



ORIGINAL ARTICLE

Study the Ability of *Saccharomyces cerevisiae* to Remove Methyl Green Dye from Water as a Pollutant Agent

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ABSTRACT: Biosorption ability of commercial dried *Saccharomyces cerevisiae* (Baker's yeast), to remove the Methyl green dye from water, was studied. This dye was chosen due to wide using of it in the different industries which is dumped into wastewater. With a view to explore the optimum conditions for adsorption of dye, Batch experiments were performed under various experimental affecting conditions, which are dye concentration, temperature, contact time. The experiment's batches were held using various initial concentrations of dye from 10 to 50 mg L⁻¹, adsorbent (Baker's yeast) dosage 0.075g at diverse temperatures (20, 30 and 40°C), and pH=7. In which the results have shown when the temperature increased the adsorption efficiency increased too. The removal percentage (%R) of dye by the baker's yeast reached equilibrium after 80 minutes. As well as, adsorption isotherm models (Freundlich and Langmuir) were studied. The maximum biosorption capacity values were calculated at mentioned conditions. Furthermore, Kinetic and Thermodynamic parameters were calculated for this adsorption process, which are indicating the process is endothermic, spontaneous process in nature and follow pseudo 2. Order model.

INTRODUCTION

Ecological pollution is the major issue in this epochal from the earth's history. Because of the steady increase in the world population, there is persistent needing for growing of industries and agriculture which leading to produce waste[1]. Different chemical waste materials, which are a result from industries and agriculturally processes, are directly or indirectly discharged into the environment causing pollution. With the rapid development of various industries, In developing countries, the situation is most severe because of the absence of pre-treatment before dumping of waste [1].

In the subject of water pollution, most of the chemical waste like detergents, chloroform, fats, grease, fuels, petroleum hydrocarbons and heavy minerals are directly dumped into rivers. Dumping wastes without pre-treatment procedures caused water pollution and threatened biology life [1].

Dyes are one of these pollutants which are coloured organic chemicals, Yearly, considerable amounts of dyes are manufacturing to use in the textile industries, leather, paper, pharmaceutical, cosmetics, other industries [2]. Low concentrations of dyes in water (<1ppm) are sufficient to affect the water quality, such as the transparency of the water which leads to reducing light penetration for aquatic organisms which could be affecting photochemical activities for aquatic systems[3]. Furthermore, it could be affecting the solubility of gases in water bodies[3]. Commonly used dyes in textile and other industries having the potential to cause cancer for organisms that live in aquatic environments [3,4]. These dyes are designed to be resistant against different physical and chemical influences like light, high temperatures, oxidizing agents and enzyme degradation [5, 6]. On the other hand, the traditional systems in

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wastewater treatment unable to remove these dyes. Many processes like photolysis, aerobic and anaerobic degradation are slowly and difficult processes to completing the mineralization most of the dyes. Also, decomposition products are toxic to the aquatic organism [3, 5, 6].

Water waste treatment processes are including, adsorption, ozonation, filtration, precipitation, coagulation, oxidation, flocculation, chemical decomposition [3, 7 and 8]. Adsorption technique is vastly used to remove specific groups of pollutants from industrial wastewater due to of its high competency in this field in addition to the simplicity of this technique when compared to other methods in terms of costs and designs [9 and 10].

Bio-sorption is a biologically based adsorption technique by using biological materials like (yeast, bacteria and fungi) to remove the organic dyes from wastewater is

considered a low-cost process [11 and 12]. Microorganisms and dyes interact with each other depending on their chemical properties [11]. Many dyes have a certain affinity with many microorganisms, and one microorganism can adsorb or decompose different types of dyes [11 and 12].

An example of the microorganism that commonly used in biosorption investigations is *Saccharomyces cerevisiae* (Baker's yeast), Which had shown abilities to adsorb heavy metals and some organic dyes [13]. This yeast is cheap, readily available, safe to use, easily grown, and could be producing high biomass yields [13]. The cell wall plays an important role in adsorption [12], Which made of Mannoprotein which represents 40% of cell wall mass, While, β -1-3 glucan represent 50%, about 10% β -1-6 glucan and 1-3% chitin [14], as explained in Figure 1.

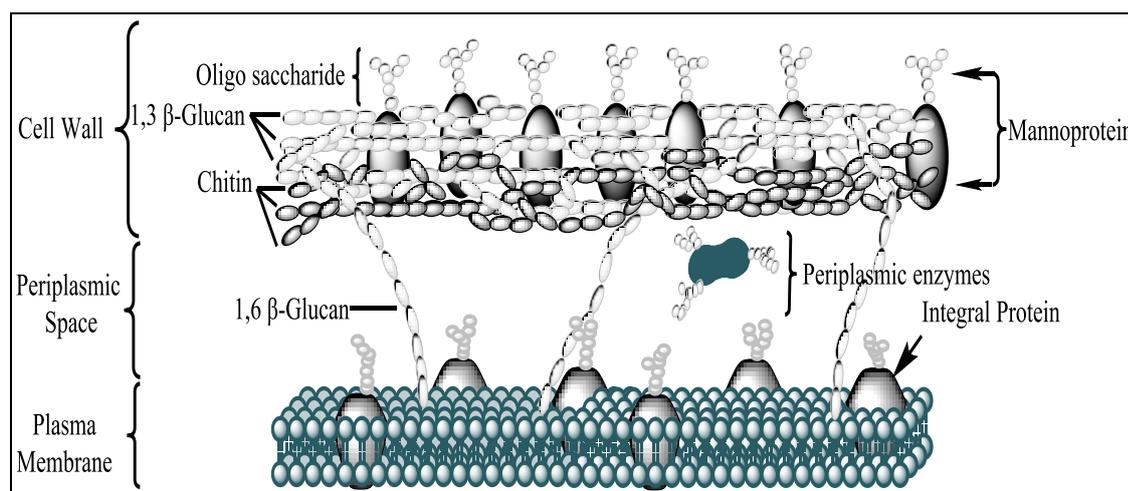


Figure 1. The Cell wall composition of *Saccharomyces cerevisiae* [15, 16, 17, 18].

The literature describes that the cell wall surface of this yeast has functional groups which are OH, COOH, NH₂, and PO₂- that could mediate biosorption [19].

On the subject of water pollution, organic dyes like Triphenylmethane are the most common pollutants, that

are used widely in textile and other industries [20]. Methyl green (MG) dye is a positively charged dye (Figure 2), which is a basic triphenylmethane-type, used in medicine, biology [20].

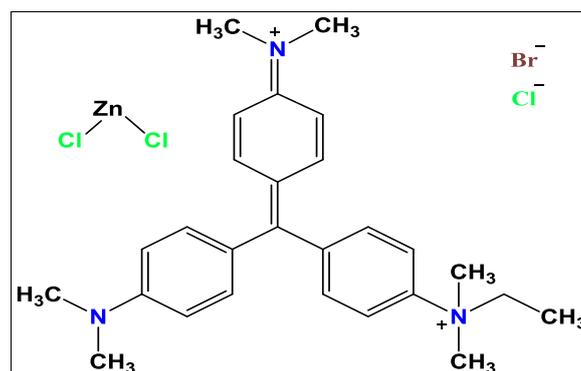


Figure 2. The chemical structure of methyl green dye.

However, the ability of Baker's yeast to adsorb pollutants and some dyes [5, 12, 13 and 19], was the reasons for conducting this current study.

This study object to probing the bio-sorption ability of *Saccharomyces cerevisiae* to eliminate the methyl green dye from an aqueous solution; including studying the dye concentration, contact time, isotherms, kinetic, thermodynamic, and temperature at equilibrium pH=7.

MATERIALS AND METHODS

Preparation of Bio-sorbent

The commercially strain of *Saccharomyces cerevisiae* (Baker's yeast) had purchased from the market under the Turkish brand (pakmaya). It was dried at temperature (60°C) to remove moisture and till a stable weight of dried biomass was obtained, Then dried biomass was grinded to a fine powder. Also, by using standard sieves, the powder was sifted to obtain constant sizes less than 1 mm.

Preparation of dye solution

Methyl green dye was bought from the company (DC Chemicals). The solution of dye was prepared as follows; 1g from dye was dissolved in 1000 ml of distilled water in order to get a concentration of 1000 mg L⁻¹. Further dilutions were made from a stock solution according to the experiment's requirement.

Determination of calibration curve

The calibration curve was determined for Methyl green dye as follows; The λ max for dye was sought and fixed

at (630nm) using different concentrations from dye, after that, the absorption has been recorded and the calibration curve plotted between absorption values and concentrations.

Adsorption batch experiments

0.075g from *Saccharomyces cerevisiae* (Baker's yeast) powder added in 100 ml conical flasks including 50 ml from methyl green dye with known concentrations. The flasks were closed with parafilm and shaking in a water bath shaker (120 rpm) at different temperatures, at intervals predetermined time. After every interval time, a specimen was taken from mixture and separated by the centrifuge for 15 minutes at 4000 rpm. Then the absorbance of the supernatant measured at 630nm. Also, negative controls without sorbent were used, in order to ensure that adsorption process was carried out by Baker's yeast. Conditions of experiments batches were explained in Table 1. The amount of Methyl green adsorbed on Baker's yeast (q_e), and the removal percentage (R%) were calculated as follows [7, 21, and 22]:

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (1)$$

$$\% R = \frac{(C_0 - C_e)}{C_0} * 100 \quad (2)$$

In which (%R) is the percentage removal of adsorbate, (q_e) is the quantity of adsorbate in mg g⁻¹, V is the total volume of the adsorbate in L, C_0 is the MG initial concentration of solution (mg L⁻¹), (C_e) is the concentration of Methyl green dye at equilibrium (mg L⁻¹) and (m) is the mass of adsorbent used (g).

Table 1. Adsorption batch experiments procedure conditions for Methyl green Bio-sorption on *Saccharomyces cerevisiae*.

Experiment	Baker's Yeast (mg)	Dye concentrations (mg L ⁻¹)	Temperature	pH	Time (min)
Contact time	75	10, 30, 50	30°C	7	20, 40, 60, 80, 100, 120, 140, 160, 180
Initial dye concentration			20°C		
	75	10, 15, 20, 25, 30, 35, 40, 45, 50	30°C	7	120
Effect of temperature			40°C		

Adsorption isotherm

The experiment was carried out using different concentrations of dye (10-50 mg L⁻¹), biomass weight was (0.075g), temperatures (20, 30 and 40°C) at pH =7. Then values of q_e of dye were calculated by using (equation 1). After that, to understand the relation between q_e and C_e of adsorption at equilibrium, their values were applied to the adsorption isotherm models.

Adsorption isotherm describes the relation between the solid-phase concentration at equilibrium (q_e) to the liquid-phase concentration at equilibrium (C_e) at the equilibrium contact time and given temperature [23]. Different isotherm equations were used for experimental data analysis of the solid-liquid sorption system like Langmuir and Freundlich.

Which are important models for describe how is a pollutant at different concentrations will interact with adsorbent surfaces, also they are helpful in optimization of using adsorbents [24].

Freundlich model

This model describes a mathematical relation between solid-liquid sorption systems. It is an empirical equation utilized in heterogeneous sorption with multilayer adsorption [21],[24]. The Freundlich equation described as following:

$$q_e = K_f C_e^{1/n} \quad (3)$$

Where K_f and n are Freundlich constants. This equation converted to linear form as following [21]:

$$\ln q_e = \ln K_f + \frac{1}{n} \times \ln C_e \quad (4)$$

When $n > 1$, gives an indication about the favorability of this process [21].

Langmuir model.

This model describes the equilibrium between adsorbate and adsorbent systems which are valid with monolayer sorption to a surface of adsorbent without interactions between adsorbed species [21, 25, and 26]. The model's equation is described as follows:

$$q_e = \frac{K_L \times C_e}{1 + a_L \times C_e} \quad (5)$$

This equation linearized as following [25, 26]:

$$\frac{1}{q_e} = \left(\frac{1}{K_L}\right) \times \frac{1}{C_e} + \frac{a_L}{K_L} \quad (6)$$

In which K_L and a_L are the Langmuir isotherm constants, K_L represent the equilibrium constant for monolayer adsorption (L g⁻¹), while a_L represent the Langmuir constant related to the rate of adsorption (L mg⁻¹) which represent the affinity binding sites. $\frac{K_L}{a_L}$ are numerically equal to the theoretical monolayer saturation capacity ($q_{\max \text{ theo}}$) mg g⁻¹ [26].

In 1974, Weber and Chakravorti described the R_L factor (dimensionless constant separation factor) the essential feature of Langmuir equation as following [27]:

$$R_L = \frac{1}{1 + a_L \times C_0} \quad (7)$$

R_L factor is indicate to the type of adsorption, when $R_L > 1$ means un favorable adsorption, $0 < R_L < 1$ means favorable adsorption, $R_L = 1$ means linear adsorption, while $R_L = 0$ means irreversible adsorption [21, 27].

Adsorption Thermodynamic

The thermodynamic parameters; Gibb's free energy change (ΔG^0), standard enthalpy (ΔH^0) and standard entropy (ΔS^0) were calculated via the next equations [7]:

$$\Delta G^0 = -RT \ln K_{eq} \quad (8)$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (9)$$

gas constant which equal to $8.314 \text{ J (mol K)}^{-1}$, T the absolute temperature and K_{eq} is the equilibrium constant which equal to the value of Langmuir equilibrium constant for adsorption K_L [26].

Finally, Standard enthalpy (ΔH^0) and standard entropy (ΔS^0) According to the van't Hoff equation [7]:

$$\ln K_L = -\frac{\Delta H^0}{R} \times \frac{1}{T} + \frac{\Delta S^0}{R} \quad (10)$$

By drawing a plot of $\ln K_L$ versus $1/T$, yields a straight line with slop equal to $-\frac{\Delta H^0}{R}$, and intercept equal to $\frac{\Delta S^0}{R}$.

Kinetic of adsorption

kinetics of the adsorption process could be defined as the removal rate of solute that rules the lodging period of the sorbate in the solid-solution interface under experimental conditions [28]. Two kinetic models; pseudo 1. and 2. Order models, were used [28, 29]. The amount of adsorbed dye on the surface of bio-sorbent at different time of process (q_t) were calculated using the following equation [29]:

$$q_t = \frac{(C_0 - q_e)}{W} \times V \quad (11)$$

Then the calculated q_t used to determine the pseudo 1. and 2. order kinetic using the following linear equations (12,13) respectively[35]:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (12)$$

$$\frac{t}{q_t} = \frac{1}{k_2 \times q_e^2} + \frac{1}{q_e} \times t \quad (13)$$

Which q_t is the amount of dye adsorbed at any time, while C_0 the initial concentration of dye (before adsorption), q_e the equilibrium concentration (after adsorption), V the volume of dye, W the weight of sorbent, t time in minute, k_1 is the pseudo 1. Order constant and k_2 is the pseudo 2. Order constant. The initial concentration C_0 was 40 m L^{-1} , volume 50ml, adsorbent 0.075mg, temperature 30°C , time 0-180min with 20 min intervals between each batch and reading absorption using UV at 630nm.

RESULTS AND DISCUSSION

Effect of contact time

In order to exploring the adsorption equilibrium conditions, the effect of contact time experiments was applied according to the procedure in the table 1. After every interval time a specimen of a mixture was taken and separated by the centrifuge for 15 minutes at 4000 rpm. Then the absorbance of the supernatant measured at 630nm. In order to determine the R%, equation 2 was used and the plot between R% versus Time were drawing (Figure 3), Also, results of total removal of dye q_t were calculated using equation 1 and 11, then a plot between q_t (mg g^{-1}) versus time (min) was drawing (Figure 4). A cursory removal fraction was noticed after 20 minutes, Which was followed gradual slower rate of sorption process by the biomass. It could be explain that as follow; In the initially of process, biomass surface contain negatively charged functional groups were unoccupied (not bonded) and facilely available for the adsorption of dye ions from the solution, Therefore; the dye ions got to bind with these functional groups [5]. it could be inferred that the process is ionic in nature [30, 31]. An equilibrium stage was attained after 80min. No further increase in bio-sorption efficiency was observed after the equilibrium was attained.

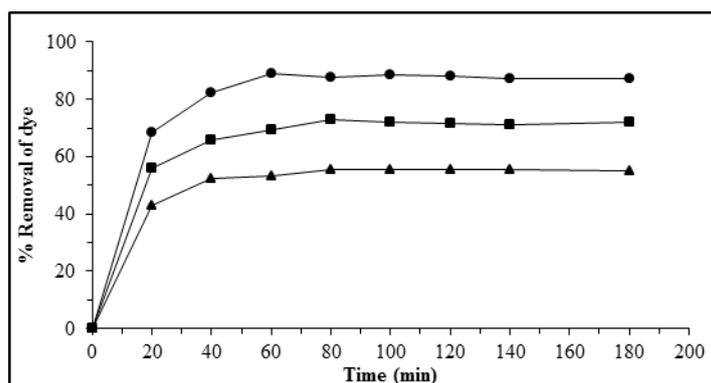


Figure 3. The relationship between time and the Removal % of Methyl green dye by the *Saccharomyces cerevisiae* (Baker's Yeast) with different initial concentrations of dye (●)10 mg L⁻¹; (■)30 mg L⁻¹; (▲) 50 mg L⁻¹, at temperature (30°C), and pH = 7.

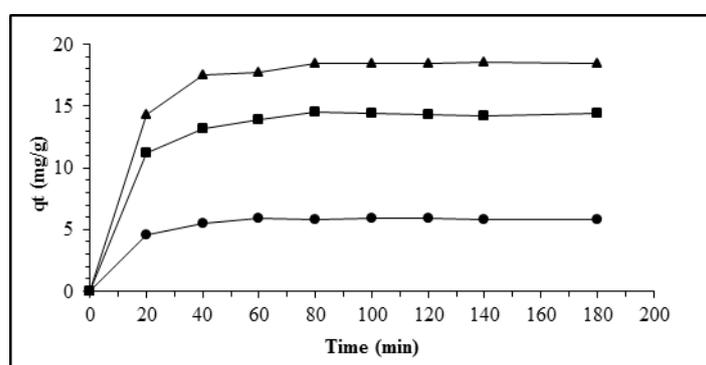


Figure 4. The amount of Methyl green dye that adsorbed on bio-sorbent at any time (q_t) on the surface of *Saccharomyces cerevisiae* (Baker's Yeast) with different initial concentrations of dye (●) 10 mg L⁻¹; (■) 30 mg L⁻¹; (▲) 50 mg L⁻¹, at 30° C, and pH = 7.

Effect of initial concentrations of dye and temperature

Different initial concentrations of methyl green dye were used in order to explore the optimum concentration at different temperature. According to the procedure in Table 1, After 120 min a specimen of a mixture was taken and separated by the centrifuge for 15 minutes at 4000 rpm. Then the absorbance of the supernatant measured at 630nm. %R and q_e were calculated, and the plots of %R versus time and q_e versus time were drawing (Figures 5 and 6), it could be inferred there is an increasing in the percentage of removal Methyl green dye with low initial concentrations at the mentioned temperatures with steady decreasing in the percentage of removal when dye's initial concentrations were increased

until reach to the equilibrium state [5]. The reason of this phenomena due to the increasing in the rivalry between dye's molecules to occupy the available reactive sites founded in the surface of adsorbent led to decreasing in the percentage of removal dye. More precisely, the amount of adsorbed dye will raise with raising the initial dye concentrations due to increasing in the effect of driving forces when absorbate concentrations gradient during the adsorption process, this means that at higher concentration of the dye, greater driving force with it to conquer the impedance of the mass transfer between the liquid and solid phase which enhancing the biosorption process [5, 31].

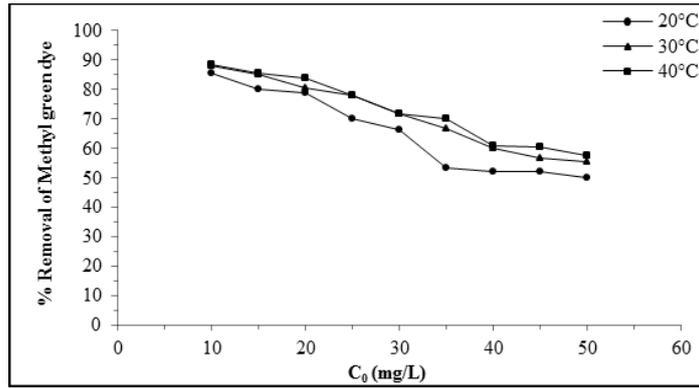


Figure 5. Effect of different initial concentration of dye (C_0) on Removal% of Methyl green by *Saccharomyces cerevisiae* at different temperatures, and pH = 7.

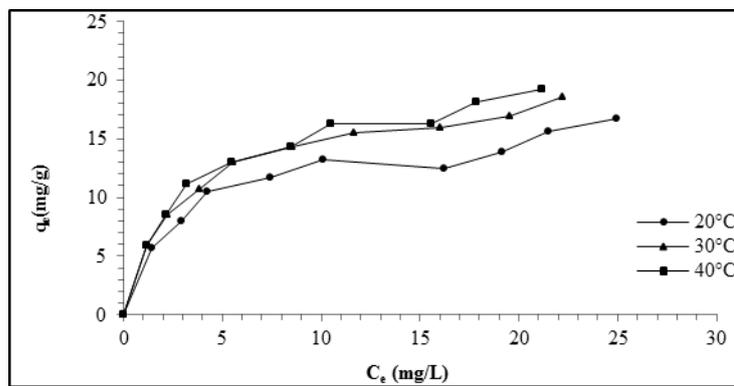


Figure 6. The amount of adsorbed dye at equilibrium (q_e) and concentration of dye at equilibrium (C_e) at different temperatures, and pH = 7.

Also, it's clear from Figures 5 and 6, the effect of increasing temperature lead to increasing in the removal percentage of dye and removal capacity by the biosorbent. This observation proposed that process is an endothermic chemisorption process due to erection of new active sites on the surface of adsorbent for extra adsorption and raising the number of molecules that earn enough energy to undergo reactions with the new active sites [5, 12, and 19].

Adsorption isotherms

The q_e and C_e values were calculated at different temperature (20, 30, 40°C), then converted to $(\ln q_e)$ and $(\ln C_e)$, The values of $\ln q_e$ (Y axis) was plotted against the values of $\ln C_e$ (X axis) in order to determining Freundlich constants (K_f and n), From the plot (figure 7), slope represent $(1/n)$, while the intercept represent $(\ln K_f)$. The constants were calculated were obtained from the plot and by using the equation 4, these data were listed in Table 2.

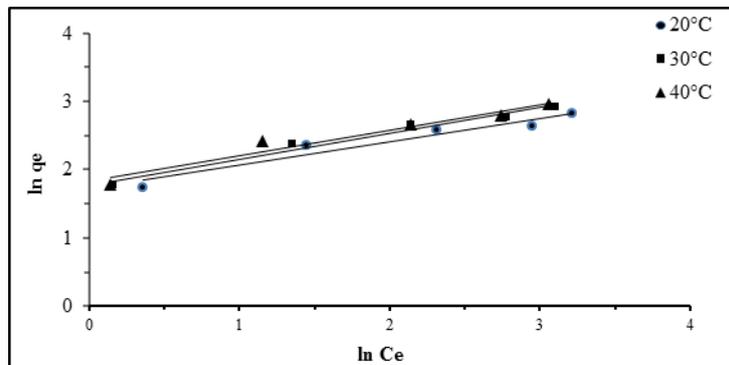


Figure 7. Relationship between $\ln q_e$ versus $\ln C_e$ with different temperatures (Freundlich isotherm model).

Table 2. parameters of Freundlich isotherm model and correlation coefficients values for Methyl green adsorption on dried biomass of *Saccharomyces cerevisiae*.

Isotherm	Parameters	Temperatures (°C)		
		20°	30°	40°
Freundlich	K_F ($\text{mg g}^{-1} (\text{L mg}^{-1})^{1/n}$)	5.57	5.92	6.21
	1/n	0.34	0.39	0.37
	n	2.94	2.56	2.70
	R^2	0.93	0.97	0.95

The K_f which is the adsorption capacity by the adsorbent, While the exponent $n > 1$ give an allusion to the favorability of this process. The $1/n$ values refer to that adsorption becoming more heterogeneous when the value fetches closer to 0 [7].

In order to determine Langmuir constants, the values of q_e and C_e were converted to $1/q_e$ and $1/C_e$, then the values of $1/q_e$ (Y axis) were plotted against the values of

$1/C_e$ (X axis) at different temperature in order to determining Langmuir constants (K_L , a_L) and $q_{\text{max the o}}$. From the plot (figure 8), slope represent $(1/K_L)$, while the intercept represent (a_L/K_L) . By using the equation 6, the constants and $q_{\text{max the o}}$ were calculated. Also, R_L were calculated (by using the equation 7). These data were listed in Table 3.

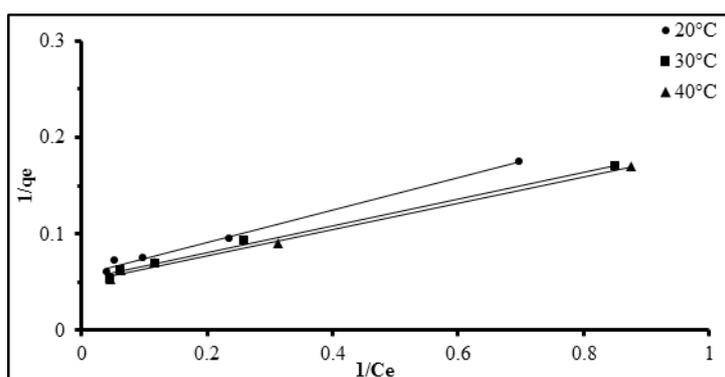


Figure 8. Relationship between $1/q_e$ versus $1/C_e$ with different temperatures (Langmuir isotherms model).

Table 3. Parameters of Langmuir, dimensionless and correlation coefficients values for Methyl green adsorption on dried biomass of *Saccharomyces cerevisiae*.

Isotherm	Parameters	Temperature (°C)		
		20°	30°	40°
Langmuir	K_L (L g^{-1})	5.98	7.15	7.37
	a_L (L mg^{-1})	0.34	0.38	0.37
	$q_{\text{max the o}}$ (mg g^{-1})	17.03	18.76	20.04
	R^2	0.99	0.99	0.99
	R_L	0.055	0.050	0.051

Values of R_L confirmed that the Baker's yeast is favorable for adsorption of methyl green dye under the experiment's conditions. The results in the Table 1 and 2. indicating that the adsorption process follows the Langmuir model better than Freundlich.

Thermodynamics of adsorption

By drawing a plot of $\ln K_L$ against reciprocal of temperatures ($1/T$). From the plot (Figure 9), the slope value is equal to the value of $(-\Delta H/R)$, and the intercept value equal to the value of $(\Delta S/R)$. Thermodynamic parameters were calculated using equations (8-10).

The results of thermodynamic parameters for adsorption process are presented in figure 9 and table 4. When the value of ΔH° is positive give an indication that adsorption process is an endothermic. Also, The positive enthalpy indicates that formatted bonds between Methyl green dye (cationic dye) and the functional groups in the

cell wall of yeast are strong enough against destroying by simple heating or shaking [5, 32]. When the value of ΔG° is negative, that indicates the spontaneous nature of the reaction [7,13], while the value of ΔS° is positive that indicate the disorder or randomness increased through the adsorption process [32].

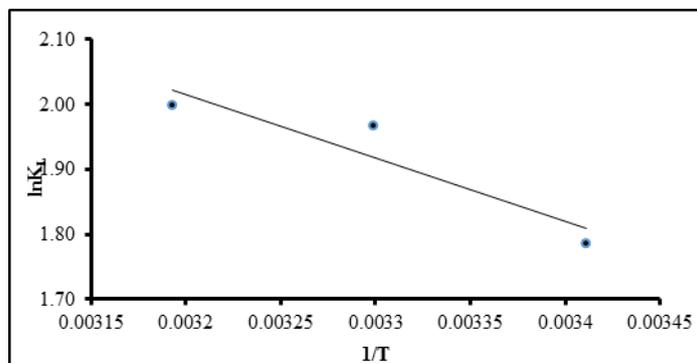


Figure 9. Van't Hoff plot for adsorption on Methyl green dye on *Saccharomyces cerevisiae*.

Table 4. Thermodynamic parameters for Methyl green dye adsorption on *Saccharomyces cerevisiae* powder at different absolute temperatures.

Temperature (K)	ΔG (KJ mol ⁻¹)	ΔH° (KJ mol ⁻¹)	ΔS° (J (mol.K) ⁻¹)
293.15	-4350.6614	8164.4913	42.6920
303.15	-4958.9458		43.2902
313.15	-5200.3351		42.6787

Kinetic of adsorption

The kinetic constants (k_1 and K_2) and q_t , q_e were determined by using equations (11, 12 and 13). Pseudo 1 order plot (figure 10) was drawn between values of $\ln(q_e - q_t)$ against time (t). The slope represents k_1 and intercept

represent the q_e , [29]. By Pseudo 2. order plot (figure 11) was drawn between values of (t/q_t) against time (t). The slope represents $1/q_e$ and the intercept $1/k_2q^2$ [29]. The results mentioned in Table 5.

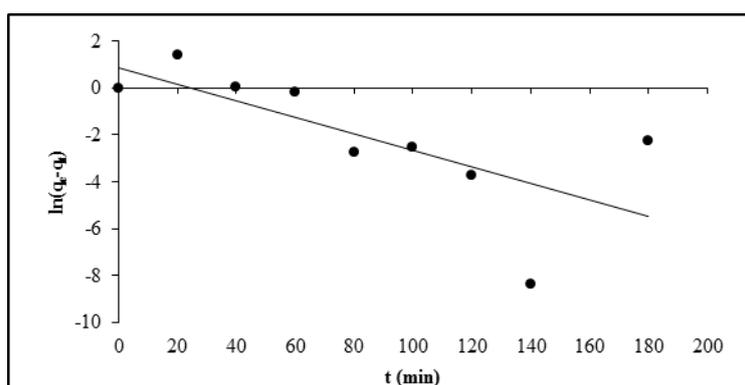


Figure 10. Pseudo 1. order kinetic model using initial concentration of methyl green dye (50 mg L⁻¹) at 30°C, and pH=7.

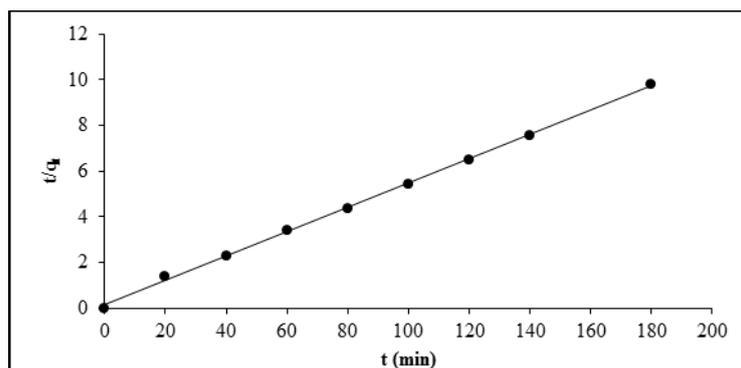


Figure 11. Pseudo 2. order kinetic model using initial concentration of methyl green dye (50 mg L⁻¹) at 30°C, and pH=7.

Table 5. The Kinetic parameters for Methyl green dye adsorption on *Saccharomyces cerevisiae* powder.

Kinetic model	qe(mg g ⁻¹)	Constants	R ²
Pseudo 1. order	2.349	K ₁ (min ⁻¹)	-0.0002
Pseudo 2. order	18.797	K ₂ (g.(mg min) ⁻¹)	0.99

From the mentioned results, it could be inferred that biosorption of methyl green dye follows the pseudo second order.

CONCLUSIONS

The biosorption of methyl green dye by the *Saccharomyces cerevisiae* (Baker's yeast) increased with raising in temperature. The maximum theoretical adsorption capacity ($q_{\max \text{ theo}} = 20.04 \text{ mg g}^{-1}$) at examined experimental conditions of adsorption (40°C, pH=7, Dried biomass= 0.075g, Initial concentration= 50 mg L⁻¹ and equilibrium contact time=80 minute). The process results follows Langmuir models better than the Freundlich model. Also, the biosorption process is chemisorption and spontaneous, endothermic in nature and follow kinetic pseudo 2. Order model. Finally, denoting the dried biomass of *Saccharomyces cerevisiae* could be counted as a good and cheaply sorbent to remove methyl green dye from wastewater of different industries that in using this dye.

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Conflict of interests

The authors advertise, there is no conflict of interest.

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