J. Mater. Environ. Sci. 5 (2) (2014) 633-640
ISSN : 2028-2508
CODEN: JMESCN

Biosorption of textile dyes Basic Yellow 2 (BY2) and Basic Green 4 (BG4) by the live yeast *Saccharomyces Cerevisiae*

Hodabalo Kelewou[1]*, Mohammed Merzouki[1], Abdelhadi Lhassani[2]
[1] *Laboratory of Biotechnology, Faculty of Science Dhar El Mahraz, PO Box 1796 Atlas Fez, Morocco.*
[2] *Laboratory of Applied Chemistry, Faculty of Science and Technology of Fez, PO Box: 2202 Fes Saiss Morocco.*

Received 11 Oct 2013, Revised 21 Nov 2013, Accepted 21 Nev 2013
** Corresponding author. E mail: keith2fr@yahoo.fr ; +212 664 64 89 80*

Abstract
Removal of two basics dyes namely Basic Green 4 and Basic Yellow 2 by the yeast *Saccharomyces cerevisiae* has been studied in a culture medium prepared from sucrose. The experiments were carried out to determine discoloration. Parameters such as pH, initial dye concentration, the biomass amount, the temperature effect on dye removal were studied. The study showed that the discoloration is best for an acid pH (5), low initial dye concentration and a temperature of 30°C respectively 96% for the Basic Green 4 (BG4) and 93% for the Basic Yellow 2 (BY2). However, the yeast rate to obtain a better removal dye is 1.5%. Finally, a study on the discoloration kinetics allowed us to conclude that the discoloration of Basic Green 4 and Basic Yellow 2 follows a first order kinetic model. We observed that the processes taking place during the two dyes discoloration is called bioaccumulation.

Keywords: Bioaccumulation, biosorption, textile dye, Saccharomyces Cerevisiae, kinetic

Introduction
The textile industry is one of the largest polluters in the world with the discharge of waste dyes and auxiliary products to receptors such as groundwater, rivers environments. They can also be toxic to some aquatic life due to the presence of metals, chlorides etc… in these effluents [1]. Existing physico- chemical methods for the removal of dye effluents suffer these strict constraints such as low efficiency and high operation cost [2]. Innovative technologies, such as bioremediation are needed while alternatives to conventional methods to find inexpensive ways of removing color for large volumes of effluents continue [3]. For the past two decades, considerable work has been done in order to use microorganisms as bioremediation agents in the dye wastewater treatment. Microorganisms are effective bioremediateurs for removing dyes via biodegradation or bioaccumulation mechanisms. The dye removal by cellular mechanisms either independently culture metabolism and temperature or is dependent metabolism is defined as the bioaccumulation [4-6]. In the bioaccumulation process, there is a first step in a rapid buildup of contaminants on the cell surface and which is independent of temperature and metabolism. A second process that depends on metabolism and which, allows the accumulation of a large amount within the cells follows this step. The bioaccumulation can take place for the removal of different kinds of dyes for textiles if their growth, the cells are sufficient sources of carbon and nitrogen assimilable easily instead of dyes in the growth medium. The use of cell culture provides the advantage over dead cells or resting because fading and cell growth takes place in order to avoid simultaneous and cell output separately before treatment. However, the main limitations to the use of systems for cellular growth bioaccumulation dyes that are nutrient media, which are required for cell growth is prevented when the dye concentration is too high. Moreove, other components or constraints wastewater can also be toxic to living cells , such as extreme pH and high salt concentration. If the problem of dye toxicity for cell cultures is overcome by employing dyes resistant microorganisms, the system auto-replenishment can operate continuously for extended periods [7-10].
Yeast has long been known to be capable of the fast metal ions bioaccumulation of solution , but very little work has been carried out on the ability of yeast to act as textile dyes bioaccumulative wastewater [10 -12] . The yeasts have many advantages compared to bacteria and filamentous fungi. Yeasts are inexpensive and readily available source. Yeasts can adapt and grow in various extreme conditions of pH, temperature and availability of nutrients as well as high concentrations of pollutant. They develop rapidly as bacteria; as the filamentous fungus, they also have the ability to withstand adverse environment [13].

In our study, we worked with live yeast *Saccharomyces cerevisiae* with nutriment, which is none other than sucrose. The first part of our work was to determine if dye Basic Yellow 2 (BY2) and Basic Green 4 (BG4) could inhibit the growth of yeast.
Subsequently we studied the pH, the amount of biomass (yeast), the initial dye concentration and temperature effect on dyes removal. Finally, a study of discoloration kinetics was conducting to determine which model best explained the phenomenon at stake in this type of process.

2. Materials and methods

2.1. Yeast preparation

Yeast that was used in our study is *Saccharomyces cerevisiae*. Lesaffre Company specializing in the manufacture of baker's yeast produces the strain used. The yeast was purchased commercially as pressed pulp 1€ per kilogram, which offers a relatively low cost for integration into the effluent treatment on an industrial scale. This yeast has the advantage of easily mixing in solution and provides a high surface area. For its use, masses ranging from 1-6 g of pulp were mixed with 200 ml of the solution to be treated. The yeast was preserved at the refrigerator at a temperature of 4°C.

2.2. Preparation of the solutions

Two textile dyes, Basic green 4(BG4) and Basic Yellow 2(BY2) were used in this study. Stock solutions of two dyes were made by dissolving 1 g of each dye in 1000 mL of distilled water. By diluting the standard solutions of two dyes, various concentrations (20–100 mg/L) were made. The pH was adjusted using NaOH (1M) and HCl (1M) supplied by Aldrich. The culture medium used to examine the germicidal effect of the dyes consisted of a meat broth and glucose (at 20 g / l); the whole was sterilized before use. Thereafter the sucrose was used as carbon source.

2.3. Experimental procedure

The germicidal effect of the dyes was checked by observing the yeast growth in a medium containing dyes and a nutritional source consisting of glucose and meat broth. Thus in three 500ml Erlenmeyer, 1 g of yeast was contacted with 200ml of the culture medium; which was added 8 mg of each dye respectively in the first and second Erlenmeyer. The third acts in order to compare the dyes effect. The whole was stirred at 120 rpm and each time a sample is taken to measure the amount of yeast in solution. The yeast growth rate was calculated from the formula (1).

$$\boldsymbol{yeast\ growth}(\%) = \frac{\boldsymbol{A_h - A_0}}{\boldsymbol{A_o}} \times \mathbf{100} \qquad (1)$$

Where A_h: absorbance at time "h"; A_0 the absorbance of the solution at the beginning of culture
Regarding bleaching experiments, an amount of 1- 6g yeast was brought in contact with solutions of dyes BG4 and BY2 whose concentration varies from 20-100 mg / L in an Erlenmeyer flask. In all experiments discoloration, 2g sucrose was added as a nutrient source, and the mixture was stirred at 120rpm for 4 hours followed by 24 hours of settling. The supernatant is assayed for determine the residual dye amount.
The Spectrophotometry UV (Hach 2000) was used for the analytic monitoring. We observe that the wavelength of maximum absorbance (λ_{max}) for BY2 is 421nm and 620nm for BG4.
Spectrophotometry UV was used for analytic monitoring of the growth of yeast. The maximum wavelength for the solution of yeast, mix yeast+ BY2 and yeast + BG4 is respectively 560nm, 562nm and 620nm.
The abatement rate D (%) of the dyes is calculated by formula (2):

$$\boldsymbol{D}\ (\%) = \frac{\boldsymbol{C_i - C_f}}{\boldsymbol{C_i}} \times \mathbf{100} \qquad (2)$$

Where C_i and C_f are respectively the initial and final dye concentrations in solution and are expressing in mg/l.

3. Results and discussion

3.1. Dye effect on the yeast growth

To determine the dye effect on the yeast growth, we removed each time 1 ml of yeast culture solutions to measure its absorbance. Studies have shown that the optical density of a solution changes linearly with the amount of the microorganism in solution [14]. The results obtained for 24 hours have allowed us to trace the evolution of the yeast growth as a function of culture time Fig1.
We note that there is 2 hours of standby time followed by 3 hours of rapid growth, more growth is slower. We can say that the yeast grow by continued for 24 hours, this means that the nutrient source used was not exhausted during the incubation time.
We can conclude that the presence of BY2 and BG4 does not inhibit the yeast growth. The *Saccharomyces cerevisiae* Growth in the BY2 presence is almost identical to its absence, while the BG4 presence reduces yeast growth. We note that when BG4 is added to the solution, growth reached 50% for 24 hours, whereas yeast only believes in more than 300%.

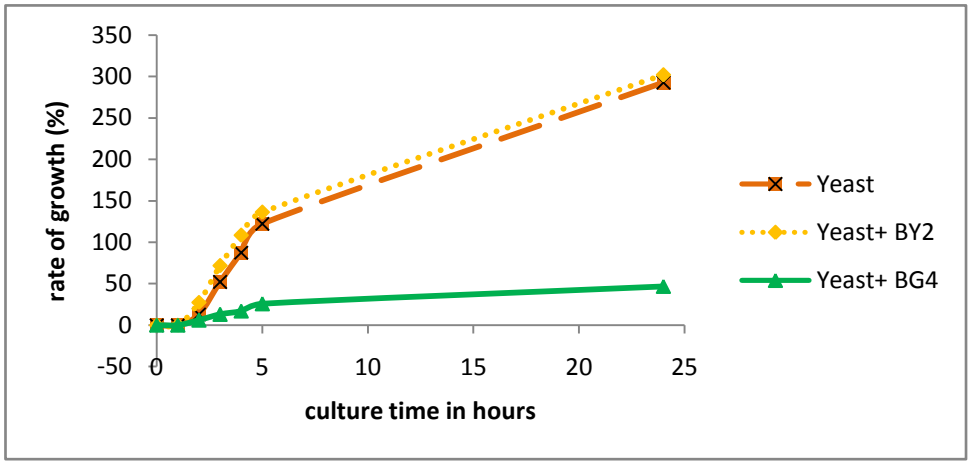

Figure 1: Growth rate of *Saccharomyces cerevisiae* according to the culture time. (Conditions: pH = 8, T = 20 ° C, stirring rate = 120 rpm)

3.2. Effect of stirring time

This test realized to determine the time that is required to have an optimal abatement rate of the dyes by the yeast *Saccharomyces Cerevisiae*.

After stirring for 1 hour, a rate reduction of approximately 50% was observing for the dye and even BY2 and BG4. The abatement rate for the two dyes increases so significantly with stirring time up to 4 hours. Beyond 4 hours, we see a stabilization of the reduction rate (Fig.2).

The optimal abatement rate for the two dyes can be obtaining after 4 hours of incubation. The stirring time has an influence on the bioaccumulation of dyes by *Saccharomyces cerevisiae* as stated by [15-17].

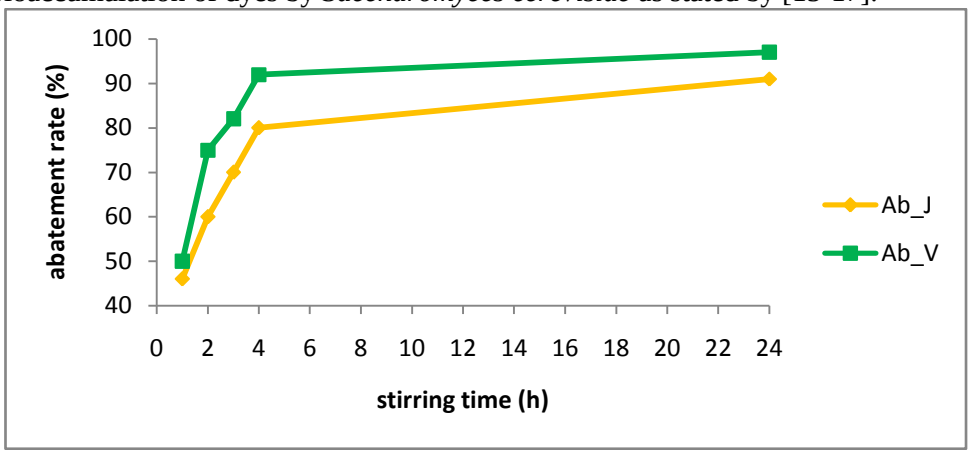

Figure 2: effect of stirring time on BG4 and BY2 bioaccumulation by the yeast *S. cerevisiae*. (Conditions: C_0= 20 mg / l; T = 20 ° C, pH = 8; Stirring rate: 120rpm); Ab_J: abatement rate of BY2 and Ab_V is the abatement rate of BG4

3.3. Influence yeast amount

The experimental results of the change in the yeast concentration are shown in Figure 3. The abatement rate of the two dyes BY2 and BG4 increases significantly between yeast rate 0.5 % and 1.5%; it passes from 87% to 95% for the BG4 while for the BY2, it is from 76% to 91%.

However, from a yeast rate of 2%, the abatement rate of dyes drops significantly. The decrease in abatement rate from 2% is probably due to low activity of yeast caused by an insufficient amount of nutrient. In addition, the optimal yeast rate for maximum abatement rate is 1.5%, which is what is confirming by studies [17]. In the continuation of this work, the yeast rate will be fixing at 1.5%.

3.4. Effect of initial dye concentration on the dye reduction

The influence of initial dye concentration on their disposal by the *Saccharomyces cerevisiae* is shown in Figure 4. We note that in terms of abatement rate, when the dye concentration in the solution increases, the removal rate also increases significantly. As against, by observing the residual dye amount in the treated water (symbolized here by absorbance), increasing the dye concentration results in lower down the elimination of dyes as observed

by several studies [17]. The reduction rate is 86 % to 95 % for the BG4 and 81 % to 91 % for the BY2 when changing the concentration of 20-100 mg / L. At the same time, the absorbance of the solutions treated evolves from 0.03 to 0.06 for the BG4 and 0.12-0.15 for BY2. This decrease is probably due to saturation of the active surface of the cell membrane sites, but also to inhibition cell growth caused by the increase of the concentration of dye. We know that during the bioaccumulation metabolism plays a major role in the absorption of pollutants within the cell.

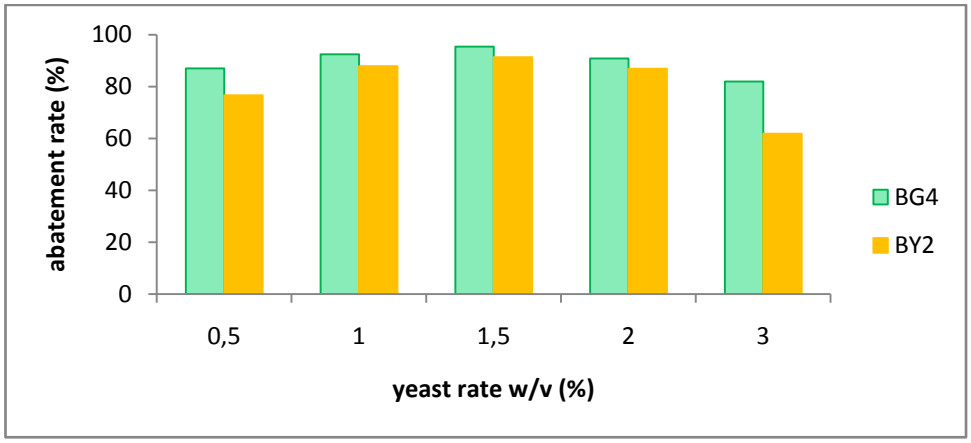

Figure 3: Effect the load of the yeast on dye removal. (Conditions: C_0= 40 mg / l; temperature = 20 ° C, pH = 8; Stirring time= 4 hours; Stirring rate=120 rpm).

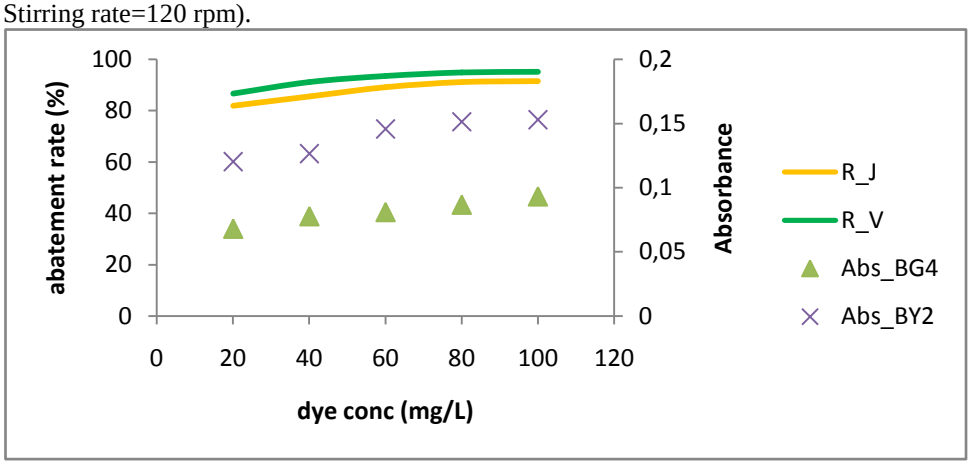

Figure 4: effect of the initial dye concentration on dye removal by the yeast *S. cerevisiae*. (Conditions: C_0: 20 - 100 mg / l; biosorbent load=1.5 %; pH = 8, temperature=20 ° C; stirring rate= 120 rpm; stirring time= 4hours); R_J: abatement rate of BY2 and R_V is the abatement rate BG4.

3.5. Effect of temperature

The experiences were carried out at three various temperatures with knowing 20, 30 and 40°C. The temperature has a significant influence on the dyes removal (Fig 5). The abatement rate is better for the two dyes when the temperature is of 30°C; on the other hand, it drops significantly beyond the temperature 40°C. However, when the temperature is of (20°C), the abatement rate is weak respectively 81% and 89% for the BY2 and the BG4.The temperature has a significant influence on the yeast activity. The yeast activity is weak with 20°C and 40°C and higher with 30°C. All the studies [17-20] showed that the temperature has an influence on the bioaccumulation. The particularity of our work is that we show the stronger yeast growth with 30°C.

3.6. Effect of pH

The pH solution of dye plays an important role in the biological process. The pH of the solute (dye) is 8. We wanted to investigate the influence of pH's changes on the dye removal. We followed the evolution of removal rates of both dyes BG4 and BY2 by yeast into five pH values (Fig 6). We had noted that the abatement rate was significantly higher for an acid pH for the two dyes that for a basic pH. Our results are in conformity with those obtained by [17, 19 - 24]. However, the explanation of a better elimination in acid medium is due primarily to a strong yeast activity. Thus, there is an optimal growth with acidic pH.

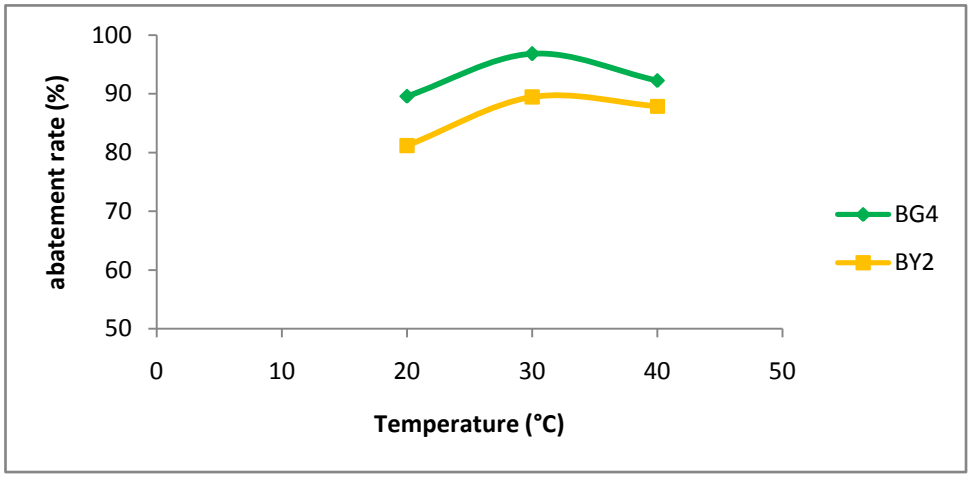

Figure 5: Temperature effect on the bioaccumulation of dyes by the yeast *S. cerevisiae*. (Conditions: C_0=40mg / l; biosorbent load= 1.5%; pH = 8; stirring rate=120 rpm; stirring time= 4hours); Ab_J: abatement rate of dye BY2 and Ab_V is the abatement rate of BG4.

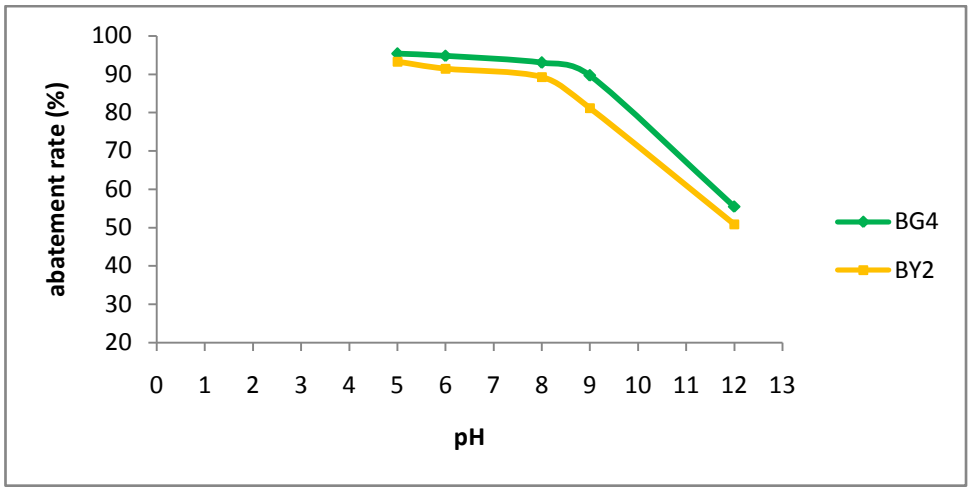

Figure 6: pH effect on dye removal by yeast *S. cerevisiae*. (Conditions: C_0 = 40mg /; l biosorbent charge= 1.5%; stirring rate= 120 rpm; stirring time= 4hours]

To understand the phenomena involved in the basic dyes BY2 and BG4 removal by *Saccharomyces cerevisiae*, we compared the spectra UV of the solutions before and after treatment (Fig 7). We note that there is no appearance of new peak on the spectra after treatment for both dyes. Therefore, the structure of the dye has not been probably destroyed and no new compound appeared during the bleaching process. In addition, an observation to optical microscope (Fig 8) showed a staining of the surface and inside cells. This leads us to conclude that the processes taking place during the discoloration is a biosorption to the cell surface followed by accumulation inside. The phenomenon observed is thus referred bioaccumulation is a two-step process: the first is that the biosorption to the cell surface and the second step is the accumulation within the cells [19, 25]
The biosorption by *Saccharomyces Cerevisiae* is due to the presence of active groups on cell surface such as polysaccharides, lipids, amino acids and other cellular components of the organism [26-28].
To complete our understanding of the accumulation process of BG4 and BY2 within yeast, we conducted additional experiments. Thus, we evaluated the process of discoloration in the absence of a nutrient source (sucrose). We noticed that in the absence of a nutrient source, the BY2 and BG4 discoloration is only 10 %. The presence of a nutrient source for the discoloration rate is 10% to over 90 %. Thus, we can say that bioaccumulation is due to cellular activity (metabolism) in the presence of a nutrient source. Bioaccumulation is better when cellular activity increases.
The stirring rate has an influence on the oxygen and carbon dioxide dissolved in the surrounding solution. The concentration of dissolved oxygen in the culture medium is an important parameter that will guide (depending on the strain used, the driving mode,) the metabolism of the microorganism concerned [29]. Thus, when the stirring time is long, the cellular activity is better and positively influences the dyes removal.

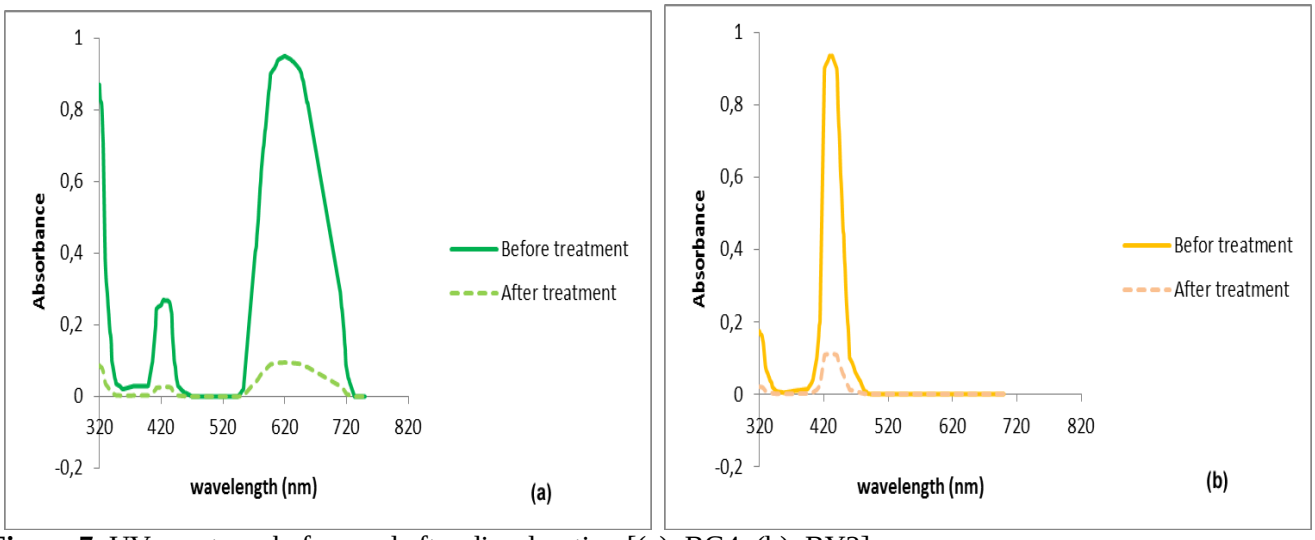

Figure 7: UV spectrum before and after discoloration [(a): BG4; (b): BY2]

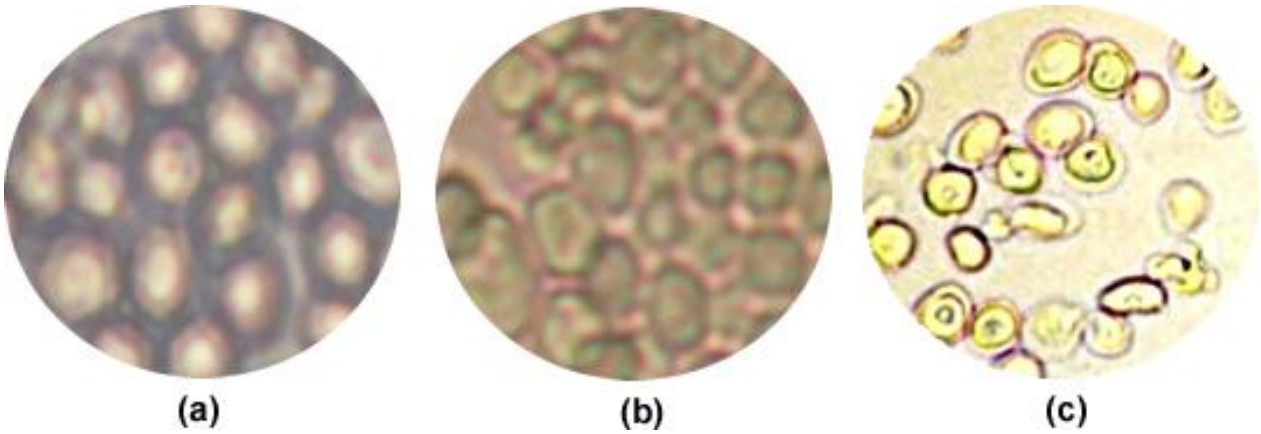

Figure 8: Yeast observation an optical microscope [(a): yeast; (b): yeast after BG4 discoloration; (c): yeast after BY2 discoloration

Accordance with the laws of thermodynamics, temperature influences the biological reactions. However, like any living organism, yeast can only work in a range of "optimal" temperature until a critical temperature beyond which it cannot survive. Several cultures of *Saccharomyces cerevisiae CBS8066* in fed-batch mode, under conditions of controlled temperature ranging from 27 ° C to 39 ° C [29]. The maximum growth rate was obtained at a temperature of 30 ° C. The best growth of temperature 30 ° C can explain the higher abatement rates for BY2 and BG4 dye at 30 ° C during testing compared to 20 ° C and 40 ° C.

The pH range reported in the literature to maintain growth of yeast *Saccharomyces cerevisiae* are between 2.4 and 8.6, with an optimum pH between 4.5 and 4 (Jones et al 1981). In addition, the initial pH of the solution has a real influence on the bioaccumulation of dyes. The pH of the solution changes the surface charge of the powder and promotes the adsorption of certain ions compared to other [30]. Beyond pH = 9, the *Saccharomyces cerevisiae* activity of is low, hence a very significant decrease in the dye removal rate at a pH above 9.

Dye concentration and salinity play an important role in the growth and viability of yeast. It is often represented by the osmotic pressure. In the field applied to biology, the osmolarity is the concentration of any solute in an aqueous solution, often expressed in osmol (or milliosmol) this corresponds to the mole number of particles per liter of solution [31] . Osmotic pressure is the minimum pressure necessary to prevent the passage of a solvent of a less concentrated to a more concentrated through a rigid semi- permeable cell membrane (permeable to water but not to ions) solution. The availability of water is one of the most important factors that affect the microorganism growth and survival. A variation of osmotic pressure causes a movement of the water towards the osmotic gradient, causing a swelling or bursting (in hypotonic or hypo-osmotic media) or plasmolysis (in the hyper-osmotic environments).

4. Discoloration kinetic

To determine the discoloration kinetic of the two basic dyes, solutions containing 50mg / L of initial dye concentration and 10 g / L of substrate was contacted with 1.75 g/L of yeast rate at a temperature of 30 ° C. Residual concentrations were measured at various incubation times namely 1 to 4 hours.
To determine the kinetics of discoloration that best meets the bioaccumulation of Basic Green 4 and Basic Yellow 2, several models of different order (zero, first and second order) [19, 24] were tested using the following equations:

- Order zero:
  $-\frac{dc}{dt} = K_0$, By integrating this equation with the boundary conditions gives: $C_t = C_0 - K_0 t$
- First order :
  $-\frac{dc}{dt} = K_1 c$, Integration with the boundary conditions gives: $C_t = C_0 e^{-K_1 t}$
- Second order :
  $-\frac{dc}{dt} = K_2 c^2$, integration with the boundary conditions in this case provides: $\frac{1}{C_t} = \frac{1}{C_0} + K_2 t$

The boundary conditions for the three equations are $C = C_0$ at $t = 0$ and $C = C_t$ for all "t" (t = time).
The constants K_0, K_1 and K_2 are respectively determined by plotting Ct versus time; ln (Ct) as a function of t (Fig 9) and ($1/C_t$) versus t. Constants K_0, K_1 and K_2 are shown in Table 1. From the data plotted curves, the kinetic constants obtained with a high coefficient of regression (R^2> 97%) were accepted and correspond to the models that best explain the process of Basic Green 4 and Basic yellow 2 bioaccumulation by *Saccharomyces cerevisiae*. We note that only the coefficient K1 was determined with a correlation coefficient greater than 97%. Thus, we can conclude that the bioaccumulation kinetics of the two dyes BY2 and BG4 is the first order; this is consistent with the results obtained by [19].

Table 1: Kinetic constant for zero-order models, one and two for Basic Green 4 and Basic Yellow 2 discoloration

Kinetic	Kinetic constant		Correlation coefficient (R^2)	
	BG4	BY2	BG4	BY2
Order zero	K_0=8,64	K_0=7,36	0,858	0,866
First order	K_1=0,607	K_1=0,38	0,987	0,977
Second order	K_2=0,066	K_2= 0,023	0,854	0,963

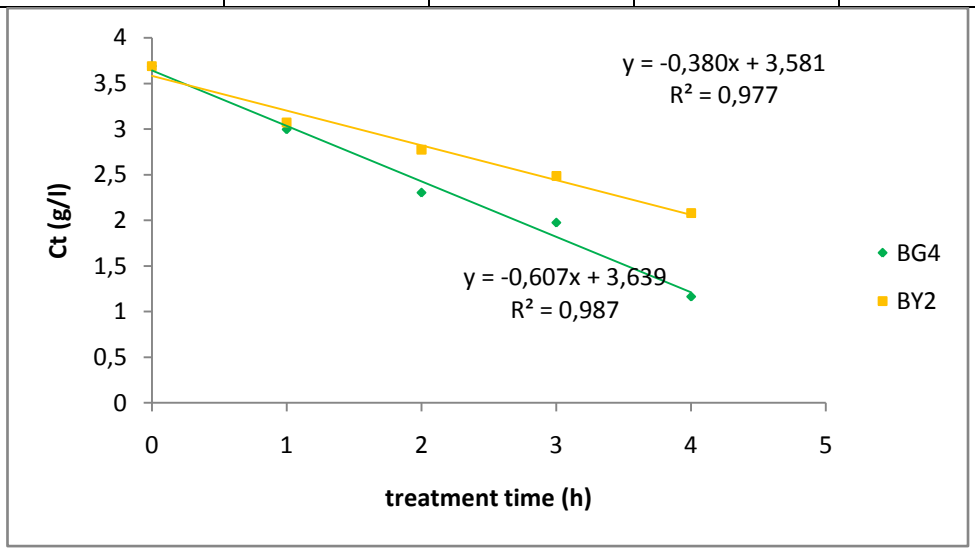

Figure 9: BG4 and BY2 Kinetic of discoloration *by Saccharomyces cerevisiae*

Conclusion

The results indicate that *Saccharomyces cerevisiae* is able to discolor very convincingly dye Basic Green 4 and Basic Yellow 2. Parameters such as the pH of the solution, yeast rate, temperature, and the initial dye concentration have a significant influence on the discoloration process. In optimum conditions, discoloration BG4 solution by *Saccharomyces*

cerevisiae can be reach 96 % while that of Basic Yellow 2 can approaches 93%. Microscopic observations and spectrophotometric analysis allowed us to infer that the mechanism taking place during discoloration is a bisorption process to the cell surface followed by accumulation inside, so bioaccumulation process. In conclusion, we can say that the kinetics of bioaccumulation of Basic Green and Basic Yellow by *Saccharomyces cerevisiae* following a first-order model, the residual dye concentration evolves in inverse exponential function of time.

Acknowledgements-We thank all those who contributed directly or indirectly in the development of this work in particular the textile factory MultiWash (factory of dyeing in Fès).

References

1. Clarke E.A., Anliker R, Hand-book of Environmental Chemistry. Part A: Anthropogenic Compounds, Springer, (1980) 181.
2. Khehra M.S., Saini H.S, Sharma D.K., Chadha B.S., Chimni S.S., *Water Res*. 39 (2005) 5135.
3. Parshetti G.K., Kalme S.D., Gomare S.S., Govindwar S.P., *Bioresource Technol.* 98 (2007) 3638.
4. Preetha B., Viruthagiri T., *Biochem. Eng. J*. 34 (2007) 131.
5. Gikas P., Romanos P., *J. Hazard. Mater*. 133 (2006) 212.
6. Sadettin S., Dönmez G., *Process Biochem*. 41 (2006) 836.
7. Renganathan S., Thilagaraj W.R., Miranda L.R., Gautam P., Velan M., *Bioresource Technol*. 97 (2006) 2189–2193.
8. Aksu Z., Donmez G., *Process Biochem*. 40 (2005) 2443.
9. Dursun A.Y., Uslu G., Cuci Y., Aksu Z., *Process Biochem*. 38 (2003) 1647.
10. Malik A., *Environ. Int*. 30 (2004) 261.
11. Donmez G., Aksu Z., *Process Biochem*. 35 (1991) 35.
12. Skountzou P., Soupioni M., Bekatorou A., Kanellaki M., Koutinas A.A., Marchant R., *Process Biochem*. 38 (2008) 1479.
13. Pajot H.F., de Figueroa L.I.C., Farina J.I., *Enzyme Microb. Technol*. 40 (2007) 1503.
14. Taniguchi L., De Fátima Faria B., Rosa R. T., *J. Microbiol. Meth*. 78 (2009) 171.
15. Liu C., Yuan H. L., Yang J. S., Li B. Z., *Afr. J. Biotechnol*. 73 (2011) 16626.
16. Wang B. E., Hu Y.Y., Xie L., Peng K., *Bioresource Technol.* 99 (2008) 794
17. Kumari K., Abraham T. E., *Bioresource Technol.* 98 (2007) 1704.
18. Ncibi M. C., Mahjoub B., Seffen M., *J Toilets Sci.* 4 (2008) 441.
19. Anjaneya O., Santoshkumar M., Nayak Anand S., Karegoudar T.B., *Internat. Biodeter. & Biodegr.* 63 (2009) 782–787.
20. Aksu Z., *Process Biochem*. 40 (2005) 997.
21. Akar T., Celik S., Akar S. T., *Chem. Eng. J*. 160 (2010) 466.
22. Janos P. Sedivy Mr. Grotschelova S., *Chemosphere.* 59 (2005) 881.
23. Abdallah R., Taha S., *Chem. Eng. J*. 195–196 (2012) 69–76
24. Aksu Z., Karabayır G., *Bioresource Technol*. 99 (2008) 7730-7741.
25. Aksu Z., *Process Biochem*. 38 (2003) 1437-1444.
26. Suarit R., Gopal P.K. & Sherped Mr. G., *J. Genet. Microbiol*. 134 (1988) 2359-2368.
27. Tan CY., Li G., Lu XQ., Chen ZL., *Ecol. Eng.* 36 (2010) 1333–1340.
28. Ashkenazy R., Gottileb L., *Biotechnol. Bioeng*. 55 (1997) 1-10.
29. Brady D., Stoll A., Duncan J.R., *Environ. Technol*. 15 (1994) 429-435.
26. Fu Y.,Viraraghavan T., *Bioresource Technol.* 82 (2002)139-145.
27. Volesky B., May P., *Appl. Microbiol. and Biotech*. 42 (1995) 797-806.
28. Sari A., Tuzen M., Citak D., Soylak M., *J. Hazard. Mater.* 149 (2007) 283.
29. Aguilera J., Van Dijken JP., De Winde J. H., Pronk J.T., *J. Biochem*. 391(2005) 311-316.
30. Devlina D., Charumathi D., Nilanjana D., *J. Hazard. Mater*. 183 (2010) 497–505.
31. Yokozeki A., *Applied Energy* 83 (2006) 15-41.