Bio removal of Methylene Blue Dye Solution by Saccharomyces cerevisiae

الإزالة الحيوية لمحلول صبغة ازرق الميثيلين بواسطة سكارواميسس سرفيسيا

By

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Abstract:

Management of dyes in the habitat causes considerable damage which can be dangerous in certain marine ecosystems because of their product disintegration, it could be harmful to some aquatic organisms. The textile wastewater coloring pigments may have an esthetic appearance and affect plant photosynthesis. There are several disadvantages to chemical or physical methods that a biological process that saves costs and is environmentally friendly can solve. Absorbents such as bacteria, fungi, cell membranes can be biological methods. Yeast may be less expensive and better than other microorganisms. This research attempted to investigate the decolorization of blue coloring in methylene blue by Saccharomyces cerevisiae. The study was carried out to find the optimum conditions in which the maximum decolorization occurred. The highest decolorization was monitored at 30^{o}C and a concentration of 100 ppm (0,01g/100ml), during which a maximum decolorization of 95,95% occurred after the 20 h incubation period. In the next assay at concentrations of 100 ppm (0.01g/100 ml) and pH 7 for 20 h, a cumulative decolorization of 98.90% has been obtained at dosages of 0.1 gram/l with 30^{o}C, which is considered as the best conditions. Saccharomyces cerevisiae was able to handle the textile wastewater in optimal conditions.

Keywords: Decolorization; *Saccharomyces cerevisiae;* Methylene blue; Baker's yeast; Scanning Electron Microscope

المستخلص :

يُعد التخلص من الصبغات المستخدمة في النسيج في مياه الصرف الصناعي يسبب أضرار كبيرة في النظام البيئي ويسبب إحلالها إعطاء نواتج اكثر ضررا علي الكائنات المائية وبالرغم من انها توفر مظهر تجميلي للأنسجة الملونة إلا إنها تؤثر علي عملية التمثيل الضوئي للنباتات وهناك العديد من العيوب للطرق الكيميائية او الفيزيائية التي يمكن تفاديها بإستخدام عملية بيولوجية قليلة التكاليف

وتصبح صديقة للبيئة و ذلك بإستخدام الكائنات الحية الدقيقة مثل البكتريا والفطريات. وفي هذه الدراسة سنُسلط الضوء علي الخميرة كأحد الطرق الحيوية الاسرع وأقل كفاءة .في هذه الدراسة تم إجراءبعض التجارب للحصول علي أعلي نسبة إزالة للون الأزرق لصبغة الأزرق ميثيلين في أقل وقت ممكن تحت الظروف المثلي .حيث لوحظ أنه تحت الظروف المثلي من درجة حرارة و رقم الأس الهيدروجيني و تركيز صبغة الأزرق ميثيلين و كمية خلايا الخميرة تم الحصول علي أعلي نسبة إزالة للون تصل الي ٩٥,٩٥% بتركيز صبغة ١٠٠ جزء في المليون بعد ٢٠ ساعة ثم بعد إجراء بعض التجارب للحصول علي أعلي نسبة إزالة تحت أفضل الظروف كما أدي إستخدام تركيز صبغة ١٠٠ جزء في المليون وتحت تاثير درجة حموضة = ٧ خلال ٢٠ ساعة بجرعة خلايا الخميرة ٠,١جم او مللتر في درجة حرارة ٣٠ درجة مئوية ، نحصل علي نسبة ازالة ٩٨,٩٠% . وتوثيقا للنتائج تم إستخدام الميكروسكوب الإلكتروني الماسح لرؤية التغيرات التي طرأت علي خلايا الخميرة قبل وبعد المعالجة ، وقد أوضحت الصور حدوث تغيير في شكل الخلايا نتيجة التعرض للصبغة في وسط واحد أيضا لوحظ ادمصاص الصبغة علي سطح الخلايا .

1 Introduction

In developing countries, most industrial effluents are heavily charged with xenobiotic compounds like dyestuff. These compounds are recalcitrant to biodegrade difficultly and are toxic to marine organisms, animals, and humans. Generality dyes are long-lasting and resistant to microbial, physical, and chemical treatments within the textile industry. Owing to the pollutants in nearby aquatic environments because of their excessive solubility inside the water as soon as they are discharged without previous treatment. The discharge of textile dyeing wastewater (TWWs) causes the surface of the water to paint in a black shade.

Synthetic dyes are widely employed in numerous applications which include textile, medical, leather, cosmetics to affect varied substrates **(Vikrant et al 2018)**. Some suggest that 100,000 varieties of dyes are distributed throughout the industry with an annual global demand of over seven tons **(Chittal et al 2019)**. In terms of unique chemical structure, synthetic dyes can

be divided into several categories, including anthraquinone dyes, azo dyes, phthalocyanine dyes, triaryl methane dyes, and so on. Among them, the most used dyes are anthraquinone and azo dyes, with content ranging from 70% to 90%. The entire synthetic dyes market.

Industries that consume dyes, especially the textile industry, are the major consumers of water and produce large amounts of TWWs for this reason **(Eslami et al 2016)**. Here, because of the ineffective operation of the thickener, 10%-15% of the dye is released into the drainage source. This comprises the dyes, dissolved solids, inorganic salts, raw substances, and other auxiliary substances. **(Zhou et al 2019)**. reported that the sewage generated from clothing contains various, often toxic, and long-lasting dyes, which, if not properly taken care of, pose a threat to the environment and population. Methylene blue (MB) is a fragrant dye that can be used for many purposes such as medicine, biology, clothing, dyeing paper, temporary hair dyeing, cotton dyeing, wood, and paper coatings. It additionally has an oxidizing photosensitizer in microorganisms, chemistry, diagnostics, and organic pollutants.

Although certain side effects may cause pulsation, vomiting, shock, purple, necrosis, and jaundice, it is not very toxic. For example, it is important to treat wastewater with this coating because it is harmful to water absorption. The biological discoloration mechanism of textile dyes has relied on the chemical structure of the dye and the microorganisms used **(Pajot et al 2011).**

Various research has displayed that dye residue treatment can show the hope of microbial degradation. The dye is broken down by many microorganisms (including yeast). Yeast has many advantages, not only can grow fast but also can withstand adverse environmental conditions, such as filamentous fungi.

Yeast is very useful in the treatment of organic wastewater of high concentrations including food, molasses, and oil **(Yang et al 2003; Martorell et al 2012)**. The baker's yeast *Saccharomyces cerevisiae* can be a useful pattern microbe for researching the response of cells to many types of stress. Microbial bleaching methods are widely used to reverse the effects of fabric manufacture through biodegradation.

This method is achieved by increasing the average yield and bio-sorption rate of live or dead biomass. Microorganisms, such as bacteria, yeast, algae, and fungi, can extract different dyes **(Gottlieb et al 2003; Lucas et al 2007)**. Subsequent research has displayed that the cultivation of *Debaryomyces polymorpha*, *Candida tropicalis*, and *Issatchenkia occidentals* **(Ramalho et al 2004)**. *C. oleophilic* **(Lucas et al 2006)**, yeast **(Jadhav et al 2007)** discolor various azo dyes. Many yeast studies are related to bio-sorption. In this example, biomass is employed accordingly as an adsorbent to concentrate and take off methylene blue dye. Because of their physical and chemical properties, biomass adsorbents have a high potential. Provides a promising solution for the traditional recycling process. They are very concerned about the analysis of the de-colorization and biodegradability of dyes by microorganisms because the effective cleaning of ecological substances is essential to the microbial process. They change the color of microorganisms in two ways: through the adsorption of microbial biomass or the biodegradation of dyes by cellular enzymes. Therefore, recent biological methods have focused on color degradation and fading. Besides high concentrations, yeast can also adapt and grow into different concentrations of pollutants under various environmental and chemical conditions.

Besides this is because of the cell wall structure of *Saccharomyces cerevisiae*, which contains traces of glucan,

mannan, and chitin **(Zhou et al 2008)**. Recently, intensive research has been conducted on yeast biological removal of dyes. Sharing or adding to the ongoing maintenance process has become a promising option.

The dye degradation rate and dye degradation efficiency of dyes are full of enthusiasm for many operating parameters of the degradation of organic molecules such as temperature, pH, dye structure and amount, oxygen, and auxiliary substrates (glucose, starch, molasses, and peptone. The cationic dye methylene blue was selected for this study. A model compound was used to test the possibility of removing baker's yeast from the batch system. Therefore, the effect of MB removal depends on the former amount of MB, pH, temperature, contact time, and the dosage of bio-absorbent. from the batch system. Therefore, the effect of MB removal depends on the former amount of MB, pH, temperature, contact time, and the dosage of bio-absorbent.

2 Materials and Methods

In the biodegradation study was accomplished for 24 to 72 hours in a 250 ml Erlenmeyer flask with a working volume of 100 ml, stirred at 150 rpm and 30°C, and intermittently in the same medium reactivate at 30°C for 24 hours. After this stage, the biomass was collected for 20 minutes at a speed of 8000 rpm under aseptic conditions. The yeast was isolated and tested by a plate test to determine the decolorizing activity. YMPD agar medium was with 50 ppm MB solution and inoculated with the obtained strain.

The plates have been incubated at 30°C for 48 h. The clearance zone as an indicator of dye degradation was observed in the plate assay.

2.1 *Saccharomyces cerevisiae* strain

The microorganism evaluated in this study is a commercial strain of baker's yeast, derived from topically local

active dry yeast, which was activated with YMPD medium, consisting of (yeast extract 3, malt extract 3, peptone 5, dextrose 10 and agar 20 g/l, pH 6). After 48 h incubation period at 30°C, the resulting yeast cells were seeded on four YMPD agar plates. First, select each colony according to the colony morphology to select colonies with different morphologies. Each selected colony was harvested and store it on the YMPD agar slope at 4°C for further studies. Use standard morphological and physiological tests to classify yeast **(Spencer et al 2011)**. The agar plate colonies under a microscope were examined to determine the morphology and surface, edge, color, and shape characteristics of the yeast. To optimize the bioremediation of MB from its aqueous solution and impurities, strain obtained from *Saccharomyces cerevisiae* and YMPD agar were used. Optimal conditions for removing MB by culturing organisms in batches.

The MB biological removal device is designed to determine the effects of carbon and nitrogen sources, temperature, pH number and MB concentration on the MB biological removal rate. All the chemicals used in the experiment are reactive.

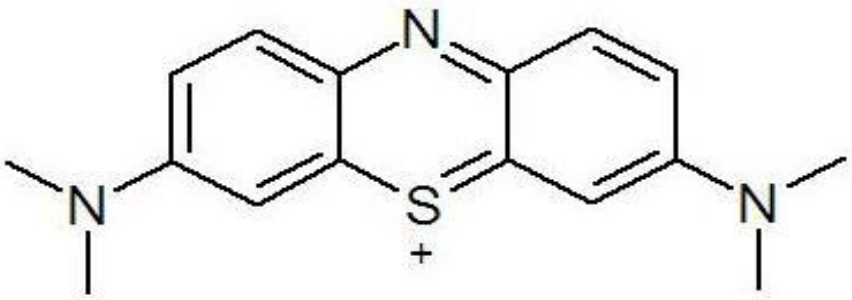
2.2 Chemicals

All the chemicals used in the experiment are reactive. **(Table 1).**

2.3 Stock solution and methylene blue validation curves

Methylene blue (basic blue 9), about C.I.52015; Chemical Formula is C16H18CIN3S.3H2O; and molecular weight, (319.9 g mol −1). The original dye solution was prepared by dissolving 1000 mg MB in each liter of distilled water, and the empirical solution was made by diluting the original dye solution with

distilled water.



A chemical compound of Mb

The GENESYS 10S UV-VIS spectrophotometer has been employed for the monitoring of double-beam UV-vision spectrophotometers (Kubota 6930, Japan) with concentrations of 50, 100, 200, 500, 1000 mg/L, spectrum behavior from 200 nm to 800 nm, to achieve full wavelength and calibration of the MB. The dilution of original MB solution with distilled water was used to obtain empirical solutions with the desired concentrations.

2.4 Treatments procedure

In this research, sugar cane molasses and candy whey were used as a cheap material and as an alternative to YMPD medium ingredients. One hundred mL of the reaction mixture (MB solution, modified YMPD medium, and yeast inoculum) was provided at different experimental elements such as the incubation temperature, MB dye concentration, yeast inoculum dose, and pH number as shown in **(Table 2)**. All experiments in a 250 mL Erlenmeyer distilled flask group and room temperature 30°C have been carried out.

2.5 Examination of *Saccharomyces cerevisiae* cells (biosorbent) by scanning electron microscopy (SEM)

Under ideal conditions, SEM micrographs are taken on the yeast cells before or after processing textile wastewater and MB solutions at different magnifications (2000X, 3000X and 5000X). Previous literature has shown that Saccharomyces cerevisiae cells usually have elliptical regions with a large and small diameter of 5 to 10 μm and 1 to 7 μm, respectively **(Walker 1998).**

2.6 Assay the MB concentrations of samples

For all samples obtained and after the biosorption process was completed, the reaction solution was subjected to centrifugation at 10,000 rpm for 7 minutes to remove the medium. Finally, spectrophotometer was used to measure the optical density at a wavelength of 660 nm in the ultraviolet visible range (HACH) (DR/2010-Canada), and the amount of MB in the sample is based on the standard curve and determined according to the following equation:

$$\%\, Decolorization = \frac{Initial\ absorbance - Final\ absorbance}{\text{Initial absorbance}} \times 100$$

where, initial absorbance = initial dye conc. (mg/L), Final absorbance =residual dye conc. (mg/L).

2.7 Biosorption of Methylene blue

The bio-sorption test was done in a 250 ml Falcon conical test tube including 45 ml of solution with dose of 0.1, 0.2, 0.3, and 0.4 g of inert cells (commercial) and viable cells which were cultivated in the laboratory. MB solutions at various concentrations of 100 and 200 mg/l were incubated at 30°C for 24 hours and centrifuged at 150 rpm.The variable measured was the absorbance by the spectrophotometer. Negative controls (without MB) were conducted, and the samples were performed in duplicate.

2.8. Determination of the biosorption capacity and biosorption efficiency

The bio-sorption strength (QE, mg/g) and bio-sorption efficiency were calculated by determining the absorbance of the number of adsorbed dyestuffs before and after the biosorption process.

$$QE\ (biosorption) = V * (Ci - Ce) / M$$

Equation 1

$$\%\ R = \frac{Ci - Ce}{Ci} * 100$$

Equation 2

where, QE (sorption) is the amount of dye absorbed per gram of sorbent (yeast) at equilibrium (mg/g), R (%) is the removal efficiency (%), Ci and Ce are the initial and equilibrium MB dye concentrations in the solution (mg/L), respectively, V is the MB solution volume (L) and M is the yeast mass (g).

2.9 Statistical analysis

All statistical analyses were done using SPSS 16.0. Statistical evaluation was of the biosorption, and adsorption data was made through one-way assessment of variance (ANOVA)

3 Results and Discussion

3.1 Factors affecting the bio-decolorization process

After preparing dry yeast cells (*S. cerevisiae*), which enriched, purified, isolated a pure colony, and then studied the morphological and physical tests as mentioned.

3.1.1 The impact of the initial pH

The data shown in **Fig. 1** presents that the buffer solution has a dye concentration of 100 mg/L for the removal of methylene blue in the batch balance system, initial biomass of 0.1 g, and an incubation temperature of 30°C, continuous test for 8 h. After the end of the incubation period at a pH value of 5, the discoloration rate was 90.44%. while at a pH value of 7 and 9, the discoloration value is significantly reduced. This means that the load of biomass between the anion and the surface of the

biomass is positive, and the pH is minimized. The electrostatic stretch ability of bio absorbable surface dyes may decrease as the pH value increases. Lower the pH value and reduce the free negative adsorption sites, which can be used to adsorb negatively charged dyes. During the decolorization process, the suspension will spontaneously change the pH value, thereby affecting the decolorization process. Therefore, the pH must be maintained at the required level.

3.1.2. Effect of initial Temperature

As shown in **Fig. 2** the maximum rate of methylene blue discoloration and cell growth were observed in the temperature of 25°C and 30°C (93.02 and 95.95%, respectively; however, the discoloration stopped completely at 40°C) after 20 h, recording (83.01%) accompanied by a sharp drop in cell growth. This result is normally due to a lack of cell viable and the denaturation of the dye enzyme taking charge of the bio-removal of the dyestuff **(Wang et al 2009)**. The temperature effect of the discoloration rate is not a pattern, but depends on the microorganism, properties. The best results for yeast are obtained between 25°C and 30°C.

3.1.3 Effect of initial different concentrations of methylene blue

Data in **Fig. 3** display that the best dye concentration became 50mgL, (100%), observed through 100mgL, (98.14%) then 200mgL, (92.67%) after sixteen hours of the incubation period. In contrast, each dye concentration, 500 and 1000mgL were recorded consequences much less than the preceding dye concentrations recording 86.88 % and 70.60%, respectively, with experiments.

The preliminary dye concentration and the actual quantity of dye adsorbed are multiplied. This approach that the adsorption is restricted to the dosage of bio-sorbents. Other researchers

stated it and attributed the phenomenon as the driving forces of the gradient multiplied and the preliminary concentration of dyes multiplied. This could enhance the adsorption method through a better preliminary dye concentration **(Çetinkaya et al 1999).**

3.1.4 Effect of initial biomass weight

The desired yeast weight was added to a 100 ml dye solution with a concentration of 100 ppm and pH 5 and incubated at 30°C for 24 h. The results showed that by increasing the dosage of the bio-absorbent, the absorption percentage or discoloration percentage of methylene blue can be increased wherever, 0.1 g yeast cells /100 ml dye solution is greater than 0.2, 0.3 and 0.4, and the absorption of dye g/g dry weight (Y d/x) decreases with the increase of adsorbent weight. The absorbance was 56, 27, and 35. It can be explained that the doses 56.27, 35.00, 22.00, and13.00 mg/g dry weight at biomass doses 0.1, 0.2, 0.3, and 0.4g, respectively are due to the protective effect and the lower migration rate of yeast particles when stimulated to high concentrations **(Fig. 5)**.

3.2. Determination of the biosorption capacity and biosorption efficiency

In the biological absorption experiment, the maximum biological absorption capacity (QE) under ideal conditions is 4.32 mg/g, the dye removal rate using 0.1 g biomass and 100 mg/L MB is 92.11%, while 3.89 mg/g and 87.00% using 0.2 g of yeast biomass under the same situations. This result may be related to the size of the yeast particles, which are small and can provide better contact between the dyes, so the yeast has a larger setting area, thus producing better MB bio-absorption results.

3.3 Examination of *Saccharomyces cerevisiae* as a biosorbent for methylene blue by Scanning Electron Microscope.

SEM was conducted with different amplifications prior to or after methylene blue cell treatment for ***Saccharomyces cerevisiae***. After interacting with the pigment, the control and

exposure of the cells were checked the changes in the cells. **Fig. 5A in Table 3** represents normal yeast cells has not been dyed like a monitor. **Fig. 5B in Table 3** shows the analysis of cells exposed for 19 h.

A dye that indicates the presence of a translucent layer on yeast cells. The colored deposits in the yeast cells are clearly crystalline. Therefore, SEM micrographs show that yeast cells have an increased affinity for staining. This has been confirmed by other researchers, for example **(Mahmoud et al 2017).**

The conclusion is that in recent years, many experiments have focused on microbial discoloration (**Aravindhan et al 2007; Wang et al 2008; Aksu et al 2008 ; Hu et al 2010)**. The most effective method of absorbing dye includes good performance, cost-effectiveness, maintainability, high productivity, and easy biodegradation methods **(Aksu 2005)** .

The first batch of studies on bio-sorbents focused on non-polluting toxic microorganisms that do not require a continuous supply of nutrients **(Crini 2006).**

Further studies on the use of live microbial biomass in wastewater management has recently been carried out. One of the advantages of living biomass is that it does not require pretreatment such as inactivation or bacterial grinding to reduce operating costs **(Fu et al 2001 ; Yu et al 2005 ; Xin et al 2012)**.

The spherical shape that changes rapidly during stress detection will cause rapid water loss due to changes in physical and chemical conditions: first, the roughness of the cell surface increases, then gradually loses stability, and then visually visible artifacts shift. As shown in **Fig. 5 (A and B)**, The baker's yeast cytomembrane consists of an external layer rich in O- and N-mannosylated proteins, imaged within the microscope as the layer adjacent to the cytomembrane, consisting mainly of an electro-dense part β-1,3 to a certain degree branched glucans β -

1,6 connections and how much smaller chitin interconnects the polymers and other walls in a load bearing matrix. **(Smith et al 2000; Klis et al 2006)**.

Saccharomyces cerevisiae can change its cell membrane and rapidly change its volume in response to shock **(Martínez et al 1996)**. It reflects the unit volume of the cell, and the lateral scattering reflects the roughness of the cell surface.

Under SEM, some cells can be felt damaged and have wrinkled and wrinkled surfaces, indicating subsequent sensitivity to osmotic pressure. A possible explanation for this may be that the most sensitive cells are the youngest, and their cell walls may be softer **(Saldaña et al 2021)**.

4 Conclusion

This study shows that Saccharomyces cerevisiae is more susceptible to discoloration of methylene blue. When studying the factors affecting bio-sorption, such as dye concentration, pH number, temperature, and biomass weight, the pigment element of methylene blue dye is absorbed by Saccharomyces cerevisiae. The best results are selected from previous experiments and conditions under which the maximum absorption percentage can be determined and used in subsequent treatments. When checking the change in dye concentration, it was found that the total absorption rate was 50 and 100 ppm, and the maximum absorption was observed when yeast was added to the intermediate solution after 16 and 20 h. The maximum absorption percentages were 100 % and 98.90%. Regarding the change in the pH number of MB medium yeast additive, the highest absorption ratio was 95.81% at pH 5 and 20 h incubation period. The total dose reduction occurred at a 93% concentration using the yeast inoculum of 0.1 g live cells and MB concentration of 200 ppm. The above steps show that an acidic pH of 5 is the best, the time required to reach the

maximum absorption is 20 h, the added yeast is 0.1g, and the concentration of MB is 200ppm.

Scanning electron microscopy observations display that the mechanism that happens throughout the discoloration technique is a few sorts of organic absorption.

Table 1. General characteristics of methylene blue

Name of dye	**Methylene blue**
Chemical formula	C16H18ClN3S
The molar mass	319.9 g/mol
Color index name	Basic blue 9
λmax (nm)	660

Table 2. Various operation parameters utilized to decolorize MB solution by *Saccharomyces cerevisiae*

Serial code	Variable factor	Fixed factors
3.1.1	Initial pH ranged from 5 to 9.	Temperature = 25°C, MB concentration 100 ppm, yeast cells weight = 0.1% w/v and various incubation periods = 0 – 20 h.
3.1.2	Various temperature ranged from 25 to 40°C.	MB concentration 100 ppm, yeast cells weight = 0.1% w/v. pH = 7 and various incubation periods = 0 – 20h.
3.1.3	MB concentration ranged from 50 to 1000 ppm.	Primary pH = 7, temperature = 25°C, yeast cells weight = 0.1 % w/v and various incubation periods = 0 – 20h.
3.1.4	Various yeast cells weight ranged from 0.1 to 0.4 % w/v.	Primary pH = 7, temperature = 25°C, MB concentration 100 ppm, and various incubation periods = 0 – 20h.

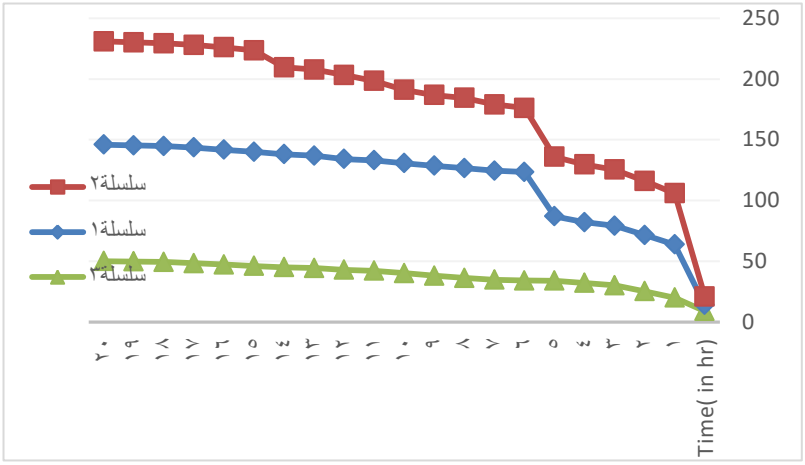


Fig. 1. Effect of primary pH on the MB bio-removal operation by *Saccharomyces. cerevisiae.*

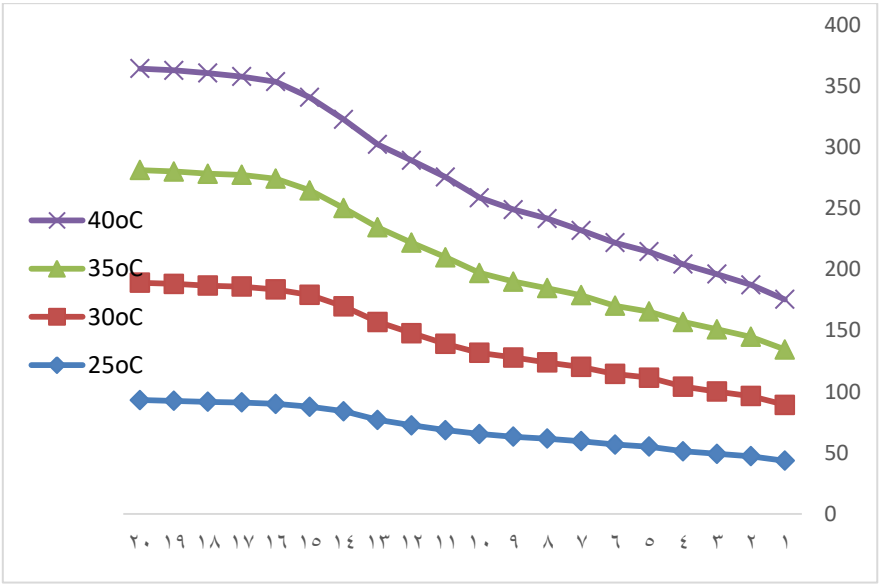


Fig. 2. Effect of primary Temperature on the MB bio-removal operation by *Saccharomyces. cerevisiae*.

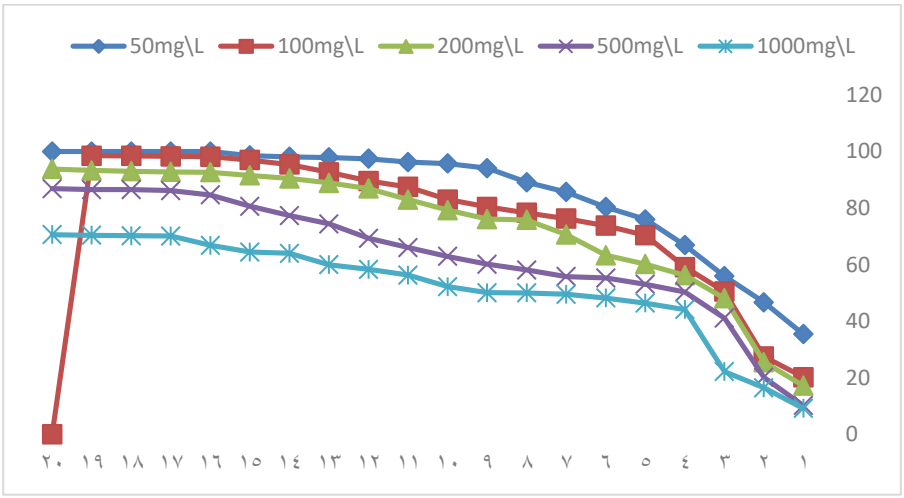


Fig. 3. Effect of primary concentrations of methylene blue on bio-removal operation by *Saccharomyces cerevisiae*.

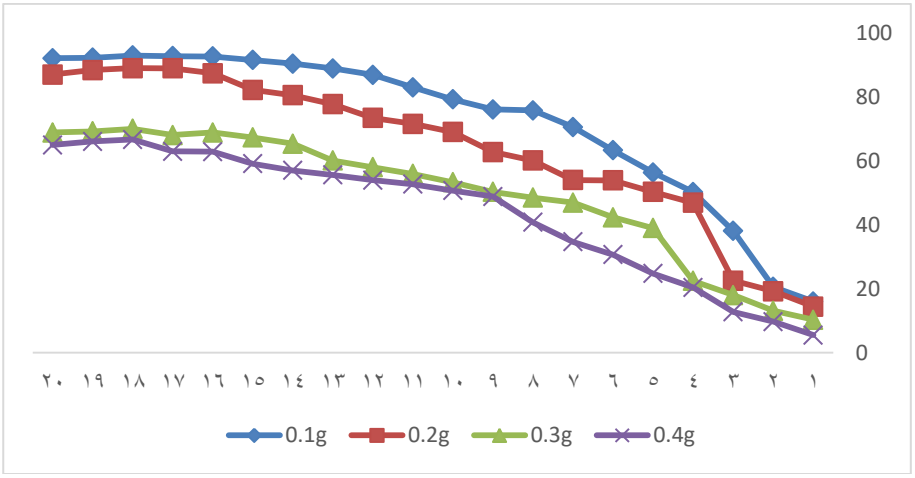
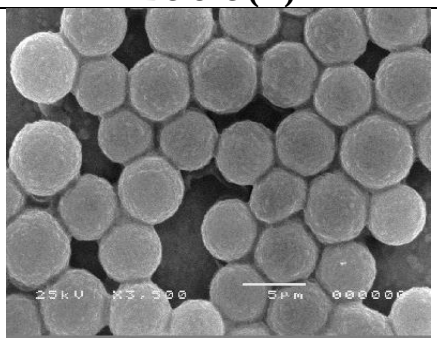
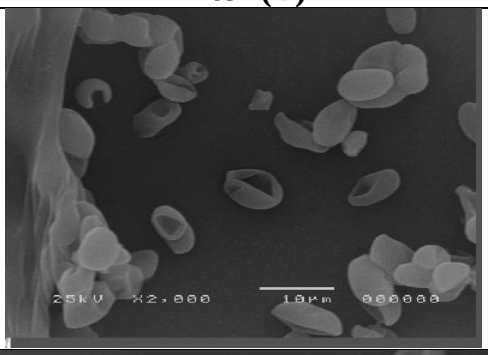
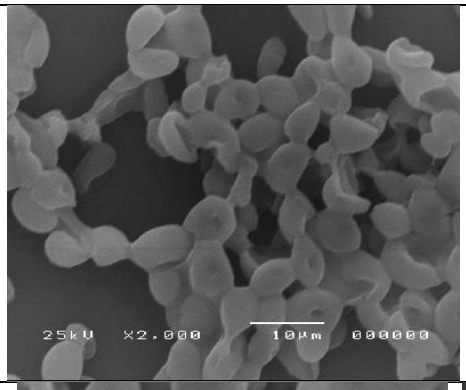
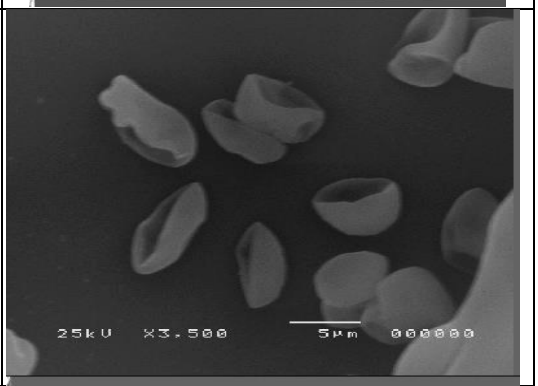
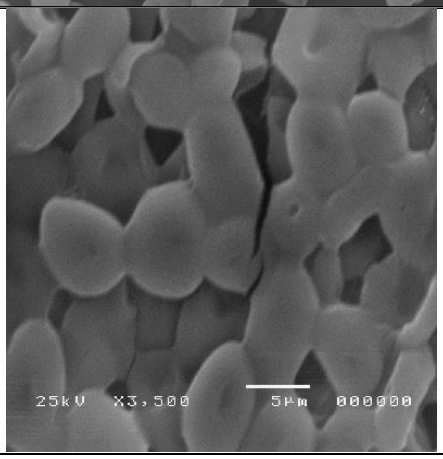
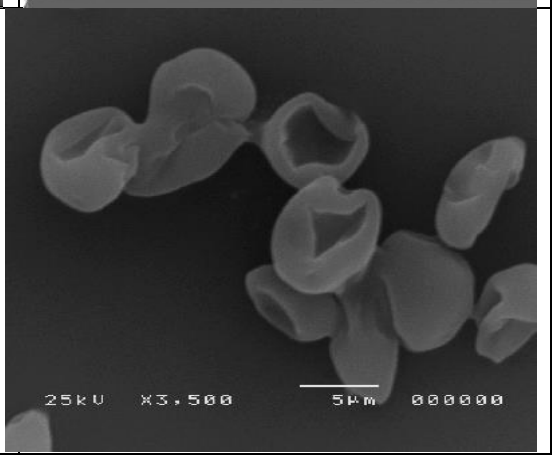


Fig. 4. Effect of primary yeast cells weight on the MB bio-removal operation by *Saccharomyces cerevisiae*.

Fig. 5 Results of Decolorization of methylene blue by using *Saccharomyces cerevisiae* at optimum factor (PH 7, 30^{o}C , 0.1 g biomass and 100mg/L dye concentration) .

Before (A)	After (B)
	
	
	

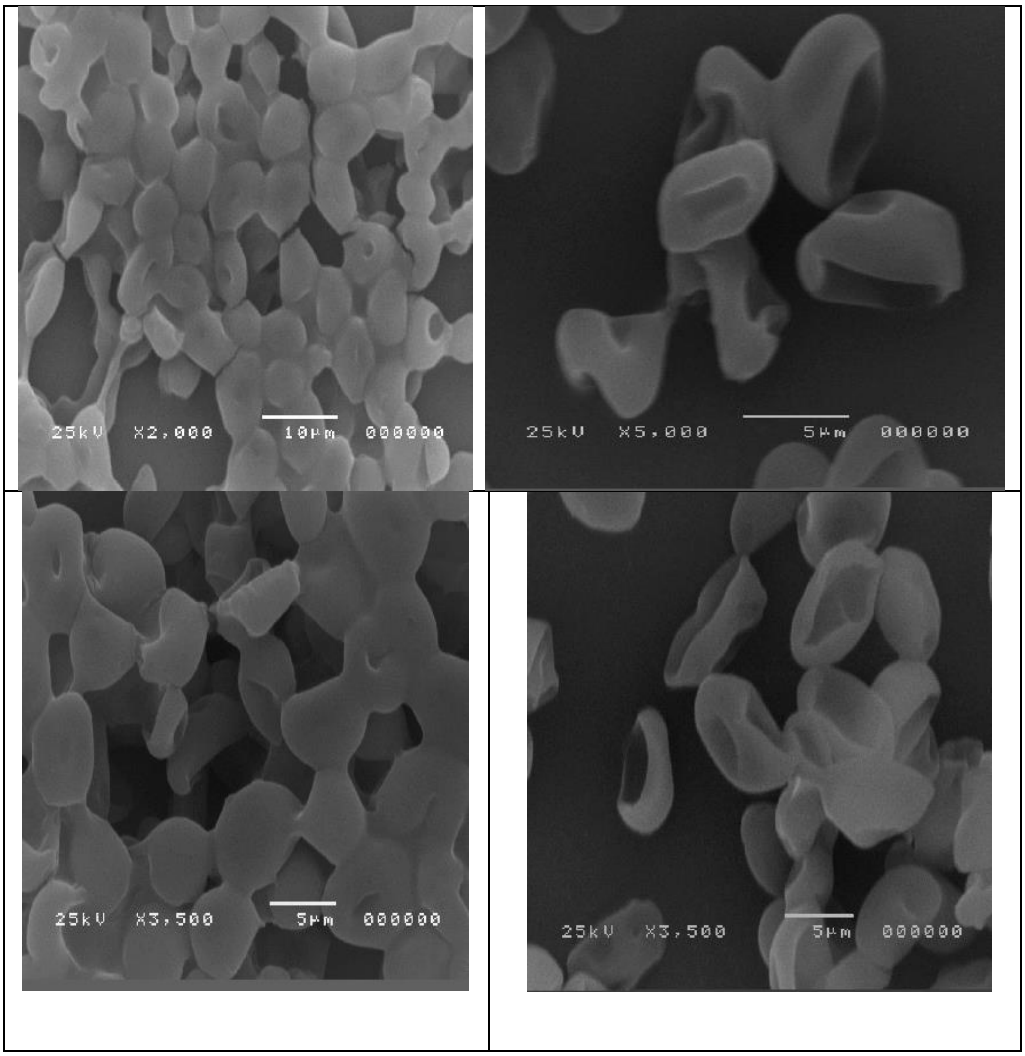


Fig. 6 Results with the aid of using scanning Electron Microscope (SEM) permitted us to deduce that the mechanism taking region at some stage in discoloration is a bio-sorption process.

References:

Aksu, Z (2005) "Application of Biosorption for the Removal of Organic Pollutants: A Review." *Process Biochemistry* 40, 997–1026.

Aksu, Z; Göknur, K (2008) "Comparison of Biosorption Properties of Different Kinds of Fungi for the Removal of Gryfalan Black RL Metal-Complex Dye." *Bioresource Technology* 99, 7730–41.

Aravindhan, R; Jonnalagadda, RR; Balachandran, UN (2007) "Removal of Basic Yellow Dye from Aqueous Solutions by Sorption on Green Alga Caulerpa Scalpelliformis." *Journal of Hazardous Materials* 142, 68–76.

Çetinkaya Dönmez, G; Aksu, Z; Öztürk, A; Kutsal, T (1999) "A Comparative Study on Heavy Metal Biosorption Characteristics of Some Algae." *Process Biochemistry* 34, 885–892.

Chittal, V; Magaly, G; Anagha, A; Purbasha, S; Bhaskara VK Rao (2019) "Biodecolorization and Biodegradation of azo dye Reactive Orange-16 by Marine Nocardiopsis Sp." *Iranian Journal of Biotechnology* 17, e1551–e1551.

Crini, Grégorio (2006) "Non-Conventional Low-Cost Adsorbents for Dye Removal: A Review." *Bioresource Technology* 97, 1061–85.

Eslami, A; Mahsa, M; Farshid, G; Vahid, V (2016) "Electrochemical Catalytic Degradation and Biodegradability Enhancement of Real Textile Wastewater by Anodic and Cathodic Reactions." *Environmental Engineering and Management Journal* 15, 2319–2326.

Fu, Y; Viraraghavan, T (2001) "Fungal Decolorization of Dye Wastewaters: A Review." *Bioresource Technology* 79, 251–62.

Gottlieb, A; Chris, S; Alan, S; Andrew, W; Stephen, F (2003) "The Toxicity of Textile Reactive Azo Dyes After

Hydrolysis and Decolorization." *Journal of Biotechnology* 101, 49–56.

Hu, Z; Hui, C; Feng, J; Shoujun, Y (2010) "Removal of Congo Red from Aqueous Solutions by Cattail Root." *Journal of Hazardous Materials* 173, 292–97.

Jadhav, JP; Parshetti, GK; Kalme, SD; Govindwar, SP (2007) "Decolorization of Azo Dye methyl red by Saccharomyces Cerevisiae MTCC 463." *Chemosphere* 68, 394–400.

Klis, F; Andre, MB; Piet, WJ (2006) De Groot. "Cell Wall Construction In Saccharomyces cerevisiae." *Yeast* 23, 185–202.

Lucas, M; Carla, S; Ana, A; José, S; Peres; A; Albino, AD (2006) "Biodegradation of the Diazo Dye Reactive Black 5 by a Wild Isolate of Candida Oleophila. "*Enzyme and Microbial Technology* 39, 51–55.

Lucas, M; Albino, S; Dias, A; Ana, S; Carla, A; José, AP (2007) "Degradation of a Textile Reactive Azo Dye by a Combined Chemical–Biological Process Fenton's Reagent-Yeast." *Water Research* 41, 1103–1109.

Mahmoud, MS; Samah AM (2017) "Calcium Alginate as an Eco-Friendly Supporting Material for Baker's Yeast Strain in Chromium Bioremediation." *HBRC Journal* 13, 245–54.

Martínez, D; Marañon, I; Marechal, PA; Gervais P (1996) Passive response of *Saccharomyces cerevisiae* to osmotic shifts: cell volume variations depending on the physiological state. Biochem Biophys Res Commun 227, 519–523.

Martorell, M; Hipólito, M; Pajot; F; Lucía, IC; Figueroa, D (2012) "Dye-Decolourizing Yeasts Isolated from Las Yungas Rainforest. Dye Assimilation and Removal Used as Selection Criteria." *International Biodeterioration and Biodegradation* 66, 25–32.

Pajot, H; Osvaldo, D; Lucía, C; Figueroa; D; Juliam, F (2011) "Unraveling the Decolourizing Ability of Yeast Isolates from Dye-Polluted and Virgin Environments: An Ecological and Taxonomic Overview." *Antonie van Leeuwenhoek* 99, 443–456.

Ramalho, Patrícia, A; Helena Cardoso, M.; Cavaco-Paulo, A; Teresa Ramalho, M. (2004) "Characterization of Azo Reduction Activity in a Novel Ascomycete Yeast Strain." *Applied and Environmental Microbiology* 70, 2279- .

Saldaña, CC; Villava, JRV; Verónica, MT; Juan, CGJ; Chávez, S; Teresa, GG (2021) "Rapid and Reversible Cell Volume Changes in Response to Osmotic Stress in Yeast." *Brazilian Journal of Microbiology*, January 21,

Smith, JS; Brachmann, CB; Celtic, I; Kenna, MA; Muhammad, S; Sarai, VJ; Avalos, JL (2000). "A Phylogenetically Conserved NAD+-Dependent Protein Deacetylase Activity in the Sir2 Protein Family." *Proceedings of the National Academy of Sciences* 97, 6658–6663.

Spencer, J; Rawling, S; Stratford, M; Steels, H; Novodvorska, M; Archer, DB; Chandra, S. (2011) "Yeast Identification: Reassessment of Assimilation Tests as Sole Universal Identifiers." *Letters in Applied Microbiology* 53, 503–508.

Vikrant, K; Balendu, SG; Nadeem, R; Kangkan, R; Ki-Hyun, K; Birendra, NR; Ram SS (2018) "Recent Advances in Bioremediation of Dye: Current Status and Challenges." *Bioresource Technology* 253, 355–367.

Walker, JA (1998) "Visual Culture and Visual Culture Studies." *The Art Book* 5, 14–16.

Wang, BE; Yong, YH; Lei, X; Kang, P (2008) "Biosorption Behavior of Azo Dye by Inactive CMC Immobilized Aspergillus Fumigatus Beads." *Bioresource Technology* 99, 794–800.

Wang, Z; Mark, G; Michael, S (2009) "RNA-Seq: A

Revolutionary Tool for Transcriptomics." *Nature Reviews Genetics* 10, 57–63.

Xin, B; Yunting, X; Yang, Z; Hina, A; Changhao, L; Shi, C (2012) "A Feasible Method for Growing Fungal Pellets in a Column Reactor Inoculated with Mycelium Fragments and Their Application for Dye Bioaccumulation from Aqueous Solution." *Bioresource Technology* 105 100–105.

Yang, Q; Min, Y; Karin, P; Ayfer, Y; Alexandra, H; Michael, S; Antonius, K (2003) "Decolorization of Synthetic Dyes and Production of Manganese-Dependent Peroxidase by New Fungal Isolates." *Biotechnology Letters* 25, 709–713.

Yu, Z; Xianghua, W (2005) "Screening and Identification of Yeasts for Decolorizing Synthetic Dyes in Industrial Wastewater." *International Biodeterioration & Biodegradation* 56, 109–114.

Zhou, Q; Aaron, F; Gerald N Flerchinger; Alejandro N Flores (2019) "Examining Interactions Between and Among Predictors of Net Ecosystem Exchange: A Machine Learning Approach in Semi-Arid Landscape." *Scientific Reports* 9, 2222.

Zhou, Y; Zhen, L; Yanxin, W (2008). "Decolorization and COD Removal of Secondary Yeast Wastewater Effluents by Coagulation Using Aluminum Sulfate." *Desalination* 225, 301–311.