Removal of water-soluble dye (methylene blue) by yeast *Saccharomyces cerevisiae.*

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Abstract

Saccharomyces cerevisiae was studied for the removal of methylene blue (MB), a reactive dye, from its aqueous solutions in two different approaches, the first one was determining the removal of MB by biosorption. Factors that affect the biosorption process such as dye concentration, biomass concentration and pH were investigated. The maximum percentage of colour removal, 83.50% in 24 h, was obtained on using cultivated yeast concentration of 0.2 g in 45 mL at 30 º C and pH 3.5, with this result it was concluded that the dried biomass of *S. cerevisiae* can be considered as a good biosorbent material for reactive dyes as MB. Also, a desorption process was made giving as result a desorption percentage of 65.47% in 120 min with methanol used as a solvent. The second approach was to evaluate the biodegradation of the MB dye with the yeast and, due to the nature of the dye, it is suggested that the yeast only performs biosorption and was unable to take MB as the carbon source for its growth.

1. Introduction

Large amounts of dyes are annually produced and applied in textile, cosmetics, paper, leather, pharmaceutical, food, and other industries. The textile industry accounts for two-thirds of the total dye market, consuming a large proportion of reactive dyes due to the high demand for cotton fabrics with brilliant colors. Even a very small amount of dye in water (10 – 50 mg/L) affects the aesthetic value, water transparency, and gas solubility of water bodies [1, 2]. Controlling pollution is the main concern of the society today. There are 10,000 types of dyes throughout the world and approximately $7x10^5$ tones of these are produced every year [3].
A remarkable amount of the dyes is lost during dyeing processes. These losses are mainly disposed into aquatic environment by textile and dyeing industries and through some other means. Wastewater from textile industries constitutes a threat to the environment in most parts of the world, as the degradation products of textile dyes are often carcinogenic. In addition, light absorption hindered by textile dyes creates problems to photosynthetic aquatic plants and algae. The most important pollutants in textile effluents are recalcitrant organic compounds, color, inhibitory compounds, surfactants, and chlorinated compounds. During processing, 5–20% of the used dyes are released into the wastewater [4, 5].
Furthermore to their visual effect and their adverse impact in terms of chemical oxygen demand, many synthetic dyes from the industries have a serious impact on the environment because their disposal into water bodies causes considerable damage to both aquatic biota and humans by inducing mutagenic and carcinogenic effects [6]. Methylene blue is the most commonly used substance for dying cotton, wood, and silk. When inhaled it can cause difficulty in breathing, while direct contact may cause permanent injury to the eyes of human and animals, as well as burning sensations, nausea, worming, profuse sweating, mental confusion and methemoglobinemia [7]. The potentially deleterious effects of MB on human health drove the interest in its removal promptly, bioprocesses are an effective and ecofriendly for removing this dye [8].

Removal of reactive dyes is especially problematic because they can easily pass through conventional treatment systems and without much change [9].Therefore, proper techniques and processes are needed in the industry for efficient removal of these toxic chemicals from water bodies. There are a number of techniques that have been proposed or applied for the treatment of dye-containing wastewater, which cover methods using biological,

physical and chemical approaches [10]. Moreover, the application of some physical and chemical methods are restricted because of high operating cost, excessive use of toxic chemicals or strict application conditions [11]. Adsorption as a traditional physical method has been proved to be an effective method for dye wastewater treatment [12].

Researchers used adsorbents made from natural materials or industrial wastes. For example, peanut shell, apple peel, sawdust, cattail root, peat, bentonite, iron/steel waste, and clay have been used to remove dyes from aqueous solutions [13, 14, 15, 16]. However, these materials generally have low adsorption capacity and thus high amount of adsorbents are needed. It is always worth to develop low-cost and high-efficiency adsorbents which combine biological, chemical and physical functions together. In recent years, a large number of studies are focused on microbial decolorization [17, 18, 19, 20]. Biosorption by microorganisms has advantages such as good availability, cost effectiveness, easy operation, high efficiency and ready biodegradation, which has been proved to be one of the most promising dye adsorption methods [21].

Early research on biosorbents mainly focused on inactivated microorganisms which are not affected by dye toxicity and also do not require continuous addition of nutrients [14]. Recently, more research is focused on the application of live microbial biomass for wastewater treatments [22, 23, 24]. One advantage of live biomass is that they do not need pre-treatments such as bacteria inactivation and grinding, which can further save operational costs [25, 26, 21].

The interactions between microorganisms (yeast, bacteria and/or fungi) and dyes depend on the chemical properties of all the reaction compounds. The yeasts have many advantages compared to bacteria and filamentous fungi. Yeasts are a better raw biosorbent material for the removal of reactive dye due to their unicellular nature and high growth rate. Yeast cells can be easily cultivated into inexpensive growth media and are a readily available source of biomass that has potential for bio-remediation of wastes [3].

The yeast *Saccharomyces cerevisiae* is one of the most ubiquitous biomass types available for bioremediation of textile dyes [2]. It is known that *S. cerevisiae* is inexpensive, safe, easily growth, readily available and produces high yields of biomass [27]. Yeasts can adapt and grow in various extreme conditions of pH, temperature and availability of nutrients as well as high concentrations of pollutant [28]. *S. cerevisiae* strains are genetically diverse, largely as a result of human efforts to develop strains specifically adapted to different process that have very different purposes [29].

A considerable attention has been directed in evaluating the capability of microorganism in decolorization and biodegradation of dyes because microbial processes play a vital role in the safe clean-up of environmental messes [30]. Nevertheless, the effluent because of transformation of dyestuffs could be toxic [31]. For environmental safety, the microbial toxicity of the byproducts produced during the decolorization process should be evaluated. Microorganisms have the ability to decolorize the dye solution through two ways: either adsorption on the microbial biomass or biodegradation of the dyes by the cellular enzymes. Therefore, the biological method is the focus of recent studies on dye degradation and decolorization. Consequently, the objective of this study is to evaluate the removal of MB in water with the yeast *Saccharomyces cerevisiae* with two different approaches, the first one determining the removal of MB by biosorption and the second approach by evaluating the biodegradation of the dye MB with the yeast.

2. Materials and methods

2.1. Strain of Saccharomyces cerevisiae.

The microorganism that was evaluated in this project was a commercial baker's yeast strain of *Saccharomyces cerevisiae* (Levapan®), which was kept cryopreserved to maintain the purity of the strain and for be use in the project and was used in different ways, for the biosorption experiments was used as following: inactive yeast, I, (direct from the product purchased in the market), and as active yeast, C, from the cryopreservation, activated with YPD medium [32],(glucose 20 g / L, peptone 20 g / L and yeast extract 10 g / L) for 24 hours in an Erlenmeyer of 2000 L with 1000 L of work volume and it was shaken at 150 rpm at a temperature of 30 ºC, after this time the biomass was harvested by centrifugation at 4500 rpm for 15 minutes and it was dried in Falcon 50 mL for 48 hours. Then it was powdered using mortar for use as a biosorbent. For the biodegradation analysis the yeast was activated also with the YPD medium and used as the section 2.6. describes it.

2.2. Stock solution and calibration curves for methylene blue.

A stock solution of MB (Cirumedics S.A.S.) of 750 mg/L was prepared for the experiments. Spectral sweeps were performed for concentrations of 0, 0.5, 1, 5, 10, 15, 20, 25 and 30 mg/L of MB to observe its behavior in the UV/Vis spectrum from 200 nm to 800 nm in the spectrophotometer GENESYS 10S UV-VIS, in this way the maximum wavelength of MB was obtained and the calibration curve was assembled.

2.3. Biosorption of Methylene blue

The biosorption experiment were performed in 250 ml conical falcon tubes containing 45 ml of the solution of with 0.2, 0.4, 0.6, and 0.8 g of inactive cells (commercial) and active cells, cultured in the laboratory at 30 ° C for 24 h at 150 rpm. The MB solution was evaluated in different concentrations, 15 and 20 mg/L. The variable measured was the absorbance by the spectrophotometer. Negative controls (without MB) were carried out. The samples were made in duplicate.

The biosorption capacity (qe, mg/g) and biosorption efficiency were calculated by determining the absorbance of the amount of adsorbed dyestuff before and after biosorption process.

$$qe\ (biosorption) = \frac{V*(Ci-Ce)}{M} \quad \textit{Equation 1}$$

$$\%\ R = \frac{Ci-Ce}{Ci} * 100 \quad \textit{Equation 2}$$

where, qe, sorption is the amount of dye biosorbed per gram of sorbent (yeast) at equilibrium (mg/g), R (%) is 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).

The biosorption experiments were conducted under different pH. The pH of the solution was adjusted by adding HCl (0.1 M) or NaOH (0.1 M)
Statistical analysis was performed with Minitab software in which analysis of variance (ANOVA) and analysis of means by Fisher's Least Significant Difference (LSD) were included.

2.4. Desorption experiments

To assess the desorption of the dye that had been adsorbed by *S. cerevisiae*, the methanol extraction method was used to acquire the MB adsorbed by the biomass collected after completing the biosorption process. This test was performed according to the work of Hosseini Koupaie et al (2013). Once the biosorption process was done in 45 ml of water with 15 and 20 mg/L of MB at 150 rpm for 24 h, the biomass of *S. cerevisiae* were collected by centrifugation at 4500 rpm for 15 min. Then, a solution of 45 ml of methanol: distilled water (ratio 1: 4) was added to the cell mass and mixed

thoroughly and left at 150 rpm for 24 h. Finally, the separation was carried out by centrifugation and its absorbance was measured by spectrophotometry.

The desorption capacity (qe, desorption, mg/g) was calculated as follows:

$$qe\ (desorption) = \frac{V*Cf}{M} \quad \textit{Equation 3}$$

where, qe (desorption) is the amount of dye desorbed from biomass per gram of dye saturated biosorbent at equilibrium (mg/g), Cf is the MB dye concentration in the desorbing solution (mg/L), V is the eluent solution volume (L) and M is the dye saturated yeast weight (g).

Desorption efficiency (%) of MB dye was calculated using

$$\%\ D = \frac{qe\ (desorption)}{qe\ (biosorption)} * 100 \quad \textit{Equation 4}$$

where, D % is desorption efficiency (%) and qe, desorption and qe, sorption are desorption and sorption capacity (mg/g), respectively.

2.5. Growth kinetics

The kinetics for the growth of *S. cerevisiae* with different types of nutrients were carried out to evaluate if the yeast cells could assimilate MB as a possible carbon source and make a biotransformation.

a. YPD kinetics
   The medium of this kinetic contains the basic nutrients of the YPD media as described in the 2.1. section.
b. YMG kinetics
   The medium of this kinetic contains glucose (1 g/L), peptone (20 g/L), yeast extract (10 g/L) and 15 mg/L of MB.
c. YMB kinetics
   The medium of this kinetic contains peptone (20 g /L), yeast extract (10 g /L) and 15 mg/L of MB.
d. 15MB kinetics
   The medium of this kinetic contains 15 mg/L of MB.
e. 20MB kinetics
   The medium of this kinetic contains 20 mg/L of MB.

Each of the growth kinetics was carried out in Erlenmeyer of 250 ml with a work volume of 50 ml, containing the medium and an *S. cerevisiae* inoculum with an optical density of 1, each of the Erlenmeyer had conditions of 30 ºC, 150 rpm, were covered to avoid possible degradation of MB with the light. Sampling was done every 12 hours for 144 hours in total. The samples were made in duplicate. The samples were used to measure pH, dry weight, absorbance of the supernatant. The pH measurement was done in the HANNA instruments pH211 Microprocessor pH meter. The dry weight measurement were performed by centrifugation in UNIVERSAL 32R (HETTICH), under the conditions of 22ºC, 14000 rpm for 10 minutes, after the biomass was dried at 40 ° C in the OF-02G oven (JEIO TECH) for 48 hours and the were weighed on the SATORIUS BP 301S scale. The supernatant was taken after centrifugation for the dry weight, and its absorbance was measured in the GENESYS 10S UV-VIS spectrophotometer.

3. Results and discussion

3.1. Calibration curves and maximum wavelength

After performing the spectral sweeps for different concentrations of MB, 660nm was defined as the maximum wavelength in the UV / Vis spectrum from 200nm to 800nm.

3.2. Saccharomyces cerevisiae as biosorbent of MB in water

MB biosorption capacity and efficiency were studied as a function of initial dye concentration (15 and 20 mg/L), while the initial pH, temperature and contact time were kept constant, but the concentration of biomass where in the range of 0.2 to 0.8 grams in 45 mL.

Figure 1. (a) Cultivated yeast. (b) Inactive yeast.

In the biosorption experiment it was clear that the cultivated yeast had the best results for the removal of MB, the maximum biosorption capacity, qe, was 3.01 mg/g and a colour removal percentage of 83.50% with 0.2 g of biomass and 15 mg/L of MB, compared with a 1.68 mg/g and a 46.74 % with the inactive yeast at the same conditions, this can be observed in the Figure 2, this results could be due to the particle size of the cultivated yeast that was between 300 microns and 75 microns, this particle size was smaller than the inactive yeast and this gives better contact between the dye and the yeast because it has a lot more surface area to be in touch, giving the result of a better biosorption of MB. This was achieved when the cultivated yeast was powered with a mortar and the inactive yeast was not treated and was used as the original granulated product, as seen in

Figure 1 .

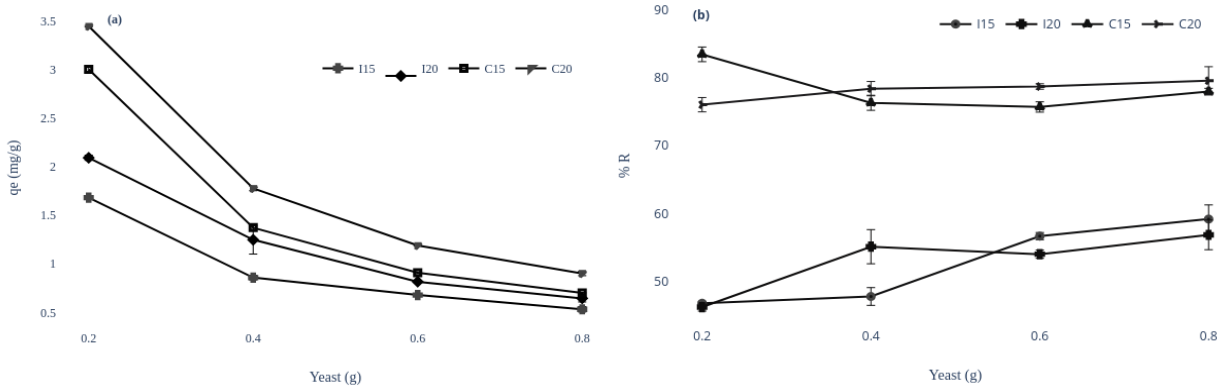

Figure 2. (a) Effect of biosorbent capacity on dye decolorization with different concentration of dyes I15, I20, C15 and C20, being I inactive yeast and C cultivated yeast, with 15 or 20 mg/L of MB. (b) MB % of colour removal with different concentrations of dye.

In the Figure 2 it can be observed that the 0.2 grams of yeast with 15 mg/L of concentration of MB has the better conditions for the color removal. The removal of the colorization depends on the structure and molecular weight of dye used, in this case MB is a heterocyclic aromatic chemical compound with the molecular formula of $C_{16}H_{18}N_3SCI$ and molecular weight of 319.85 g / mol [34], according to results reported in the literature [35], the molecular weight of MB is in the range of values of other dyes that have presented high percentages of biosorption, and this coincides with the results found in this work. The molecular weight of a dye is important in a biosorption process, at low molecular weights there will be a greater biosorption of the dye, as demonstrated in the work of H.M. El-Hennawi et al (2013), anthraquinone and metal complex vinyl sulphone azo dye were the ones with the highest percentage of color removal (92.8% and 92.6% respectively) and these have a molecular weight of 208.21 g / mol and 118.16 g / mol respectively [36, 37], meanwhile the lowest percentage of color removal are azo vinyl shulpone reactive yellow and red dyes (62.5% and 62.86% respectively of removal) with a molecular weight of 682.8 g / mol and 496.4 g / mol respectively [38, 39, 35].

The importance of the structure of the molecules to be adsorbed has been studied by H.E.Reynel-Avila et al (2016), where the performance of the adsorption has varied according to the molecular volume and weight of the dyes. They concluded that the smallest dye molecules have the highest adsorption capacity, suggesting that the steric factors involved in dye removal appear to be dependent on the residence time of contact of the biosorbent with the dye. In other words, the molecular properties have an impact on the mass transfer phenomena in the absorption of dyes where it is expected that the lighter and smaller molecules are favored during the first hours of contact time of the discoloration process, and large molecule adsorption requires long contact times to reach equilibrium [40].

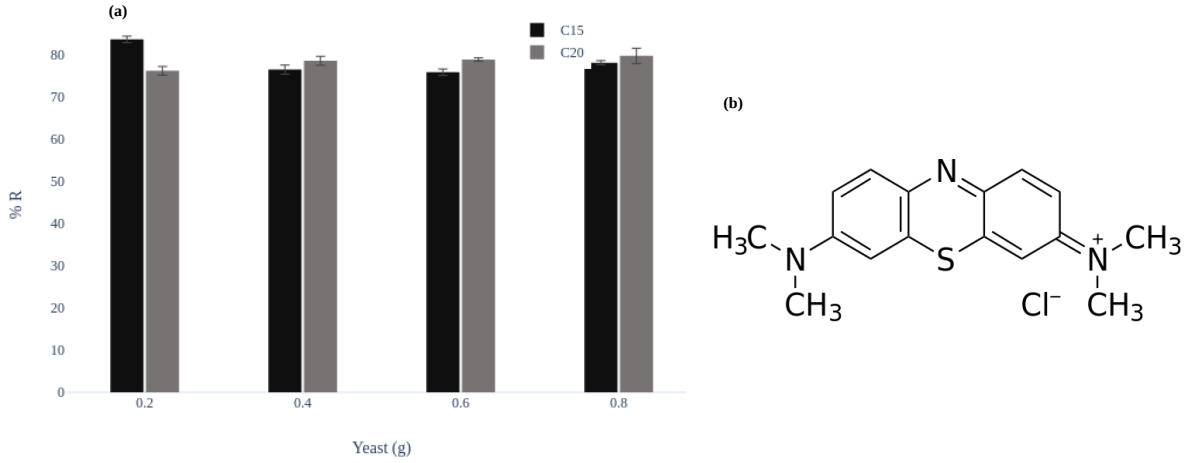

Figure 3. (a) Effect of concentrations of dried cultivated biomass on its capacity of MB biosorption. (b) Chemical structure of MB.

The initial dye concentration plays an important role to control the mass-transfer resistance of the dye, because the presence of concentration gradient of dye in liquid (aqueous solution) and solid (biosorbent) phases is the driving force for the biosorption process **[41]** and this behavior is shown in Figure 1. This phenomenon should be caused by the attractive forces between the dye molecule and the adsorbent such as van der Waals forces and electrostatic attraction. Afterward, fast diffusion onto the external surface was followed and the chemisorption of dye continued until reaching the equilibrium. The increase in loading capacity of the adsorbent for dye ions, like seen in Figure 3 with the sample C15 is probably due to a higher driving force for mass transfer. Then, a slower biosorption rate would occur as the surface biosorption sites gradually decrease **[42]**. A proposed mechanism of the process is that the biosorption of the dye MB on the surface of baker's yeast is a monolayer biosorption. The biosorption may be occurred through electrostatic attraction and hydrogen bond interaction **[43]**.

Different analysis of variance were performed. The first ANOVA was with biomass concentration as factor and the biosorption capacity of the yeast (qe) as response variable where a P value of 0 was obtained, to corroborate this an analysis of means (LSD) was made giving the conclusion that there were groups significantly different in the factors studied, and the factor of 0.2 g of yeast was the one with the highest biosorption capacity of MB.
For the analysis of variance of the biomass concentration factors and the MB concentrations factors with the removal percentage as response variable, P values greater than alpha were obtained for both cases, and corroborating it with an analysis of means (LSD) it was obtained that the groups are homogeneous .

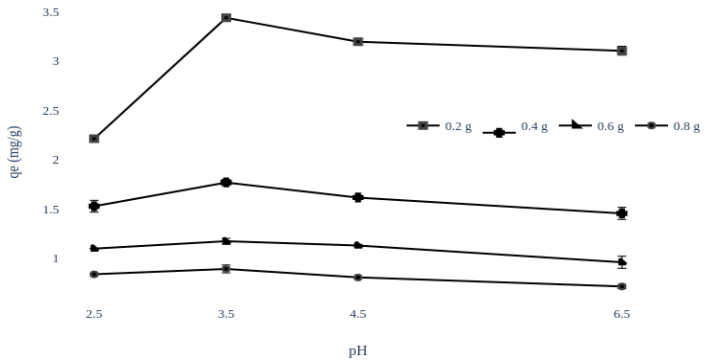

Figure 4. Effect of pH variations in the biosorption capacity of the cultivated yeast.

The pH is one of the most important factors controlling the adsorption of dye onto suspended particles, provides a critical role in adsorption of dye from the solution because it affects the solubilization of dye and concentration of ions present on the adsorbent surface [44]. To study the effect of pH on percentage of dye removal, the reactions were carried out at different pH ranges (2.5, 3.5, 4.5 and 6,5) with 15 mg/L of initial dye concentration and 0.2, 0.4, 0.6, 0.8 g of the yeast for 120 min. This experiments also had negative controls (without MB) to ensure that the biosorption was only by the yeast and that any sorption effect of the dye on the wall of the conical flasks or the yeast itself could be ruled out. Maximum dye removal was observed at pH 3.5 for the dye tested, in the Figure 4 can be seen that the amount of yeast indicated for the biosorption process is 0.2 g of cultivated yeast and the pH with the maximum MB removal was 3.5, but still the process did not have a lot of difference in this range of pH, in the work of Z. Aksu (2003), decided that the optimal pH was 3 and the biomass exhibited maximum dye uptake due to its positively charged nature at acidic pH and the anionic nature of the reactive dyes, as the MB.

For the statistical analysis of the pH, the pH was taken as a factor and the biosorption capacity of the yeast (qe) as the response variable, the P value obtained was 0, and to corroborate this an analysis of means (LSD) was made giving the conclusion that there were groups significantly different in the factors studied, and the pH range of 2.5-3.5 was the one with the highest biosorption capacity with yeast.

3.3. Desorption of MB

For the first experiments of desorption with methanol/water solution (1:4) for 24 h, low desorption efficiencies were obtained as show on table 1.

Table 1. Low dye desorption efficiency (D%) with cultivated cells of yeast with solution of methanol/water 1:4

C15		C20	
Yeast (g)	% D	Yeast (g)	% D
0,2	1,081	0,2	0,784
0,4	0,397	0,4	0,474
0,6	0,266	0,6	0,264
0,8	0,224	0,8	0,265

After having low efficiency in the desorption process with methanol / water solution (1: 4), in a literature review it was found that in the work of Ming-Xia Wang et al (2015) solutions containing methanol, ethanol, acetone, 0.01M HCl, 0.01M NaOH and 0.01M Na_2SO_4 were used and their results conclude that methanol and ethanol were the eluents with the highest efficiency for the desorption process [45].

Therefore, a second desorption process was carried out with methanol and ethanol as solvents. Biosorption assemblies were made with 0.2, 0.4 0.6 and 0.8 g of yeast with a pH 3.5 for 120 min in constant agitation and 30ºC, after this biosorption process, desorption was executed with methanol and ethanol, the solutions were stirred at 150 rpm at 30ºC for 120 min. Yeast biomass was separated from the mixture by centrifuging at 4000 rpm for 20 min.

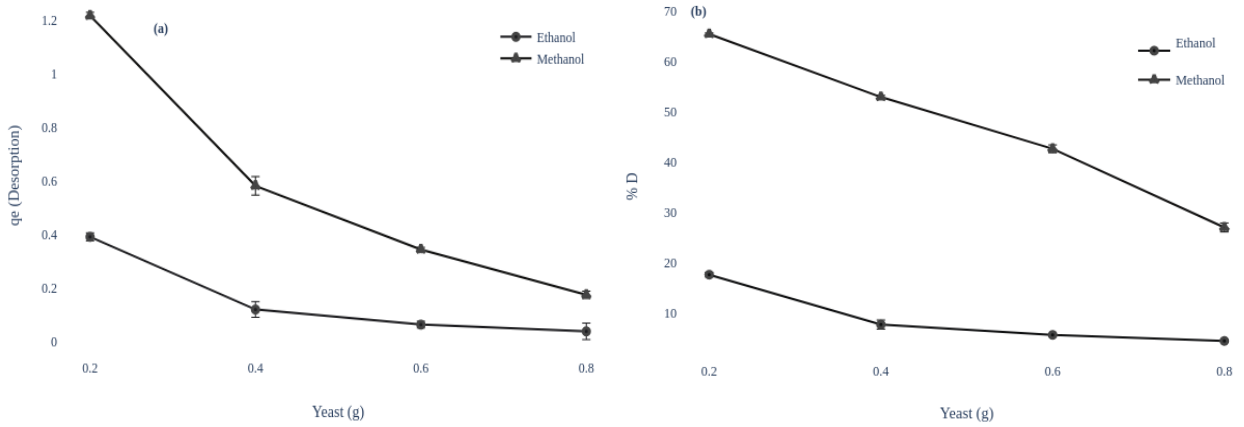

Figure 5. (a) Desorption capacity of the cultivated yeast with ethanol and methanol as solvents. (b) Desorption percentage with ethanol and methanol.

The results showed in Figure 5 suggest good efficiency, these experiments were performed only for 120 min and not in 24 h as the previous desorption with the methanol and water solution (1:4). This gives us good expectation of the efficiency of methanol as a solvent for the desorption of MB from yeast cells, the percentage of desorption with methanol was the highest (65.47%) with 0.2 g of cultivated yeast, meanwhile ethanol had a low desorption capacity (17.60%).

Generally, dyes can be biosorbed both on the surface of cell walls and in the inner cell structures when using live biomass. The biosorption mechanism can also be investigated through the dye desorption process. Here two different solvents were used and the results show that dyes adsorbed on the yeast can be washed out by organic compounds, which suggest that electrostatic attraction was a decisive factor in biosorption process. Meanwhile, in the results of Ming-Xia Wang et al (2015) , shows that organic solvents were good solutions for the desorption of dyes from the pellets of the yeast. For example, in their results, the desorption efficiency of methanol was approximately 11 times higher than that of Na_2SO_4 solution. The reason may be that the lipid bilayer of cell walls can be damaged by organic solvents, which made it easier for the molecule to move out of the cell structures [46, 47].This can also suggest that the dyes were adsorbed into the inner cell structure [45].

3.4. Saccharomyces cerevisiae as biodegradant of MB

Figure 6 shows growth kinetics of the biomass and the behavior they had, the yeast *S. cerevisiae* developed an exponential growth in the three media showed until 36 hours and then stabilized starting at the 60th hour without available carbon sources until the 144th hour. For the YMG and YMB media there are marked variations after 72 hours due to possible variations of the yeast in a medium with toxic components, such as the MB. The maximum production of biomass was 13.45 g/L at the 132nd hour for the YPD medium. For the YMG and YMB medium there was a result of 6.15 g/L and 5.40 g/L of biomass production respectively. These biomass values are low because when carbon sources other than glucose are used, gluconeogenesis is required, where hexose-type storage carbohydrates are generated, for maintenance within the metabolic pathway of hexose monophosphate, in which leads to the synthesis of the necessary ribose in the anabolic processes and the subsequent generation of ribose 5-phosphate that leads to the pentose phosphate route [48].This markedly reduces the performance of the process, which under normal conditions of glucose consumption is estimated between 85% and 90% of the substrate conversion [49]. It should also be noted

that the media containing the MB solution had components such as peptone, yeast extract and only for YMG 0.1% glucose, and observing the growth of the yeast in the kinetics, it can be suggested that it was due to the fact that the yeast was fed of these nutrients.

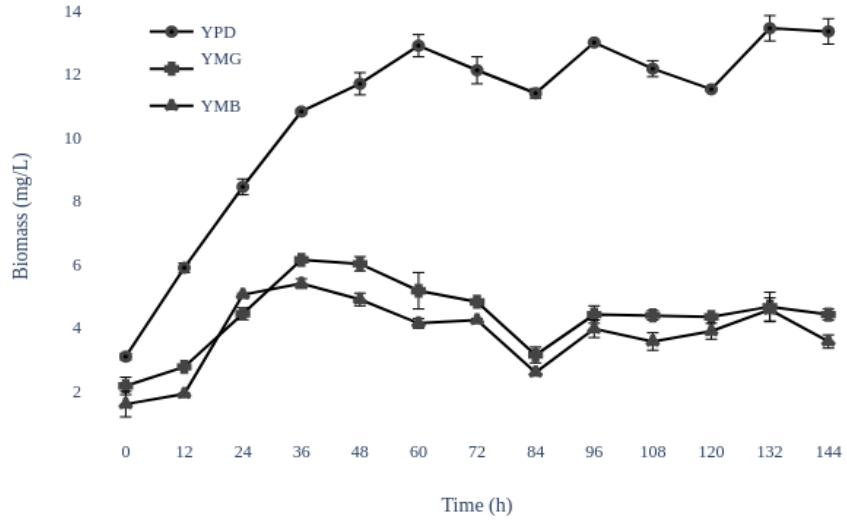

Figure 6. Growth kinetics of yeast with different media.

The removal of MB is congruent with the exponential phase that is show in the Figure 6, the maximal removal of colour is directly proportional to the biomass that was in the media. The results taken after the 48h, show variations in the concentration of MB due to possible reactions between the compounds of the medium and the MB. This hypothesis was proven when doing an experiment of equal montage with the YMG and YMB media without yeast inoculum. It was discovered that when mixing the components of the YMG and YMB media, which were peptone, yeast extract and for YMG 0.1% of glucose, together with the MB, there was a reaction that when completing the kinetic time, 144 hours, the intense blue color of MB changed to light green, see Figure 7, and it was concluded that there was a reaction that produced new compounds without having the yeast in the solution, also spectral sweeps were made and it was verified that there were new compounds on the solution. These results indicated that the yeast was not actually biodegrading MB, but that all compounds in the solution reacted with each other and therefore changed the color of the solution. This result gave us indications that there was only one biosorption process carried out by the yeast to MB in this experiment.

It is well known that the carbon and nitrogen source (like glucose, peptone, and others) are the essential nutrient required by microorganisms for their growth and metabolism, a statistical analysis was performed by Saurabh Mishra et al (2018), where in a two-level factor analysis, it is considered that when supplementing glucose and peptone together, the percentage of discoloration decreases , indicating the negative influence of peptone in the discoloration process, and they no longer used it in the medium to be supplemented with microorganisms [50]. Further study is suggested for the understanding of the products that can create the reactions that contain Methylene with peptone and yeast extract, these are still unclear because they are complex substrates without exact composition, hydrolyzed and have more compounds than just protein, and their interaction is not reported yet.

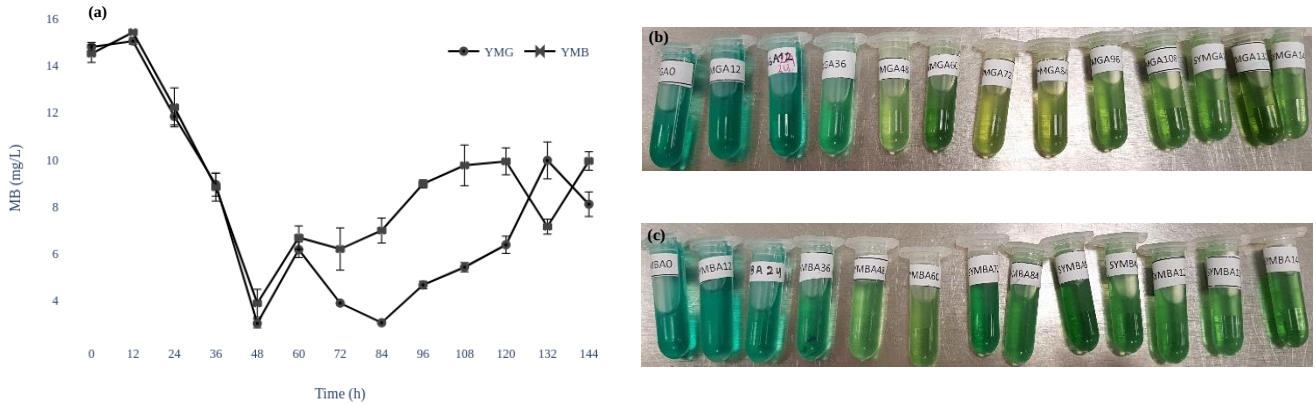

Figure 7. (a)Variation in MB concentration over time. (b) Samples of the kinetics of YMG medium with MB, without added yeast. (c) Samples of the kinetics of YMB medium with MB, without added yeast.

The concentration of yeast as well as the time of operation are crucial factors since they diminish the pH by means of the possible production of acid reagents [51]. The impact to the pH with the possible alkalizing compounds, that were created by a suggested reaction between the peptone and the yeast extract with the MB, was plotted in Figure 8 (b,c) during a test period of 144 hours. Significant differences were demonstrated between the YPD medium and the media containing MB. A qualitative analysis of the odor of the cultures was carried out in the course of the kinetics, at first the solutions had a characteristic odor of the yeast and the components of the medium, but as the hours progressed it became a strong bad smell, this could be because available oxygen is consumed and the pH reaches a plateau, since the organism changes to anaerobic respiration [52].Therefore, derived from the energy metabolism of *S. cerevisiae*, only one third of carbon dioxide is synthesized during anaerobic fermentation. In addition, due to the decrease in the pH of the surrounding medium, the production of organic acid of *S. cerevisiae* is inhibited [53]. A notable indication of the anaerobic pathway is the alcoholic odor of the dough samples with a progressive duration of fermentation [51]. The cellular operation is maintained by the constant pumping of protons from the cell; the concentration gradient of hydrogen ions together with the electrical potential of the cell membrane, which determine the motor force of protons, is influenced by pH variations, affecting its composition and nature after the dissociation of acids and bases, also affecting final products of anaerobic metabolism [54].The presence of organic acids increases the proton flux of the dissociated acids, this implies modifications of the general behavior, in case of the decrease of the glucose yield due to the high concentrations of acids [55]. There is a dependence of the growth rate with the pH, since the functioning of the different intracellular and extracellular components are influenced by the pH values. Based on this it has been shown that yeasts prefer a slightly more acidic environment between 4.0 and 5.0 [56], this is represented in the Figure 8 by the YPD pH which remains with an average of 5.67. For the YMG and YMB medium the pH values were higher than YPD with an average of 8.41 and 8.55, respectively.

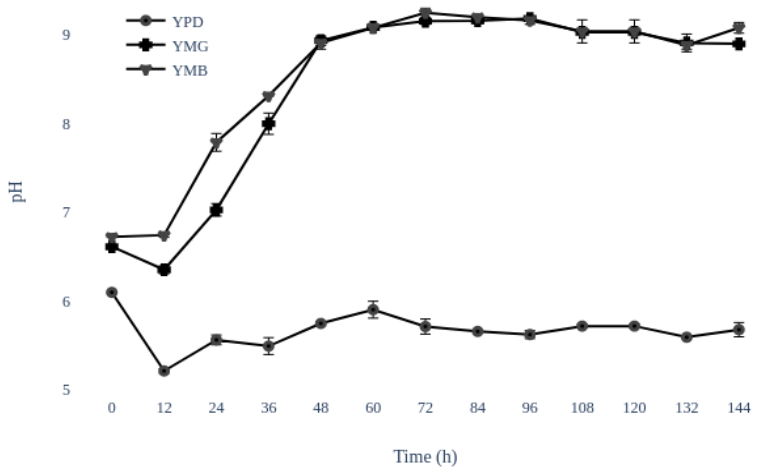

Figure 8. pH of media YPD, YMG, YMB in the growth kinetic.

For the 15 MB and 20 MB medium, the results coincided with the biomass growth kinetics. In Figure 9, the little or no growth that this had when trying to take MB as the main source of carbon can be detected. Note that the units are actually small and the changes it has are irrelevant for the dry weight.

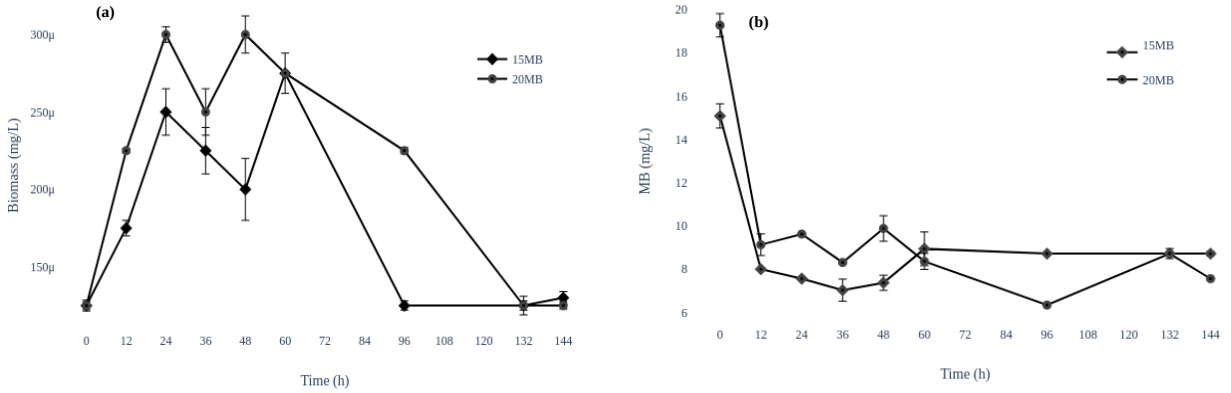

Figure 9. (a) Growth kinetic of the yeast with 15MB and 20MB as media. (b) Variation in MB concentration over time with yeast.

The yeast was not able to carry out the biodegradation process of the dye due to the complexity of the structure of the MB producing difficulty in taking carbon as a nutrient and for this reason there was no growth in biomass in kinetics. The inoculum of yeast that were added to the medium carried out a biosorption process and then stabilized until there was a minimum remaining average of 8.14 mg/L of MB in the solution, as seen in Figure 9. The pH of the solution was stable throughout the kinetics always in the range of 5.0 to 5.9.

4. Conclusions

The cultivated dried yeast *S. cerevisiae* could be used as biosorbent for reactive dyes, like MB, from an acuose medium. The yeast was not successful when taking carbon as nutrient of the MB and there was no biodegradation of the dye. The suitable conditions for the elimination of the dye by means of the biosorption process concluded in this project were yeast cultures of particle size between 300 microns and 75 microns to have a greater surface area and facilitate contact with the dye, the pH with higher response of the yeast for the biosorption capacity were in the range of 2.5-3.5, and it must be taken into account that the percentage of color removal varies by concentration of the yeast, the pH and the initial concentration of the dye. The results in this work suggest that *S. cerevisiae* is possibly an efficient biomass for the treatment of textile wastewater due to its considerable percentage of removal of MB (83.50%) in 24 h, also once the biomass is saturated with the dye , it can be easily regenerated, is inexpensive, and readily available source, given the results of the desorption process with methanol as solvent, obtaining a desorption percentage of 65.47% in 120 min. The biosorption process could be a viable methodology in terms of cost and presents flexibility, simple design and ease of operation in textile water treatment systems.

5. References

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