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Extraction, growth and immobilization of Yarrowia lipolytica yeast cells for dye effluent treatment

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Abstract: The removal of organic dyes from industrial wastewater remains a problem, both technically and economically. In this study, *Yarrowia lipolytica* yeast cells were isolated from poultry meat and immobilized using alginate. The immobilized *Yarrowia lipolytica* yeast was used as biosorbent to remove methylene blue (MB) dye from synthetic effluent water. The results show that maximum adsorption capacity under optimum conditions was 66.67 $mg \cdot g^{-1}$. The equilibrium adsorption data fitted well onto the Freundlich adsorption isotherms with $R^2>0.99$. Adsorption kinetics was of pseudo-second order process suggesting that the adsorption was a chemisorption. FTIR spectra identified typical absorption bands of a biosorbent. Sorption of MB dye on *Yarrowia lipolytica* yeast cells was exothermic with weak sorption interaction.

Introduction

Years of chemical industrial manufacturing and related human activities have led to widespread organic and inorganic contamination of the environment. The use of dyes for coloring is one of the major contributors to the contamination of the environment. Removal of organic dyes from industrial waste and naturally occurring waters still remains a challenge both technically and economically (Yesiladali et al. 2006). In most developed and developing countries, a number of environmental regulatory measures, with regard to contaminants discharged from industrial operations, are being introduced (Rajaram and Das 2008). However, due to the high cost of removing dyes from industrial wastewater, many industries in developing countries continue to pollute the environment because they avoid the effluent treatment process. Organic dyes are toxic, carcinogenic and discharge of organic dyes from industries into water bodies also results in reduced dissolved oxygen concentration causing anoxic conditions, which subsequently affect aerobic organisms (Chander and Arora 2007, Djordjevic et al. 2014). Most synthetic organic dyes also affect the aesthetic merit and water transparency. Major sources of organic dye pollutants are food processing, hospitals, printing and packaging, plastics, rubber, schools or universities laboratories and photography shops (Chen et al. 2003, Choudhary et al. 2014).

Synthetic dyes are used because they are brighter, color-fast, easier to apply and cheaper to produce. Methylene Blue (MB), a cationic dye, is the most widely used dye in many industrial applications. Research has shown that MB causes eye burns which are responsible for permanent injury to both human and animals. When inhaled MB can give rise to short periods of both rapid and difficult breathing (Unal et al. 2003). When MB is ingested through the mouth, it produces a burning sensation and will cause nausea, vomiting, profuse sweating, mental confusion and methemoglobinemia (Dragan and Lghin 2013).

Most organic dyes are soluble in water and cannot be removed effectively by most physical separation processes. Chemical reduction, precipitation, electro chemical treatment, ion-exchange and reverse osmosis are also the commonly used procedures for organic dye removal from solutions (Babu et al. 2007, Ong et al. 2011, Zou et al. 2015). However, due to the significant disadvantages of incomplete dye removal, high energy requirements, toxic sludge or other waste products generation and more over the high expense of chemicals, there is an increasing demand for eco-friendly technologies using low cost alternatives. Exploiting high biosorption capability exhibited by many alga species, bacteria and yeasts cell might be an alternative cost-effective way of treating such waters (Gadd 2009, Dogan et al. 2016). Biosorption studies of organic dyes have been carried out by a number of researchers. A summary of selected studies is shown in Table 1. Biosorbents based on bacteria or algae suffer from poor mechanical properties and hence immobilizing techniques are being developed (Mareno-Garrido 2008).

Materials and methods

Isolation and growth of Yarrowia lipolytica yeast cells

The streaking techniques along with the aseptic techniques were used to isolate and culture *Yarrowia lipolytica* yeast cell from poultry meat. Yeast extract, glucose chloramphenicol

Table 1. Selected examples of studies on the removal of methylene blue using different biosorbents

Adsorbent	q_{max} (mg·g^{-1})	Reference
Sunflower seed husk	4.757–23.196	Ong et al. 2010
Rice husk	40.58	Vadivelan and Kumar 2005
Wheat shell	16.56–21.50	Bulut and Aydin 2006
WH Root powder	8.04	Soni et al. 2012
Water Hyacinth	17.58–46.35	Murali and Uma 2016
Yarrowia lipolytica yeast	55.56–66.67	This study

(YGC) agar growth media and yeast extract peptone dextrose (YPD) agar both manufactured by Merck (Pvt.) Ltd., were used as media. Poultry meat was incubated for 72 hours at 28°C. Using an inoculating needle, swabs were collected from the poultry meat and placed on the solidified YGC growth media. The streaked yeast cells were then placed in an incubator and the temperature was maintained at 28°C for another 72 hours. For screening, growth samples were then serially diluted. 100 µl from each dilution was again cultivated on a specific agar of yeast extract (YGC). The incubation was done at 28°C for another 48 hours (Mirbagheri et al. 2012). Qualitative screening of *Yarrowia lipolytica* yeast cells was performed on yeast extract YPD medium solution for three to five days. The yeast colonies and YPD growth media solution were then filtered. Isolated yeast cells were identified using assimilation, fermentation and molecular tests as further reported by Mirbagheri et al. 2012. The filtered yeast colonies were deactivated by placing them in a drying oven at 50±2°C for two hours before being immobilized as explained below.

Immobilization of Yarrowia lipolytica yeast cells

The immobilization method was adapted from Berger et al., 2015. A 200-cm^3 solution of sodium alginate was prepared by adding 4 g of the powder. The solution was used in less than 24 hours. To 30 cm^3 sodium alginate solution, 3 g of deactivated yeast cells were suspended. The yeast-alginate mixture was then added dropwise to a 1% solution of $CaCl_2$. As each drop fell through, a bead containing immobilized *Yarrowia lipolytica* yeast cells was formed at the bottom of the 1% $CaCl_2$ solution. The beads were filtered off and washed with distilled water until the filtrate tested negative to chloride test.

Preparation of methylene blue sorbate

A stock solution of concentration 100 mg·L^{-1} was prepared by dissolving 0.1000 ± 0.0001 g of analytical grade methylene blue (MB), supplied in powder form by Merck (Pvt) Ltd. in a 1000 cm^3 volumetric flask. Solutions of 20, 40, 60 and 80 mg·L^{-1} concentrations were prepared by serial dilution. Each prepared solution was shaken slowly so as to obtain homogeneity. For pH adjustment, solutions of 0.1 M hydrochloric acid and 0.1 M sodium hydroxide were also prepared. The concentrations of the stock solutions were quantified using a Thermo Fisher Scientific Genesys 10S UV-Vis spectrophotometer. The calibration curve was obtained by measuring absorbances of standard solutions at λ_{max} = 650 nm.

Adsorption study

Batch experiments of the adsorption studies were first conducted with 50 mL of dye at 25°C in a 250 mL screw-cap plastic container at an agitation speed of 110 rpm. Adsorption parameters were first optimized in terms of contact time (5, 30, 60, 180 and 360 min), initial dye concentration (20, 40, 60 and 80 mg·L^{-1}), pH (3, 5, 7, 9 and 11) and biosorbent dosage (0.1, 0.5, 1, 1.5 and 2 g). As control, pure calcium alginate adsorbent was used to determine its contribution in adsorption and results showed that the removal efficiency was less than 9 mg·g^{-1} for an initial concentration of 80 mg·L^{-1}. Dye concentrations before and after adsorption were measured using a UV/Vis spectrophotometer. The quantity of MB adsorbed by a unit mass of an adsorbent at equilibrium (q_e) was calculated using equation 1.

$$q_e = \frac{C_o - C_e}{m} V \tag{1}$$

where C_e is the concentration of adsorbate at equilibrium in mg·L^{-1}, C_t is the concentration of adsorbate at time t, C_0 is the initial concentration, m is the mass of the adsorbent and V is the volume of the adsorbate.

Equilibrium, thermodynamics and kinetics studies were also conducted at the optimum adsorption conditions. For equilibrium and thermodynamics studies, 0.1 g beads were used with 50 mL of MB dye solutions at pH 5 of 20, 40, 60 and 80 mg·L^{-1} initial dye concentrations at 25, 35 and 45°C, all agitated at 110 rpm. To determine the biosorption kinetics, 0.1 g beads were suspended in 50 mL of 80 mg·L^{-1} MB solution at pH 5 and 25°C. The suspensions were agitated at 110 rpm. Absorbances of samples were measured at time intervals of 5, 10, 15, 20, 30, 40, 50, 60, 90, 150 and 180 min.

Results and discussion

FT-IR characterization of biosorbent

FTIR spectra of alginate, chicken skin and chicken thigh yeast are shown in Figure 1. A broad absorption band around 3200 cm^{-1} can be assigned to an –OH stretching vibration, while the sharp absorption bands at 2900 and 2700 cm^{-1} may be due to C–H symmetric and asymmetric stretching respectively. Peaks that are found around 1000–1100 cm^{-1} indicate the presence of C–O or ether group. The C=C stretching is found at 1534.38 and 1626 cm^{-1} while the C=O stretch is recorded at 1708 cm^{-1}. Generally the FT-IR spectrum of both *Yarrowia lipolytica* yeast cells indicates the presence of –COOH group and –OH group.

Effect of initial dye concentration and contact time

The results on the effect of initial MB concentration and contact time on the adsorption capacity of the *Yarrowia lipolytica* yeast

cells based biosorbent are illustrated in Figure 2. Steep rises in the first 30 minutes irrespective of initial MB concentration can the attributed to available active adsorption sites and the concentration gradient. Time to reach equilibrium was much shorter for lower initial MB concentration as compared to higher initial MB concentration. In one of our previous works with silica gel immobilized *Chlorophyta hydrodictyon africanum* based biosorbent a similar trend was observed (Muzarabani *et al.* 2015). Similar results have also been reported in the literature (Tsai et al. 2008, Elass et al. 2010).

Effect of pH

It has been demonstrated that pH has a strong influence on the adsorption mechanism of biosorbents and hence also on the adsorption capacity (Rubin et al. 2005, Barka et al. 2011). The results on the effect of pH on the MB biosorption capacity of alginate immobilized *Yarrowia lipolytica* yeast based biosorbent are illustrated in Figure 3. The low adsorption capacity at low pH has been attributed by a number of researchers to a high degree of protonation of adsorbent surface due to the presence of amino groups (Yan and Wang 2013, Pavan et al. 2008, Yao et al. 2009, Ahmad et al. 2009). As expected, this results in the repelling of the diprotonated cationic dye molecules. In the alkaline pH, hydroxyl ions form complexes with the cationic MB preventing their adsorption on the biosorbent.

Effect of biosorbent dosage

The results on the effect of biosorbent dosage on the MB adsorption capacity are illustrated in Figure 4. The values were found to decrease with increase in adsorbent dosage. The decrease maybe due to the fact that as the amount of adsorbent is increased, the total surface area that is available for the adsorption of MB is reduced, due to overlapping and aggregation of the beads (Nsami and Mbadcam 2013). The highest equilibrium adsorption capacity q_e value was observed when using 0.1 g of *Yarrowia lipolytica* yeast adsorbent.

Adsorption Isotherms studies

An adsorption isotherm describes the relationship between the adsorbate in the liquid or gas phase and the adsorbate

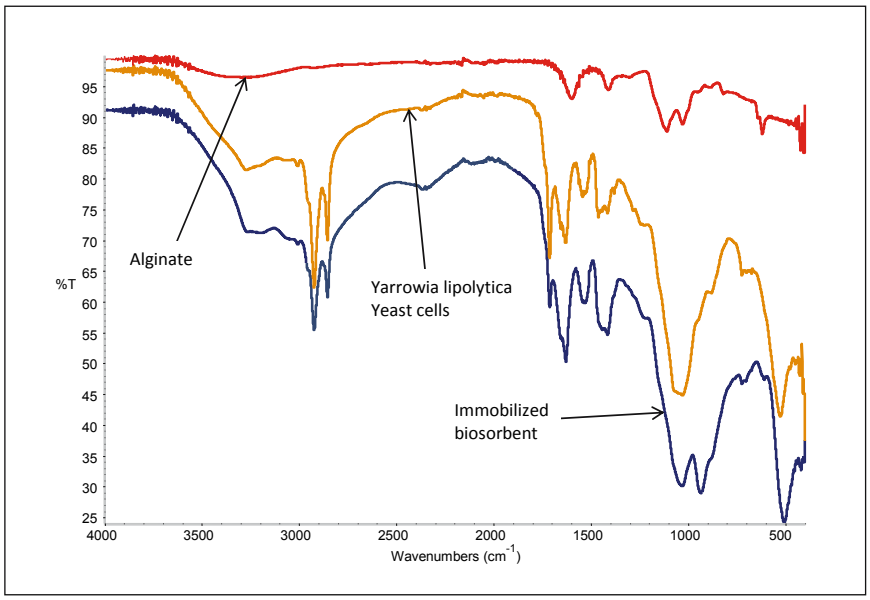

Fig. 1. FT-IR spectra of *alginate, Yarrowia lipolytica* yeast cells and biosorbent

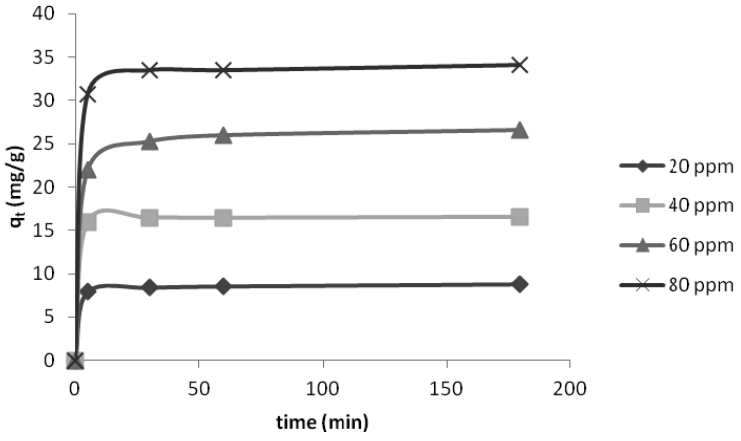

Fig. 2. Effect of initial concentration and time on the sorption of MB by immobilized *Yarrowia lipolytica* yeast (T= 25 °C, pH= 7, dosage= 0.1 g, agitation= 110 rpm)

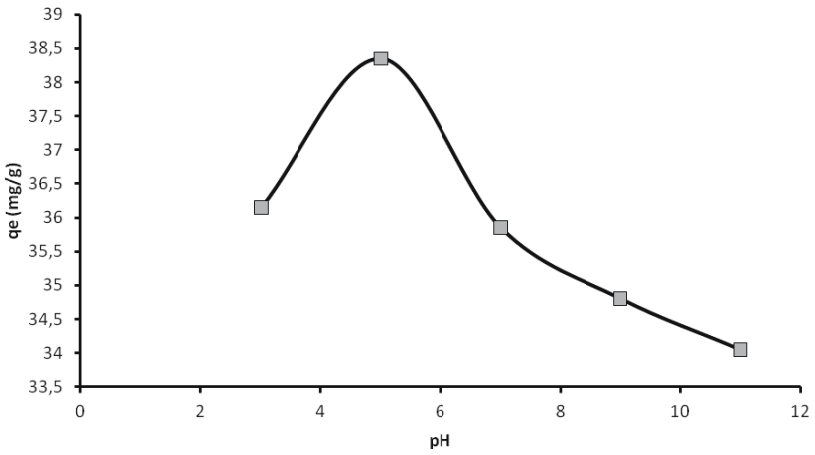

Fig. 3. Adsorption capacity of immobilized *Yarrowia lipolytica* yeast adsorbent (T= 25°C, dosage = 0.1 g, C_o= 80 mg·L^{-1} agitation= 110 rpm

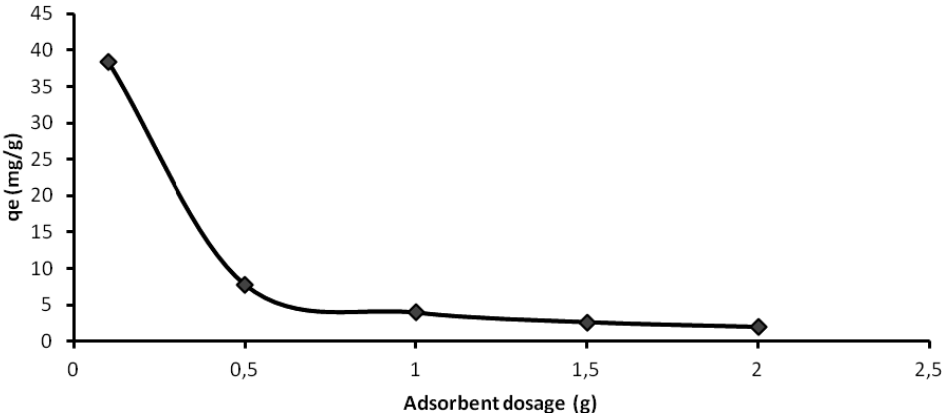

Fig. 4. Effect of immobilized *Yarrowia lipolytica* yeast for the uptake of MB (C_o= 80 mg·L^{-1}, T= 25°C, pH= 5, agitation= 110 rpm)

adsorbed on the surface of the adsorbent at equilibrium at constant temperature (Dabrowski 2001). The Langmuir and Freundlich isotherms are the two most well-known isotherms which have been used to describe the equilibrium of a number of adsorption systems. Langmuir adsorption isotherm assumes that adsorption occurs at specific homogeneous sites of equal energy within the adsorbent. The Langmuir isotherm can be represented as equation 2:

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \tag{2}$$

where: q_e is the adsorption capacity at the equilibrium solute concentration in mg·g^{-1}; C_e is the equilibrium concentration of adsorbate in solution in mg·L^{-1}; q_m is the maximum adsorption capacity corresponding to complete monolayer coverage in mg·g^{-1} and K_L is the Langmuir constant related to energy of the adsorption.

Equation 2 can be linearized to give equation 3:

$$\frac{c_e}{q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m} \tag{3}$$

The values of q_m and K_L can be obtained from the slope and intercept of the linear equation by plotting C_e/q_e against C_e.

The Freundlich adsorption isotherm assumes a multi-layer adsorption and is represented by equation 4.

$$q_e = K_f C_e^{1/n} \tag{4}$$

where K_f and 1/n are empirical constants dependent on the nature of sorbent, sorbate and the temperature. The values K_f and 1/n are important in selecting an adsorbent as a separating medium, in which K_f(mg·g^{-1}) is the over-all adsorption capacity (q_e at C_e = 1 mg·L^{-1} or *log* C_e = 0) and 1/n is the heterogeneity factor. The heterogeneity factor (1/n) indicates the strength of bond energy between sorbate and sorbent.

This equation is conveniently used in the linearized form by taking the logarithm of both sides as

$$lnq_e = lnK_f + \frac{1}{n} lnC_e \tag{5}$$

A plot of (*ln* q_e) against (*ln* C_e) yielding a straight line indicates the confirmation of the Freundlich isotherm for adsorption. The constants can be determined from the slope and the intercept (Itodo 2011). Experimental findings for the Langmuir and Freundlich adsorption isotherms are summarized in Table 2 below.

From the experimental data summarized in Table 2, the Freundlich isotherm for *Yarrowia lipolytica* yeast cells fitted very well with higher correlation coefficient (R^2>0.99) compared to the ones obtained from Langmuir isotherm. This suggests that the surface area of biosorbent contains heterogeneous sites that are of different energy levels. For the Freundlich isotherms, the parameter *n* which measures preferential adsorption of one adsorbate to other indicates a favorable adsorption as the values were in the region of 1 < n < 10. The maximum adsorption capacity q_{max} calculated from the Langmuir isotherm was 66.67 mg·g^{-1}.

Kinetic studies

Pseudo-first-order and pseudo-second-order models were used to study adsorption of MB. The pseudo-first order kinetics can be described by equation 6.

$$\frac{dq}{dt} = k_1(q_e - q_t) \tag{6}$$

where k_1 is the pseudo-first order kinetic constant, q_e and q_t the amount of MB adsorbed at equilibrium and time t respectively. Linearizing the above equation gives equation 7.

$$ln(q_e - q_t) = lnq_e - kt \tag{7}$$

Plots of $ln(q_e–q_t)$ versus *t* were constructed to give a liner relationship from which the values of k_1 can be obtained.

A pseudo-second-order kinetic process can be described by equation 8 and the linearized form by equation 9.

$$\frac{dq}{dt} = k_2(q_e - q_t)^2 \tag{8}$$

$$\frac{1}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_t}t \tag{9}$$

where k_2 is the pseudo second order kinetic constant. Plots of t/q_t against *t* should give a linear relationship. The comparison of the pseudo-first and pseudo-second order parameters is shown in Table 3. Correlation coefficients (R^2=1) suggest that adsorption kinetics was better described by the pseudo-second order kinetics model. This indicates chemisorption. The results agree with the findings by other researchers who worked with similar biosorbents (Caparkaya and Cavas 2008, El Jamal and Ncibi 2012).

Thermodynamics parameters

Thermodynamic parameters $\Delta G°$, $\Delta H°$ and $\Delta S°$ (all in kJ·mol^{-1}·K^{-1}) were calculated using equations 10–12.

$$K_0 = C_{solid}/C_{liquid} \tag{10}$$

$$\Delta G° = -RTln(K_0) \tag{11}$$

$$logK_0 = \Delta S°/(2.303R) - \Delta H°/(2.303RT) \tag{12}$$

where: ΔG is the free energy change, ΔH is enthalpy change, ΔS is entropy change and K_0 is thermodynamic equilibrium

Table 2. Comparison of the Langmuir and Freundlich Adsorption Parameters

	Langmuir parameters				Freundlich parameters			
Temperature	R^2	q_{max}	K_L	R_L	R^2	*1/n*	*n*	K_f
25°C	0.916	66.67	1.333	0.0093	0.992	0.306	3.268	1.855
35°C	0.969	55.56	1.611	0.0077	0.999	0.338	2.959	1.745
45°C	0.993	66.66	3.60	0.0035	0.995	0.379	2.639	1.931

Table 3. Comparison of pseudo-first and pseudo-second order kinetic parameters

	Pseudo-first order			Pseudo-second order		
(ppm)	q_{exp}	k_1	R^2	q_{exp}	k_2	R^2
20	9.80	0.041	0.943	10.10	0.678	1.000
40	19.65	0.0447	0.918	20.00	0.104	1.000
60	29.40	0.0556	0.976	30.30	0.011	1.000
80	38.65	0.031	0.989	40.00	0.658	1.000

Table 4. Thermodynamic parameters for the sorption of MB dye on immobilized Yarrowia lipolytica yeast

ΔH° (kJ·mol^{-1})	ΔS° (kJ·mol^{-1}·K^{-1})	ΔG° (kJ·mol^{-1})	
		298 K	318 K
- 60.74	- 0.19	- 5.87	- 3.21

constant (Vijayakumar *et al.* 2012). Thermodynamic parameters are summarized in Table 4 below. The negative ΔH value conformed to the experimentally observed exothermic sorption nature of MB dye and the negative values for ΔS indicate a decrease in the randomness at the solid-liquid interface when sorption of MB proceeded towards equilibrium. The values obtained were in line with weak hydrophobic forces which are hydrogen, van-der Waals and π-interactions which are common for hydrophobic organic compounds with few polar groups (Unuabonah *et al.* 2008).

Conclusion

The sorption results shade light on the understanding of the relationship between organic dyes and *Yarrowia lipolytica* yeast cells. MB sorption reached equilibrium fast as this was done in 60 minutes. The rate determining step of MB dyes sorption was the boundary layer control between the sites on yeast cell wall and MB molecules in solution. pH also had a reciprocal effect on MB dye adsorption. The adsorbent showed adsorption properties, with maximum adsorption capacity of 66.67 $mg \cdot g^{-1}$. The experimental data fitted well onto the Freundlich adsorption isotherm and adsorption processes followed the pseudo-second order. Sorption of MB on *Yarrowia lipolytica* yeast cells was exothermic and enthalpy values indicated a chemo-sorption.

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