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## Adsorption of Crystal Violet by deoiled-residue of Jatropha: Kinetics and Isotherm studies Crystal Violet Jatropha Adsorption studies

B. Subramanyam

School of Civil Engineering, SASTRA University, Thanjavur-613401, Tamil Nadu, India

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### ABSTRACT

*The increasing demand for biofuels using non-edible oil seeds (primarily jatropha curcas) has assumed a gigantic proportion in a tropical country like India. However, one of the lee-side of this ambitious project is problems of handling the significant amount of deoiled cakes. These are essentially non-edible even for animals, although production of residue is often quite high. The present work aims to investigate the potential of deoiled cake as an adsorbent for the treatment of dye containing wastewaters. The model compound used was crystal violet dye. The batch studies indicate that at an equilibrium time of 3.5 h, 90% adsorption was observed at 400 ppm initial concentration. The kinetic modeling studies indicate that adsorption phenomenon seemed to be a chemisorption process. Among the isotherm models, Sip and Redlich-Peterson isotherm models are found to be best suited to the adsorption process. The deoiled cake studied demonstrated high potential for the removal of dyes from aqueous solution.*

**Key words:** Adsorption isotherms; crystal violet removal; kinetic modeling; deoiled cake.

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### INTRODUCTION

Synthetic dyes have been increasingly been used, over natural counterparts, in the textile and dyeing industries[1]. Numerous synthetic dyes are produced globally, leading to severe wastewater pollution[2]. The dye not only carcinogen to human health, but also results in reduction in gas solubility as well as increase in turbidity[3 and 4]. Dye removal has been amply discussed in the literatures and there are reports on dye removal using low cost adsorbents [5 and 6]. However, the use of deoiled cakes for adsorption has been of rather recent use. The aim of the present work is to explore the potential of Jatropha deoiled cake, being utilized as adsorbent for the removal of Crystal violet (CV), a water soluble dye from its aqueous solution.

Jatropha curcas is an important industrial crop belongs to Euphorbiaceae family [7 and 8]. In India, Thailand and other Southeast Asian countries are using Jatropha seeds as a major source of biodiesel fuel [9]. Jatropha seeds produce an average 500g of deoiled cake per kg of seed used for biodiesel production [10]. The deoiled cake cannot be used as cattle feed, unlike other oilseeds due to presence toxic phorbol esters [11]. The potential utilization / safe disposal of huge amounts of Jatropha deoiled cake needs to be taken care.

Studies on the adsorptive capacities of the selected Jatropha deoiled cake was carried out under optimal adsorbent dosage ( $w$ ), contact time ( $t$ ) as well as initial pH and initial concentration ( $C_0$ ). The dye sorption capacity for the

adsorbent has also been studied using several adsorption isotherms (viz., Freundlich, Langmuir, Temkin, Redlich–Peterson, and Dubinin–Radushkevich isotherms) to obtain the best isotherm-model.

### MATERIALS AND METHODS

The water-soluble CV was obtained from M/s Merck and its stock solution was prepared in double distilled water. Solutions of desired concentrations of the adsorbate were prepared from stock solution, and double distilled water was used for necessary dilutions. All reagents used in the investigation were of analytical grade. Fresh stock solution, as required, was prepared every day and was stored in a brown color glass reservoir of 5L capacity to prevent photo-oxidation. The  $C_0$  was ascertained before the start of each experimental run by using UV Spectrophotometer.

Jatropha deoiled cake was crushed thoroughly, washed with distilled water, and then dried in an oven. The dried material was then treated with hydrogen peroxide solution and left for 24h to oxidize the adhering organic impurities. The residue was then dried at 110 °C for 1h in a vacuum oven. The dried adsorbent was grinded and sieved to desired particle size of 125 micron and stored in separate vacuum desiccators for further studies. Jatropha deoiled cake was found to have the physical characteristics of protein 63%, fat 0.5%, ash 10%, fibre 6% and carbohydrates 20%.

For each study, a 100 ml synthetic dye solution (of varying concentrations, ranging from 50 to 600 ppm) was prepared in 250ml Erlenmeyer flask and a known amount of adsorbent was added to each bottle, at optimum pH. The solution was equilibrated for 24h at room temperature, followed by filtration of the adsorbent and subsequent analysis of filtrate for dye concentrations. The percentage removal of dyes and equilibrium adsorption uptake,  $q_e$  (mg/g), was calculated using the following relationships:

$$\% \text{ Removal} = \frac{100(C_0 - C_e)}{C_0}, \quad (1)$$

$$\text{amount adsorbed (mg/g)} q_e = \frac{(C_0 - C_e)V}{w}, \quad (2)$$

Where  $C_e$  the equilibrium adsorbate concentration (ppm),  $V$  the volume of the solution (l) and  $w$  is the mass of the adsorbent (g).

CV concentration was estimated using Systronics Double Beam spectrophotometer.

### RESULTS AND DISCUSSION

pH is a very important factor in wastewater treatment. It is especially important in adsorption as it affects the surface charge of the adsorbents, besides controlling the degree of ionization of pollutants. The adsorption of CV by Jatropha deoiled cake was studied at various pH values of the dye solution, obtained by addition of varying proportions of 0.1N NaOH or 0.1 N HCL. As the structure (Figure 1) shows, CV is cationic in nature. At acidic pH,  $H^+$  ions may compete with the dye cations for adsorption sites, resulting in lower adsorption. The basic pH yields a lower surface charge density thus causing the reduced electrostatic repulsion between the cationic dye and adsorbent surface. Consequently higher adsorption may result at high pH. However, dye and adsorbent interactions are complex as is influenced by various forces. The amount of dye adsorbed shows a declining trend with higher as well as with lower pH, with maximum removal of dyes (90.44% by the adsorbent) observed at pH 4.8.

The effect of the adsorbent dose was studied by varying the adsorbent concentrations from 1 to 8 g/L. The initial concentration of dye was fixed at 300 ppm. It was particularly significant during 1 – 4g/l. However, increasing the dosage beyond 4 g/l reduced the uptake of dye. This could be due to overcrowding by adsorption particles. The optimum adsorbent doses were thus fixed at 4 g/L.

The effect of initial dye concentration in the range of 50 to 600 ppm on adsorption has been studied. The percentage dye adsorption increased from 64.5 % to 90.5 % at 400 ppm and further decreased to 84.5 % at 600 ppm. However,

the amount of dye adsorbed increases from 8.5 mg/g to 126.4 mg/g as concentration increases from 400 ppm to 600 ppm.

The adsorption experiments were carried out at different contact times with fixed adsorbent dose (4 g/L of Jatropha deoiled cake), temperature 30 °C, pH 4.8 and adsorbate concentration of 400 ppm. It is evident from the experiments that the rate of dye removal seemed to be rapid during the initial 30min and a steady decrease thereafter. There was a gradual slow down as it approached equilibrium, with more or less a constant rate of adsorption at the intermediate stage. No significant change in dye removal was observed after about 3.5h. It was also found that the adsorptive removal of the dye probably ceased after 3.5h (or 210min).

Adsorption kinetics is important to control the process efficiency. Various kinetic models were studied in the present paper like pseudo-first-order[12] and pseudo-second-order models were studied [13].

#### Pseudo-first-order model

The pseudo-first-order rate equation of Lagergren is generally described by the following equation:

$$\frac{dq_t}{dt} = k_t (q_e - q_t) \quad (3)$$

Where  $k_t$  is the pseudo-first-order rate constant. After integration, by applying the conditions,  $q_t = 0$  at  $t = 0$  and at  $t = t$ ,  $q_t = q_t$ , equation (3) becomes

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

Where  $q_e$  is the amount of dye adsorbed at equilibrium in mg/g. The value of  $k_t$  can be calculated from the plots of  $\log(q_e - q_t)$  versus  $t$  for different concentrations of crystal violet.

#### Pseudo-second-order model

The pseudo-second-order kinetic model is expressed as follows:

$$\frac{t}{q} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (5)$$

Where  $k_2$  is the second order rate constant (g/mg min). Values of  $k_2$  and  $q_e$  can be calculated from the intercept and slope of the plots of  $t/q$  versus  $t$ . The results of  $k_2$  and correlation coefficients ( $r^2$ ) are shown in Table 1.

#### The Elovich Equation

The Elovich is one of the useful models, it can be expressed as [14](S.H.Chien, W.R.Clayton,1980)

$$\frac{dq_t}{dt} = \alpha \exp(-\beta q_t) \quad (6)$$

Where,  $\alpha$  is the initial adsorption rate ( $\text{mg g}^{-1} \text{min}^{-1}$ ) and  $\beta$  is the desorption constant ( $\text{g mg}^{-1}$ )

To simplify the Elovich equation, it is assumed that  $\alpha\beta t \gg 1$  and by applying boundary conditions,  $q_t = 0$  at  $t = 0$  and  $q_t = q_t$  at  $t = t$  Eq(6) becomes :

$$q_t = \left(\frac{1}{\beta}\right) \ln(t + t_0) - \left(\frac{1}{\beta}\right) \ln t_0 \quad (7)$$

Where  $t_0 = 1/(\alpha\beta)$ . If  $t$  is much larger than  $t_0$ , Eq (7) can be simplified as:

$$q_t = \frac{1}{\beta} \ln(\alpha\beta) + \frac{1}{\beta} \ln(t) \quad (8)$$

A plot between  $q_t$  versus  $\ln(t)$  yield a linear relationship with a slope of  $(1/\beta)$  and an intercept of  $(1/\beta) \ln(\alpha\beta)$ . The term  $1/\beta$  indicates the number of sites available for adsorption. The values of  $\alpha$ ,  $\beta$  and correlation coefficients are shown in Table 1.

The first order and second order kinetic constants along with Elovich model parameters,  $\alpha$  and  $\beta$  are presented in Table 1. The rate-limiting step is an important factor in the adsorption process. Kinetic studies help to identify the adsorption process and predicting the economical design mechanism.

As shown in Table 1, the correlation coefficients for pseudo-first-order and pseudo-second-order models, obtained at all initial dye concentrations were nearly equal, but for pseudo-first-order,  $Q_{cal}$  and  $Q_{exp}$  values showed striking differences. Therefore, based on kinetic reaction, the adsorption was found to favour pseudo-second-order reaction. Thus, based on these results, the pseudo-second-order kinetic model was found to be the rate limiting step. The Elovich kinetic model was also used to explain the present adsorption phenomena. The values of the two constants ( $\alpha$  and  $\beta$ ) of this Elovich equation, were obtained from the slope and intercept of a plot of  $q_t$  versus  $\ln(t)$ , which have the good linearity ( $r^2$  values varies from 0.9230 to 0.984). As observed from the data (Table 2), the values of  $\alpha$  and  $\beta$  showed a general trend of variation (increase in case of  $\alpha$  and decrease in case of  $\beta$ ) with an increase in initial dye concentration. Secondly, as noted before with increasing the initial dye concentration (from 50 to 400 mg/l), the  $\beta$  – values showed a general trend of reduction (0.875 to 0.067 g/ mg). Thus,  $1/\beta$  (which is apparently indicative of the number of sites available for adsorption, as per the second assumption) showed a distinct increase with increase in concentration, reinforcing the occurrence of chemisorptions in the deoiled-adsorbate system.

### Isotherm study and error analysis

Various adsorption isotherm equations have been used to study the nature of adsorption, with the basic idea of optimizing the design of an adsorption process. In the present study, adsorption of dyes was evaluated and compared with popular two and three parameter single-solute isotherm models. For three parameter models (R-P and Sip isotherm), MATLAB (v 7.0) was used to determine the unknown parameters, correlation coefficient and the percentage deviation, using the values obtained in the laboratory study on dye adsorption on to the Jatropha deoiled cake. The percent deviation was calculated as follows:

$$\text{Percent deviation} = \frac{q_{e,exp} - q_{e,calc}}{q_{e,calc}} \times 100 \quad (9)$$

Table 1. The adsorption kinetic rate constants for the CV adsorption on Jatropha

Initial Concentration (ppm)	Experimental	Pseudo first-order		Calculated	Pseudo second-order			Calculated	Elovich model		
	qe (mg/g)	$k_1$	$r^2$	qe (mg/g)	$k_2$	h	$r^2$	qe (mg/g)	$\beta$	$\alpha$	$r^2$
50	10.483	0.053	0.995	9.183	0.037	4.202	0.9980	10.638	0.876	107.777	0.923
100	21.244	0.048	0.991	18.030	0.016	7.519	0.9970	21.739	0.395	107.059	0.934
200	41.364	0.051	0.991	36.728	0.008	15.873	0.9980	43.478	0.224	444.575	0.958
400	90.435	0.032	0.994	82.414	0.002	20.408	0.9940	100.000	0.067	559.054	0.974

The equation for bi-parametric Langmuir's isotherm equation can be expressed as follows:

$$q_e = \frac{q_m b C_e}{1 + b C_e} \quad (10)$$

$q_m$  the maximum adsorption capacity (mg/g) and  $b$  is the constant related to the free energy of adsorption (L/ mg).

The Freundlich equation is an empirical model that considers heterogeneous adsorptive energies on the adsorbent surface and the equation expressed as follows:

$$q_e = K_F C_e^{1/n} \quad (11)$$

Where  $K_F$  is a constant, indicating the relative adsorption capacity of the adsorbent ( $(\text{mg}^{1-(1/n)} \text{L}^{1/n}) / \text{g}$ ) and  $n$  is a constant, representing the intensity of adsorption.

### Modified Langmuir – 2

$$q_e = \frac{b C_e}{1 + b C_e} \left( \frac{1 + \sigma^2 (1 - b C_e)}{2(1 + b C_e)^2} \right) \quad (12)$$

Where  $q_e$  and  $C_e$  represent their usual connotations (as given above) and  $\sigma$  is the isotherm constants related to the degree of sorption.

**Dubinin-Radushkevich (D-R)** isotherm is often used to estimate the characteristic porosity in addition to the apparent free energy of adsorption. Thus, for evaluating these parameters the Dubinin- Radushkevich isotherm is being used in the following form:

$$q_e = K_{DR} \exp \left[ b \left( RT \ln \left( 1 + \frac{1}{C_e} \right) \right)^2 \right] \quad (13)$$

Here,  $K_{DR}$  relates to free energy of sorption, and  $b$  is the D-R isotherm constants related to the degree of sorption and  $R$  is the universal Gas constant.

**Temkin** isotherm model considered that the effect of adsorbate interactions on adsorption isotherms and suggested that because of these interactions the heat of adsorption of all the molecules in the layer would decrease linearly with coverage. The Temkin isotherm expressed in the following form:

$$q_e = \frac{RT}{b} (\ln AC_e) \quad (14)$$

$A$  and  $b$  are the isotherm constants.

#### Langmuir-Freundlich isotherm

$$q_e = \frac{q_{mLF}(K_{LF}C_e)^{m_{LF}}}{1+(K_{LF}C_e)^{m_{LF}}} \quad (15)$$

Where  $q_e$  is the adsorbed amount at equilibrium (mg/g),  $q_{mLF}$  is the Langmuir-Freundlich maximum adsorption capacity (mg/g),  $C_e$  is the adsorbed equilibrium concentration (mg/L),  $K_{LF}$  is the equilibrium constant for a heterogeneous solid and  $m_{LF}$  Langmuir-Freundlich heterogeneity parameter lie between 0 and 1. The Langmuir-Freundlich isotherm model at low sorbate concentrations effectively gets reduced to Freundlich isotherm and thus does not follow Henry's law. At high sorbate concentrations, it behaves as monolayer sorption and shows the Langmuir isotherm characteristics.

#### Redlich-Peterson Model

The Redlich-Peterson (1959) model is a three-parameter model given by:

$$q_e = \frac{x}{m} = \frac{AC_e}{1 + BC_e^\beta} \quad (16)$$

where,  $x$  = Moles of adsorbate adsorbed (mg)

$m$  = Mass of adsorbent (g)

$C_e$  = Final adsorbate concentration (mg/l)

The three constants are  $A$ ,  $B$ , and  $\beta$ . The parameter ' $A$ ' has the units of mg/g, ' $B$ ' has the units of (l/g), and  $\beta$  lies between 0 and 1 (Redlich and Peterson, 1959).

Sip isotherm model equation is expressed as follows:

$$q_e = \frac{(K_S C_e)^\beta}{[1 + (\alpha_S C_e)^\beta]} \quad (17)$$

Where  $K_S$  (L/g) and  $\alpha_S$  (L/mg) $^\beta$  are the Sips isotherm constants and  $\beta$  is the exponent which lies between 1 and 0.

The Figure. 2 (comparison of Langmuir, Freundlich, Modified Langmuir-2, D-R and Temkin) and Figure. 3 (comparison of Redlich-Peterson, Langmuir-Freundlich and Sip isotherm) represent the comparison of experimental and calculated amount of dye adsorbed onto the Jatropha deoiled cake. The suitability of the models was evaluated based on study of correlation coefficient, absolute sum of squares and standard deviation of residuals (Sy.x).

For Crystal Violet dye, all the models discussed in preceding paragraphs were found to represent the experimental data well. Langmuir isotherm model was found to fit the data reasonably, with a correlation coefficient 0.998, absolute sum of squares 24.17 and standard deviation of residuals 2.458. Freundlich isotherm model was found to

have good agreement between the experimental and observed data, with a correlation coefficient of 0.9925. The absolute sum of square value is 91.33, indicating slight divergence from the experimental data. Modified Langmuir-2 isotherm model was found to have not in good agreement between the experimental and observed data, with a correlation coefficient of 0.7585. The absolute sum of square value was 2942. D-R isotherm model was found to have correlation coefficient of 0.923 and absolute sum of squares value as 938.3. Temkin adsorption isotherm model, on the other hand has fitted the data reasonably well with a correlation coefficient 0.9949 and absolute sum of squares value as 62.10. The fitted parameter values for all the isotherms used are shown in Table.2.

Table 2. The Adsorption Isotherm parameters with correlation coefficients

Isotherm	Adsorbent	Isotherm	Adsorbent
<b>Langmuir Best fit values</b>		<b>Redlich-Peterson Best fit values</b>	
B	0.006559	$K_{RP}$	2.667
$q_m$	453.4	$\alpha RP$	0.001374
Std. Error		$\beta$	1.269
b	0.0004685	Std. Error	
$q_m$	19.73	$K_{RP}$	0.234
$R^2$	0.998	$\alpha RP$	0.00217
Absolute Sum of Squares	24.17	$\beta$	0.2806
a standard deviation of residuals(sy.x)	2.458	$R^2$	0.9985
		Absolute Sum of Squares	18.66
		the standard deviation of residuals(sy.x)	2.494
<b>Freundlich Best fit values</b>		<b>Sip Best fit values</b>	
$K_F$	8.985	$K_S$	391.9
n	1.546	$\beta$	0.00865
Std. Error		$\alpha$	1.098
$K_F$	1.268	Std. Error	73.86
n	0.07322	$K_S$	0.003005
$R^2$	0.9925	$\beta$	0.1458
Absolute Sum of Squares	91.33	$\alpha$	
the standard deviation of residuals(sy.x)	4.778	$R^2$	0.9983
		Absolute Sum of Squares	20.91
		the standard deviation of residuals(sy.x)	2.64
<b>Langmuir Modified -2</b>		<b>Langmuir-Freundlich model</b>	
B	0.2903	QM	391.9
Z	-0.1379	K	0.00865
Std. Error		M	1.098
B	0.02003	Std. Error	
Z	0.002505	QM	73.86
$R^2$	0.7585	K	0.003005
Absolute Sum of Squares	2942	M	0.1458
the standard deviation of residuals(sy.x)	27.12	$R^2$	0.9983
		Absolute Sum of Squares	20.91
		Sy.x	2.64
<b>D-R</b>			
KDR	220.4		
B	260.9		
Std. Error			
KDR	12.46		
B	45.49		
$R^2$	0.923		
Absolute Sum of Squares	938.3		
Sy.x	15.32		
<b>Temkin</b>			
B	0.02554		
A	0.06284		
Std. Error			
B	0.0009141		
A	0.003706		
$R^2$	0.9949		
Absolute Sum of Squares	62.1		
Sy.x	3.94		

Isotherm	Adsorbent
<b>Redlich-Peterson Best fit values</b>	
K <sub>RP</sub>	2.667
α <sub>RP</sub>	0.001374
β	1.269
Std. Error	
K <sub>RP</sub>	0.234
α <sub>RP</sub>	0.00217
β	0.2806
R <sup>2</sup>	0.9985
Absolute Sum of Squares	18.66
the standard deviation of residuals(sy.x)	2.494
<b>Sip Best fit values</b>	
K <sub>s</sub>	391.9
β	0.00865
α	1.098
Std. Error	73.86
K <sub>s</sub>	0.003005
β	0.1458
α	
R <sup>2</sup>	0.9983
Absolute Sum of Squares	20.91
the standard deviation of residuals(sy.x)	2.64
<b>Langmuir-Freundlich model</b>	
QM	391.9
K	0.00865
M	1.098
Std. Error	
QM	73.86
K	0.003005
M	0.1458
R <sup>2</sup>	0.9983
Absolute Sum of Squares	20.91
Sy.x	2.64

Since for *Jatropha* deoiled cake, all the eight isotherm models (five two parameters and three three parameters) studied showed high  $r^2$  values (more than 0.9) except for Modified Langmuir-2 ( $r^2$  0.7585), it possibly indicates simultaneous validity of multiple isotherm models and corresponding axioms. It was also observed that the distribution of the average percent deviation was reduced from two parameter models to three parameter models (Figure.4 and 5). On comparison of all the three fitness parameters (namely, the correlation coefficient, absolute sum of squares and standard deviation of residuals), the three parameter models namely Langmuir-Freundlich, Redlich-Peterson and Sip seemed to be most suitable for understanding the adsorption mechanisms of *Jatropha* deoiled cake studied. Hence, the best fitting order of the isotherm models for both the soil is determined to be Sip (=Redlich-Peterson) > Langmuir-Freundlich > Langmuir > Freundlich > Temkin > D-R > Modified Langmuir-2.

### CONCLUSION

In the present study, adsorption batch studies of crystal violet dye onto *Jatropha* deoiled cake were carried out. The studies indicate *Jatropha* deoiled cake as an effective adsorbent for removal of dye from aqueous solution. Based on the kinetic modeling (pseudo-second-order model and Elovich model) the adsorption phenomenon seemed to be a chemisorption process. Although all the eight adsorption isotherms models (namely, the Langmuir, Freundlich, Modified Langmuir-2, D-R, Temkin, Langmuir-Freundlich, Sip and Redlich-Peterson) were obeyed fairly well by *Jatropha* deoiled cake except Modified Langmuir-2 isotherm model, yet the best models explaining the adsorption were found to be the Sip and Redlich-Peterson isotherm models. Further modifications in deoiled cake are in progress to assess their increased potential as adsorbents for removal of toxic chemicals.

### REFERENCES

- [1] S. Couto Rodríguez, J. Osma Toca, J. L. Herrera. *Eng. Life Sci.* **2009**, 9, 116–123.  
 [2] T. Deveci, A. Unyayar and M. A. Mazmanci. *J. Mol. Catal., B Enzym.*, **2004**, 30: 25-32.

- [3]C. Novotny, N. Dias, A. Kapanen, K. Malachova, M.Vandrovcova, M.Itavarra,N. Lima. *Chemosphere*, **2006**,63, 1436-1442.
- [4]HR.Kariminiaae-Hamedani,K, Kanda,F Kato. *J Biosci Bioeng*. **2004**; 97:39–44.
- [5]Z. Kyzas George. *Materials*,**2012**, 5(10), 1826-1840.
- [6]G.Crini. *Bioresource & Technology*, **2006**,97, 1061-1085.
- [7]P.Sirisomboon,P. Kitchaiya, T.Pholpho and W.Mahuttanyavanitch. *Biosystems Engineering* ,**2007**,97, 201–207.
- [8]Heller, Joachim. Physic nut. *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops. 1. Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute,**1996**, Rome.
- [9]D.Saetae, W.Suntornsuk. Antifungal activities of ethanolic extract from *Jatropha curcas* seed cake. , **2010**,20(2):319-24.
- [10]R.Zanzi, JAS.Perez, PB.Soler. Production of biodiesel from *Jatropha curcas* in the region of Guantanamo in Cuba. In: Proceedings of the 3rd international congress university-industry cooperation, Ubatuba, **2008**,Brazil.
- [11]O.Aiyelaagbe, K. Adesogan,O. Ekundayo and JB. Gloer. *Phytochemistry*,**2007**, 68, 2420 – 2425.
- [12]Y.S.Ho, G. McKay. *Process Biochemistry*, **1999**, 34,451–465.
- [13]Ho Y.S, J.C.Y.Ng and G.McKay. *Sep. Purif Methods*, **2000**, 29,189-232.
- [14]Chien and Clayton. *Soil Science Society of America Journal*,**1980**, 44 (2), 265–268.