

Optimization of environmental parameters on the decolorization of Reactive Brilliant Blue dye by yeast isolates from textile effluent

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Abstract

The present study was conducted to investigate the decolorization and degradation of Reactive Brilliant Blue dye using yeasts isolated from the effluent treatment of the textile industries and the identified yeast strains under optimal conditions using the standard methods. Among twenty-four yeast strains, only five yeasts have the ability to decolorize the dye (2.8%). Three yeast strains; *Rhodotorula glutinis*, *Candida utilis(1)* and *Candida sphaerica* as well as the local two yeast isolates which were identified as *Rhodotorula rubra* and *Cryptococcus albidus* showing high decolorization rate, they were used for the decolorization of Reactive Brilliant Blue dye in a medium containing glucose and yeast extract as a best carbon and nitrogen sources, the pH of medium varied among the yeasts, *C. utilis(1)*, *R. rubra* and *C. albidus* was 4, while *C. sphaerica* and *R. glutinis* was 6 and 5.5, respectively. All yeast strains were incubated for 18 h at 25°C except *C. utilis(1)* at 37°C. *C. utilis(1)*, *R. glutinis* and *C. sphaerica* showed high decolorization rate under static aerobic conditions. While *R. rubra* and *C. albidus* showed decolorization under static anaerobic conditions. According to the potentiality of yeast strains; *C. sphaerica* could achieved a removal ratio of 68.83%, while *C. albidus* 68.40%, *R. rubra* 67.75%, *R. glutinis* 66.88% and *C. utilis(1)* 63.85% of Reactive Brilliant Blue dye in a concentration of 10 mg/L. The highest biodegradation of the dye by the five yeast strains was confirmed by using plain distilled water as a decolorization medium. In conclusion, yeast strains could be used for the biodegradation of dye- polluted waters including rate of degradation of anthraquinone dye.

Key words: Yeast- Reactive Brilliant Blue dye- Decolorization- Optimization

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1.Introduction

Textile dyes are engineered to be resistant to environmental conditions. During recent years the treatment of textile dye effluents has been the focus of significant research because of the potentially low cost of the process (**Pajot *et al.*, 2011**). Many synthetic dyes belong to xenobiotic chemicals that are degraded with difficulty in nature, and therefore their removal from aqueous effluents from the textile industry has been receiving considerable environment research attention (**Borchert and Libra, 2001**). Large amount of synthetic structurally varied dyes are being used extensively in textile , paper, printing, leather, paints, plastic, cosmetic, food industries and dye houses due to their ease of production, fastness and color variety as compared to natural dyes(**Cristovao *et al.*, 2009; Waghmode *et al.*, 2011**).The disposal of the effluent from these industries and dye houses into the environment causes change in pH, increase COD(chemical oxygen demand), TOC(total organic carbon) and also affects photosynthetic activity of aquatic life due to reduced light penetration and gas solubility in water bodies which leads to adverse effects on aquatic life(**Aksu and Donmez, 2003; Saratale *et al.*, 2009**. A large number of synthetic dyes with specific chemical groups (azo, acid, base, anthracene, triphenylmethane etc.) are widely used in the textile, cosmetic and pharmaceutical industry(**Pavko, 2011**),in addition, a significant amount of these compounds are discharged into the environment through effluents (**Christiane *et al.*, 2013**). Because color in waste water is highly visible and affects esthetics, water transparency and gas solubility in water bodies and especially because many dyes are made from known carcinogens such as benzidine and other aromatic compounds, waste waters with dye have to be treated (**Dong *et al.*, 2003 and Ayed *et al.*, 2011**). The presence of very low concentration of dyes in effluent is highly visible and undesirable (**Nigam *et al.*, 2000; Robinson *et al.*, 2001**).The excessive discharge of the effluents from the textile industries contains toxic chemicals such as azo dyes and reactive dyes which adversely affect the natural resources, soil fertility and aquatic organisms and disturb the integrity of the ecosystem (**Puvaneswari *et al.*, 2006 and Sudha *et al.*, 2014**). Conventional wastewater treatment system is inefficient because of the recalcitrant nature of dyes (**Waghmode *et al.*, 2011**). Existing physical and chemical technologies are expensive, time consuming and produce a large amount of sludge or cause secondary pollution ((**Telke *et al.*, 2009 and Tamboli *et al.*, 2010**).

Mechanisms of biological decolorization of textile dye depend greatly on the chemical structure of the dye and the microorganisms used (**Pajot *et al.*, 2011**). Numerous studies have indicated that biodegradation by microorganisms is a promising approach for treating dyes

contained in wastes (Fu and Viraraghavan, 2001). Over past decades many microorganisms have been found to be capable of degrading dyes; these include bacteria (Sani and Banerjee, 1999), filamentous fungi (Swamy and Ramsay, 1999; Balan and Monteiro, 2001), yeasts (Martorell *et al.*, 2012), actinomycetes (Zhou and Zimmermann, 1993) and algae (Dilek *et al.*, 1999). Filamentous fungi are well recognized for dye decolorization, while the reports on textile dye decolorization mechanisms of yeasts have been scarce (Pajot *et al.*, 2011). Fungal ligninolytic enzyme system (lignin peroxidase, manganese peroxidase and laccase) may also be involved in the bio-oxidation of dyes (Gold and Alic, 1993). However, the requirement for low pH (Swamy and Ramsay, 1999) for optimum enzyme activity and the long hydraulic retention time for complete decolorization (Swamy and Ramsay, 1999) are major disadvantages in using fungi. In addition, growth of filamentous fungi is slow compared with most single-cell microorganisms and the production of mycelium often makes filamentous fungi poorly adaptable to wastewater treatments (Yu and Wen, 2005). Compared to bacteria and filamentous fungi, yeasts have many advantages. They not only grow rapidly like bacteria but also filamentous fungi have the ability to resist unfavorable environments (Martorell *et al.*, 2012). Besides, yeasts have been found to be very efficient in treating high strength organic wastewaters, such as food, molasses and oil manufacture industrial effluents (Yang *et al.*, 2008 and Martorell *et al.*, 2012).

Only a few reports on the degradation of azo dyes or anthraquinone dyes by yeasts have appeared (Yu and Wen, 2005; Pajot *et al.*, 2011).

In the present study, we have focused our attention on the use of local yeasts isolated from the effluent treatment of the textile industries. These identified yeast strains were used for the bioremediation purpose, especially for the textile effluent treatment containing Reactive Brilliant Blue as an industrial dye under optimum conditions.

2-Materials and methods

2.1. Collection of samples

The samples were collected from the effluent treatment of the textile industries and soil surrounding areas of Atlas and Miser Spain industries located in Shobra El-khiema, where the coloured effluent was used as the source of yeast isolation in the present study. The samples were kept in sterile glass bottles and preserved at 4°C in refrigerator and were tested within 24 h of the collection time.

2.2. Isolation, identification and maintenance of dye degrading yeasts

Pour plate technique was used for the isolation of dye decolorizing yeast on Yeast Extract Peptone Dextrose agar medium (YEPD) (Chen *et al.*, 2002), contains (g/L); glucose, 20; peptone, 20; yeast extract, 10; agar, 20. Well grown yeast colonies were picked and further purified by streaking method. The isolated strains were maintained at 4°C on universal agar slants at pH 4.7 and contains (g/L); glucose, 10; peptone, 5; yeast extract, 3; malt extract, 3 (Suzuki *et al.*, 1989). Identification of the yeast isolates was carried out using morphological and biochemical characteristics (Barnett *et al.*, 2000) and integral system plus. In addition to ten yeast strains, obtained from Microbiological Resources Center (MIRCEN), Ain Shams University, Cairo, Egypt. All yeast strains were tested as dye degrading yeasts.

2.3. Dye stuff

Antraquinone dye (Reactive Brilliant Blue) was obtained from Sigma Aldrich and was employed in this work by dissolving powdered dye stuff in hot ethyl alcohol up to a concentration of 10 mg/L.

2.4. Screening of dye degrading yeast

2.4.1. Inoculum preparation

Yeasts were individually tested for their growth and decolorization ability on agar medium of pH 5- 6 (Yu and Wen, 2005) and contains (g/L): glucose, 10; KH₂PO₄, 1; (NH₄)₂SO₄, 1; MgSO₄·7H₂O, 0.5; yeast extract, 0.2 and supplemented with 10 mg/L of Reactive Brilliant Blue dye and the plates were incubated at 25°C till decolorization zone formation and secondary screening was performed with the same procedure on broth medium.

2.4.2. Dye decolorization experiments

Dye decolorization experiments were carried out in 50 ml Erlenmeyer flasks containing 10 ml of broth decolorization medium inoculated with 1 ml of the yeast suspension (OD at 620 nm = 0.3) which prepared from an old yeast culture of 18 h. The inoculated flasks were incubated under static aerobic conditions for a period of 24 h at 25°C. Uninoculated decolorization medium was also incubated under the same conditions as a control to check abiotic decolorization of the dye. After incubation, the samples were centrifuged for 10 min at 6000g and the decolorization was assessed by measuring the absorbance of the supernatant at 620 nm (Yu and Wen, 2005).

2.4.3. Decolorization assay

Dye decolorization assay was measured in terms of percentage decolorization using UV-spectrophotometer. The percentage was calculated from the following equation, % Decolorization= (Initial OD- Final OD X 100) / Initial OD

Each decolorization value is the mean of three parallel experiments (**Christiane *et al.*, 2013**).

2.5. Optimization of environmental parameters that affect the decolorization process

Various factors were optimized to achieve the highest decolorization rate of Reactive Brilliant Blue using the selected yeasts. All the experiments were conducted in triplicate.

Decolorization experiment was performed for all the following parameters as described before in **2.4.2**

2.5.1. Temperature

Decolorization of Reactive Brilliant Blue by the selected yeasts was studied at different temperatures such as 20, 25, 30, 37, 45 and 55°C. Decolorization medium was used for this purpose containing, 0.1 g/ L of the dye. Decolorization experiment was performed as described before.

2.5.2. Incubation time

The effect of different incubation times 2, 4, 6, 12, 18, 24, 48, 72 and 96 h were carried out on decolorization efficiency of the selected yeasts. Incubation temperature was at 25 °C except one yeast strain at 37°C.

2.5.3. PH value

Effect of different pH values of the medium in ranging from 3 to 6.5 (0.5 intervals) was examined on the decolorization efficiency of the selected yeast strains. The incubation temperature was maintained at 25°C except one yeast strain at 37°C for 18 h. The pH was adjusted by using 0.1N HCL and 0.1N NaOH solutions.

2.5.4. Aerobic and anaerobic conditions

Aerobic and anaerobic conditions during incubation time of the selected strains were used to find the best condition for maximum decolorization. Two groups of flasks containing decolorization medium that was enriched with 0.1 g / L of the dye adjusted at optimum pH for biodegradation. One group of flasks was incubated under static aerobic condition and the second group was incubated under static anaerobic condition at 25°C except one yeast strain at

37°C for 18h. Anaerobic conditions were achieved by adding sterile paraffin oil on the surface of the broth medium.

2.5.5. Inoculum size

Different inoculum concentrations of 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 % (v/ v) were used to find the best inoculum concentration to achieve the maximum decolorization efficiency. The different inoculum concentrations were added to the decolorization medium (10ml) containing 0.1 g/L of Reactive Brilliant Blue dye .The liquid medium was adjusted at optimum pH for biodegradation. The flasks were incubated at 25°C except one yeast strain at 37°C under static aerobic conditions except two yeast strains under anaerobic conditions for 18h.

2.5.6. Dye concentration

Six levels of Reactive Brilliant Blue dye 10, 50, 100, 200, 300 and 500 mg/l were used to find the best concentration for maximum decolorization. Four ml of yeast suspension (OD at₆₂₀= 0.3) were prepared from an old yeast culture of 18 h and were added to 10 ml of decolorization medium. The flasks were adjusted at optimum pH for biodegradation and were incubated at 25°C except one yeast strain at 37°C. The flasks were incubated under static aerobic conditions except two yeast strains were incubated under anaerobic conditions for 18h.

2.5.7. Carbon source

In order to assess the effect of different carbon sources on yeast decolorization, four different carbon sources, namely sucrose, lactose, maltose and starch were used by replacing D- glucose in decolorization medium. Four ml of yeast suspension (OD at₆₂₀= 0.3) prepared from an old selected yeast culture and were added to 10 ml of decolorization medium containing 0.01g/L of Reactive Brilliant Blue dye. The flasks were adjusted at optimum pH for biodegradation and were incubated at 25°C except one yeast strain at 37°C. The flasks were incubated under static aerobic conditions except two yeast strains were incubated under anaerobic conditions for 18h.

2.5.8. Nitrogen source

The effect of five different nitrogen sources yeast extract, peptone, ammonium nitrate, sodium nitrate and Beef extract was examined on the decolorization efficiency of the selected yeast isolates. . Four ml of yeast suspension (OD at₆₂₀= 0.3) were prepared from an old selected yeast culture of 18 h and were added to 10 ml of decolorization medium containing

0.01g/L of Reactive Brilliant Blue dye. The decolorization medium was adjusted at optimum pH for biodegradation. The flasks were incubated at 25°C except one yeast strain at 37°C. All selected yeasts were incubated under static aerobic conditions except two yeast strains under anaerobic conditions for 18h.

2.5.9. Type of medium

Different types of media; decolorization medium, plain distilled water, distilled water with 5% glucose and distilled water with 1% glucose plus 0.1% peptone plus 0.1% yeast extract were used to know the best recommended medium for maximum decolorization. Four ml of yeast suspension (OD at 620= 0.3) were prepared from an old selected yeast culture of 18 h and were added to 10 ml of decolorization medium containing 0.01g/l of Reactive Brilliant Blue dye. The decolorization medium was adjusted at optimum pH for biodegradation. The flasks were incubated at 25°C except one yeast strain at 37°C. The flasks were incubated under static aerobic conditions except two yeast strains were incubated under anaerobic conditions for 18h.

2.6. Statistical analysis

All values and data points presented in this work are the means of at least triplicate determinations of independent assays. Data were analyzed using (Two-way ANOVA) two-way analysis of variance (Field, 2013).

3. Results and Discussion

3.1 Results

3.1.1. Screening of dye degrading yeast

All of the 14 isolates and 10 strains of the yeast were screened for their efficiency to remove anthraquinone. The results indicated that out of the 24 yeasts, 5 yeast isolates (2,8%) showed variable potential to decolorize 10 mg /l Reactive Brilliant Blue in decolorization medium. In addition to the three yeast strains namely; *Candida utilis* 1, *C.sphaerica*, *Rhodotorula glutinis*. Two yeast isolates (6, 7) were isolated from the effluent treatment of the textile industry and were identified as *Rhodotorula rubra* and *Cryptococcus albidus*. These yeast isolates were selected for further experiments in this study, based on their remarkable abilities to remove this dye color (Table 1).

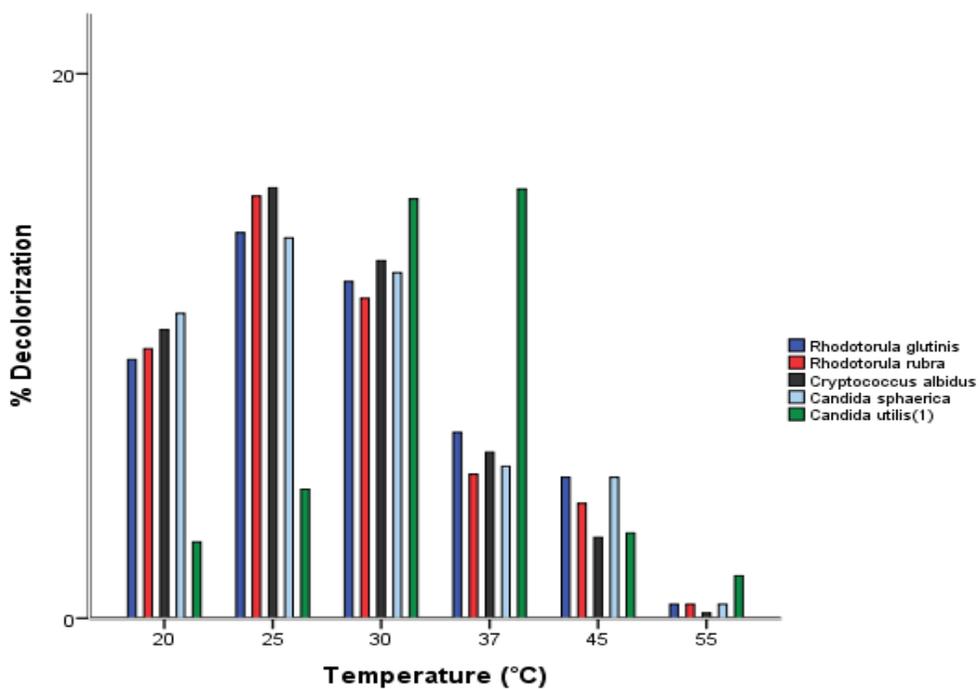
Table 1: Decolorization screening on solid and broth media for twenty four yeasts with decolorization medium mixed with Reactive Brilliant Blue (10 mg/L) grown at 25 °C for 24h.

Tested yeast	Solid medium	Broth medium
	Zone decolorization (mm)	Colour reduction
<i>Candida utilis</i> (1)	30	+ve
<i>Candida utilis</i> (2)	-ve	-ve
<i>Candida utilis</i> (3)	-ve	-ve
<i>Candida utilis</i> (22)	-ve	-ve
<i>Saccharomyces cerevisiae</i> (43)	-ve	-ve
<i>Saccharomyces cerevisiae</i> (44)	-ve	-ve
<i>Rhodotorula glutinis</i>	35	++ve
<i>Candida sphaerica</i>	60	+++ve
<i>Candida famata</i>	-ve	-ve
<i>Cryptococcus albidus</i> (1)	-ve	-ve
Isolate 1	-ve	-ve
Isolate 2	-ve	-ve
Isolate 3	-ve	-ve
Isolate 4	-ve	-ve
Isolate 5	-ve	-ve
Isolate 6	38	++ve
Isolate 7	43	++ve
Isolate 8	-ve	-ve
Isolate 9	-ve	-ve
Isolate 10	-ve	-ve
Isolate 11	-ve	-ve
Isolate 12	-ve	-ve
Isolate 13	-ve	-ve
Isolate 14	-ve	-ve

3.1.2. Environmental parameters that affect the decolorization process

3.1.2.1. Temperature

Incubation temperature in a range from 20 to 55°C was used to find out the optimal temperature for the decolorization of Reactive Brilliant Blue dye using the selected yeast strains in broth medium. It was observed that an increase in the temperature from 20 to 37°C had a significant effect on the decolorization of Reactive Brilliant Blue dye (Fig.1). However, the optimal temperature for decolorization was at 25°C for all the tested yeast strains except for *Candida utilis* (1) was at 37°C.

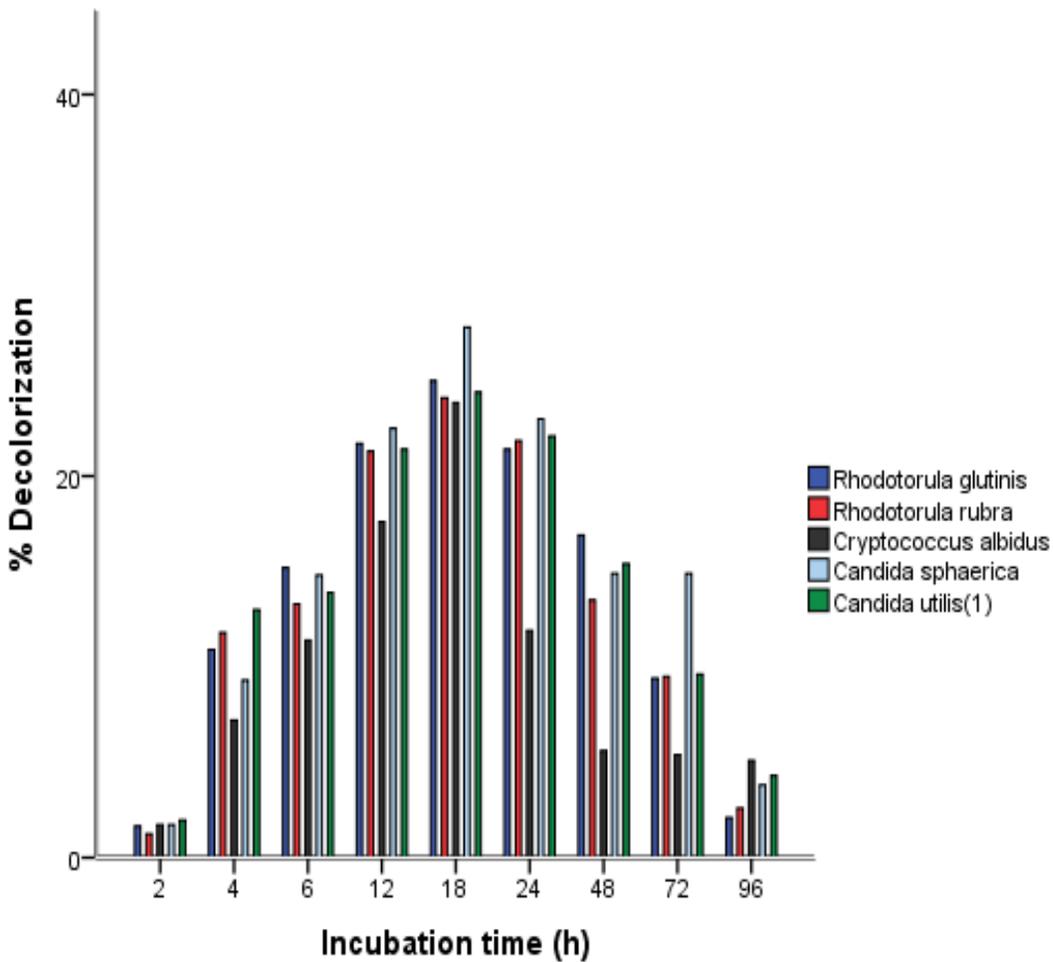


LSD at 5% > 0.002

Fig.(1): Effect of incubation temperature on the decolorization by the selected yeast strains

3.1.2.2. Incubation time

Incubation times in a range from 2 to 96h were used to know the optimal incubation time for the decolorization of Reactive Brilliant Blue dye using the selected yeast strains in broth medium (Fig. 2). It was observed that the increase in incubation time from 2 to 18h had a significant effect on the decolorization rate of the dye. However, the optimal incubation time to decolorize the dye for all tested strains was 18h. The decolorization rate in all yeast strains was dropped sharply as the incubation time increased from 24 to 96h.

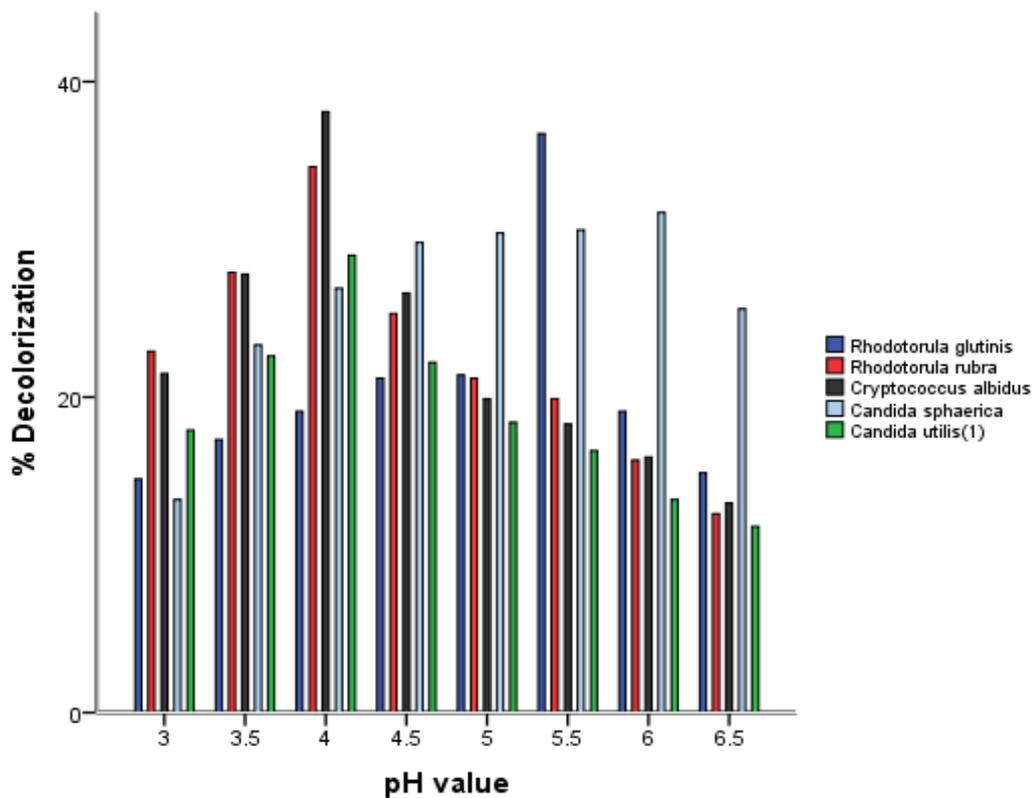


LSD at 5% → 0.006

Fig.(2): Effect of incubation time on the decolorization by the selected yeast strains

3.1.2.3. pH value

PH range from 3 to 6.5 was used to know the optimal pH value for dye degrading yeasts that achieve the highest decolorization of Reactive Brilliant Blue dye. It was observed that the optimum pH of the five selected strains was different (Fig. 3). In case of *Candida sphaerica*, the increase in pH from 3 to 6 had a significant effect on the decolorization of dye and the optimal pH for decolorization was at 6. However, the decolorization rate dropped 6.5. In case of *Cryptococcus albidus*, *Rhodotorula rubra* and *Candida utilis* (1), there was an increase in the rate of decolorization from 3 to 4 and the optimal pH for decolorization was 4. While, in case of *Rhodotorula glutinis*, the optimal pH for decolorization was 5.5.

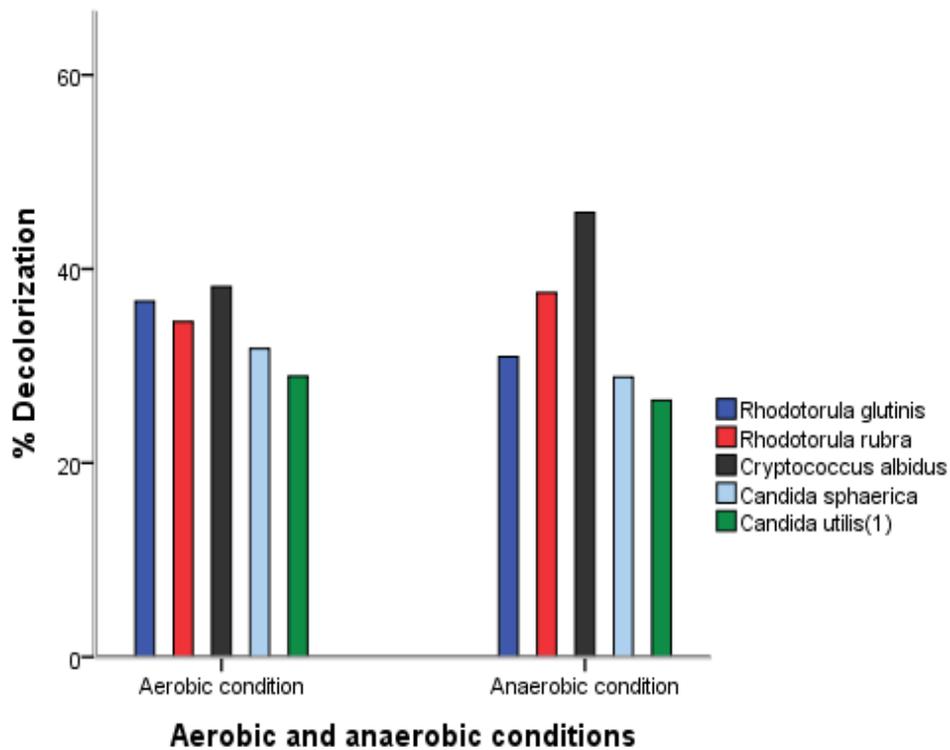


LSD at 5% → 0.01

Fig.(3): Effect of PH value on decolorization rate

3.1.2.4. Aerobic and anaerobic conditions

The decolorization rate of Reactive Brilliant Blue dye was increased significantly when *Candida sphaerica*, *Rhodotorula glutinis* and *Candida utilis*(1) were incubated under aerobic conditions. Whereas, the decolorization rate of *Cryptococcus albidus* and *Rhodotorula rubra* was increased significantly under anaerobic conditions (fig. 4).

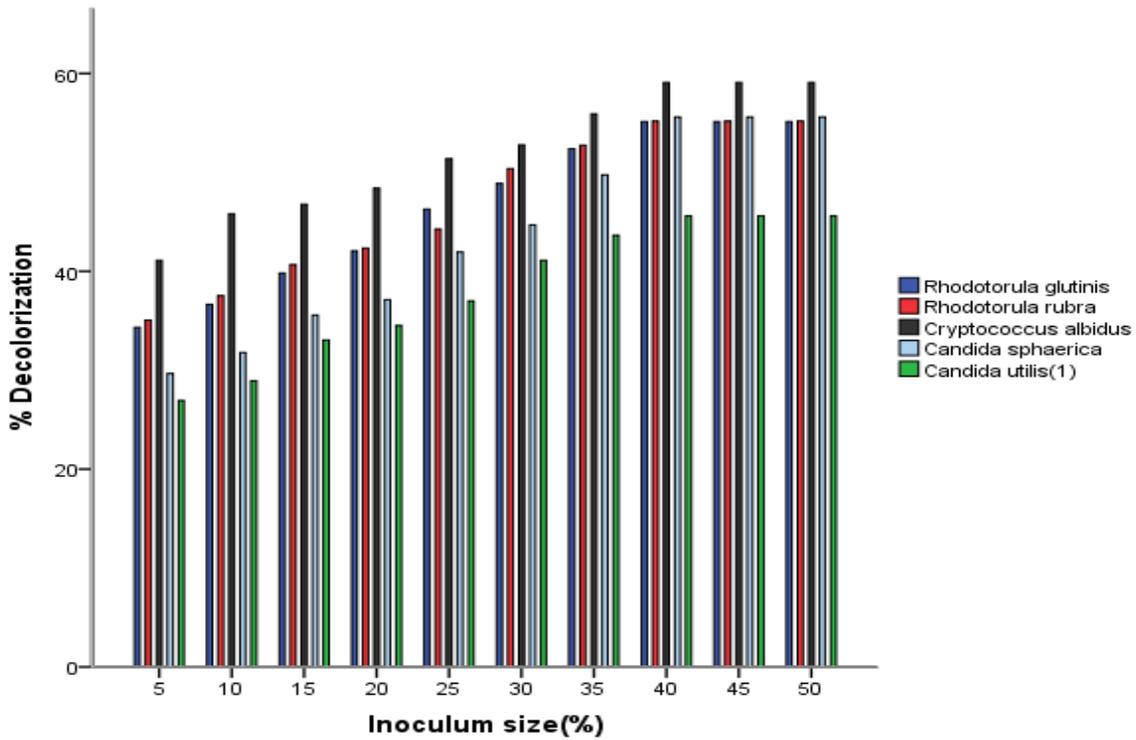


LSD at 5% → 0.205

Fig.(4): Effect of aerobic and anaerobic conditions of incubation on the decolorization by the selected yeast strains

3.1.2.5. Inoculum size

From Fig. (5), shows the increasing of inoculum size from 5 to 40% (v/v) of all selected yeast strains had a significant increase on the decolorization of Reactive Brilliant Blue dye. However, the optimal inoculum size of all yeast strains to decolorize dye was 40%. The inoculum size has significantly affected the decolorization.



LSD at 5% → 0.009

Fig.(5): Effect of inoculum size on the decolorization by the selected yeast strains

3.1.2.6. Dye concentration

The effect of initial concentration of Reactive Brilliant Blue dye on the % decolorization was investigated and the percentage of decolorization decreased with an increase in the initial concentration (fig. 6). Maximum decolorization was observed at a concentration of 10 mg /L for all the selected yeast strains. However, decolorization rate in all yeast strains was dropped sharply from 200 to 300 mg /L but no decolorization was observed at 500 mg/ L. Dye concentration significantly has affected the decolorization.

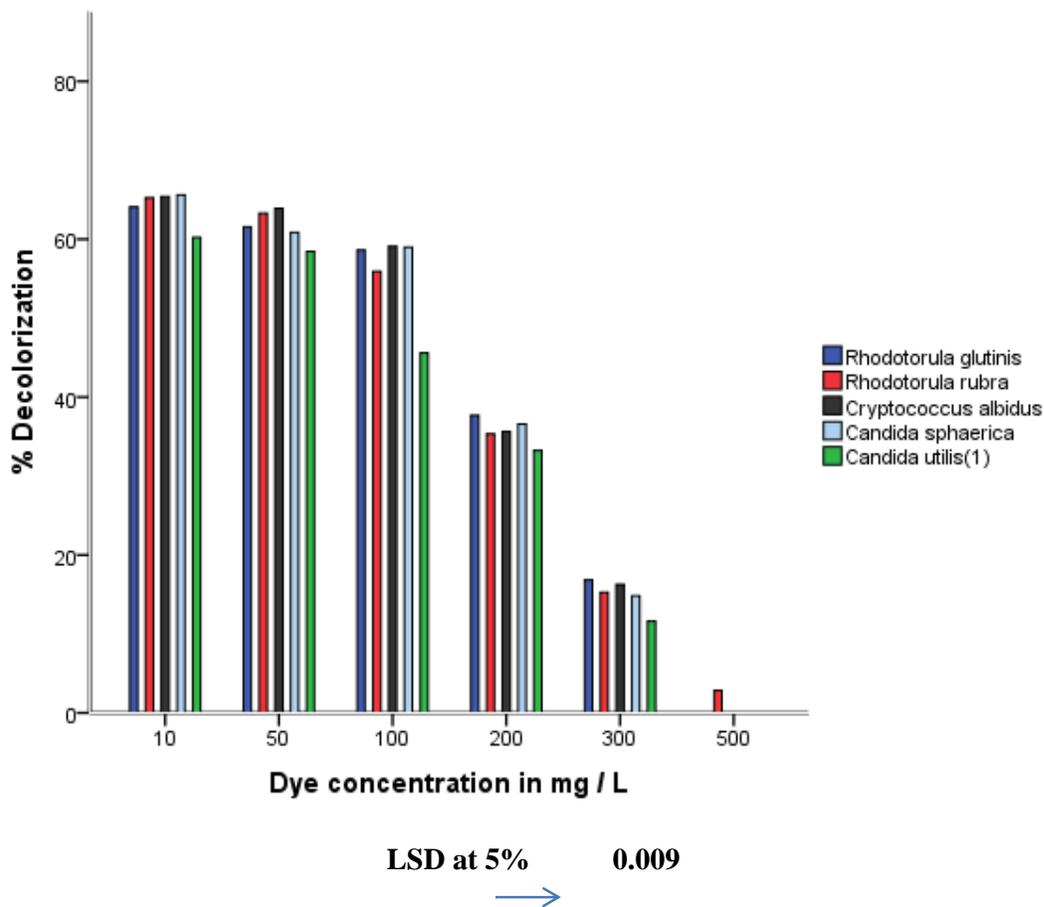
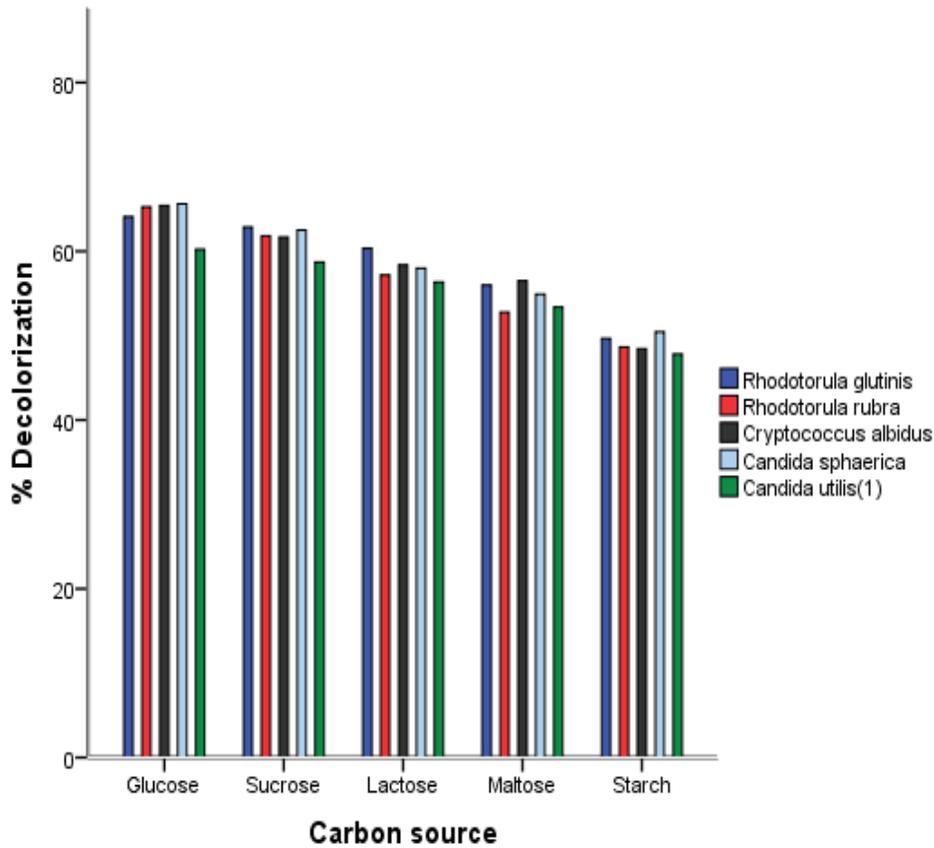


Fig.(6): Effect of dye concentration on the decolorization by the selected yeast strains

3.1.2.7. Carbon source

The results of decolorization rate indicated that glucose is the best carbon source for all yeasts that decolorize Reactive Brilliant Blue dye (fig.7). However, the rate of decolorization by all the selected yeast strains was lower when starch was supplied as a carbon source.

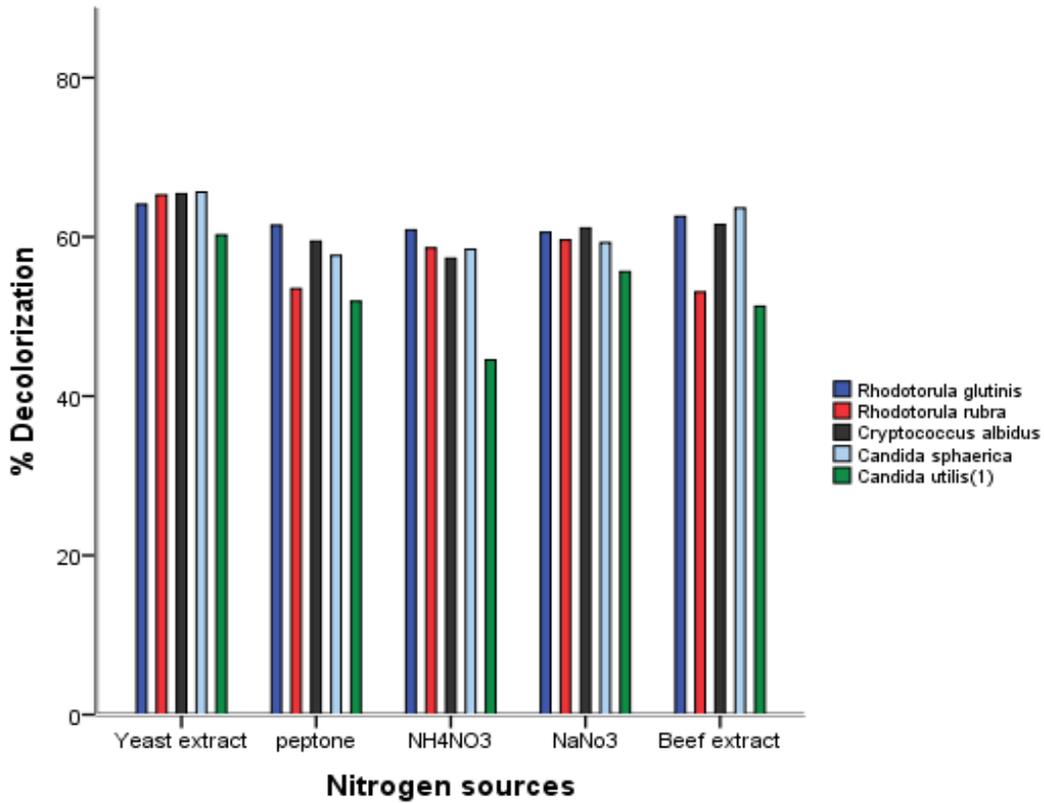


LSD at 5% → 0.018

Fig.(7): Effect of carbon source on the decolorization by the selected yeast strains

3.1.2.8. Nitrogen source

The results in figure(8) showed that Yeast extract is the optimum and best nitrogen source for decolorization of Reactive Brilliant Blue dye for all the selected yeast strains in comparing with other tested nitrogen sources. However, the decolorization rate in case of other nitrogen sources is different according to the yeast strain.

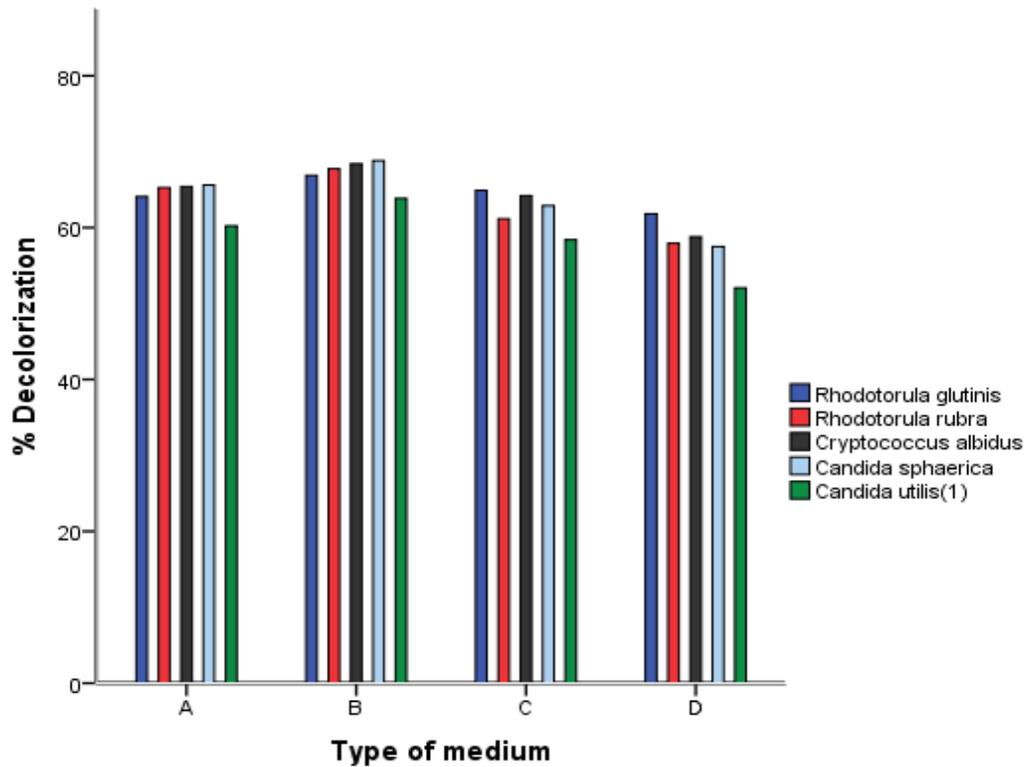


LSD at 5% → 0.001

Fig.(8): Effect of nitrogen source on the decolorization by the selected yeast strains

3.1.2.9. Type of medium

Different types of media were used for culturing yeasts to know the best medium composition for the decolorization of Reactive Brilliant Blue dye. All types of used media showed a decolorization of dye. However, the best results were obtained when plain distilled water was used (fig. 9).



LSD at 5% → 0.003

Fig.(9): Effect of medium composition on the decolorization by the selected yeast strains

Note:

(A) Decolorization medium, (B) plain distilled water, (C) Distilled water with 5% glucose and (D) Distilled water with 1% glucose, 0.1% peptone and 0.1% yeast extract.

3.2 Discussion

Industrial effluent is not stable and it varies often in a wide range depending upon the process practiced. South Asian countries are experiencing in severe environmental problems due to the rapid industrialization. This phenomenon is very common where the polluting industries like textile dyeing, leather tanning, paper and pulp processing, sugar manufacturing, etc. thrive as clusters. Among these the textile industries are large industrial consumers of water as well as producers of waste water. The effluent discharged by this industry leads to serious pollution of groundwater and soils and ultimately affects the livelihood of the poor (**Jenkins, 1982 and Shah et al., 2013**). Some studies have shown that yeast species acted as a promising dye adsorbent capable to uptake higher dye concentration such as *Galactomyces getrichum*, *Saccharomyces cerevisiae* and *Trichosporon beigilli*, etc. (**Jadav et al., 2008 and Lavanya et al., 2014**). In this study, out of twenty-four yeast isolates and strains, only five strains (2.8%) have the ability to decolorize Reactive Brilliant Blue dye.

Decolorization of Reactive Brilliant Blue by the selected yeast strains was investigated over a temperature range of 25-55°C. The increase in temperature from 25 to 37°C caused an increase in decolorization within 18 h of incubation. However, further rise in temperature from 37°C to onward had negative effect on decolorization of Reactive Brilliant Blue. Decrease in decolorization at higher temperature may be due to the thermal deactivation of the enzyme responsible for decolorization (**Shah et al., 2013**). It is very likely that all selected yeast strains were mesophilic yeasts because they all showed better decolorization in temperature range of 25 to 37°C. The mesophilic range is traditionally used since it is generally thought that maintaining high temperature would be uneconomical, while degradation within the psychrophilic range is too slow (**Varel et al., 1980**). Similar optimum temperature was observed in the decolorization of Remazole black B by *Kluyveromyces maxianus* IMB3 (**Meehan et al., 2000**) in case of *Candida utilis*1 but the remaining strains in contrast with this result.

Textile effluent has alkaline pH due to substantial presence of salts during dyeing process. High pH level of textile effluents is one of problems in their biological treatment. Therefore, tolerance to high PH is important to make this technology valuable (**Shah et al., 2013**). Another limiting factor for microbial activities and anthraquinone dye decolorization is the PH of medium. Optimum pH for growth and decolorization of the selected yeast strains is different. It is very likely that all selected yeast strains were acidophilic yeasts because they all

showed better decolorization in pH range of 3 to 6. It is also likely that pH might also have affected the enzymatic activity involved in decolorization of dye, in addition to the cellular growth of inocula. It was observed that rate of decolorization increased with increase in inoculum concentration. Maximum rate of decolorization was observed at a 20% (v/v) inoculum concentration. There was no proportionate increase of decolorization with increase inoculum concentration when inoculated in textile effluent (**Moosvi et al., 2005**).

Decolorization rate of all the selected yeast strains was reduced when the concentration of dye was more than 200 mg/L, this might be due to the toxic effect of the dye against the yeast growth or enzymatic activity responsible for the degradation of the dye or inadequate biomass concentration for the uptake of higher concentrations of dye (**Jadhav et al., 2007**). In literature, the concentrations usually used are less than 0.2 g/L (**Bibi and Bhatti, 2012; Moreira-Neto et al., 2013**). Previous investigations have shown that the dye concentration does affect the rate of biodegradation and the optimum dye level could also vary from microbial species to species and in general higher color removal efficiencies have been observed at medium dye concentrations (**Sponza and Isik, 2005; Khalid et al., 2008**).

Different carbon and nitrogen sources were investigated for their impact on the decolorization of Reactive Brilliant Blue dye by the selected yeast cultures. Among these glucose and yeast extract supported the highest biodegradation reaction. This observation could be explained that glucose acted as a co-substrate and electron donor which favored the yeast growth and Reactive Brilliant Blue decolorization. In contrast addition of glucose as a carbon source seemed to be effective to promote the decolorization, probably due to the preference of the cells in assimilating the added carbon sources over using the dye compound as the carbon source. Hence the foregoing results suggested that the addition of glucose and yeast extract in synthetic media showed maximum decolorization of Reactive Brilliant Blue by all the selected yeast strains. Similar results of decolorization and biodegradation of textile Navy blue HER by *Trichosporon beigeli* NCIM-3326 for nitrogen source but in contrast with the results of carbon source (**Saratale et al., 2009**).

Effect of type of medium on the decolorization of Reactive Brilliant Blue was assessed and the best results were obtained, when distilled water was used as the medium. The results indicate the influence of type of medium and faster biodegradation of Reactive Brilliant Blue than decolorization medium. Similarly the results were obtained by (**Jadhav et al., 2007**) for the decolorization of azo dye methyl red by *Saccharomyces cerevisiae* MTCC 463.

4. Conclusion

The present study revealed that the selected five yeasts can be used for decolorizing Reactive Brilliant Blue dye. The cultures exhibited maximum decolorization ability under optimum environmental conditions. The decolorization rate increased up to 69% in some selected strains. Yeast strains can be further studied for bioremediation of dye-polluted waters including the degradation of anthraquinone dye.

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ألملخص العربي

المعايير البيئية المثلى لتحلل صبغ Reactive Brilliant Blue بالخمائر المعزولة من النفايات السائلة لاصباغ المنسوجات

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اجري هذا البحث بغرض دراسة تأثير دور الخمائر المعزولة من النفايات السامة الناتجة من صناعات الغزل والنسيج واثتان من سلالات الخميرة المعرفة باستخدام الطرق النموذجية والنامية تحت الظروف المثلى على تحليل صبغة Reactive Brilliant Blue.

أظهرت النتائج أنه من بين أربعة وعشرون سلالة من الخميرة خمسة أنواع (2.8%) فقط قادرة على إزالة لون الصبغة. تم تعريف ثلاث منها على أنها *Rhodotorula glutinis* و *Candida utilis(1)* و *Candida sphaerica* بالإضافة إلى السلالتين المعرفتين وهما *Rhodotorula rubra* and *Cryptococcus albidus*.

تم استخدام السلالات الخمسة في تحليل الصبغة باستخدام بيئة تحتوي على الجلوكوز ومستخلص الخميرة كمصدرين لعنصري الكربون والنيتروجين على التوالي . وقد لوحظ أن درجة الحرارة المثلى للتحليل الحيوي للصبغة للأربع أنواع من الخمائر كانت ٢٥ ° م بعد ١٨ ساعة بينما كانت عند ٣٧ ° م للسلالة الخامسة (*C. utilis 1*). وقد كان معامل الأس الهيدروجيني الأمثل مختلف بالنسبة للسلالات المختلفة ، فقد كان معامل الأس الهيدروجيني ٤ عند استخدام السلالات *C. utilis(1)* و *R. rubra* و *C. albidus* بينما كان ٦ عند استخدام *C. sphaerica* و ٥،٥ عند استخدام *R. glutinis* .

ووجد أن السلالات *C. utilis(1)* و *R. glutinis* و *C. sphaerica* قد أظهرت معدل عال للتحليل تحت الظروف الهوائية بينما السلالات *R. rubra* و *C. albidus* قد أظهرت معدل مرتفع تحت الظروف اللاهوائية.

أوضحت الدراسة أن نسبة تحلل الصبغة كان ٦٨,٨٣ % لسلالة *C. sphaerica* و ٦٨,٤٠ % لسلالة *R. rubra* و ٦٧,٥ % لسلالة *R. glutinis* و ٦٣,٨٥ % لسلالة *C. utilis(1)* . كما لوحظ أن نتائج التحلل الحيوي للصبغة كانت معنوية عند استخدام الماء المقطر كبيئة لتحلل الصبغة .

في الختام، يمكن استخدام سلالات الخمائر للتحلل الحيوي للصبغة الملوثة لمياه الصرف الصناعي متضمنة تحلل صبغة anthraquinone.