
S = 0.251408	$R^2 = 100.00\%$		R^2(adj) = 99.96%	
Mandarin peel (MP)				
Constant		7.855	0.07692	0.006
Main factors				
C_0	−4.142	−2.071	0.07692	0.024
pH	8.461	4.231	0.07692	0.012
t	7.603	3.802	0.07692	0.013
Interaction of two factors				
C_0.pH	−2.151	−1.075	0.07692	0.045
$C_0.t$	−1.006	−0.503	0.07692	0.097
pH.t	6.020	3.010	0.07692	0.016
Interaction of three factors				
C_0.pH.t	−0.700	−0.350	0.07692	0.138
Central point (cp)		−3.026	0.17200	0.036
S = 0.217565	$R^2 = 99.99\%$		R^2(adj) = 99.89%	

Estimated effects and coefficients for % removal (coded units) for both biosorbents. Full 2^3 factorial design. The effects and coefficients are given in coded units.

Analysing Fig. 2A and B and the values of Table 4 for the PFP biosorbent, it can be inferred that the time of contact between the dye and PFP biosorbent was the most important variable of the overall biosorption procedure. The positive value of its coefficient means that the removal of MB dye by PFP biosorbent wastes was favoured at high times of contact ($t = 10$ h). As the attained MB dye removal was only 48.43% (see Table 1), it is necessary to explore other levels of this variable in a future factorial design. The second important factor for overall optimization of the batch system was the pH. An increase of the pH from 4.0 to 9.0 led to a remarkable increase in the removal of MB promoted by PFP biosorbent. As MB is a basic dye (see Fig. 1), i.e., it is cationic, this dye would be electrostatically attracted to the biosorbent surface charged negatively at pH 9.0. The third factor important to the overall optimization of the biosorption process is the initial concentration of MB. It is clearly noted that the dye removal is higher for lower concentration of MB. It is known that the removal capacity of a biosorbent usually decreases for higher dye concentration [1]. Garg et al. [1] also reported a significant decrease of MB removal using treated Indian Rosewood sawdust as biosorbent. In further experiments of the new factorial design, the C_0 of MB will be fixed at 3.20 mg l^{-1}. The fourth important factor for overall optimization of the biosorption system is the interaction of two factors $C_0.t$, followed by pH.t and then C_0.pH.t and the last in importance is C_0.pH.

For MP as biosorbent, the results in Fig. 2C and D and Table 4 indicate that pH is the most important variable for the overall optimization of the batch biosorption procedure. This result is different from that obtained with the previous biosorbent, for which the most important factor was the time of contact. On the other hand, if the absolute values of the standardised effects were taken into account, the effect of pH for PFP presents a higher absolute value when compared with MP. From the results in Table 1, it can be inferred that for the levels of the factors chosen until now, the PFP biosorbent is a more effective biosorbent for MB removal from aqueous solution, when compared with MP. The second important factor for MB removal from aqueous solution using MP as biosorbent was the time of contact. The absolute value of this standardised effect is less than half of those obtained with the PFP biosorbent. It can be inferred that the kinetics of biosorption of MB on MP is faster than the biosorption of

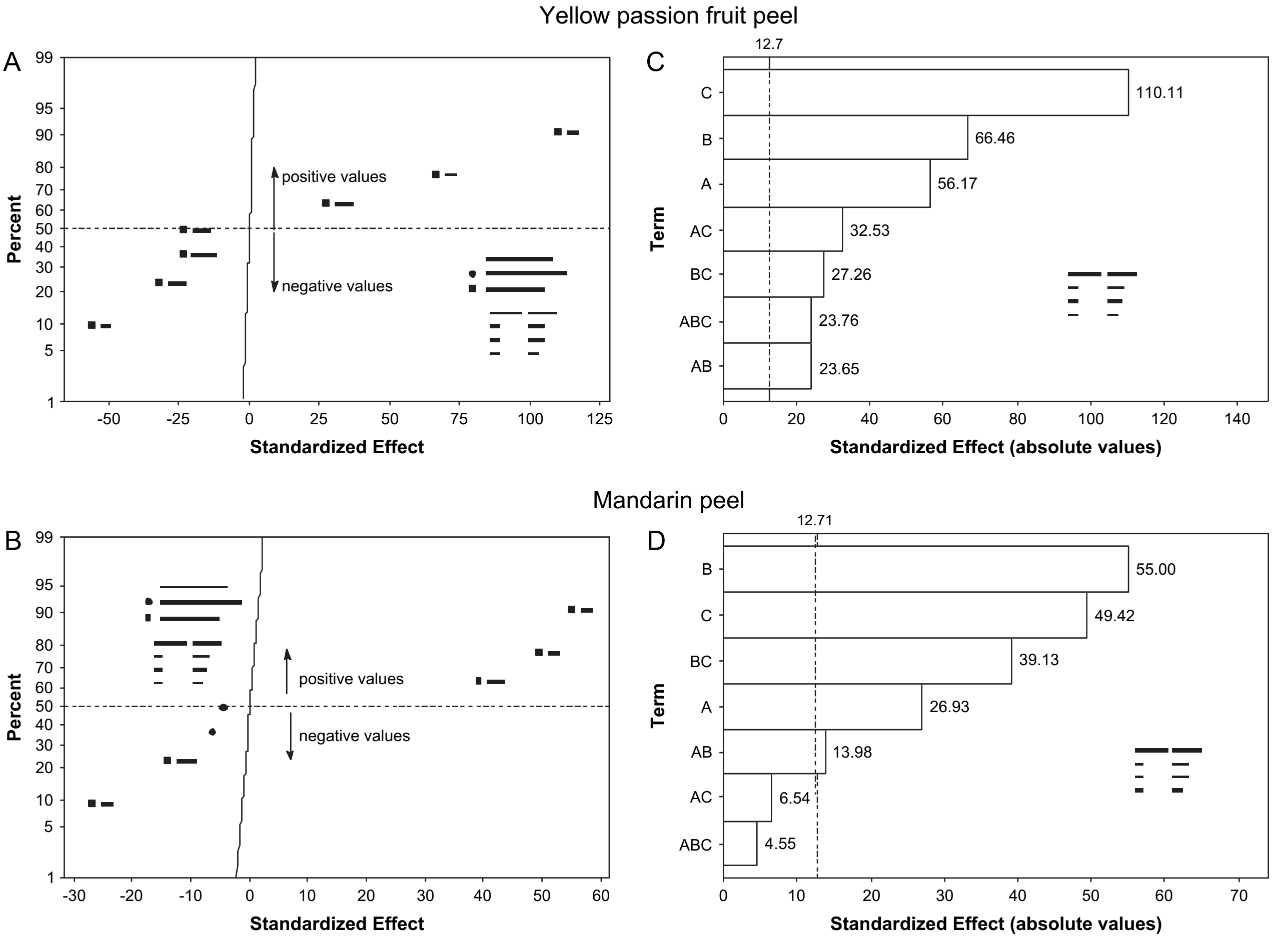

Fig. 2. (A,B) Normal probability plots of standardised effects at $p = 0.05$, for PFP and MP biosorbents, respectively. The dotted line at 50% divides the negative effects from the positive ones. (C,D) Pareto plots of standardised effects at $p = 0.05$, for PFP and MP biosorbents, respectively.

MB on PFP. However, other experiments should be carried out in order to verify this effect. In this paper these experiments were not performed, because the aim of this work is to optimize the best conditions for MB removal using PFP and MP as biosorbents, and not to compare the kinetics of adsorption of the dye using different biosorbents. The third more important factor for the overall optimization of removal of MB using MP as biosorbent was the interaction of pH.*t* whose absolute value was higher than the main factor C_0. Only the achievement of this result justifies the use of the statistical design of experiments over the conventional univariate process of optimization of the system. This information would not be acquired in a univariate optimization of the biosorption system. Otherwise, if the system were being optimized by using univariate procedure, small increase of the pH of the solution associated with a small increase in the contact time (t) could lead to a misinterpretation of the results achieved with univariate procedure. Probably the experimenter would remake the measurements, because a small positive variation of the pH of the solution associated with a small increase in the contact time between the dye and the biosorbent would lead to an unexplained increase in the dye removal, which could not be explained by using the univariate procedure of optimization of the system. The fourth important factor to overall optimization is the initial concentration of the dye (C_0). This result showed that the initial concentration of MB should be decreased in order to achieve the highest response (% removal), and this factor was fixed at 3.20 mg l^{-1} in the further surface response analysis design. The last important factor for the optimization of the batch removal of MB using MP as biosorbent was $C_0.t$. The two unimportant interactions at 5% probability level were C_0.pH and C_0.pH.t.

Table 5 presents the analysis of variance for full 2^3 factorial design with two centre points, for each biosorbent. For PFP biosorbent, it was observed that, the main factors, two-way interactions and three-way interactions were significant at a 5% probability level ($p < 0.05$), as discussed above. In addition, this analysis of variance showed that this model did not present a curvature, since its probability was 0.149. For MP as biosorbent, it was observed that, the main factors and two-way interactions were significant ($p < 0.05$), and the three-way interactions were not significant ($p = 0.138$). In addition, the analysis of variance showed that this model presented a curvature, since its probability was 0.036.

Fig. 3 shows the mean changes that occur in the response (% of dye removal) when the levels of the factors were changed from low level (−) going on to high level (+), passing through the central point (0), for both biosorbents. As can

Table 5
Analysis of variance for percentage of dye removal using PFP and MP as biosorbents for full 2^3 factorial design (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	*F*	*P*
Yellow passion fruit peel (PFP)						
Main effects	3	1244.95	1244.95	414.984	6565.56	0.009
Two-way interaction	3	149.21	149.21	49.735	786.87	0.026
Three-way interaction	1	35.69	35.69	35.686	564.60	0.027
Curvature	1	1.11	1.11	1.111	17.58	0.149
Residual error	1	0.06	0.06	0.063		
Pure error	1	0.06	0.06	0.063		
Total	9	1431.02				
Mandarin peel (MP)						
Main effects	3	293.133	293.133	97.7110	2064.27	0.016
Two-way interaction	3	83.761	83.761	27.9204	589.85	0.030
Three-way interaction	1	0.981	0.981	0.9813	20.73	0.138
Curvature	1	14.646	14.646	14.6461	309.42	0.036
Residual error	1	0.047	0.047	0.0473		
Pure error	1	0.047	0.047	0.0473		
Total	9	392.569				

DF, degree of freedom; Seq SS, sequential sum of squares; Adj SS, adjusted sum of squares; *F*, factor *F*; *P*, probability.

be seen, for the biosorption of MB on PFP, the percentage of removal at the central point corresponds to the average of the response values to all factors studied. On the other hand, the biosorption of MB on MP, the response at the central point does not correspond to the average of the response value for all the main factors studied, showing the presence of a curvature of the responses between the maximum and the minimum values of the levels of the factors. Based on these results, in order to achieve the maximum removal of MB using the two biosorbents, there is a necessity to carry out different strategies. For obtaining the maximum removal of MB using PFP, it is recommended to continue the factorial design with other values of the levels established in this first statistical design of experiment. Otherwise, for obtaining the maximum removal of MB using MP, a surface analysis design is recommended, due to the presence of curvature.

3.2. Optimization of removal of methylene blue employing yellow passion fruit peel as biosorbent

For the overall optimization of removal of MB using PFP as biosorbent, the experiments given in Table 2 were performed. In this set of experiments, the initial MB concentration was fixed at 3.2 mg l^{-1}, the pH was ranged from 9.0 to 11.0 and the time of contact between the MB and PFP biosorbent ranged from 12.0 to 48.0 h. Main factors, interaction effects, coefficients of the model, standard deviation of each coefficient, and probability for the full 2^2 factorial designs are presented in Table 6. As can be seen, the effect of pH was not significant when the levels of this factor were increased from 9.0 to 11.0 ($P > 0.05$). This result indicates that pH 9.0 should be fixed for the overall optimization of the removal of MB using PFP as biosorbent. Otherwise, the factor time was significant ($P = 0.004$). Interactions between the factors were not significant; however, the central point was significant. As the pH (9.0–11.0) is not important for the overall removal of MB from aqueous solution employing PFP, this result indicated that the factor time was levelled out, which means that the equilibration time was achieved for 48 h of contact between MB and PFP. This fit model presents an adjusted square correlation coefficient (R^2 adjusted) of 99.98%, which is very well fitting with the statistical model.

Fig. 4 shows the distribution of the residual values, defined as the differences between the predicted values (model) and the observed values (experimental). As can be seen, the residuals follow a normal distribution, with three negative residuals (below 50%) and three positive residuals (above 50%). In addition to this, the maximum and minimum deviation of these values from zero is only ± 0.3235, which indicates that the model presents a minimal deviation of the fitted value from the observed value.

Based on the statistical design of experiments, it can be concluded that the best conditions for MB removal from aqueous solution employing PFP as biosorbent is pH 9.0, *t* 48 h, and using the fixed conditions as follows: C_0 3.20 mg l^{-1}; temperature of 25 °C; mass of biosorbent 200.0 mg; and shaking speed of 60 rpm. The total number of experiments to arrive to this conclusion was only 16.

3.3. Optimization of removal of methylene blue employing mandarin peel as biosorbent

After performing a screening of factors using a full 2^3 factorial design, a central composite response surface design (containing 13 experiments, divided into four cube points, five centre points in cube, four axial points) was carried out according to experiments described in Table 3, in order to achieve the highest MB removal from aqueous solution by the MP. The levels of the chosen factors were set based on the previous factor analysis described above, using acidities of the solution ranging from pH 9.0 to 11.0, and increasing the contact time between the MB and MP upto 48 h. In these experiments the MB initial concentration was fixed at

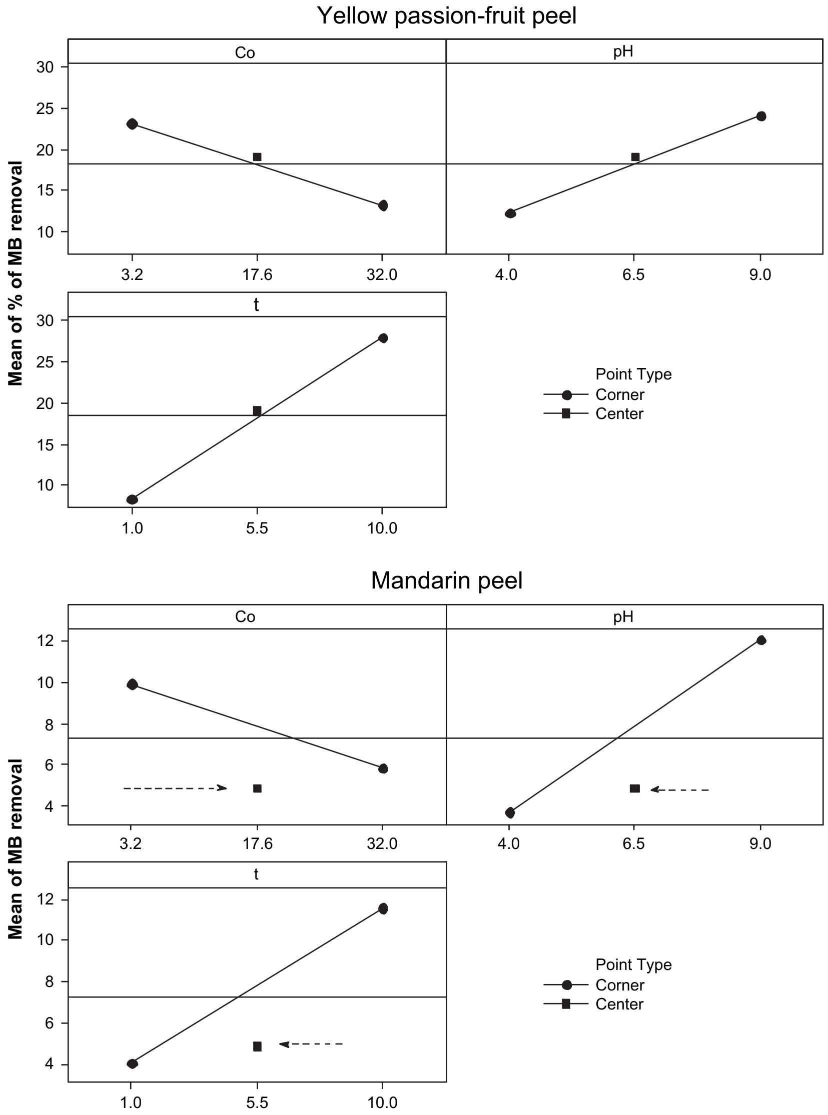

Fig. 3. Main effects of the plot (data means) for methylene blue removal by PFP and MP as biosorbents. The arrow indicates the curvature, when the factor is increased from low value (−) to the higher level (+) passing through the centre point (0). The units of C_0 and time are expressed in mg l^{-1} and h, respectively.

3.20 mg l^{-1} at 25 °C and with a speed of shaking of 60 rpm, using 200.0 mg of MP as biosorbent.

Fig. 5A shows the contour plot of the response (% MB removal) for time of contact versus pH. As can be seen, the highest percentage of removal occurs when the time of contact is higher than 36 h. On the other hand, when the pH ranged from 9.0 to 11.0 the effect on the overall removal of MB from aqueous solution using MP as biosorbent was not so

Table 6
Factorial fit: percentage of removal of MB versus pH and *t*

Term	Effect	Coefficient	SE of coefficient	*P*
Yellow passion fruit peel (PFP)				
Constant		61.2625	0.2287	0.002
Main factors				
pH	−0.3669	−0.1835	0.2287	0.570
t	69.7231	34.8615	0.2287	0.004
Interaction of two factors				
pH.*t*	−1.5897	−0.7949	0.2287	0.178
Central point (cp)		24.4266	0.3962	0.010
$S = 0.457456$	$R^2 = 100.00$		R^2(adj) = 99.98	

Estimated effects and coefficients for % removal (coded units) for PFP biosorbent. Full 2^2 factorial design. The effects and coefficients are given in coded units.

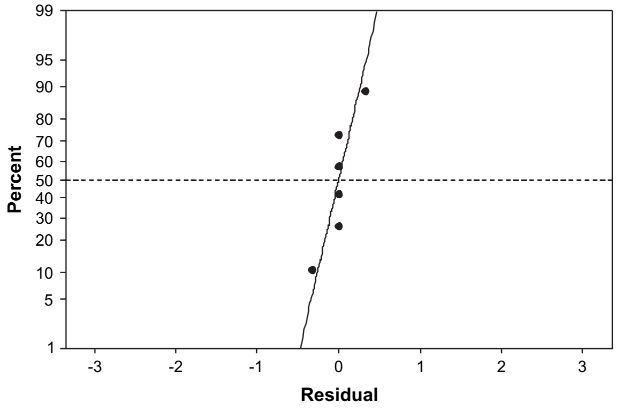

Fig. 4. Normal probability plot of the residual for MB removal using PFP. The dotted line at 50% divides the negative residuals of the fitting from the positive ones.

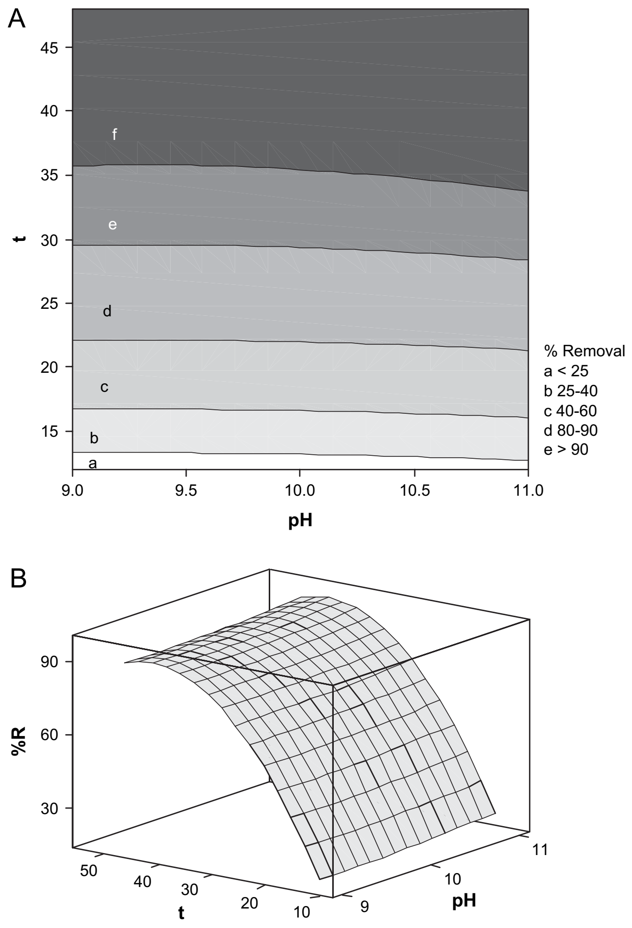

Fig. 5. (A) Contour plot of t versus pH for the removal of MB using MP. The regions of the contour plot are divided into several regions, where the response (% of removal) is expressed in percentage. (B) Surface plots of removal of MB using MP. The units of time are expressed in h.

Table 7
Response surface regression for MB removal using MP as biosorbent

Term	Coefficient	SE of coefficient	P
Constant	81.2142	0.5353	0.000
pH	1.2706	0.5263	0.046
t	36.4862	0.5263	0.000
pH^2	0.9171	0.7757	0.276
t^2	−25.4472	0.7757	0.000
pH.t	−0.0807	0.6446	0.904
$S = 1.289$	$R^2 = 99.9\%$	R^2 (adjusted) = 99.8%	

Full quadratic model. P, probability; SE, standard error of coefficient. The coefficient values are given in coded units.

much significant. Fig. 5B shows the surface plot of the percentage of removal of methylene blue as a function of t and pH. As can be seen, the pH (9.0–11.0) has not so much significance on the overall optimization of MB removal from aqueous solution employing MP as biosorbent, whereas, the time of contact was significant and it was levelled out for times higher than 36 h, indicating that at this time, the equilibration between the biosorbate (MB) and biosorbent (MP) was reached.

By analyzing Table 6, it was observed that at the levels of the factors chosen in the experiments given in Table 3, the significant effects were pH, t and t^2 ($P < 0.05$), and also, it was observed that the interaction between pH and time of contact (pH.t) and pH^2 were not significant. In addition, at 5% probability level, it can be seen that the full quadratic model fits properly (R^2 adjusted 99.8%), indicating that overall optimization of the MB removal from aqueous solution employing MP was achieved (Table 7).

The removal of MB using MP as biosorbent follows the equation given below. By employing this equation, complete removal of MB using MP as biosorbent occurs at pH 11.0 with a contact time of 42.9 h. This predicted response presented a composite desirability of 82.11% and a correlation coefficient of 0.9642, which is a fairly good fit of the proposed model. The total number of experiments to overall optimization of the removal of MB from aqueous solution using MP as biosorbent was only 23.

$$\%\ \text{MB removal} = 81.21 + 1.27\,\text{pH} + 36.49t - 25.44t^2, \tag{2}$$

being the values in coded units, and the pH in the interval of 9.0–11.0 and time in the interval of 12.0–48.0 h. The fixed conditions are C_0 of 3.20 mg l^{-1}, temperature of 25 °C, and shaking speed of 60 rpm.

4. Conclusion

Full 2^3 factorial designs were employed for screening the factors that would influence the overall optimization of MB removal from aqueous solution employing PFP and MP as biosorbents. This optimization showed that the best initial conditions for both biosorbents were pH 9.0, contact time higher than 10 h, initial concentration (C_0) of MB as low as 3.20 mg l^{-1}. Biosorbent mass was fixed to 200.0 mg, temperature to 25 °C and shaking speed to 60 rpm.

For PFP biosorbent a curvature of the response (percent of MB removal) for the selected levels of all factors was not verified. Based on these results a new full 2^2 factorial design with two central points were carried out. Analysing these results, it was verified that the best conditions for complete removal of

MB from aqueous solution employing PFP biosorbent was pH 9.0, t 48 h, keeping constant C_0 3.20 mg l^{-1}, mass of PFP biosorbent 200.0 mg, temperature 25 °C, shaking speed of 60 rpm. The total number of experiments for this optimization was only 16.

For MP biosorbent a central composite surface response design was carried out, investigating other limits of the previous values attained with the full 2^3 factorial design, and the best conditions for MB removal from aqueous solution were pH 11.0, t 42.9 h, keeping constant C_0 3.20 mg l^{-1}, mass of MP biosorbent 200.0 mg, temperature 25 °C and shaking speed of 60 rpm. The total number of experiments to arrive at these conditions was only 23.

The optimization of the conditions for MB removal from aqueous solutions employing PFP and MP biosorbents, associated with the use of statistical tools (factorial design and central composite surface analysis) reduced remarkably the total number of experiments, when compared with isotherms and kinetic curves of adsorption of dyes using different adsorbents. Methylene blue achieved a 95% removal from aqueous solution using PFP (see Table 2) and MP (see Table 3), confirming the ability of the statistical tools to arrive at these conditions.

The hypothesis that the kinetics of biosorption of MB on MP is faster than the biosorption of MB on PFP acquired with the screening of variables was confirmed in the overall optimization of the system. Complete removal of 3.20 mg l^{-1} MB using PFP will require at least 48.0 h at pH 9.0, while this task would be performed in 42.9 h using MP at pH 11.0 and keeping constant all the other conditions as described above.

The mechanism of biosorption of MB in both biosorbents should be the electrostatic attraction of positive MB (see Fig. 1) with the negatively charged biosorbent.

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