Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 07, pp.1815-1822, July, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

BIOREMOVAL OF TRIPHENYLMETHANE DYE MALACHITE GREEN BY *Pseudomonas stanieri*: KINETIC AND EQUILIBRIUM STUDIES

[1]Ilayaraja, M., [2]Kulandaivel, S. and *,[1]Sayee Kannan, R.

[1]Department of Chemistry, Thiagarajar College, Madurai-625009, Tamil Nadu, India
[2]Department of Zoology and Microbiology, Thiagarajar College, Madurai-625009, Tamil Nadu, India

ARTICLE INFO

Article History:
Received 29th April, 2013
Received in revised form
06th May, 2013
Accepted 15th June, 2013
Published online 18th July, 2013

Key words:
Malachite green, *Pseudomonas stanieri*, Biodecolorization, Kinetics, Isotherms.

ABSTRACT

An isolated marine bacterium *Pseudomonas stanieri* was studied for its biodecolorization potential using Malachite Green (MG) as a model dye pollutant. Various carbon sources (Sucrose and Starch) and nitrogen source (peptone) were optimized for bacterial growth and decolorization of dye. Maximum rate of MG decolorization (50 ppm) was observed within 60 h by the isolated bacterium in the culture medium by plate assay method. The decolorization was confirmed by UV-VIS spectrophotometer. The initial dye solution showed high peak at the wavelength of 619 nm. The decolorization dye showed disappearance of peak. The decolorization rate was in direct proportion to the biomass concentration. The effect of dye concentration (50-250 ppm), pH (6-9), temperature (30 -50˚C) and inoculums size (5-25 ml) on dye removal potential of the bacterium was studied. Kinetic data were described by pseudo-first and second-order models. Langmuir and Freundlich adsorption models were applied to analyze the isotherms and isotherms constant.

Copyright, IJCR, 2013, Academic Journals. All rights reserved.

INTRODUCTION

Dyes are considered to be particularly dangerous organic compounds for the environment. Now a days, many countries like India consume approximately 600 thousand tons of dyes per annum (Ishikawa *et al*., 2000). The total annual global textile dye production is estimated to be 800 tons. All dyes used in textile industry are designed to resist fading upon exposure to sweat, light, water, many chemicals including oxidizing agents and microbial attack. During processing, up to 15% of the dyestuff is released into the water (Vaidya and Datye, 1982). Malachite Green is a basic dye and water soluble. It dissociate into anion and coloured cations. It belongs to triphenyl methane class and it contains two amino groups. Malachite Green is used extensively in textile industries for dyeing nylon, wool, silk, leather, and cotton (Culp and Beland, 1996). Although MG is not approved by the U.S. Food and Drug Administration, its worldwide use in aquaculture will probably continue due to its relatively low cost, ready availability and efficiency. Therefore, potential human exposure to MG could result either from the consumption of treated fish or from working in the dye and aquaculture industries (Chang-jun *et al*., 2001). Malachite Green is highly toxic to mammalian cells by promoting hepatic tumor formation in rodents and also causes reproductive abnormalities in rabbits and fish (Fernandes *et al*., 1991; Rao, 1995). These industrial effluents are toxic and are characterized by high chemical oxygen demand (COD), biological oxygen demand (BOD), suspended solids and intense color. These are highly conjugated and can be extremely injurious to life. Synthetic dyes are classified by their chromophore, have different and stable chemical structure to meet various coloring requirement and often not degraded or removed by conventional, physical and chemical processes such as adsorption, coagulation, flocculation, oxidation, filtration and electrochemical methods (Calabro *et al*., 1991). Now a days biological decolorization has been investigated as a method of transfer, degrade or mineralize dyes (Bunut *et al*., 1996). Biodegradation capacities are due to highly non-specific, free-radical mediated processes resulting from the activities of several enzymes secreted by fungi and bacteria such as Laccase, Manganese peroxidase and lignin peroxidase (Fu and Viraraghavan, 2001). But fungal treatment of effluents is usually time consuming process. A wide range of bacteria were studied for their ability to decolorize synthetic dyes, including *Aeromonas hydrophila* (Chen *et al*., 2003), *Pseudomonas luteola* (Chang *et al*., 2001) and *Escherichia coli* NO_3 (Chang and Kuo, 2000). Aerobic degradation of triphenylmethane dyes has been demonstrated repeatedly. However, these dyes resist to degradation in activated sludge system (Yatome *et al*., 1993; Parshetti *et al*. 2006). The present study aims to investigate the potential of *Pseudomonas stanieri* isolated from marine water for decolorizing a solution containing MG dye. Correlation of the kinetic properties with dye concentration and other rate-dependent environmental parameters like temperature, pH, dye concentration, inoculums size and culture media were characterized. The kinetic data were analyzed by pseudo-first- and pseudo-second-order models. To predict the nature of the adsorption process, the non-linear Langmuir and Freundlich isotherms were investigated to determine the best fit for predicting the nature of the adsorption process from equilibrium adsorption data.

Corresponding author: Sayee Kannan, R.
Department of Chemistry, Thiagarajar College, Madurai-625009, Tamil Nadu, India

MATERIALS AND METHODS

Chemicals and Media

The triphenyl methane dye used in this study was malachite green (Fig. 1). (Sigma chemical company). All microbiological media and medium ingredients were purchased from HiMedia laboratories (Mumbai, India). All the reagents used in this study were of AR grade.

Isolation and screening of dye decolorizing bacteria

Marine water sample was collected from seashore of Rameswaram, Tamil Nadu. The dye decolorizing bacteria was isolated from marine

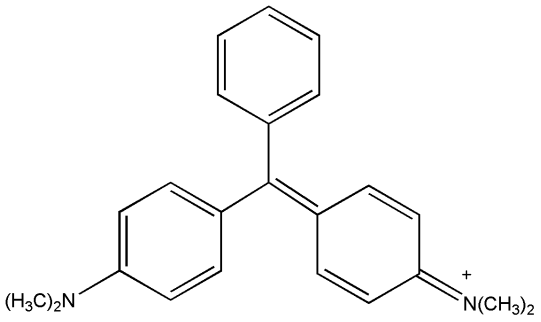

Fig.1. Chemical structure of malachite green

water by serial dilution technique and plating appropriate dilutions on marine agar medium containing (g/L): Sodium Chloride 19.40, Potassium Bromide 0.08, Magnesium Chloride 8.80, Strontium Chloride 0.034, Peptone 5.00, Boric Acid 0.22, Sodium Sulfate 3.24, Disodium Phosphate 0.008, Calcium Chloride 1.80, Sodium Silicate 0.004, Yeast Extract 1.00, Sodium Fluoride 0.0024, Potassium Chloride 0.55, Ammonium Nitrate 0.0016, Sodium Bicarbonate 0.16, Agar 15.00 and Ferric Citrate 0.10. Final pH was adjusted to 7.6±0.2 at 25˚C. The isolated cultures were studied by inoculating them in nutrient agar medium containing (g/L): peptone-5, beef extract-3, yeast extract-2, sodium chloride-5 at pH-7.0.

Identification of dye degrading bacteria

Plate assay was performed for detection of dye decolorizing activity of bacteria. The nutrient agar and malachite green (500 mg/L) was autoclaved at 121˚C for 15 min. The isolated cultures were inoculated on nutrient agar medium. The plates were wrapped with parafilm and incubated at 37˚C for seven days. The plates were observed for clearance of the dye surrounding the colonies. Dye degrading bacteria was identified on the basis of morphological and biochemical tests according to Bergey's Manual of Systematic Bacteriology (Sneath *et al*., 1984) and it was further confirmed by 16S rRNA sequencing.

Evaluation of dye decolorization

Decolorizing activity was performed in 100ml of medium-I (1% starch and 1% peptone) and medium-II (1% sucrose and 1% peptone) containing 5 mg of malachite green and 10 ml of inoculums. Uninoculated dye medium served as control. The flasks were incubated for three days under stationary and shake culture condition. About 2 ml samples were withdrawn aseptically and centrifuged at 8000 rpm for 10 min. The clear supernatant was used for measuring absorption at 619 nm using UV-Vis spectrophotometer (HITACHI, JAPAN). The percentage of decolorization of dye was determined by using the formula:

$$\text{Decolorization (\%)} = \frac{(I-F)}{I} \times 100$$

Where, I= Initial absorbance and F= Absorbance of decolorized medium.

Optimization of dye decolorization

Decolorization of malachite green dye by the isolate was optimized with respect to the effect of 1% starch and peptone, 1% sucrose and peptone, different concentration of dye (50-250 mg/L), inoculums size (5-25 ml), pH (5-8), temperature (30-50˚C) and at different culture conditions such as agitation and stationary conditions. Every 12 h interval, the samples were withdrawn and analyzed for % of decolorization.

Kinetic modeling

The data obtained from the contact time- dependent experiments were further used to evaluate the kinetics of the adsorption process of Pseudo-first-order (Lagergren, 1898; Saha *et al*., 2010) and Pseudo-second-order (Ho and McKay, 1999; Saha *et al.*, 2010). Kinetics models were tested to obtain the rate constant and equilibrium adsorption capacity at different inoculums.

Adsorption isotherm

The equilibrium sorption isotherm is fundamental in describing the interactive behavior between sorbates and sorbent and for the design and analysis of sorption system. In the present investigation, isotherm study of MG was conducted at different concentrations by keeping the inoculums size fixed at 10 ml (3 mg)**.**

RESULTS AND DISCUSSIONS

Isolation, Screening and Identification of Dye Degrading Bacteria

An isolated bacterium was identified by microscopic and biochemical tests. Further it was confirmed as *Pseudomonas stanieri* by 16S rRNA gene sequencing and it was deposited in NCBI gene bank (Accession no. eu732701). Normally textile dye effluent sample was collected from the disposal site for screening of microbes for all the researchers. But we collected the sample from marine water, it decolorize the dye in very high efficiency. *Pseudomonas sp.* and *Shewanella* strains were isolated from dye effluent due to its efficient dye removal capacity and has been studied extensively for Azo dye decolorization (Hong *et al.*, 2007., Xu *et al.*, 2007). However there is no information on its resistance to salinity was available. So we isolated marine microbes. Meng *et al*. (2012) reported that initial growth rate of *Shewanella* strain decreased with the increase in NaCl concentration. The growth of microbes in the presence of 1-3% NaCl after 23 hrs was 17.6-27.6% higher than that observed under NaCl free condition.

Evaluation of dye decolorization

Effect of culture condition on dye decolorization

The isolated *Pseudomonas staineri* grown under agitation conditions demonstrated a better growth and dye removal than that under static conditions. Agitate culture exhibited apparent decolorization (85.4%) of MG within 60hrs in medium-I. In anaerobic or static conditions, the bacterial decolourizing was less due to less cell growth than that under aerobic conditions. Bacterial biomass, cell surface are important for uptake and removal of dye effectively (Guang-feiLiu *et al*., 2006). Analysis if the product of MG dye reduction process, using spectrophotometer, photographic evidence was used to monitor the colour change taking place during treatment. We got two peaks (425 nm and 573 nm) for standard dye (Fig. 2). After 60 h incubation of dye with bacterial culture, the degradation products of dye was analyzed in the visible regions, λ_{max} 515 nm and 612 nm were obtained. The microorganisms can degrade MG to less harmful product and can absorb or decolorize the compound through fixation and secretion of secondary metabolites (enzymes and organic acid) by the functional groups present on the cell's surface. Ponraj *et al.* (2011) reported that the range of activity on decolorization of orange 3R supplemented with sucrose was 87.8%, 72.36%, 86.18% and 80.49% with *Bacillus sp., Klebsiella sp., Salmonella sp., and Pseudomonas sp*., respectively. The nitrogen source peptone was used in this study, it helps the growth of microbes more ultimately, and the range of activity on decolorization of MG with 1% starch and peptone was 85.4% and 1% sucrose with peptone 82.8%. When the concentration was increased, the decolorizing activity decreased because of high concentration reduce the distance between the bacterial cells, affecting the charge distribution of their neighbors and altering the ability of the bacteria to migrate to the biomass transfer, resulting in reduced adsorption. But in low concentration, this

unexpected increase of removal percentage observed could be explained by the increase on the biomass concentration, turbidity, probably motivated by the presence of a carbon and protein sources. Fig.3 reveals that displays image in the culture medium, standard MG solution and the image of the dye solutions that had been treated with bacterial cells at 60 h incubation period.

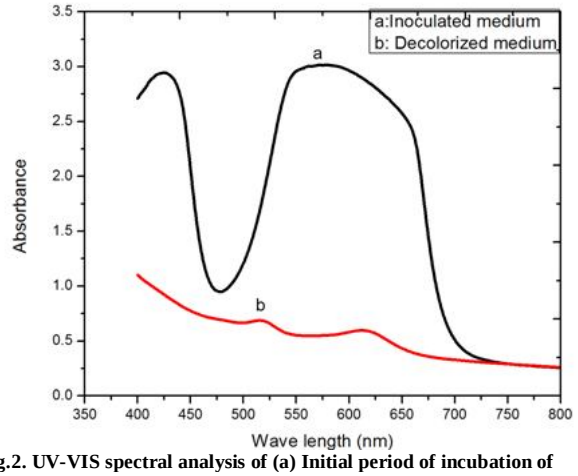

Fig.2. UV-VIS spectral analysis of (a) Initial period of incubation of inoculated medium (b) Decolorized medium (after 60 h incubation)

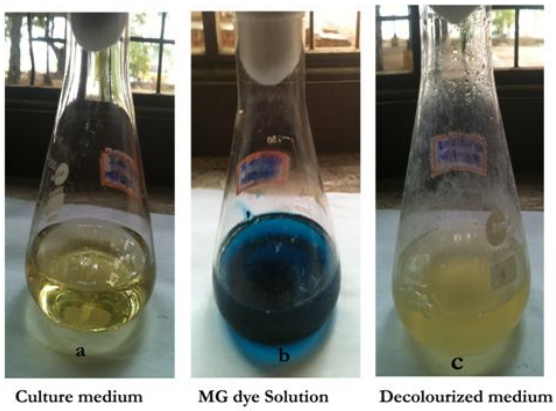

Fig.3. Showing the (a) Culture medium (b) MG dye solution (b) Decolorized medium

Effect of dye concentration on decolorization

The percentage of color removal from liquid medium shows the best bio-decolorization activity. The effect of dye concentration on decolorization under agitation and stationary condition was shown in Table 1. Maximum decolorization was observed at 50 ppm (85.4%) in the medium-I and 82.8% were occurred in medium-II at agitation condition.

Effect of inoculums on dye decolorization

In this study, the ability of *Pseudomonas stanieri* in biodegradation of MG was investigated. The percentage of decolorization increased with increase in the inoculums size, reaching maximum 94.1% at 20 % (v/v) of inoculums size (Table 2). The maximum decolorizing rate was reached (0.78 mg/l/h) at 20 % (v/v) inoculums size. But decreases further, Verma and Madamwar (2003) reported that the Direct red 81 decolorization rate was increased with increase in the inoculums size 2.53 mg/l/h at 20% inoculums size in medium-I and 0.80mg/l/h in medium-II. AR27 reduction required the presence of bacterial cell and faster decolorization was observed in the presence of higher initial cell concentration. Meng *et al.* (2012) reported that 4.5% and 54% AR27 were removed in 12 h when the biomass was 0.04 and 0.18 g/L respectively. In our study 62.6% and 55.7% dye were removed in 12 h, when the biomass concentration 0.06 g/L in the medium-I and medium-II respectively. The reduction rate generally decreased as time went on at all cell concentration, which might be due to the gradually decreased dye concentration. Lamia Ayead *et al.*, (2009) reported that the dye removal increased significantly along with an increase in the biomass concentration 82.4%. Similar observation was reported during decolorization of MG by micro algae *Cosmarium sp* (Daneshvar *et al*., 2007). Parashetti *et al*., (2006) reported that 13% of MG decolorization was observed at 70 mg/L after five hours by *Kocuria rosea* MTCC 1532. In our study decolorization activity of *Pseudomonas stanieri* has 96.5% in 10% of inoculums at 60 h. Ponraj *et al*., (2011) reported that *Pseudomonas sp* have highly decolorize 50% with 10% inoculum at 144 h. Our isolate was highly efficient strain compared to others. Kumar *et al*. (2009) used a mixed culture for decolorization of reactive azo dye and reported 98% at 10% inoculums size.

Effect of pH on decolorization of dye

pH is a major factor for the growth of bacteria. So we optimized the pH for the dye decolorization. Maximum decolorization activity was observed at pH 8.0 (90.9%, 88.4%) in the medium-II and medium-I

Table 1. Effect of dye concentration on dye decolorization under Agitation and stationary condition

Condition	Time (h)	% of decolourization									
		Medium-I (ppm)					Medium-II (ppm)				
		50	100	150	200	250	50	100	150	200	250
Agitation	12	52.6	49.3	46.5	39.5	35.4	46.3	42.5	38.6	34.5	29.8
	24	62.5	58.6	52.4	48.7	39.9	57.2	54.6	52.8	47.4	45.1
	36	66.5	62.1	58.7	56.5	52.1	65.1	62.9	58.4	52.6	48.4
	48	82.2	72.9	68.5	64.5	61.1	78.1	72.5	68.9	59.9	57.1
	60	85.4	77.0	72.3	68.4	65.3	82.8	78.1	72.7	66.5	60.5
Stationary	12	39.6	25.5	22.9	18.9	16.5	34.8	29.1	22.5	16.9	12.1
	24	42.7	38.9	32.6	25.4	20.6	41.6	38.4	29.5	22.1	19.8
	36	58.9	42.6	38.7	32.6	28.5	53.6	48.4	40.9	35.6	29.2
	48	69.7	66.5	60.1	54.7	49.5	67.7	62.5	58.7	50.1	46.5
	60	78.6	72.9	67.5	59.6	49.8	71.3	67.5	60.6	57.5	49.1

Table 2. Effect of inoculums size on dye decolorization in agitation condition

S. No	Time (h)	% of decolourization									
		Medium-I					Medium-II				
		5 ml	10 ml	15 ml	20 ml	25 ml	5 ml	10 ml	15 ml	20 ml	25 ml
1	12	23.0	41.6	55.2	62.6	60.6	25.0	38.0	43.2	55.7	35.9
2	24	25.5	73.4	76.0	79.1	76.2	31.2	70.3	75.1	79.6	74.2
3	36	30.2	78.0	86.4	90.3	88.5	86.4	86.9	91.1	94.2	91.1
4	48	65.6	77.6	88.5	90.8	90.1	88.5	89.0	91.6	95.3	94.7
5	60	68.4	81.7	92.8	94.1	93.5	94.5	96.5	97.1	97.1	95.3

respectively. Our result were coincided with the findings of Ponraj *et al*., (2011) reported that *Pseudomonas sp.* shows the highest decolorization (89.09% and 86.72) at pH 6 and pH 8.0 (Table 3). The optimum pH for color removal is between pH 6.0 to 10.0 in most of the dyes (Chen *et al*., 1999). The pH tolerance of discoloring bacteria is quite importance because of reactive MG bind to cotton fibers by addition or substitution mechanism under alkaline conditions (Aksu, 2003). The decolorization rate value decreased as pH was increased further from 7.0 (1.244 mg/l/h) to 8.0 (1.129 mg/l/h) (Gurulakshmi, 2008). *Pseudomonas luteola* exhibited best decolorization at pH 7.0 with constant decolorization rate up to pH 9.5 (Chang and Lin, 2001). The pH value between 6.0 to 9.0 was optimum for decolorization of triphenylmethane and azo dye by *Pseudomonas sp.* (Mali, 2000).

Table 3. Effect of pH size on dye decolorization in agitation condition

S. No	Time (h)	% of decolourization									
		Medium-I					Medium-II				
		5	6	7	8	9	5	6	7	8	9
1	12	2.4	6.3	48.9	53.4	28.3	3.4	9.1	51.2	29.9	56.1
2	24	7.8	15.2	56.5	59.1	32.5	9.2	14.8	61.4	31.2	61.2
3	36	12.4	27.2	62.8	65.8	37.9	11.8	27.2	68.7	38.6	67.9
4	48	23.8	44.5	71.9	77.4	39.2	22.1	43.4	70.8	41.4	75.5
5	60	32.7	49.1	82.3	89.4	41.3	29.9	50.3	84.5	41.3	90.9

Table 4. Effect of temperature on dye decolorization in agitation condition

S. No	Time (h)	% of decolourization					
		Medium-I			Medium-II		
		30 ˚C	40 ˚C	50 ˚C	30 ˚C	40 ˚C	50 ˚C
1	12	50.2	54.8	49.1	51.5	54.5	47.8
2	24	54.5	61.4	50.3	52.2	59.6	52.2
3	36	60.1	69.1	55.2	54.6	73.2	55.7
4	48	71.8	80.5	61.4	65.8	75.4	64.5
5	60	86.5	91.6	69.8	65.4	89.8	68.1

Table.5. Pseudo-first-order and Pseudo- second-order kinetic models for the adsorption of MG dye onto *Pseudomonas stainieri* bacteria at various cells mass

	Inoculums size (ml)	Medium-I			Medium-II		
		q_e cal (mg/g)	$k_1\times10^{-3}$	R^2	q_e cal (mg/g)	$k_1\times10^{-3}$	R^2
First-order-kinetic	5	6.54	0.008	0.118	13.21	0.013	0.617
	10	154.17	0.045	0.794	36.89	0.016	0.544
	15	81.47	0.055	0.950	62.51	0.050	0.859
	20	20.98	0.051	0.940	14.68	0.047	0.957
	25	1.67	0.023	0.927	1.46	0.024	0.984
		q_e cal (mg/g)	$k_1\times10^{-3}$	R^2	q_e cal (mg/g)	$k_2\times10^{-3}$	R^2
Second-order-kinetic	5	303.0	2.0096	0.104	101.3	2.3092	0.007
	10	65.35	1.1366	0.971	96.15	3.4122	0.955
	15	47.84	1.7834	0.997	58.13	7.9770	0.966
	20	34.36	3.6826	0.998	39.6	1.7765	0.986
	25	31.74	1.6627	0.917	39.8	7.8914	0.894

Effect of temperature on dye decolorization

The dye decolorization activity of our culture was found to increase with increasing incubation temperature (Table 4) from 30˚C to 50˚C with maximum activity obtained at 40˚C (4.58 mg/l/h). Further increase in temperature resulted in drastic reduction in decolorization activity of the bacterial culture *Pseudomonas stanieri.* Decline in decolorization activity at higher temperature can be attributed to the loss of cell viability or the denaturation of MG reductase enzyme. Maximum dye decolorization activity of the bacterial consortium NBNJ6 was noticed at 37˚C (Junnarker *et al*., 2006). Ponraj *et al*., (2011) reported that the range of activity on decolorization of orange 3R with 37˚C was 72.22% with *Pseudomonas sp.* Lamia Ayed *et al*., (2009) proved that no thermal inactivation of decolorization activity under operational temperature indicating that *S. paucimopilis* are active at a broad range of temperature which led to their use for the treatment of dyeing waste water.

Kinetics studies

cording to Froehner *et al*., (2009a), various steps may control the kinetics of the adsorption phenomena on bacteria such as mass transfer of solute through the boundary film, internal diffusion of solute and sorption of solute onto sites. Some of them are fast such as sorption onto sites and others are slow, depending on some parameters like agitation and surface homogeneity. Several models can be used to describe the overall mechanism. The kinetic experiments were performed using only microorganisms for initial dye concentrations of 50 ppm and using microorganism mixed with different inoculums size between 5 ml to 25 ml. The experimental kinetic data were fitted by pseudo-first- and pseudo-second-order models. The linearized forms of the pseudo-first-order model and pseudo-second-order (Quintelas *et al*., 2011) are shown below in Eqs. (1) and (2), respectively:

$$\log (q_e - q_t) = \log q_e - k1 \,.\, t \quad (1)$$

$$t/q_t = 1/(k_2.q_e^2) + t/q_e \quad (2)$$

where, q_e is the amount of solvent sorbed at equilibrium per mass unit of sorbent (in milligrams per gram), q_t is the amount of MG sorbed at time t per mass unit of sorbent (in milligrams per gram), k_1 the pseudo-first-order rate constant (in hour) and k_2 is the pseudo-second-order rate constant (in grams per milligrams per hour). The rate constants, predicted uptakes and corresponding correlation coefficients are summarized in Table 5. The plots of both, first- and second-order models are shown in Fig 4.

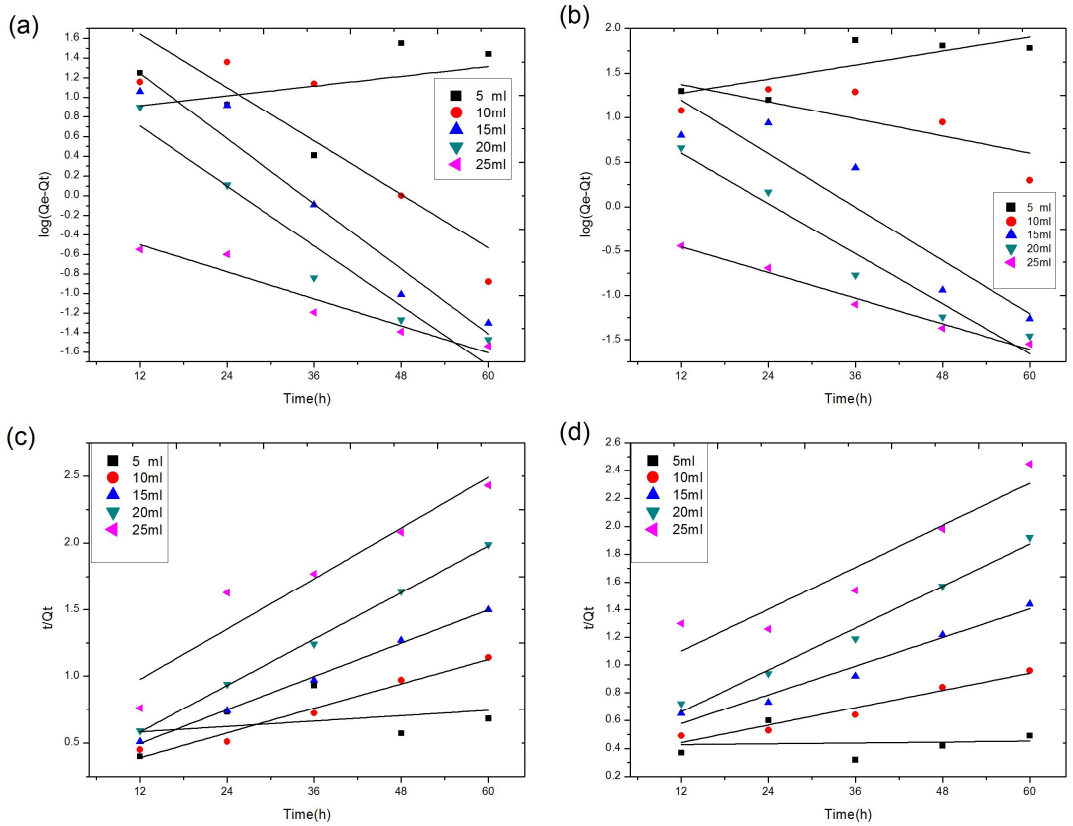

Fig.4. Pseudo-first-order and Pseudo- second-order kinetic models for the adsorption of MG dye onto *Pseudomonas stainieri* bacteria at various cells mass-medium I and medium II

For all studied systems, straight lines with high correlation coefficients were obtained. The pseudo-first-order model assumes that the reaction rate is limited only by one step of sorption on a single class of sites and that all sites are time dependent (Fonseca *et al*., 2009). Pseudo-second-order kinetics model assumes that the rate-limiting step of the process may be chemisorptions (Kumar *et al*., 2011), involving valence forces through the sharing or exchange of electrons between the biomass and MG, complexation, coordination and /or chelation (Suazo-Madrid *et al*., 2011). The results showed that the correlation coefficients (R^2) obtained at different inoculums size for the pseudo-first-order kinetic model is in the range of medium-I (0.118 - 0.927) and medium-II (0.617 - 0.984). In addition, the theoretical and experimental equilibrium adsorption capacities, q_e obtained from this kinetic model varied widely at all inoculums size. These findings suggest that adsorption of MG on *Pseudomonas stanieri* cannot be described by the pseudo-first-order kinetic model. Conversely, the kinetic data exhibited an excellent compliance with pseudo-second-order kinetic equation. The plots of t/q_t against t at different inoculums size showed excellent linearity (Fig. 4). The pseudo-second-order rate constant k_2, the calculated q_e values and corresponding linear regression correlation coefficients values R^2 are given in Table 5. As seen in Table 5, the calculated q_e values agree with experimental q_e values well, and also, the correlation coefficients for the pseudo-second-order kinetic plots at all the studied inoculums sizes are significantly higher (R^2>0.99). The best correlation for the system provided by the pseudo-second-order model suggests that chemical sorption involving forces through sharing or exchange of electrons between adsorbent and adsorbate might be significant.

Equilibrium studies

The equilibrium models are extensively used to investigate the amounts of MG dye sorbed by a certain biomass. The distribution of MG dye between solution and biomass is a measure of the position of equilibrium and can be expressed by one or more isotherms. The Langmuir adsorption isotherm has been successfully applied to many pollutants adsorption process and has been the most widely used sorption isotherm for the sorption of solute form a liquid solution (Langmuir, 1918). The common form of Langmuir isotherm is:

$$q_e = q_{max}\, b\, C_e / 1+bC_e \quad (3)$$

Where 'q_e' is the amount of MG dye sorbed at equilibrium (mg/g), 'q_{max}' is the monolayer sorption capacity (mg/g), 'b' is Langmuir constant 'C_e' is concentration of MG dye in solution at equilibrium. The linear form is

$$C_e/q_e = C_e/q_{max} + 1/b\, q_{max} \quad (4)$$

When a plot of (C_e/ q_e) versus C_e is drawn Fig. 5.

The MG maximum adsorption capacity of *Pseudomonas stanieri* was in medium-I and medium-II (15.625 and 13.333 mg/g) respectively. In addition, the coefficients of determination R^2 of the Langmuir equation demonstrated that the adsorption of MG onto *Pseudomonas stanieri* follows the Langmuir's model. Freundlich isotherm is on empirical equation describing adsorption on to a heterogeneous surface. The Freundlich isotherm (Freundlich, 1907): is commonly presented as

$$q_e = K_F\, C_e^{\,1/n} \quad (5)$$

Where K_F and $^{1/n}$ are the Freundlich constants. The linear form is as under

$$\ln q_e = \ln K_F + 1/n \ln C_e \quad (6)$$

Table 6. Langmuir and Freund lich isotherm parameters for the adsorption of MG dye onto *Pseudomonas staineri* bacteria at various concentrations

	Conc. (ppm)	Medium-I			Medium-II		
		Q_{max} (mg/g)	b	R^2	Q_{max} (mg/g)	B	R^2
Langmuir isotherm	50	15.625	0	0.974	13.333	0	0.961
	100	0.488	2.1051	0.241	12.239	6.4335	0.958
	150	14.705	5.2310	0.982	12.345	4.5002	0.954
	200	12.626	3.7715	0.966	10.438	3.0904	0.944
	250	10.992	3.0229	0.973	8.478	2.3925	0.945
		K_F(mg/g)	1/n	R^2	K_F(mg/g)	1/n	R^2
Freundlich isotherm	50	13.580	0.3821	0.943	12.089	0.4683	0.925
	100	13.511	0.5280	0.960	14.554	0.7414	0.938
	150	16.117	0.6292	0.980	14.554	0.7414	0.938
	200	28.569	1.0199	0.277	14.842	0.9602	0.939
	250	15.559	0.96	0.971	14.421	1.20	0.950

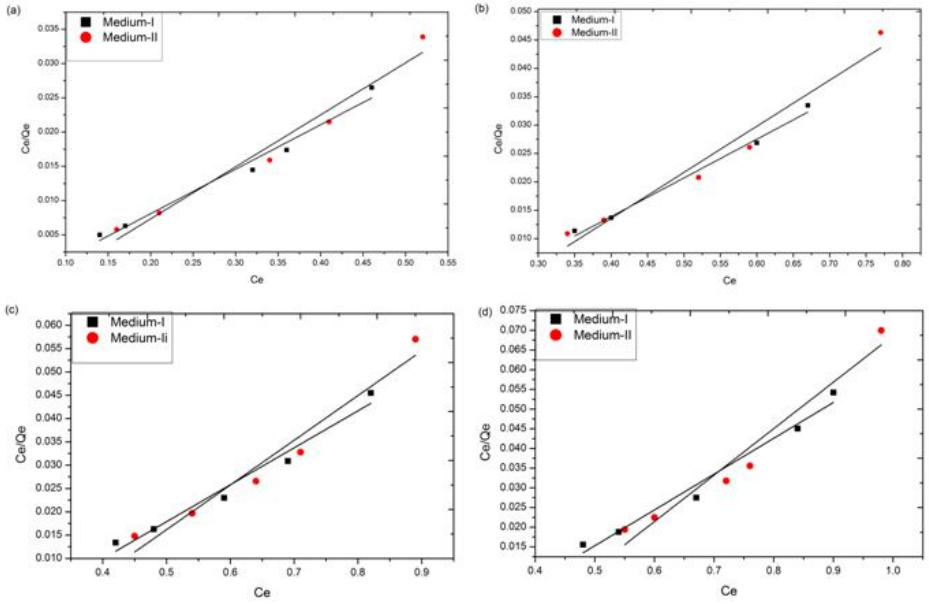

Fig.5. Langmuir isotherm models for the adsorption of MG dye onto *Pseudomonas staineri* bacteria at a) 50 ppm b)150 ppm c) 200 ppm d) 250 ppm concentrations at different medium

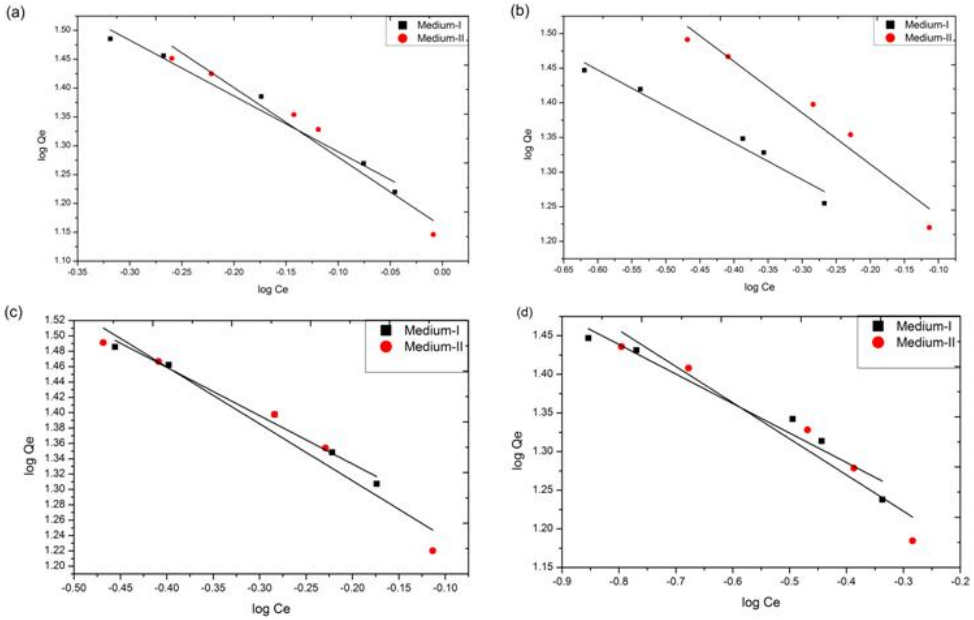

Fig.6. Freundlich isotherm models for the adsorption MG dye onto *Pseudomonas staineri* bacteria at a)50 ppm b)100 ppm c) 150 ppm d) 250 ppm concentrations at different medium

A plot of 'ln q_e' versus 'ln C_e' should yield a straight line with '1/n' as slope and 'lnK_F' as intercept, related to the adsorption capacity and adsorption intensity of the adsorbent respectively. The adsorption of MG onto *Pseudomonas stanieri* was drawn in Fig. 6. It was found that correlation coefficient values were less than 0.99 at both medium. It indicates that Freundlich model was not applicable to present study. The Langmuir and Freundlich adsorption constants and the corresponding correlation coefficients are shown in Table 6. The adsorption of MG was well fitted to the Langmuir isotherm model with the higher R^2 (0.982). It indicated the adsorption took place at specific homogeneous sites within the adsorbent forming monolayer coverage of MG at the surface of the *Pseudomonas stanieri*.

Acknowledgement

The authors wish to acknowledge The management and Department of Chemistry, Thiagarajar college, Madurai-9, for providing lab facilities.

Conclusion

The present study revealed the ability of *Pseudomonas stanieri* to decolorize MG. The result showed that the decolorization depend on dye concentration, initial inoculums size, pH and temperature. The optimal decolorization of MG dye was attributed at 50 ppm dye concentration, 20 ml of inoculums size, pH 8.0 and temperature 40˚C. The spectrum of MG in visible region exhibited a main peak with the maximum at 619 nm. The decrease of absorption peak of MG at 619 nm indicated a rapid degradation of the dye. According to Chang and Kuo, (2000) decolorization of dyes can be due to adsorption to biomass or biodegrading**.** If the dye removed is attributed to biodegradation either the major visible light absorbance peak will disappear or a new peak will appear. These strains have also ability to decolorize other dyes including textile dyes. Based on the obtained correlation coefficient (R^2), the pseudo-second –order equation was the model that best fit for the experimental kinetic data, suggesting chemical sorption as the rate limiting step of the adsorption mechanism. Equilibrium isotherm data were fitted using two parameter models. Among these models, Langmuir model is in good agreement with the experimental data with high R^2 value.

REFERENCES

Aksu, Z., Do¨nmez, G., 2003. A comparative study on the biosorption characteristics of some yeasts for Remozal Blue reactive dye. Chemosphere 50, 1075–1083.

Bunat, I.M., Nigam, P., Singh, D., Marchant, R., 1996. Microbial decolorization of textile dye containing effluents: a review. Bioresour. Technol. 58, 217-227.

Calabro, V., Drioli, E., Matera, F., 1991. Membrane distillation in the textile waste water treatment. Destination. 83, 209-224.

Chang, J.S., Chou, C., Chen, S.Y., 2001. Decolorization of azo dyes with immobilized Pseudomonas *luteola*. Process Biochem. 36,757–763.

Chang, J.S., Kuo, T.S., (2000). Kinetics of bacterial decolorization of azo dye with Escherichia *coli* NO3. Bioresour. Technol. 75, 107–111.

Chang, J.S., Lin, Y.C., 2001. Decolorization of Recombinant *E.coli* strain harboring azo dye decolorizing determinants from *Rhodococcus sp*. Biotechnology Letters. 23, 631-636.

Chang-jun, C., Daniel-Doerge, R., Carl-Cerniglia, E., 2001. Biotransformation of Malachite Green by the fungus *Cunninghamella* elegans. Appl. Environ. Microbiol. 67, 4358–4360.

Chen, K.C., Huang, W.T., Wu, J.Y., Houng, J.Y., 1999. Microbial decolorization of azo dyes by *Proteus mirabilis*. J.Inst. Microbiol and Biotechnol. 23, 686–690.

Chen, K.C., Wu, J.Y., Liou, D.J., 2003. Decolorization of the textile dyes by newly isolated bacterial strains.J.Biotechnol.101,57–68.

Chowdhury, S., Saha, P., 2010. Sea shell powder as a new adsorbent to remove basic green (Malachite green) from aqueous solutions: Equilibrium, kinetic and thermodynamic studies. Chemical engineering journal. 164, 168-177.

Culp, S.J., Beland, F.A., 1996. Malachite Green: a toxicological review. J Am Coll Toxicol. 15, 219–238.

Daneshvar, N., Khataee, A.R., Rasoulifard, M.H., 2007. Biodegradation of dye solution containing Malachite Green: optimization of effective parameters using Taguchi method. J. Hazard. Mater. 143, 214–219.

Fernandes, C., Lalitha, V.S., Rao, K.V.K.,1991. Enhancing effect of Malachite Green on the development of hepatic pre-neoplastic lesions induced by N-nitrosodiethylamine in rats. Carcinogenesis. 12, 839–845.

Fonseca, B., Maio, H., Quintelas, C., Teixeira, A., Tavares, T., 2009. Retention of Cr(VI) and Pb(II) on a loamy sand soil: kinetics, equilibria and breakthrough. J. Chem. Eng. 152, 212–219.

Freundlich, H., 1907. Ueber die adsorption Loesungen. Journal of physical chemisty, 7,385-470.

Froehner, S., Cardoso da Luz, E., Maceno, M., 2009a. Enhanced biodegradation of naphthalene and anthracene by modified vermiculite mixed with soil. Water Air Soil Pollut. 202,169–177.

Fu, Y.,Viraraghavan, T., 2002. "Dye biosorption sites in *Aspergillus niger*". Bioresour. Technol. 82, 139-145.

Guang-fei Liu., Ji-ti Zhou., Jing Wang., Zhi-Yong song., Yuan-yuan, Q.V., 2006. Bacterial decolorization of azo dyes by *Rhodopseudomonas palustrips*, World J. microbiol. Biotechnol. 22, 1069-1074.

Gurulakshmi, M., Sudarmani, D.N.P., Venba, R., 2008. Biodegradation of leather acid dye by *Bacillus subtilis*. Advanced biotech. 2-8.

Ho, Y.S., McKay, G., 1999. Pseudo-second-order model for sorption processes. Process Biochem. 34, 451-465.

Hong, Y.G., Guo, J., Xu, Z.C., Xu, M.Y., Sun, G.P., 2007. Respiration and growth of *Shewanella decolorationis* S12 with an azo compound as the sole electron acceptor. Appl. Environ. Microbiol. 73, 64–72.

Ishikawa, Y., Ekar, T., Leder, A., 2000. Chemical economics hand book: Dyes. Menlo Park (CA):SRI chemical and Health Business Services.

Junnarker, N., Murty, D. S., Nikhil, S. B., Datta, M., 2006. Decolorization of diazo dye Direct Red 81 by a novel bacterium consortium, World J. microbiol. Biotechnol. 22, 163-168.

Kumar, K., Dastidar, M.G., Sreekrishnan, T.R., 2009. "Effect of process parameters on aerobic decolorization of reactive azo dye using mixed culture". World Academy of Science, Engineering and Technology. 58, 962-965.

Kumar, P.S., Ramalingam, S., Sathishkumar, K., 2011. Removal of methylene blue dye from aqueous solution by activated carbon prepared from cashew nut shell as a new low-cost adsorbent. Korean J. Chem. Eng. 28,149–155.

Lagergren, S., 1898. Zur theorie der sogenannten adsorption geloster stoffe, Kungliga Svenska Vetenskapsakademiens, Handlinger. 24, 1-39.

Lamia Ayed., Kamel Chaieb., Abdelkarim Cheref., 2009. Biodegradation of triphenylmethane dye Malachite Green by *Sphingomonas paucimobilis*. World J. Microbiol. Biotechnol. 25, 705-711.

Langmuir, I., 1918. The adsorption of gases on plane surfaces of glass, mica and platinum. Journal of American chemical Society. 40, 1361-1403.

Mali, P. L., Mahajan., M. M., Patil, D.P., Kulkarni, M . V., 2000. Biodecolorization of members of Triphenylmethanes and azo groups of dyes. Journal of Scientific and Industrial Research. 59, 221-224.

Parshetti, G., Kalme, S., Saratale, G., 2006. Biodegradation of Malachite Green by *Kocuria rosea* MTCC 1532. Acta. Chim. Slov. 53, 492–498.

Ponraj, M., Gokila, K., Vasudeo Zambare., 2011. Bacterial decolorization of Textile dye-Orange 3R. Int. J. Advanced Biotechnology and Research. 2, 168-177.

Quintelas, C., Figueiredo, H., Tavares, T., 2011. The effect of clay treatment on remediation of diethylketone contaminated wastewater: uptake, equilibrium and kinetic studies. J. Hazard. Mater. 186, 1241–1248.

Rao, K.V.K.,1995. Inhibition of DNA synthesis in primary rat hepatocyte cultures by Malachite Green: a new liver tumor promoter. Toxicol Lett. 81, 107–113.

Saha, P., Chowdhury, S., Gupta, S., Kumar, I., 2010. Insight into adsorption equilibrium kinetics and thermodynamics of Malachite green onto clay soil of Indian origin. Chemical Engineering Journal, 165, 874-882.

Sneath, P.H.A., Mair, N.S., Sharpe, M.E., Holf, J.G., 1984. "Bergey's Manual of Systematic Bacteriology". Vol. I, Williams and Wilkins, Baltimore, U. S. A.

Suazo-Madrid, A., Morales-Barrera, L., Aranda-Garcia, E., Cristiani-Urbina, E., 2011. Nickel (II) biosorption by Rhodotorula glutinis. J. Ind. Microbiol Biotechnol. 38, 51–64.

Vaidya, A.A., Datye, KV., 1982. Environmental pollution during chemical processing of synthetic fibres, Colourage. 14, 3-10.

Verma, P., Madamwar, D., 2003.Decolorization of synthetic dyes by a newly isolated strain of *Serratia marcescers*. World J. Microbiol. Biotechnol. 19, 615-618.

Xianming, Meng., Guangfei, Liu., Jiti Zhou, Q., Shiang, Fu., Guanghui, Wang., 2012. Azo dye decolorization by *Shewanella aquimarina* under saline conditions. Bioresour. Technol. 114, 95-101.

Xu, M.Y., Guo, J., Sun, G.P., 2007. Biodegradation of textile azo dye by *Shewanella decolorationis* S12 under microaerophilic conditions. Appl. Microbiol. Biotechnol. 76, 719–726.

Yatome, C., Yamada, S., Ogawa, T., 1993. Degradation of crystal violet by Nocardia corllina. Appl. Microbiol. Biotechnol. 38,565–569.

****