Int.J.Curr.Microbiol.App.Sci (2020) *9*(3): 1243-1260

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 3 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.903.145

Biodegradation of the Azo Dyes Direct Red 81 and Reactive Brilliant Red X-3B by Wild Strains of Yeasts *Meyerozyma guilliermondii* and *Naganishia diffluens*

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Keywords

Azo dyes, Yeasts, Decolorization, Degradation, *Meyerozyma guilliermondii*, *Naganishia diffluens*

Article Info

Accepted:
05 February 2020
Available Online:
10 March 2020

A B S T R A C T

Fifty yeast isolates from different habitats nearby a textile factory in Menoufia governerate, Egypt were screened for their ability to decolorize the azo dyes direct red 81 (DR81) and reactive brilliant red X-3B (RBR). Out of those, twenty two isolates displayed positive results therefore they were characterized morphologically and physiologically. The most active two were identified as *Meyerozyma guilliermondii* and *Naganishia diffluens* on the bases of 28S ribosomal RNA sequencing. The bio degradation of the dyes was confirmed by UV-visible spectra and IR analysis. The dyes decolorization percentage of 0.01 and 0.005% of DR81 and RBR reached 87.0 and 70.7% by these two yeasts respectively. Sucrose and glucose were the best carbon sources for the two yeasts whereas peptone was the best nitrogen source for both. Incubation at 30^0C for 48h of shaked culture was the best physical conditions for bio-decolorization process. The results also indicated that pH5 and 7 are the optimum pH values, increasing the concentration of NaCl resulted in a significant decreasing in dye decolorization. Elevated concentrations of Cu^{+2} or Fe^{2+} had a significant diverse effect on the ability of *M. guilliermondii* to decolorize DR81but there was a slight effect of Co^{2+}. On the other hand, there was a non significant effect of Cu^{2+} and Fe^{2+} on the activity of *N. diffluens* towards decolorization of RBR but high concentrations of Co^{2+} showed inhibitory effects on the biodegradation processes. These results were discussed in relation with their importance in the field of biotechnological application for bioremediation of azo dyes-polluted effluents.

Introduction

Controlling Pollution is the main concern of the society today. The release of harmful wastewaters in and around the heavily industrial areas is a serious environmental and health concern. Large numbers of chemically different dyes are used for various industrial applications and significant proportion appears in the form of wastewater and is spilled into the environment (Meyer, 1981). Enormous volumes of effluents are generated at different stages of textile manufacturing, as a result of the use of copious amount of chemicals and dyes. With the increased demand for textile products, the textile

industry and its waste waters have increased proportionally making it one of the main sources of severe pollution problems worldwide (Pavithra and Kousar, 2016). In addition to their visual effect and their adverse impact in terms of chemical oxygen demand, many synthetic dyes are toxic, mutagenic and carcinogenic (Chung *et al.*, 1992). Textile industries use large amount of water in every textile processing operations and waste water generated during these processes contains 5-10% of unfixed dyes (Markandeya *et al.*, 2017). It has been reported that, the direct discharge of dyes in concentrations higher than 1 mg/l, treated or not, could increase community complaints and concerns. continuous release of dye polluted waste water without proper treatment contaminates soil, ground water, sediments and surface water etc. (Markandeya *et al.*, 2017). Therefore, waste products generated can pose various environmental problems, if not properly treated before being discharged (Babu *et al.*, 2007; Mostafa, 2015). Colored effluents, when released into the nearby water bodies affect the light and oxygen penetration decreasing the photosynthetic rate which ultimately affects the flora and fauna of aquatic eco-system (Das *et al.*, 2016). Therefore, the treatment of these textile effluents is necessary There are many structural varieties of dyes with respect to the type of chromophore, such as azo, anthraquinone, acridine, aryl methane, cyanine, phthalocyanine, nitro, nitroso, quinone-imine, thiazole or xanthene dyes. There are 10,000 commercially available dyes and pigments produced every year around the world of which a great name of the dyes are composed azo (-N=N-), which are linked by an azo bridge. Azo dyes are used in various processes including tattooing, printing, cosmetics, consumer's products as well as textile dying because of their chemical stability and versatility., most of which are toxic, carcinogenic and recalcitrant in nature (Shah, 2018). Azo dyes, which are widely used in textile manufacturing and related products (aromatic amine), can cause allergies, dermatitis, skin irritation, carcinogenic and mutagenic actions as well as acute and chronic toxicity (Yaseen and Scholz, 2017). Physical and chemical methods of dyes treatment such as adsorption, membrane filtration, chemical precipitation and flocculation, zonation, photolysis, ion pair extraction, oxidation with hydrogen peroxide (Fenton's reaction) etc., have been evaluated for the removal of color from industrial effluent (Das *et al.*, 2016). However, application of each of these methods has been found to have several shortcomings such as excess amount of chemical usage or sludge generation with obvious disposal problems; costly plant requirements or operating expenses; lack of effective color reduction, particularly for sulfonated azo dyes; and sensitivity to a variable wastewater input (Fayidh *et al.*, 2011).

So that, these methods are not feasible techniques since they are very expensive, experience operational problems and formation of hazardous byproducts or intensive energy requirement (Patel *et al.*, 2006; Stephenson and Sheldon, 1996). Although physical and chemical methods provide high color removal, they are disadvantageous, since color removal efficiency varies with dye type contained in the wastewater (Kariminiaae *et al.*, 2007; Santos *et al.*, 2007). Nowadays research has indicated that biosorption, is one of the most promising technologies and the removal of dyes by different kinds of biosorbent materials has been receiving more attention. Biological wastewater treatment is often the most economical and eco-friendly alternative, relative to other physical and chemical processes. Microbial decolorization methods, such as bioremoval by growing culture in

medium and biosorption by (living or dead) microbial biomass, are commonly applied to the treatment of textile industry effluents because various microorganisms, such as bacteria, yeasts, algae and fungi are able to remove different classes of dyes (Tan *et al.*, 2013; Gul UD, 2013; Kelewou *et al.*, 2014) the interactions between microorganisms (yeasts, bacteria and/or fungi) and dyes depend on the chemical properties of all the reaction partners each dye can have affinity to various microorganisms and on the other side one microorganisms is able to bind or degrade more types of dyes.

The purpose of this study was to screen wild isolates of yeasts for decolorization and degradation of the synthetic dyes, Direct Red 81 and Reactive Brilliant Red X-3B which has a potential biotechnological applications in the field of azo dyes bioremediation.

Materials and Methods

Azo dyes

The direct red81 (DR) dye and Reactive Brilliant Red X-3B (RR) dye were obtained from a local company (Quesna Dyes Factory, Quesna, Menoufia Governorate, Egypt). These azo dyes are routinely used in the dyeing process of cotton fibers. Stock solutions of these dyes were prepared by dissolving accurately weighed dyes in distilled sterilized water to the concentration of %1.0 g/L. The experimental solutions were prepared by diluting the stock solution with appropriate volume of distilled water.

Media and culture conditions

Different growth medium were used in this study; (1) yeast- peptone Dextrose (YPD) agar consists of (g/L) yeast extract, 10; peptone, 20; dextrose, 20; Agar,15, (2) yeast extract mineral medium (YM) which consists of (g/L) glucose, 5; $(NH4)_2SO_4$, 1; KH_2PO_4, 1; MgSO4.7H2O, 0.5; yeast extract, 0.1; $CaCl_2.2H_2O$, 0.1; Agar, 15, (3) Potato Dextrose Agar (PDA) medium consists of (g/L) Potato extract (Extracted from 200g potato); dextrose, 20; Agar, 15. (4) Mineral KDP medium containing (g/L) $MgSO4.7H_2O$, 0.5; KH_2 Po_4, 1; dextrose ,10; peptone,5 was also used. The medium pH was adjusted to 5.0 unless otherwise stated. For inhibition of bacterial growth, chloramphenicol at 0.1g/L was added to the medium after autoclaving and prior pouring the plates.

Sampling and isolation of yeasts

The yeast strains used in this study were isolated from different natural sources, soil and waste water (located nearby a textile factory), vegetables, fruits peels, sugar cane bagasse, flowers, leaves and water. One ml or one gram of waste water or solid samples, respectively, was serially diluted up to 10^{-7}, and about 0.1 ml of each dilution was inoculated onto the surface of autoclaved YPD and PDA plates by using the spread-plate method. The plates were incubated for 5 days at 27^0C, and the pure yeast strains were re-inoculated on PDA slants, maintained at 4^oC and periodically sub-cultured. Characteristic features (color, margin and elevation) of the isolated yeast colonies were recorded, and the cells of a young actively growing culture (from 2-3 days at 25°C) were investigated microscopically to determine cell shape, size and east budding.

Morphological and physiological characterization of the yeast isolates

Morphological characterization of the isolated yeast strains was carried out according to Barrnett *et al.*, (1983) Kurtzman (1990). The color, margin and elevation of the isolated yeast colonies grown on YPD agar were

recorded, and the cells of 3 days -old colonies were also investigated microscopically using 40X objective lens power to determine cell shape, size and budding as described by Pitt and Hocking(1999).

Physiological characterization of the yeasts were performed according to Barrnett *et al.,* (1990) and Kurtzman *et al.,* (2011). Carbohydrate fermentation was carried out by filling test tubes with 10 ml KDP broth medium containing 0.2g of glucose, fructose, sucrose, starch both alone with inverted Durham tubes, then autoclaved at 121 Ċ for 20 minutes. Each test tube was inoculated with fresh yeast suspension then incubated at 28^0C for one week and examined frequently for bubbles of gas. For carbohydrate assimilation, KDP agar medium was used without dextrose. Different carbohydrate suspensions (glucose, fructose, sucrose, starch) were prepared, and after autoclaving and pouring into Petri dishes and inoculation with aliquot volumes of cell suspension they were incubated at 28 ^{0}C for 2 days. Assimilation of different carbohydrates was detected by the yeast growth. Assimilation of nitrogen compounds was similar to that of carbohydrate with the addition of ammonium sulphate, sodium nitrate, urea or peptone as a sole nitrogen source and the assimilation was detected by yeast growth.

Gelatin liquefaction (proteolytic enzyme) that liquefy gelatin was carried out by inoculation of yeast smear on the tube containing YPD gelatin medium and incubate the inoculated tubes along with a non inoculated medium (control treatment) at 28^0C for two weeks. To confirm that liquefaction was due to gelatinase activity, the tubes were kept in the refrigerator at 4^0C until the control tubes were solidified.

In addition to the morphological and physiological characters, the most active isolates were further identified according to 28S rRNA gene sequence at sigma-lab, 6-October City, Cairo, Egypt.

Screening for azo dye degradation by the isolated yeasts

The ability of yeasts for degradation of azo dyes was carried out according to Machado *et al.,* (2005).Triplicate 100 µl suspension (0.1 OD at 650 nm) of each yeast isolate was placed in a hole of 0.3 Cm diameter on the center of plates containing solid KDP medium supplemented with 0.01% Direct Red or Reactive Red dye. Non inoculated culture medium was also used as a control treatment. The plates were incubated at 28±2ºC for 2 days, and the diameter of any change to the dye color around the growing colonies was measured

Decolorization and degradation of the dyes by yeasts in liquid medium

500 µl of cell suspension (with 0.1OD at 650 nm) of the most active yeast strains were placed in 250ml Erlenmeyer flasks containing 50 ml of sterilized liquid KDP medium with 0.01% Direct Red or Reactive Red dye conc. The flasks were incubated at 28±2ºC for 2 days in shaking condition (120 rpm). Triplicate sets of flasks were used for each yeast isolate, and non inoculated culture media were used as a control. Yeast cells were collected by centrifugation for 5 min at 4000rpm and the supernatant was analyzed spectrophotometrically (at 509 and 536 nm for Direct Red and Reactive Red respectively) as described by Casieri *et al.,* (2008). The decolorization efficiency is expressed as the color reduction percentage (%R) and Calculated as Follows $\%R=100\ (A_o-A_t)/A_O$. Where Ao is the absorbance value of control (non treated) and A_t is the absorbance value of treated flasks at time t. The best isolates for dye decolorization were used for further studies.

Spectroscopic analysis (UV) and infra red (IR) measurements

After yeasts growth for 48h on dye-containing KDP medium some of the culture filterate was assayed by using UV- visible spectrophotometer (T-80+ UV-VIS-Spectrometer, UK, 190-1100). Infrared analysis of the filterate was also carried out to identify the structural variation using [Perkin–Elmer] infrared data station [1430 Ratio – Recording Infrared spectrophotometer]. Culture fluid without yeast cells incubated under the same conditions was used as the control.

Environmental Factors affecting degradation of dyes

Effect of temperature and aeration

These experiments were carried out by triplicate of 250 ml Erlienmeyer flasks containing 50 ml of KDP medium and inoculated with 0.5 ml suspension of the most active yeast isolates (S18 - S20). Non inoculated culture medium was used as control. All flasks were incubated in shaking incubator (120 rpm) at 28 ^{0}C for 48h under different physical and chemical conditions. The percentage of dye degradation was determined as previously indicated. Sampling was taken at different time intervals to estimate the effect of incubation periods. To evaluate the effect of the initial pH vale on degradation, the medium pH was adjusted by using 0.1N HCl and 0.1N NaOH prior autoclaving. The effect of temperature and aeration was also carried out under different temperatures and aeration conditions.

Effect of dye concentration, carbon and nitrogen sources, salinity and heavy metals

The effect of dye concentration was performed by growing the yeast isolates on KDP liquid medium supplemented with different concentrations (0.002, 0.005, 0.010, 0.015, 0.02 g%) of Reactive Red or Direct Red dye. To study the effect of nitrogen and carbon sources on dye degradation equivalent concentrations (5.0 g/L) were used in the medium supplemented with the dye concentrations 0.015% (DR) and 0.005% (RR) for growth of S18 and S20 respectively. The effect of carbon source on the degradation activity was conducted by using different carbon sources at a concentration (10g/L). To study the effect of salinity, different concentrations of NaCl (0, 3.0, 5.0 and 10.0%) and other conditions were adjusted as the obtained optimum parameters values. To investigate the effect of metal ions different concentrations (0, 20, 50, 100, 150 ppm) of Sterilized $FeCl_3$, $CoCl_2$, $CuCl_2$ and $ZnSO_2$ were incorporated in the growth medium. The medium was inoculated and incubated as indicated above

Results and Discussion

Characterization and identification of the dye-degrading yeasts

Degradation of dyes being studied was monitored by the appearance of clear zone and/or discolorization of the dye around the growing colonies. The present study revealed that out of the isolated fifty yeasts there were 22 (coded S1-S22) exhibited such activities (indicated in Table 1 and Figure 1). The morphological and physiological features of those isolates indicated that the most colonies are spherical, white to creamy, have entire margin, raised elevation and reproduced by budding (Table 2). Table 1 also shows that the isolates S20 and S18 exhibited the highest degradation activity; %87 of Direct Red dye and 70.7% Reactive Red dye respectively, therefore these two isolates were identified and selected for further investigation in this study.

The biochemical tests indicated that both S18 and S20 are able to ferment glucose and assimilate glucose, fructose, sucrose, and starch as carbon compounds, they also able to assimilate ammonium, nitrate, urea and peptone as nitrogen sources (Table 3). Additionally, the isolate S20 liquefied gelatin while S18 showed disability to do so.

Genetic identification of the isolates S18 and S20

The genetic identification of the isolates S18 and S20 on the bases of similarity of the 28S ribosomal rRNA sequences indicated isolates are *Naganishia diffluens* (MH794402.1) and *Meyerozyma guilliermondii (*MN371925.1) with homology 99.11 and 96.9% respectively.

The UV–visible spectra and IR analysis

UV-vesible spectra of both the non inoculated (control) Direct Red and Reactive Red dyes solution as well as the culture filtrate of *M. guilliermondii and N. diffluens* are indicated in Figures 2 and 3. They showed that the ג max of both dyes had been changed to high extent. The original peak of Direct Red dye (at 552 nm) completely disappeared in culture filtrate of *M. guilliermondii* and another new peak appeared at 240nm. The original peak of Reactive Red dye at 534 nm completely disappeared and another new peak appeared at 394 nm, while the peak at 244 nm remained the same as the same at 246 nm in the culture filtrate of *N. diffluens*. In terms of IR analysis Figures 4 and 5 show that the control treatment of Direct Red dye gave band at V 1123 cm^{-1} which correspond to sodium salt (SO_3Na), control of Reactive Red X-3B dye gave band at V 601 which correspond to SO_3H group in ortho position. These bands were not present in the spectrum of original dyes. This means that sodium salt in Direct Red dye structure destroyed by the action of *M. guilliermondii*, SO_3H group in ortho position of Reactive Red dye structure destroyed by the action of *N. diffluens* These results revealed the changes in the chemical composition of both dyes due to the presence of yeasts, therefore it could be suggested the degradation and/or adsorption of those dyes by the yeasts strains being studied.

Factors affecting dyes decolorization by *M. guilliermondii* and *N. diffluens*

Effect of the medium pH, temperature and aeration

Different initial medium pH values (3, 5, 7, 9, and 11) were used to evaluate the influence of pH on dyes decolourization by the yeast strains *M. guilliermondii* (S180) *and N.diffluens* (S20). Table 4 shows that the maximum decolorization percentage (80.3%) of Direct Red dye by *M. guilliermondi* was detected at pH 5 whereas *N. diffluens* degraded 70.8% of reactive dye at pH 7. These results are in agree with Parshetti *et al.*, (2007) who reported that the complete decolorization of Reactive Blue-25(100 ppm) by *Aspergillus ochraceus* NCIM-1146 occurred at pH 5.However, 87%, 81% and 70% decolorization was obtained at pH 3,7 and 9, respectively. Similar results were also obtained by EL-Sayeh (2010) who observed decreasing in the decolorization activity (47%) at PH 3 compared with (94%) at PH 5.5 for Direct violet dye by *Aspergillus fumigatus.*

Table 5 indicates that decolorization of both Direct Red and Reactive Red dyes was most efficient at 30° with 85.3% and 80.8% of color reduction by *M. guilliermondii* and *N. diffluens* respectively. Despite there was a non significant influence with increasing the temperature to 40°C, such yeast activity decreased sharply at this temperature for decolorization of reactive dye by *N. diffluens*. This might be due to the loss of cell viability

or deactivation of the enzyme responsible for decolorization as assumed by Kumar *et al.*, (2009).

Effect of aeration

The yeast decolorization activity of both dyes seemed to be significantly influenced by aeration condition as Table 6 indicates that shaking condition resulted in increasing the decolorization of direct red and reactive red by *M. guilliermondii* and *N. diffluens* from 77.38 and 23.01 to 87.91 and 69.01% respectively. Ion activity represented 65% in the case of Reactive Red dye and 92.9% in. These results are in harmony with those of El-Sayeh (2010) who reported that direct violet dye decolorization by *Aspergillus fumigatus* was more efficient in agitated cultures as compared to static conditions. The increased activity in dye decolorization in shaking conditions could be attributed to the increased transfer of oxygen and distribution of nutrients.

Effect of dye concentrations

Different concentrations of dyes (0.002, 0.005, 0.01, 0.015, 0.02%g) in KDP liquid medium were used to determine their effect on degradation and decolorization of Direct Red, Reactive Red dyes by *M. guilliermondii, N. diffluens* respectively. (Table 5) indicate that 0.005% of Reactive Red dye concentration shows the highest decolorization value 81.3%, on the other hand, 0.015 % of Direct Red dye concentration represent the highest concentration which shows maximum decolorization 88.7%.

Table.1 Decolorization of dyes by the yeast isolates after growth for 48 h on solid KDP medium supplemented with 0.01% DR81and 0.05% RBR dyes. Other conditions as shown in methods

Yeast isolates	DR81		RBR	
	decolorized zone (mm)	Decolorization %	decolorized zone (mm)	Decolorization %
S1	13.0±0.05	30.0	12.5±0.02	**53.0**
S2	20.0±0.07	28.0	16.0±0.01	**45.0**
S3	22.0±0.07	20.0	16.0±0.01	**40.3**
S4	18.0±0.06	22.0	16.0±0.01	**29.0**
S5	22.0±0.07	17.0	10.0±0.11	**35.0**
S6	50.0±0.09	44.0	20.0±0.01	**65.0**
S9	26.0±0.08	85.0	20.0±0.14	**69.0**
S10	29.5±0.81	82.0	11.0±0.03	**39.0**
S11	25.0±0.79	30.0	20.0±0.14	**61.0**
S12	24.0±0.11	16.0	11.0±0.08	**40**
S17	14.0±0.05	28.0	15.0±0.22	**49.9**
S18	12.4±0.01	29.0	34.0±0.01	**70.7**
S19	23.4±0.12	54.0	12.0±0.02	**46**
S20	54.0±0.09	87.0	15.0±0.01	**35.0**
S21	28.0±0.08	25.0	22.0±0.14	**57.0**
S22	14.3±0.03	19.0	10.0±0.07	**26.0**

Table.2 Morphological characters of colonies and cells of the most active dyes-degrading yeast isolates

Yeast isolates	Cell shape	Color	Size	Margin	Elevation
S1	Spherical- budding	White to creamy	Small	Entire	Raised
S2	Spherical- budding	White to creamy	Small	Entire	Raised
S3	Ovoid- budding	White to creamy	Moderate	Entire	Raised
S4	Spherical- budding	Orange	Small	Entire	Raised
S5	Elongated	White to creamy	Moderate	Serrate	Raised
S6	Elongated-budding	White to creamy	Small	Entire	Flat
S7	Spherical	White to creamy	Small	Entire	Raised
S8	Ovoid	Orange	Moderate	Entire	Unbonate
S9	Ovoid-budding	White-butyrous	Moderate	Entire	Raised
S10	Elongated	Orange	Small	Entire	Raised
S11	Elongated-budding	White to creamy	Moderate	Entire	Raised
S12	Spherical-budding	Light orange	Small	Entire	Raised
S13	Spherical- budding	Pink	Small	Entire	Convex
S14	Spherical- budding	Pink	Moderate	Entire	Raised
S15	Elongated-budding	White to creamy	Moderate	Entire	Raised
S16	spherical	White to creamy	Small	Entire	Raised
S17	Spherical-budding	White to creamy	Small	Entire	Raised
S18	Elongated-budding	Yellow white	Moderate	Entire	Raised
S19	Spherical-budding	Orange	Small	Entire	Raised
S20	Spherical-budding	White to creamy	Moderate	Entire	Raised
S21	Ovoid-budding	White- butyrous	Moderate	Entire	Raised
S22	Spherical-budding	White- butyrous	Small	Entire	Raised

Table.3 Biochemical characters of the yeast isolates (S18-S20)

Biochemical tests	S18	S20
Assimilation of carbon compounds		
Glucose	+	+
Fructose	+	+
Sucrose	+	+
Starch	+	+
Assimilation of nitrogen compounds		
Ammonium sulfate	+	+
Sodium nitrate	+	+
Urea	+	+
Peptone	+	+
Fermentation of carbon compounds		
Glucose	+	+
Fructose	-	+
Sucrose	-	+
Starch	-	+
Gelatin liquefaction	-	+

Table.4 Effect of initial medium pH value on degradation % of direct red (DR81) by *M. guilliermondii* and reactive dye (RBR) by *N.diffluens* after incubation for 48h at 28^0C.

Yeast strain	pH				
	3	5	7	9	11.5
M. guilliermondii	0.00±13.52	80.28±0.99	73.78±8.19	71.85±2.69	11.56±11.82
N. diffluens	0.14±5.04	54.28±3.04	70.79±1.49	57.54±4.29	32.38±3.45

Table.5 Effect of temperature on degradation % of direct red (DR81) by *M. guilliermondii* and reactive (RBR) dye by *N.diffluens* after incubation for 48h at 28^0C at pH 4 and 7 respectively

Yeast strain	Temperature		
	20	30	40
M. guilliermondii	71.20±0.68	85.29±0.62	83.81±2.39
N. diffluens	54.74±3.88	80.79±2.63	6.30±0.99

Table.6 Effect of aeration on degradation % of direct red (DR81) dye by *M. guilliermondii* and reactive red (RBR) dye by *N.diffluens* grown for 48h at 28^0C

Yeast strain	Aeration condition	
	static	shaker
M. guilliermondii	77.38±1.79	87.91±0.93
N. diffluens	23.01±5.03	69.01±3.01

Table.7 Effect of dye concentration on degradation % of direct red (DR81) dye by *M. guilliermondii* and reactive red (RBR) dye by *N.diffluens* grown for 48h at 28^0C

Yeast strain	Dye concentration (%)				
	0.002	0.005	0.01	0.015	0.02
M. guilliermondii	3.05±4.99	36.03±2.73	80.47±0.80	88.67±1.54	83.40±0.90
N. diffluens	79.88±0.65	81.25±3.64	67.84±3.62	58.24±3.68	45.75±17.26

Table.8 Effect of nitrogen source on degradation % of direct red (DR81) dye by *M. guilliermondii* and reactive red (RBR) dye by *N.diffluens* grown for 48h at 28^0C

Yeast strain	Nitrogen source				
	Peptone	Pot.nitrate	urea	Sod.nitrite	amm.nitrate
M. guilliermondii	83.88±0.65	81.25±3.64	67.84±3.62	58.24±3.68	45.75±17.26
N. diffluens	67.20±3.59	0.00±4.28	10.47±12.41	3.20±2.84	3.98±1.15

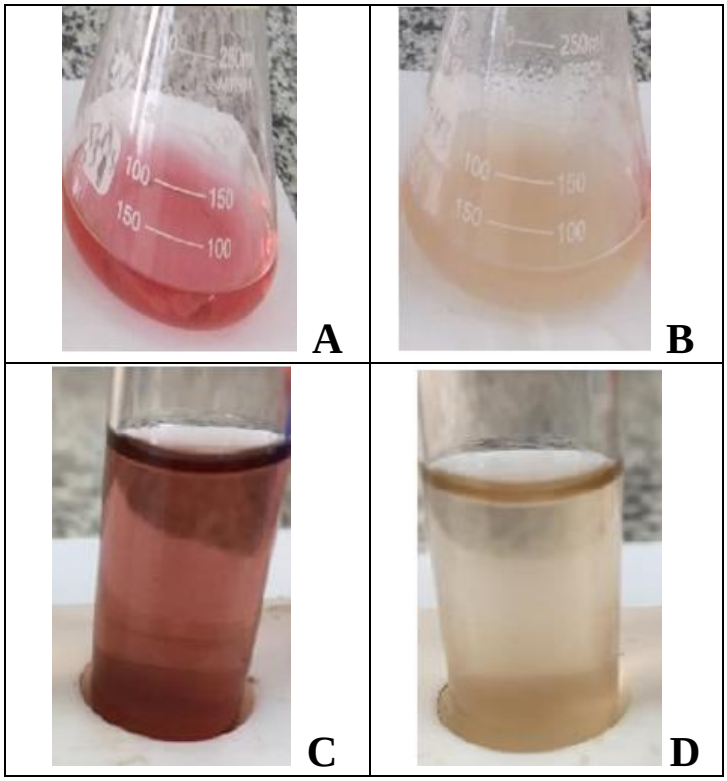
Table.9 Effect of carbon source on degradation % of direct red (DR81) dye by *M. guilliermondii* and reactive red (RBR) dye by *N.diffluens* grown for 48h at 28^0C

Yeast strain	Carbon source				
	Glucose	Fructose	Sucrose	Maltose	Starch
M. guilliermondii	63.44±2.20	55.22±1.23	77.80±3.93	61.77±1.54	13.92±15.43
N. diffluens	82.52±2.28	75.38±2.12	45.84±6.16	62.29±3.76	15.20±3.41

Table.10 Effect of salinity on degradation % of direct red (DR81) dye by *M. guilliermondii* and reactive red (RBR) dye by *N.diffluens* grown for 48h at 28^0C

Yeast strain	NaCl concentration (%)			
	0	3	5	10
M. guilliermondii	77.90±0.86	62.99±2.42	47.44±2.26	20.87±9.07
N. diffluens	53.54±10.79	19.24±3.90	15.45±9.44	7.99±1.71

Figure.1 Decolorization of (A) 0.015% Direct red (DR81), and (C) 0.005% Reactive Red (RBR) by (B) *Meyerozyma guilliermondii*, and (D) *Naganishia diffluens* grown in KDP liquid medium-containing the dyes, respectively, at 28°C for 48h.



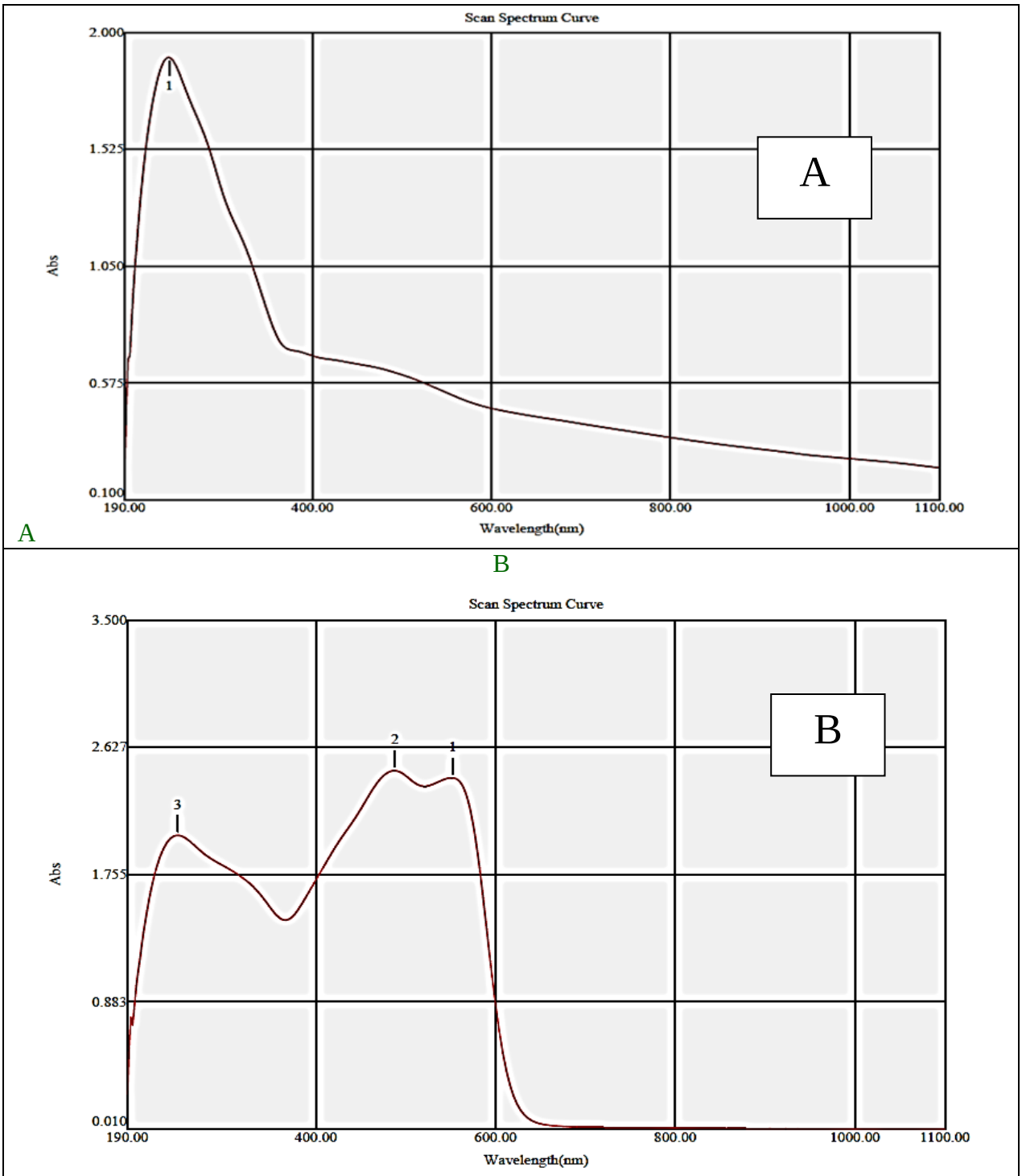


Figure.2 Absorption spectra of 0.01% direct red (DR 81) dye solution after incubation for 48h at 28 ^{0}C in (A) absence, and (B) presence of the yeast *M. guilliermondii*

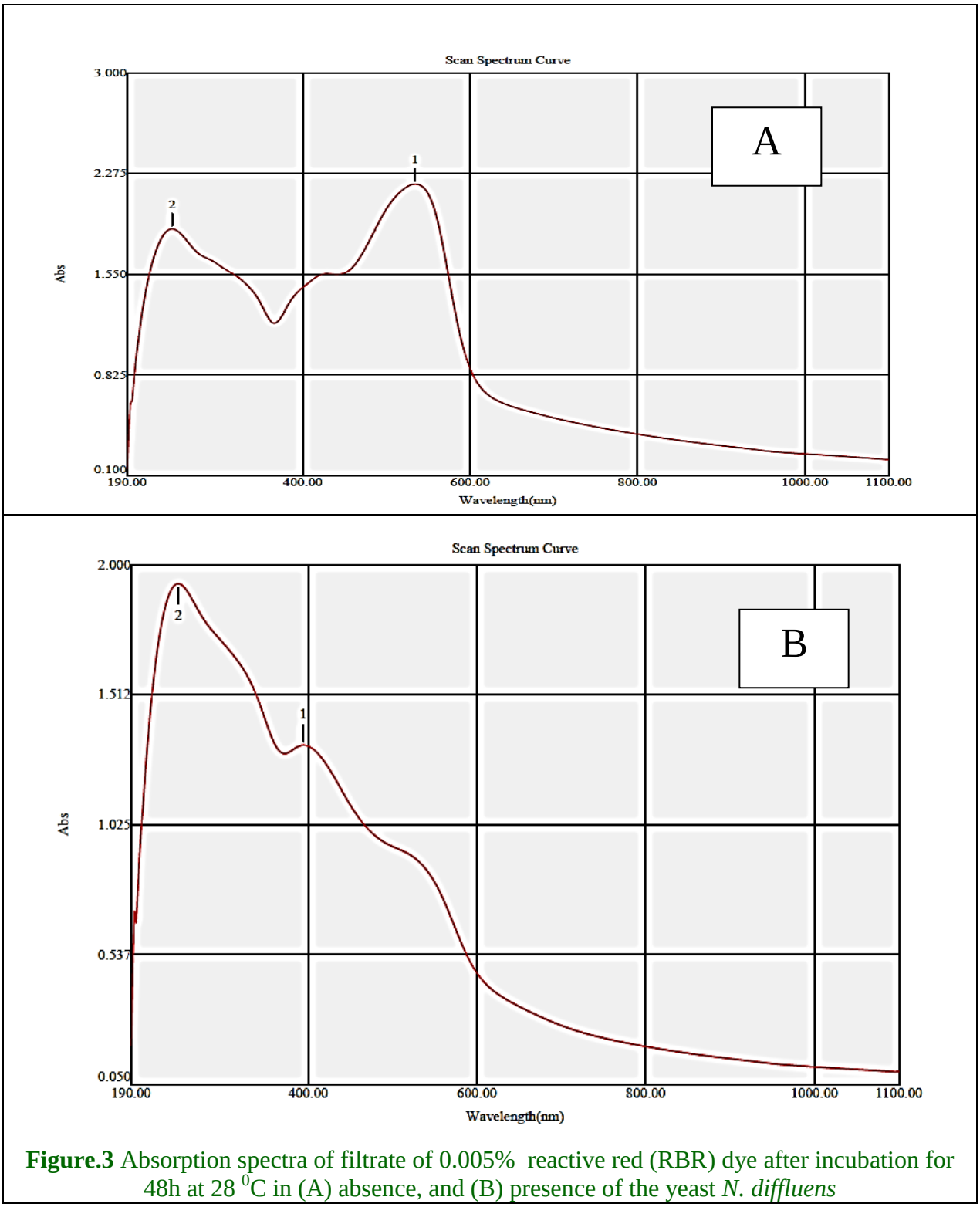
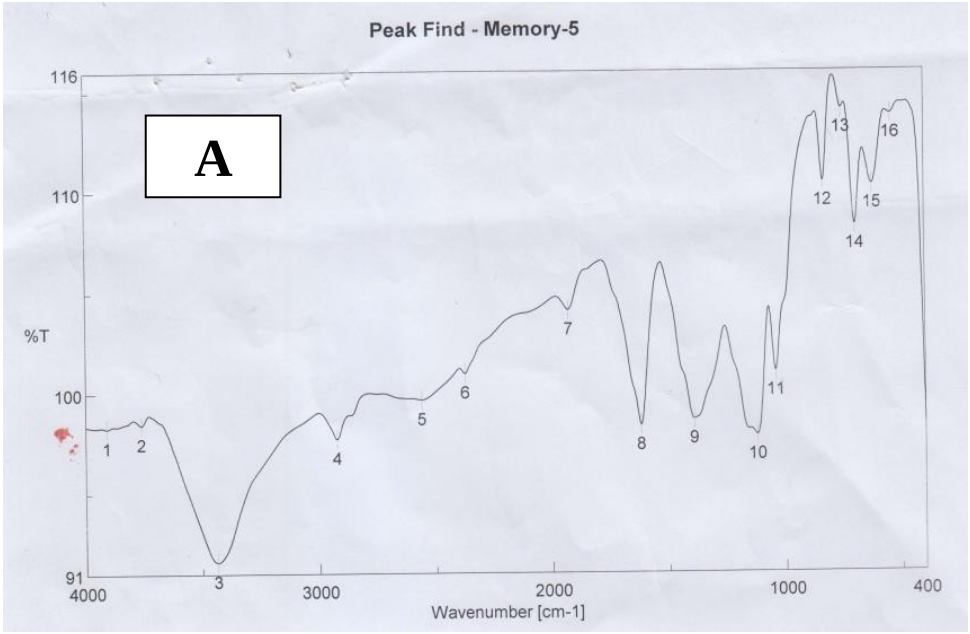


Figure.3 Absorption spectra of filtrate of 0.005% reactive red (RBR) dye after incubation for 48h at 28 ^{0}C in (A) absence, and (B) presence of the yeast *N. diffluens*



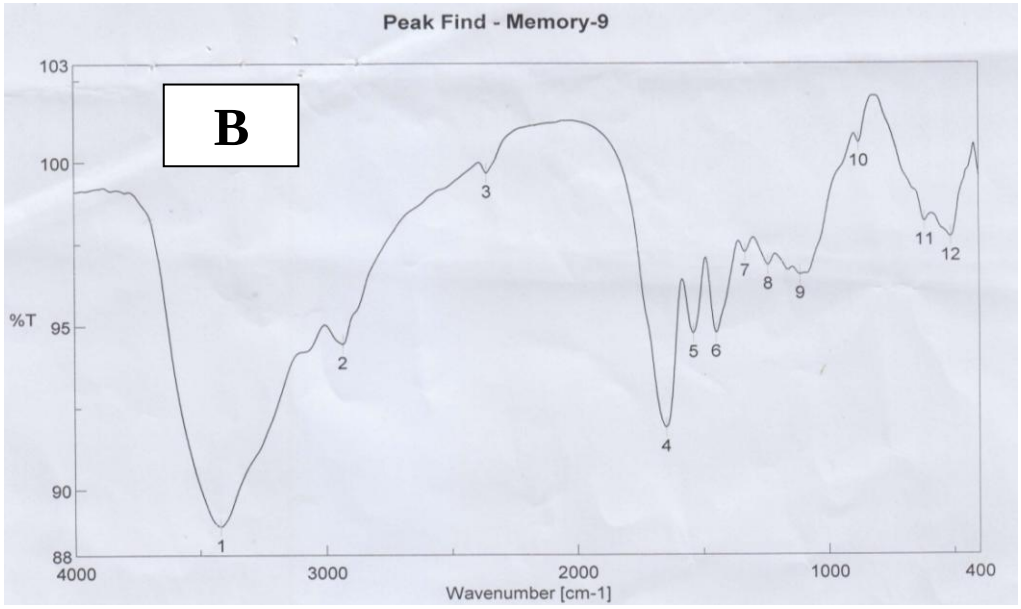
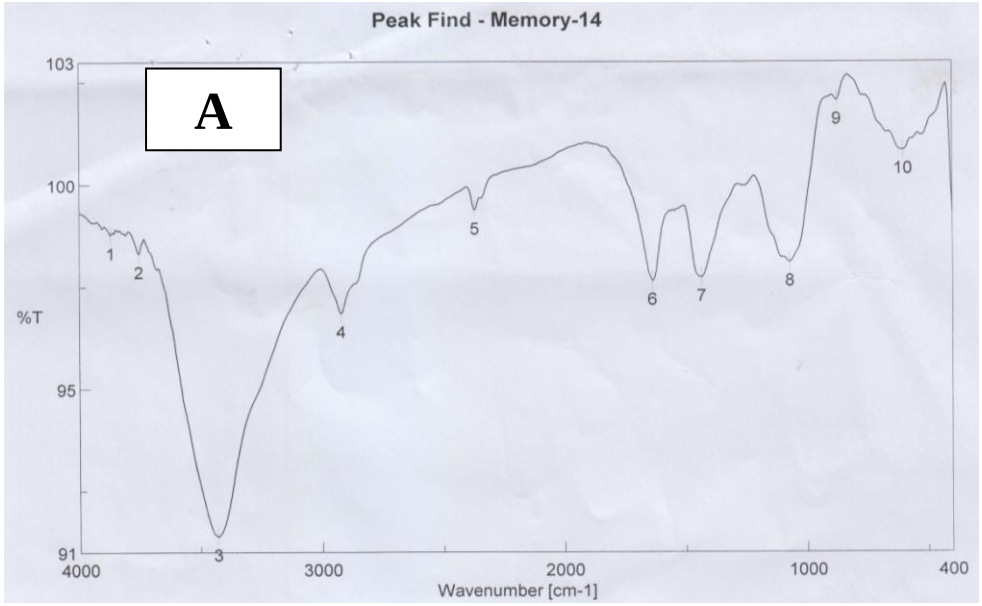


Figure.4 IR spectra of 0.01% direct red (DR) dye solution after incubation for 48h at 28 ^{0}C in (A) absence, and (B) presence of the yeast *M. guilliermondii*



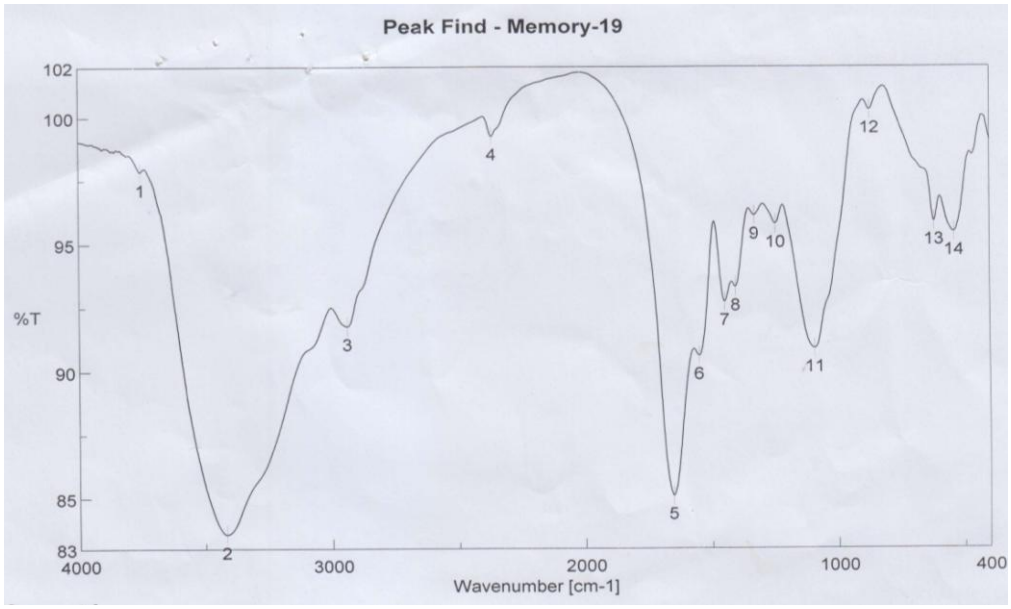


Figure.5 IR spectra of 0.01% reactive red (RBR) dye solution after incubation for 48h at 28 ^{0}C in (A) absence, and (B) presence of the yeast *N. diffluens*

Table.11 Effect of heavy metals on degradation % of direct red (DR81) dye by *M. guilliermondii* and reactive red (RBR) dye by *N. diffluens* grown for 48h at 28^0C

Yeast strain	Metal	Concentration (ppm)				
		0	20	50	100	150
M. guilliermondii	Co	89.69±1.01	86.80±2.21	82.52±1.70	78.57±0.99	74.34±2.28
	Zn	86.96±5.07	82.07±1.87	82.02±3.62	77.83±3.23	74.44±3.42
	Cu	88.68±0.32	72.30±3.52	73.56±1.96	38.31±2.17	02.26±6.80
	Fe	87.07±1.59	79.91±2.10	57.31±3.90	45.22±3.25	04.10±3.55
N. diffluens	Co	80.65±2.97	35.46±9.51	00.88±6.66	00.73±3.02	00.37±5.19
	Zn	79.88±2.77	80.91±5.06	80.20±2.65	75.89±5.30	76.61±7.72
	Cu	78.15±6.72	44.93±11.13	54.68±3.35	70.45±2.55	35.61±2.75
	Fe	79.25±9.37	58.34±7.38	55.98±4.87	53.25±8.55	51.41±1.00

These results also indicate that the biodegradation of direct dye increased significantly with increasing the dye concentration (up to 0.15%). Contrarily, increasing the concentration of reactive dye beyond 0.005 resulted in markedly decreased its biodegradation which might be due to the toxicity of dye or the breaking down products of this dye. This observation is similar to that obtained by Sumathi and Manju (2000) who reported that the reduction in the decolorization rates may be a result to toxicity of dye to *Aspergillus foetidus* and/ or inadequate biomass concentration for the uptake of higher concentration of dye. Fetyan *et al.,* (2016) found that *Saccharomyces cerevisiae* showed high efficiency to decolorize direct blue 71 reached to maximum activity (100%), and this decolorization activity decreased at the higher concentration above 200ppm which may be due to the toxic effect at these higher concentrations for the yeast.

Effect of carbon and nitrogen sources

Tables 8 shows that peptone is the best nitrogen source for decolorization of both dyes by those yeast strains as it reached to 83,88 and 67.20% of the supplemented Direct Red and Reactive Red respectively. El-Sayeh (2010) also found that the highest fungal growth and ligninolytic enzymes production with the subsequent dye bioremoval were obtained in the peptone containing medium. In terms of the influence of carbon source. The optimum value of Direct Red decolorization was detected in the presence of sucrose (Table 9) as it represented 77.8%, while in the case of Reactive Red dye, the optimum decolorization was 795% obtained in the presence of glucose. Miranda *et al.*, (1996) reported that using sucrose at an initial concentration of 10g/L produced a maximum color removal of 69% while using molasses of 5g/l equivalent to sucrose only produced a color removal of 45% for *Aspergillus niger*. Also Parshetti *et al.,* (2006) used molasses and sucrose as carbon sources for the decolorization of Malachite Green (91%) using *Kocuria rosea* MTCC 1532. Therefore the need to add carbon source depends on the organism and type of the dye to be treated.

Effect of salinity

Different concentrations (0, 3.0, 5.0 and 10.0 %) of NaCl were supplemented to the growth medium to evaluate the effect of salinity on dye biodegradation process *by M. guilliermondii* and *N. diffluens*. The results in Table 10 indicated that the decolorization of

Direct Red and Reactive Red dyes decreased from 77.9 and 53.5% to 20.87 and 7.99% by increasing the concentration of NaCl from 0.0 to 10.0% by the yeasts, respectively. This might be due to increasing the osmotic pressure outside the cell as suggested by Narjes *et al.,* (2013).

Effect of heavy metals

Since metal ions form a part of textile effluent, used to enhance the binding of the dye with the fiber, the effect of different heavy metals (cobalt, zinc, iron, copper) at different concentrations (0, 20, 50 ,100, 150ppm) were used to investigate their effects on the activity of the studied yeast strains on dye decolorization. Table 11 shows that there was a differential effect on decolorization process according to the metal ion, the dye and the yeast strain. Generally there was an adverse effect on decolorization of both dyes by increasing the metal concentration. However, the effect of copper and iron was significant on decolorization of direct red by *M. guilliermondii*. By the addition of 150 ppm $CuCl_2$ or $FeCl_3$ to the growth medium resulted in decreasing the degradation of active dye from 88.67 and 87.07% to 0.02.26 and 0.04.10% respectively.

The higher concentrations of copper or iron also had a less drastic effect on the degradation of reactive dye by *N. diffluens*, however the presence of cobalt exhibited complete inactivation to the decolorization process at moderate concentration (50 ppm). The effect of copper could be attributed to enhancement of laccase by copper. Murugesan *et al.,* (2009) reported that addition of elevated concentrations of Cu^{+2} ions (up to 1mM) enhanced the *Ganoderma lucidum* laccase activity and enhanced the decolorization process of Ramazol Black B and Ramazol Orange- 16 reactive dyes.

Acknowledgements

Authors are greatly thankful to Prof. Adel A. Nassar, professor of organic chemistry, Chemistry department, Faculty of science, menoufia University for helping in explanation of UV-visible spectra and IR analysis.

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How to cite this article:

Mohamed M. Gharieb, Azza M. Soliman and Esraa M. Hassan. 2020. Biodegradation of the Azo Dyes Direct Red 81 and Reactive Brilliant Red X-3B by Wild Strains of Yeasts *Meyerozyma guilliermondii* and *Naganishia diffluens*. *Int.J.Curr.Microbiol.App.Sci.* 9(03): 1243-1260. doi: https://doi.org/10.20546/ijcmas.2020.903.145