



Research paper

Degradation of indigo dye by a newly isolated yeast, *Diutina rugosa* from dye wastewater polluted soil

Paul O. Bankole^{a,b,e,*}, Adedotun A. Adekunle^b, Olayide F. Obidi^c, Olumide D. Olukanni^d, Sanjay P. Govindwar^e

^a Department of Science Laboratory Technology, Federal Polytechnic Ilaro, Ogun State, Nigeria

^b Department of Botany, University of Lagos, Lagos State, Nigeria

^c Department of Microbiology, University of Lagos, Lagos State, Nigeria

^d Department of Chemical Sciences, Redeemer's University, Ede, Osun State, Nigeria

^e Department of Biochemistry, Shivaji University Kolhapur, Maharashtra, India

ARTICLE INFO

Keywords:

Biodegradation

Indigo dye

Yeast

Diutina rugosa

Adsorption isotherms

ABSTRACT

Isolation, identification, and characterization of newly isolated yeast, *Diutina rugosa* capable of decolorizing indigo dye were investigated in this study. Molecular and phylogenetic analyses of 23S rRNA sequence data indicated that the yeast belonged to the new genus, *Diutina*. The optimization of physicochemical parameters such as pH of the solution (2–8), initial dye concentration (10–60 mg L⁻¹), adsorbent mass (0.1–2 g), and temperature (10–50 °C) was studied to scale-up the conditions of dye removal. Furthermore, complete decolorization (99.97%) of indigo dye (10 mg L⁻¹) was achieved at pH 2, temperature 30 °C and 2.0 g cell biomass within 5 days. Degradation was monitored through UV–vis spectrophotometric, FTIR, and GCMS analyses. The results of FTIR analysis obtained revealed the loss and shifts in spectra peaks of the experimental in comparison with the biological control. Possible degradation pathway was proposed using the intermediate metabolites; 1, 2-dihydro-3H-indol-3-one and cyclopentanone obtained through GCMS analysis. The enzyme analyses revealed significant inductions and major roles played by NADH-DCIP reductase and lignin peroxidase in the asymmetric cleavage, initial reduction and deamination of indigo dye. The equilibrium experimental data were fitted to Langmuir, Freundlich and Temkin adsorption isotherms with high adjusted coefficient of determination values; adjR² = 0.907, adjR² = 0.867, and adjR² = 0.965 respectively. However, the Langmuir and Temkin adsorption isotherms affirmed the monolayer and heterogeneous biosorption characteristics of *Diutina rugosa*. Temkin adsorption isotherm model (R² = 0.971) represented the best fit of experimental data than other isotherm models.

1. Introduction

Indigo is an organic dye with a characteristic blue colour belonging to the vat-indigoids class of dyes. Most indigo dye produced nowadays are of synthetic origin although some are still been produced naturally from plants. Indigo is mostly applied to cellulosic fibers (cotton) after reduction to leuco form in an alkaline dye bath [1]. Indigo dye is largely used over the world in the production of denim [2] and as a colorant for cloth dyeing (“adire”/“gara”) in West Africa [3]. It is used extensively

by textile industries and small scale textile dyers. A sizeable amount of the dyestuffs is released during textile manufacturing processes. The textile industry releases dye contaminated in proportional to the utilization of large quantity of water. Indigo dye, of all vat dyes, is largely soluble in solvents of organic origin. This makes indigo quite recalcitrant and difficult to mineralize on the application of most bio-treatment techniques. The presence of dyes or their degraded metabolites poses a grave environmental threat [4]. Malfunctioning and damage organs and organ systems could arise as a result of human

Abbreviations: UV–vis, ultra violet visible spectrophotometer; FTIR, fourier transform infrared spectroscopy; GCMS, gas chromatography mass spectrometry; R, correlation coefficient; R², coefficient of determination; AdjR², adjusted coefficient of determination; S.E.M, standard error of means; q_e, amount of dye adsorbed (mg g⁻¹); b_T, the adsorbent at the equilibrium; q_t, amount adsorbed by the adsorbent at any time (mg g⁻¹); C_e, concentration of the indigo dye at the equilibrium (mg L⁻¹); C₀, initial concentration of the indigo dye (mg L⁻¹); q_{max}, maximum amount adsorbed (mg g⁻¹); t, contact time (min); n, the order of adsorption with respect to the effective concentration of the adsorption active sites present on the surface of the adsorbent; R, the universal gas constant (8.314 J K⁻¹ mol⁻¹); T, the absolute temperature at 298 (K); b_T, Temkin isotherm constant; A_e, Temkin isotherm equilibrium binding constant (L g⁻¹); K_F, the Freundlich equilibrium constant (mg g⁻¹ (mg L⁻¹)^{-1/n}); n_F, the Freundlich exponent (dimensionless); K_L, Langmuir isotherm constant (L mg⁻¹); B, heat sorption constant (J mol⁻¹); Q₀, maximum monolayer coverage capacity (mg g⁻¹); DR, *Diutina rugosa*

* Corresponding author at: Department of Science Laboratory Technology, Federal Polytechnic P.M.B 50 Ilaro, Ogun State, Nigeria.

E-mail addresses: paul.bankole@federalpolyilaro.edu.ng, pbank54@yahoo.co.uk (P.O. Bankole).

<http://dx.doi.org/10.1016/j.jece.2017.08.050>

Received 11 July 2017; Received in revised form 20 August 2017; Accepted 24 August 2017

Available online 02 September 2017

2213-3437/ © 2017 Elsevier Ltd. All rights reserved.

exposure to textile dyes disposed indiscriminately to the environment [5]. Optimal circulation of oxygen and light absorption in an aquatic environment is distorted by textile dyes contamination. This in turn, drastically affect photosynthetic and other physiological processes of plants, diatoms, and algae. Chemical coagulants and flocculants alongside activated sludge process are mostly employed for complete mineralization and treatment of water insoluble vat dyes [6]. Installation, maintenance and economic sustenance of advanced chemical and physical dye biotreatment processes have proven to be costly and relatively expensive. Hence, the development of much cheaper and eco-friendly biological processes [7]. Mycodecolorization has been found very effective and efficient [8]. Meanwhile, few research reports are available on the efficiency of fungi in the degradation of indigo dye [9]. Fungi hyphae and cells exhibit tenacity on exposure to dye [10] with the ability to degrade a vast number of organic pollutants, vat dyes inclusive [11]. Fungi dissipate non-specific and non-stereoselective oxidative enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase [12]. Biodegradation of wide range of organic pollutants is made possible by the non-specific nature of fungal enzyme systems [13]. Fungal unique carbon and nitrogen metabolism system ensure their survival in virtually all habitats [14]. Interestingly, researchers are drifting towards using isolates from sites contaminated with dyes for biotreatment because of their adaptation to survive harsh conditions. Recently, the use of yeast biomass in dye bioremediation processes have witnessed tremendous preference by most researchers. Yeasts are relatively economic and cheaply produced source of biomass for bioremediation due to their ability to proliferate at low pH medium, extreme temperature and high concentration of pollutants [15]. Some yeasts have been found quite effective in treating high strength organic wastewaters and highly colored substances such as VAT dyes [16].

Yeast genera such as *Issatchenkia*, *Debaryomyces*, *Pseudozyma*, *Kluyveromyces*, *Schizosaccharomyces*, and *Saccharomyces* have been extensively studied for dye degradation potentials [17]. Specifically, Jadhav and Govindwar [18] reported 95.5% degradation efficiency of malachite green mediated through secretion of NADH-DCIP reductase and MG reductase enzymes by *Saccharomyces cerevisiae* in plain distilled water. Goodness of fit was recorded when equilibrium data obtained from the degradation of basic violet 3 by *Candida tropicalis* were subjected to Langmuir adsorption isotherm [19]. Aksu and Donmez [20] reported biosorption capacities of nine different yeast isolates on remazol blue under ambient laboratory conditions. The yeasts; *Kluyveromyces marxianus* IMB3, *Rhodotorula* sp. and *Rhodotorula rubra* has been found to be very proficient in the decolorization and degradation of different textile dyes through adsorption [21–23]. Furthermore, the dye decolorization efficiency of baker's yeast, *Saccharomyces cerevisiae* has equally been reported [4]. However, there is the paucity of information on the biodegradation potential by the yeast of the new genus, *Diutina*. In this light, this research is in furtherance of our work at discovering vat dyes biodegradation efficiency of newly isolated yeast species, *Diutina rugosa* from dye wastewater polluted soil. Scaling up of physicochemical parameters (pH, temperature, dye concentration, biomass) and adsorption isotherms (Langmuir, Freundlich and Temkin) in indigo dye degradation by *Diutina rugosa* were also studied.

2. Materials and methods

2.1. Properties of indigo dye

Indigo dye (C.I: Vat Blue I) used is of high analytical grade (98% purity) with molecular formula $C_{12}H_8O_2N_2$ and chemical structure (Fig. 1). The dye was chosen amongst other vat dyes because of its widespread and extensive use by textile industries and local textile dyers throughout the world.

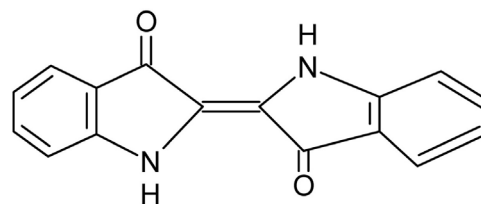


Fig. 1. Chemical structure of indigo dye. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Chemicals and dyestuff used

Yeast extract agar and broth, potato dextrose broth (PDB) and other chemicals were obtained from Kiri Industries, Ahmedabad, Gujarat, India. Aniline-2-sulfonic acid and Riboflavine-2-sulfonic acid were purchased from Kemira Chemicals, Bradford, United Kingdom. Tartaric acid, Chloranil, Dimethylformamide (DMF), 2, 6-dichlorophenol indophenol (DCIP), nicotinamide adenine dinucleotide (di-sodium salt), veratryl alcohol and others were bought from Sigma-Aldrich, USA. The dyes, vat yellow 4, vat violet 1, vat blue 3, vat green 1 and indigo dye were purchased from DyStar Company, Germany. Other chemicals are of high laboratory, purity and analytical grade.

2.3. Dye wastewater polluted soil collection

The highly colored waste water polluted soil was collected from Itoku ($7^{\circ}48'09.62''N$; $4^{\circ}28'06.63''E$), Abeokuta, Ogun State, Nigeria in an airtight plastic can and tightly stoppered. Filter paper was used in removing large suspended particles upon soil suspension in sterile distilled water and maintained at pH 7.0. It was later stored at a temperature ($4^{\circ}C$) for further experimental processes to prevent contamination by non-indigenous microbes.

2.4. 23S rRNA sequencing and documentation

Sequencing of isolated yeast's 23S rRNA gene was achieved at Laragen Incorporated, 10601 Virginia Avenue, Culver City, CA. Sequence search/comparison and alignment procedures were achieved using Basic Local Alignment Sequence (BLASTn) and CLUSTALW pairwise-multiple alignment program tools available on National Centre for Biotechnology Information (NCBI) server and Molecular Evolutionary Genetics Analysis (MEGA) 7 software respectively. The sequence was refined manually and an Accession Number; KR262715 was assigned upon submission to the NCBI using the BankIt Tool [24].

2.5. Isolation and identification of yeast

Diutina rugosa stock culture was maintained on yeast extract agar isolated previously from indigo dye waste water contaminated soil in Itoku, Abeokuta, Ogun State, Nigeria. The sampling location is known worldwide for extensive use of indigo dye in making tie and dye cloths ('Adire'). The stock culture and yeast biomass were maintained at $4^{\circ}C$ and $28^{\circ}C$ respectively on yeast extract broth containing maltose 8% (w/v), peptone, yeast extract agar 2% (w/v) and microbiological agar 4% (w/v). The collection of the yeast's cells were done after centrifugation at 10000 rpm for 10 min. The yeast's pure DNA genome was isolated through modified Cetyl Trimethyl Ammonium Bromide (CTAB) protocol. The D1/D2 domains of the larger subunits of the yeast's gene were used in amplifying the Internal transcribed spacer (ITS) region of the 23S rRNA sequence. The sequence data obtained thereafter were compared with previously documented ones available on NCBI database using blastn tool. The yeast was identified as *Diutina rugosa* based on nucleotide sequence homology, documented and accorded a strain code LAG on the NCBI library. The inference was done using the neighbor-joining tree method [25] of the phylogenetic analyses

involving 26 nucleotide sequences with a total of 5242 positions. Molecular Evolutionary Genetics Analysis (MEGA version 7 software) [26] was used in computing the tree scale, branch lengths and evolutionary distances after proper editing and deletion of all ambiguous positions from each sequence pair.

2.6. Physicochemical parameters variation during decolorization

Overall, the decolorization studies were carried out under anoxic static conditions at pH 7, ambient temperature (30 °C), indigo dye concentration (10 mg L⁻¹) and yeast biomass (2 g) cells. Decolorization experiments were carried out within 5 days ($\lambda_{\text{max}} = 680 \text{ nm}$).

2.6.1. Decolorization efficiency of *Diutina rugosa* on different vat dyes

Five different types of vat dyes (vat yellow 4, vat violet 1, vat blue 3, vat green 1 and indigo) were used to study the decolorization proficiency of *Diutina rugosa* at ambient temperature (~30 °C). The decolorization experimental set up involved Erlenmeyer flasks (in triplicates) with each containing yeast extract broth, 2.0 g biomass of *Diutina rugosa* cells supplemented with 10 mg L⁻¹ dye concentration each of all the dyes. An aliquot (2 mL) from the set up earlier withdrawn was centrifuged at 10000 rpm for 10 min in order to separate yeast's biomass cell. The absorbance of the supernatant was measured at 680 nm for indigo dye, 580 nm for vat yellow 4, 450 nm for vat blue 3, 620 nm for vat green 1 and 650 nm for vat violet 1. The changes in absorbance (λ_{max} of the dyes) was monitored (in triplicates) with UV/Vis spectrophotometer (Hitachi U-2800; Japan) daily for 5 days. Decolorization (%) was determined as follows:

$$\text{Decolorization (\%)} = \frac{\text{Absorbance (initial)} - \text{Absorbance (final)}}{\text{Absorbance (initial)}} \times 100$$

2.6.2. Effect of pH and temperature variations on the decolorization of indigo dye

Erlenmeyer flasks containing yeast extract medium and dye (10 mg L⁻¹) were kept at different temperatures 0, 10, 20, 30, 40 and 50 °C and pH 2, 3, 5, 6 and 7 adjusted with diluted H₂SO₄ and KOH for 5 days in non-shaking anoxic condition, with their abiotic controls of flasks containing dye only.

2.6.3. Effect of variations in cell biomass of *Diutina rugosa* on the decolorization of indigo dye

Immobilized cell mass (0.1, 0.2, 0.5, 1 and 2 g) were used to study the effect of *Diutina rugosa* on the decolorization of indigo dye (10 mg L⁻¹) at room temperature (30 ± 2 °C)

2.6.4. Effect of initial concentrations on decolorization of indigo dye by *Diutina rugosa*

Six different indigo dye concentrations 10, 20, 30, 40, 50, and 60 mg L⁻¹ (in triplicates) was used in scaling up the effect of initial dye concentrations. Proportionally, Erlenmeyer flasks containing different concentrations of the dye and yeast extract broth with no yeast biomass was used as biological controls.

2.7. Enzyme extraction and activities during decolorization

The raw biomass collected on the yeast extract medium {maltose 8% (w/v), peptone, yeast extract agar 2% (w/v) and microbiological agar 4% (w/v)} were used for the enzyme bioassay. The enzyme source (5 mL) contained 0.1 mL of enzyme solution homogenized through sonication by giving five strokes each of 10 s, at 1.5 min intervals, at 60 amp, and at 4 °C., 60 μM DCIP, 30 mM NADH in 60 mM potassium phosphate buffer (pH 7.4). Lignin peroxidase (LiP) activity was determined through oxidative conversion of veratryl alcohol to veratrylaldehyde by H₂O₂. The relative increase in absorbance (at 310 nm) due to the oxidation of veratryl alcohol to veratrylaldehyde was then

monitored [27]. The composition of the reaction mixture (4 mL) includes; 0.5 mL veratryl alcohol (10 mM), 3.0 mL citrate buffer (pH 2.0) and 0.5 mL enzyme. Within 5 min, the reaction mixture initiation was effected through the supply of 100 μL of 0.8 mM H₂O₂ with bovine-serum albumin (as standard) being used in the determination of protein according to Lowry et al. [28]. Relative changes in absorbance (unit min⁻¹ mg of protein⁻¹) was used in expressing the average rates of a unit of enzyme activity (in triplicates).

Phenol red (10) was used in the determination of Manganese peroxidase (MnP) activity. The reaction mixture (1 mL in total) is composed of 25 mM lactate, 0.1 mM MnSO₄, 1 mgmL⁻¹ of bovine serum albumin (Sigma), 0.1 mg of phenol red (Merck) mL⁻¹, and 0.5 mL of culture filtrate in 20 mM sodium succinate buffer (pH 4.5). The addition to a final concentration of 0.1 mM of hydrogen peroxide was used in initiating the reaction mixture. It was thereafter stopped with the addition of 50 mL of 10% NaOH after 2 min and A₆₁₀ was then measured. The absence of manganese ions (Mn²⁺) through the non-supply of Manganese tetraoxosulphate VI solution was used as phenol red oxidation control experiments. The evaluation of manganese peroxidase activity was done through subtraction of phenol red (10) values obtained in the presence and absence of manganese ions respectively. The activity was later expressed as the increase in A₆₁₀ per minute per milliliter.

The reaction mixture containing 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (12%) in 0.1 M acetate buffer (pH 4.9) was used in determining laccase activity of *Diutina rugosa*. The oxidized reaction mixture was later measured at 420 nm [29].

Salokhe and Govindwar [30] method was used with slight modifications in the determination of NADH-DCIP reductase activity. The coefficient (extinction of 19 mM⁻¹ cm⁻¹) was used in evaluating the reduction in DCIP. Relative changes in absorbance (unit min⁻¹ mg of protein⁻¹) was used in expressing the average rates of a unit of enzyme activity (in triplicates). Lowry et al. [28] method were employed in protein determination with bovine serum albumin (standard).

2.8. Metabolites analyses

Decolorization of the indigo dye was determined by UV-visible spectrophotometric analysis (Hitachi U-2800; Japan) using crude extract, Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectroscopy (GCMS). The yeasts biomass were removed after dye decolorization by filtration followed by centrifugation at 10000 rpm for 10 min in order to extract the metabolites. The metabolites were synthesized from the obtained supernatant with an equal volume of ethyl acetate; dried over anhydrous Na₂SO₄, dissolved in a small quantity of HPLC grade methanol and used for further analytical studies like FTIR and GCMS analyses.

2.8.1. FTIR analysis

The samples were prepared using spectroscopic pure KBr (93:7 ratio), pellets were fixed in the sample holder and analyzed [31]. The analysis was carried out using Shimadzu 8400 S FTIR spectrometer (Shimadzu Corporation, Japan) in mid infra-red region of 400–4000 cm⁻¹ with 16 scan speed.

2.8.2. GCMS analysis

The temperature programming mode with a Restek column (non-polar; XTI-5, 0.25 mm id, 60 m long) with ionization voltage 70 eV was used for Gas chromatography analysis. The column temperature was linearly raised from 80 °C for 2 min to 280 °C at 10 °C per minute and maintained further for 7 min. While the GCMS interface temperature 290 °C, that of injection port was kept at 280 °C. Helium gas with a flow rate of 1.0 mL min⁻¹ was used as carrier gas. Shimadzu QP 2010 GCMS Engine (Shimadzu Corporation, Japan) was used following the earlier procedure reported by Patil and Jadhav [32]. Comparison of retention time, mass spectra obtainable in the GCMS solution software and

fragmentation pattern were used in identifying the metabolites.

2.9. Adsorption isotherm studies

Biosorption efficiency of the yeast isolate at different aqueous equilibrium concentrations of the indigo dye was tested using different adsorption isotherm studies. The experimental set up involved the addition of 2.0 g biomass of the adsorbent (*Diutina rugosa* cells) differently with varying concentrations (0, 10, 20, 30, 40, 50 and 60 mg L⁻¹) of the adsorbate (indigo dye) in a static condition for 5 days. Upon withdrawal of 2 mL aliquot from the reaction medium, centrifugation at 10000 rpm for 10 min was done. From the homogeneous medium, 2 mL aliquot was withdrawn and centrifuged at 10000 rpm for 10 min. Thereafter, the UV-visible spectrophotometer was used in determining the amount of dye left from the supernatant obtained. The equilibrium value 'q_e' in (mg g⁻¹), of the amount of indigo dye adsorbed, was equally determined thus:

$$q_e = \frac{C_i - C_e}{N} \times V \quad (1)$$

where (q_e) is the mass (mg) of the indigo dye adsorbed per amount (g) dry *Diutina rugosa* cells; (V) is the total reaction volume (L), and (N) is the mass of dry *Diutina rugosa* cells (g). All physicochemical parameters were analyzed according to standard methods for the examination of water and wastewater [33]. C_i and C_e (mg L⁻¹) are the initial and equilibrium/residual concentrations of indigo dye solutions. The percentage of decolorization (% D) of indigo dye by *Diutina rugosa* is defined as:

$$\%D = \frac{C_i - C_e}{C_e} \times 100 \quad (2)$$

where C_i and C_e are the initial and the equilibrium dye concentrations (mg L⁻¹) respectively. Adsorption experiments were done in triplicates to avoid random error.

2.9.1. Langmuir adsorption isotherm

The isotherm studies were carried out to quantitatively determine the formation of a homogeneous indigo dye monolayer on the biomass cell surface of *Diutina rugosa* till no further adsorption takes place. Langmuir isotherm was used to experimentally describe the equilibrium distribution between the indigo dye and yeast's biomass cell. The isotherm is to further validate the monolayer adsorption of indigo dye molecules onto different active sites of the yeast biomass. The model assumes uniform energies of indigo dye adsorption onto the surface of yeast biomass with no relative transmigration of indigo dye in the plane of the yeast cells. Based the aforementioned experimental facts, the model was represented by the equation below:

$$\frac{1}{q_e} = \frac{1}{Q_o} + \frac{1}{Q_o K_L C_e} \quad (3)$$

Where: C_e = the equilibrium concentration of indigo dye (mg L⁻¹), q_e = the amount of indigo dye adsorbed per gram of the yeast cells at equilibrium (mg g⁻¹), Q_o = maximum monolayer coverage capacity (mg g⁻¹), K_L = Langmuir isotherm constant (L mg⁻¹). Q_{max} and K_L values were extrapolated from the intercept and slope of the Langmuir plot of 1/q_e versus 1/q_e [34].

2.9.2. The Freundlich adsorption isotherm

This model was employed to experimentally and quantitatively determine the heterogeneous adsorption process of indigo dye by *Diutina rugosa* cells. The model was used to further confirm the proportional increase in yeast biomass with increasing dye concentration in the interaction medium [35]. The model equation is expressed as follows:

$$q_e = K_F C_e^{1/n} \quad (4a)$$

Where 'C_e' is the amount of indigo dye left in the supernatant after the interaction with *Diutina rugosa* cells (mg L⁻¹), q_e is the equilibrium of adsorption (mg g⁻¹); K_F is the Freundlich constant (mg g⁻¹) (L mg⁻¹)^{1/n}. Eq. (4a) could be linearized through logarithms:

$$\text{Log}q = \frac{1}{n} \text{Log}C_e + \text{Log}K_F \quad (4b)$$

2.9.3. The Temkin adsorption isotherm

This model is to explicitly describe the interactions between the *Diutina rugosa* cells and the indigo dye. While ignoring extremely low and very high concentration, the model was used to experimentally quantify the vital role played by temperature (heat) of the adsorption interaction medium between the indigo dye and yeast biomass. The model is derived by plotting the quantity of the dye sorbed q_e (mg g⁻¹) as implied in Eq. (5) against ln C_e and the constants (A_t and b_T) were determined from the slope and intercept [36]. The model is described as follows:

$$q_e = B \ln A_t + B \ln C_e \quad (5)$$

where

$$B = \frac{RT}{b_T}$$

b_T = Temkin isotherm constant, R = Universal gas constant = 8.314 J mol⁻¹ K⁻¹, A_t = Temkin isotherm equilibrium binding constant (L g⁻¹), T = Temperature at 298 K, B = heat sorption constant (J mol⁻¹), C_e is concentration of the indigo dye in solution at equilibrium (mg L⁻¹)

3. Results and discussion

3.1. Molecular identification of *Diutina rugosa*

Accession number; KR262715.1 was assigned upon due submission of the 23S rRNA molecular sequence data (D1/D2 region) at the National Centre for Biotechnology Information (NCBI) database. Furthermore, comparative analyses of the data done with previous submission in NCBI database revealed, *Diutina rugosa* has 100% maximum homology with those of *Diutina rugosa* (KY107668.1), *Candida rugosa* strain CBS 613 (KT336717.1), *Candida rugosa* strain ATCC 10571 (GU144663.1), *Candida rugosa* strain 8 (EF375701.1) and *Candida rugosa* (AB437389.1). The phylogenetic tree further revealed that *Diutina rugosa* (KR262715) has closest homological identity with *Diutina rugosa* (KY107668.1) which are all part of the clade including *Candida rugosa* strain CBS 613 (KT336717.1), *Candida rugosa* strain ATCC 10571 (GU144663.1), *Candida rugosa* strain 8 (EF375701.1) and *Candida rugosa* (AB437389.1) with a major outgroup, *Diutina catenulata* (KY107665.1) (Fig. 2).

3.2. Variations in physicochemical parameters

3.2.1. Decolorization of different vat dyes by *Diutina rugosa*

When subjected to the same condition (10 mg L⁻¹, 30 °C), indigo dye amongst other VAT dyes showed high susceptibility to decolorization by *Diutina rugosa* in a pre-screening experiment. The results revealed decolorization (99, 91, 90, 79 and 66%) for indigo, vat green 1, vat brown 3, vat yellow 1 and vat violet 4 respectively (Fig. 3). This, however, ignited our interest to further study the effect of *Diutina rugosa* on indigo dye.

3.2.2. Decolorization at different pH

Relative decrease in decolorization efficiency was observed from acidic pH 2 to alkaline pH 8 (Fig. 4a). Although in this present study, decolorization was observed at pH 4, 5, and 6 but optimum decolorization (99.97 and 97.85%) was however observed at pH 2 and 3

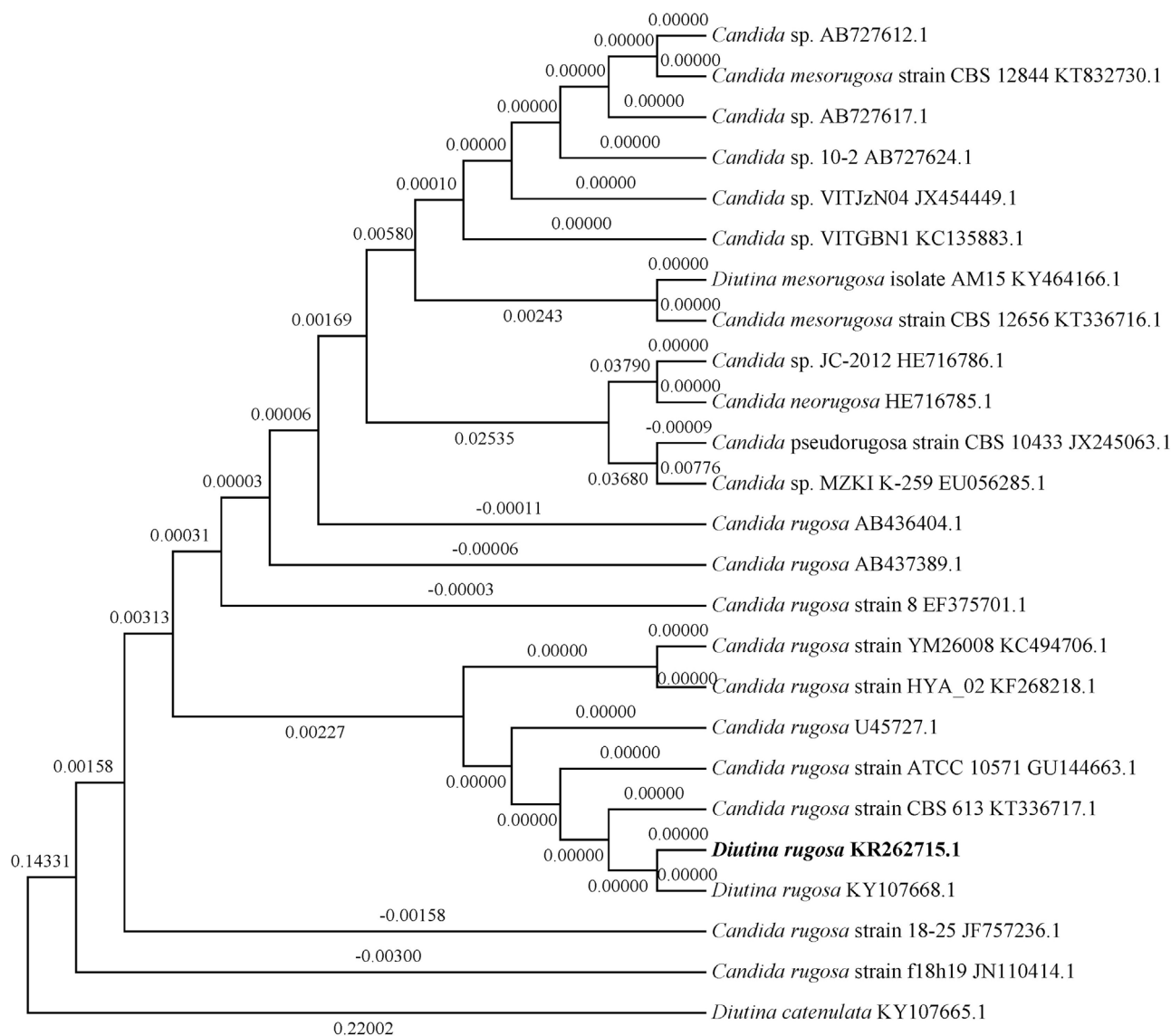


Fig. 2. Evolutionary relationships 23S rRNA sequence of *Diutina rugosa*. The numbers at the nodes indicate the levels of bootstrap support based on maximum-composite likelihood method.

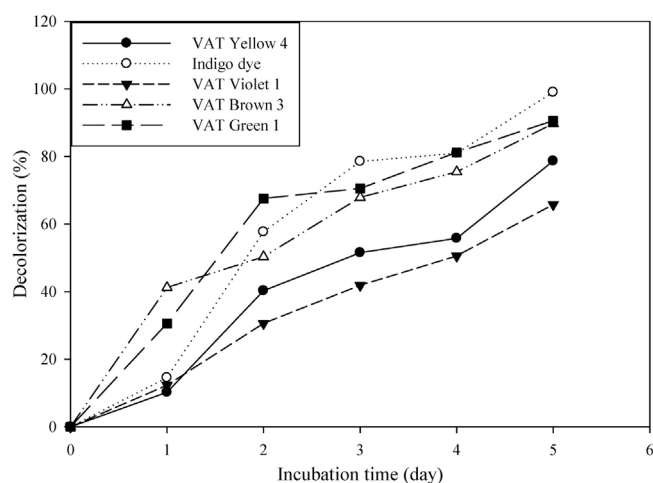


Fig. 3. Decolorization efficiency of *Diutina rugosa* on different vat dyes.

respectively. This was however in agreement with previous works reported by Pajot et al. [37] and Tian et al. [38]. Biosorption is highly favored at lower pH owing to strong electrostatic forces existing between charged dye molecules and the yeast cell [39]. This further suggested that in acidic medium, protonation of functional groups on yeast biomass surface is greatly and optimally enhanced [40]. Decolorization of dyes is largely due to the electrostatic interactions between the negatively charged indigo dye anions and positively charged *Diutina rugosa* cells. Iscen et al. [41] reported of adsorption depletion owing to electrostatic repulsion brought about by increases in the negatively charged sites on the biomass surface as a result of attendant increase in pH.

3.2.3. Decolorization at varying temperatures

While significant decolorization were recorded at lower temperatures (10 and 20 °C), optimum decolorization (99.95%) of indigo dye was observed at almost ambient temperature (30 °C) (Fig. 4b). Decolorization by biosorption is actively aided through increasing temperature of the reaction medium of the yeast biomass and indigo dye [39]. Our study characteristically showed that mycoremoval of colour usually decreases with attendant increase in temperature indication

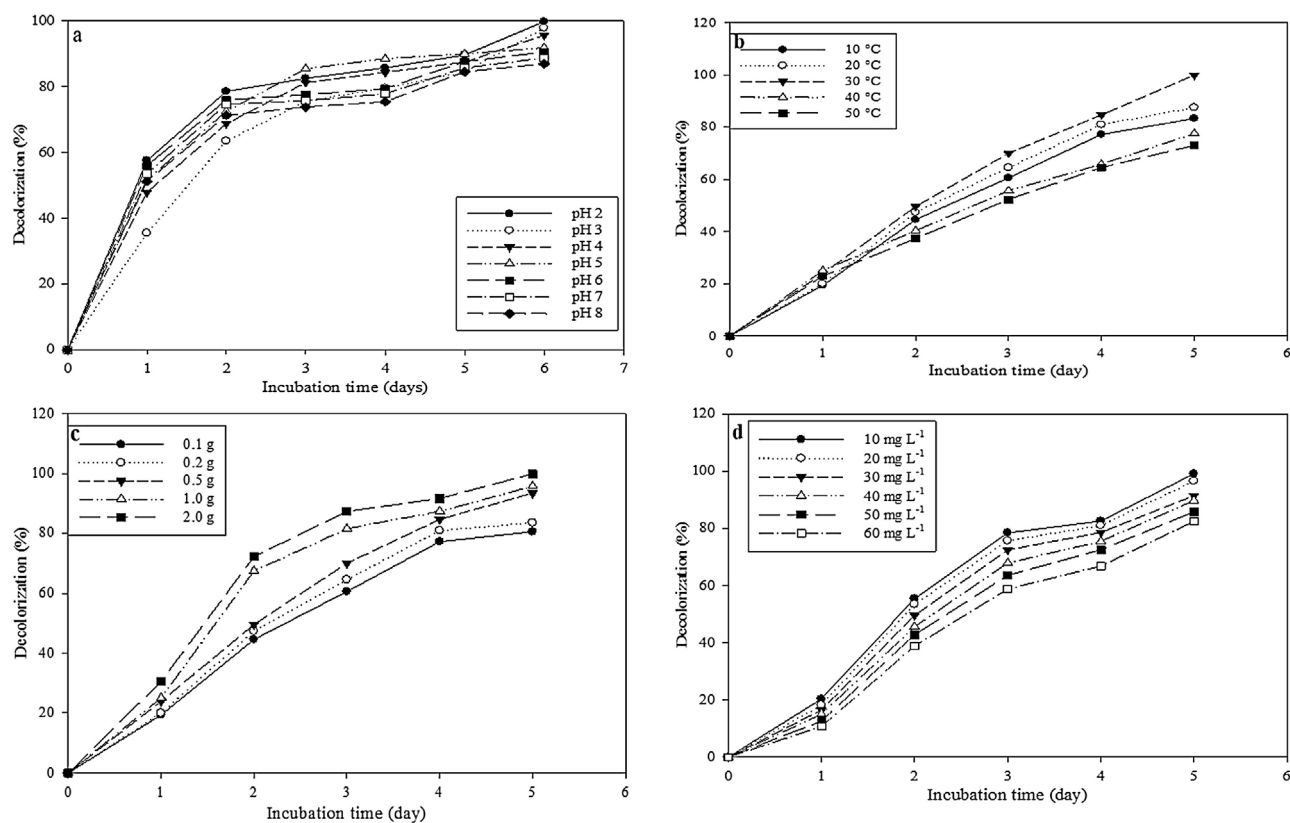


Fig. 4. Effect of (a) pH (b) temperature (°C) (c) cell biomass (g) (d) initial dye concentration (mg L^{-1}) on decolorization of indigo dye by *Diutina rugosa*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Enzyme activities (laccase, NADH-DCIP reductase, manganese peroxidase and lignin peroxidase) of *Diutina rugosa* cells at 0 h (control) and after 96 h of exposure to indigo dye.

Enzymes	Control	Experimental (after decolorization)
Laccase ^a	0.35 ± 0.002	$0.22 \pm 0.065^{**}$
NADH-DCIP reductase ^b	6.87 ± 0.11	$8.79 \pm 0.07^{***}$
Manganese peroxidase ^a	0.019 ± 0.010	$0.025 \pm 0.004^{**}$
Lignin peroxidase ^a	0.075 ± 0.002	$0.095 \pm 0.002^{**}$

Values are mean of three experiments \pm SEM. Significantly different from control cells at $^{**}p < 0.01$; and $^{***}p < 0.001$ by one-way ANOVA with Tukey-kramer comparison test.

^a Units $\text{min}^{-1} \text{ml}^{-1}$.

^b $\mu\text{g DCIP reduced min}^{-1} \text{mg}^{-1} \text{cells}$.

that the process was exclusively exothermic. Decreasing decolorization efficiency of indigo dye by *Diutina rugosa* recorded at high temperatures (40 and 50 °C) (Fig. 4b). was evidently due to the destruction of the yeast biomass surface [42]. Active sites on the cell surface of most fungi works optimally during decolorization of different dyestuffs at temperatures ranging from 25 to 37 °C [24]. It equally suggested possible denaturation of extracellular enzymes of the yeast.

3.2.4. Decolorization at varying yeast cell biomass

Microbial decolorization of dyestuff is enhanced with optimum proliferation of cells which in turn favors' enzyme production [43]. Based on the aforementioned, we decided to study the effect of different yeast biomass cells (0.1, 0.2, 0.5, 1.0, and 2.0 g) on decolorization efficiency. It was however observed in the study, that higher biomasses (of 1 and 2 g) ensured proliferation of sufficient cells which resulted in maximum decolorization of indigo dye by (95.76 and 99.87%) respectively (Fig. 4c). Chen and Ting [44] have reported the advantages of using 1 g or more biomass. This benefit is attributed to production of

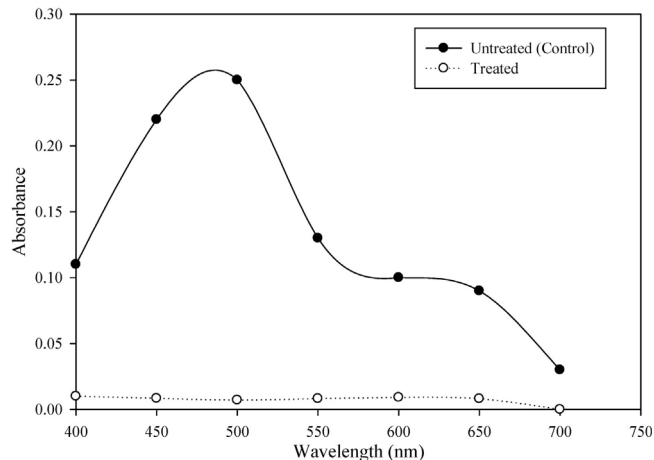


Fig. 5. UV/Vis spectra of indigo dye before and after treatment with *Diutina rugosa*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

more cells capable of secreting extracellular enzymes necessary for biodegradation [45]. This trend can be predicated to larger surface area and availability of more sorption sites [46].

3.2.5. Decolorization at varying dye concentrations

Decreasing decolorization efficiency (99.1, 96.54, 91.44, 89.67, 85.76 and 82.44%) was directly proportional with increasing concentration of indigo dye (10, 20, 30, 40, 50 and 60 mg L^{-1}) (Fig. 4d). This suggests that, at higher dye concentrations adsorption is negatively affected [47]. Lower dye concentration of indigo dye propels and favors mass transfer resistance between solid and aqueous surfaces [48]. Dye decolorization capacity of yeast is greatly depleted with increasing dye

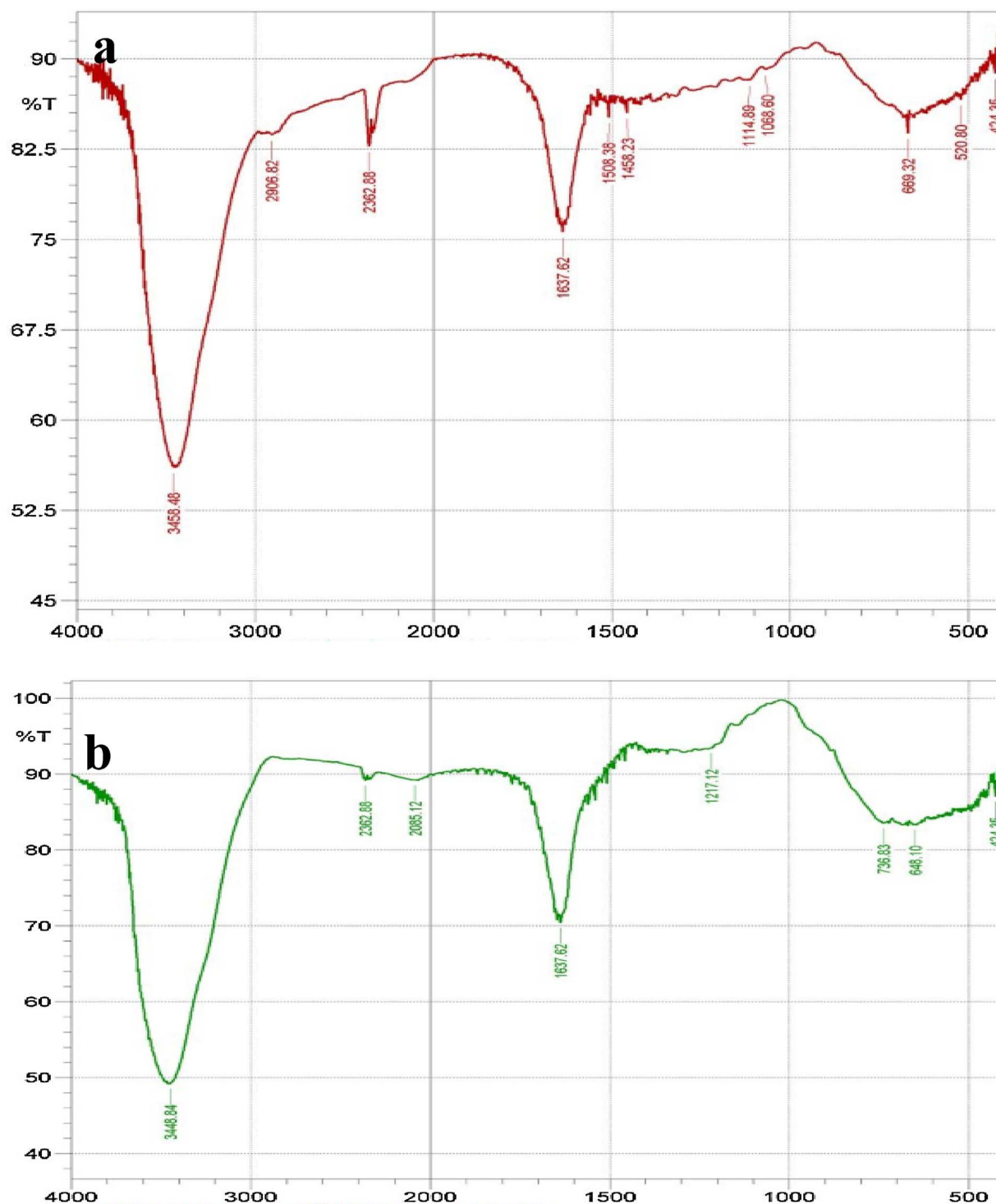


Fig. 6. FTIR spectra of (a) untreated indigo dye (b) degraded product of indigo dye by *Diutina rugosa*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

concentration due to surface saturation action on the yeast surface. Iscen et al. [41] reported similar decolorization results when RR239 concentration was raised above 250 mg L^{-1} . Furthermore, the results of this study were in concordance with diminishing percentage decolorization of Acid Red 18 and Reactive Black 5 respectively by *Schizopyllum commune* [49].

3.3. Enzyme analysis

Significant inductions of NADH-DCIP reductase and LiP reductase

enzymes was observed in the decolorization set up (control with no indigo dye) and experimental (with the indigo dye + DR) (Table 1). This suggested that the specificity of the enzymes through reductive and oxidative systems deployed in the biodegradation of indigo dye [50]. In the present study, this would be the first report on the induction of lignin peroxidase (LiP) and NADH-DCIP reductase in *Diutina rugosa* in relation to the biodegradation of indigo dye. LiP induction affirms the presence of lignin component typical of yeast cells [51]. After decolorization, significant high induction of NADH-DCIP reductase (8.79 ± 0.07) and LiP (0.095 ± 0.002) enzymes respectively

Table 2
FTIR analysis of indigo dye before and after treatment with *Diutina rugosa*.

Indigo VAT (Control)		Indigo VAT (Treated)	
Peak (cm ⁻¹)	Bond	Peak (cm ⁻¹)	Bond
3458.48	N–H Str.	3448.84	N–H Str.
2908.82	O–H Str.	2362.88	O–H Def.
1637.62	C–H Str.	1637.62	C–H Str.
1508.38	NH Vibr.	1217.12	N–H Str.
1458.23	N=N Str.	736.83	N=O Str.
1114.89	N=N Str.	648.10	C–N Str.
1068.60	N=N Str.	424.35	C–OH Str.
669.32	S=O Str.		
520.80	NO ₂ Str.		
424.35	C–H Def.		

Def – Deformation; Str – Stretching; Vibr. – Vibration.

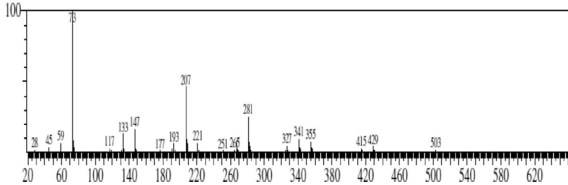
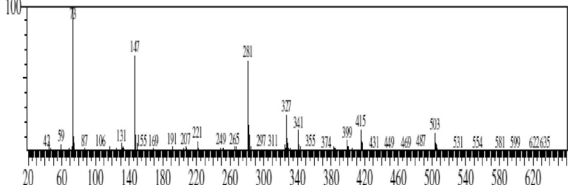
(Table 1). The vital role played by NADH-DCIP reductase in the breakdown of asymmetric ring and eventual reduction of the indigo dye cannot be over emphasized (Fig. 7) [52]. On the contrary, there were low inductions of laccase and manganese peroxidase after decolorization. The results obtained were however not in agreement with related work done by Jadhav and Govindwar [18].

3.4. Metabolites analyses

3.4.1. UV/Vis and FTIR analyses

Diutina rugosa cells provides an economical and environmental friendly alternative to the removal of indigo dye. *D. rugosa* showed complete decolorization of indigo dye within 5 days at room temperature under static condition and with no addition of organic and inorganic supplements. There was relative disappearance (at day 5) of the peak observed at 480 nm (control-day 0) in the UV/Vis absorbance spectra of indigo dye (Fig. 5). The relative reduction and disappearance of the peaks at day 5 suggested the reduction of the dye components thus decolorization. The drastic decrease in absorption peak at day 5 (Fig. 5) of the spectrum showed decolorization in concordance with the visual observation of the Erlenmeyer flasks. The results of FTIR analysis revealed the reduction of the spectra peaks in control from 10 at day 0 to 7 at day 5 (Fig. 6a and 6b). The loss of peak at 1508.38 cm⁻¹ indicates the absence of the asymmetric bond due to the action of NADH-DCIP reductase. The O–H stretching characterized by the peak at 2908.82 cm⁻¹ witnessed deformation with the formation of a new peak at 2362.88 cm⁻¹ (Fig. 6b). The loss of peaks at 1217.12, 736.83, and 648.10 cm⁻¹ suggested deformation of benzene ring (Table 2). The peak 424.35 cm⁻¹ reappearing (Fig. 6b)

Table 3
GC-MS data of obtained metabolite after degradation of indigo dye by *Diutina rugosa*.

S/N	RT (min)	m/z	MW	Area (%)	Mass spectrum
1.	23.84	133	133	78.90	
2.	20.61	87	84	21.10	

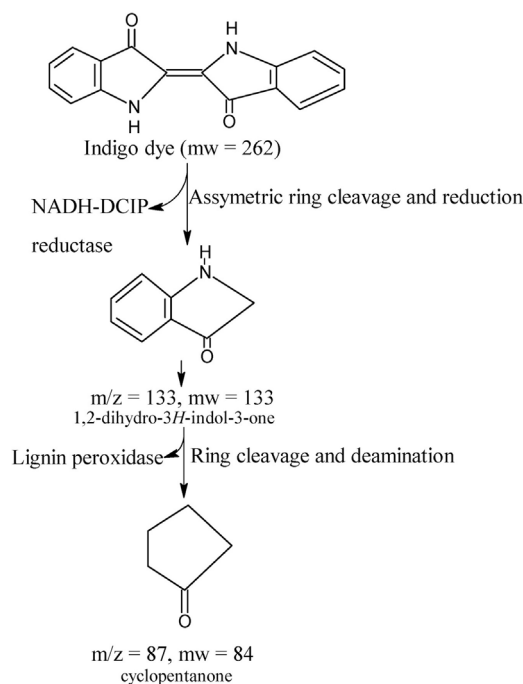


Fig. 7. Proposed schematic pathway for degradation of indigo dye by *Diutina rugosa*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

indicates the conversion of aliphatic amines to cyclopentanone. The results suggested that functional groups on the *Diutina rugosa* cell surface might have affected the biosorption process [53].

3.4.2. GCMS analyses

The GCMS analyses results (Table 3) revealed the formation of two major intermediate products with molecular weight of 133 and 87 g mol⁻¹ representing 1, 2-dihydro-3H-indol-3-one and cyclopentanone respectively (Fig. 7). The GCMS data obtained was used to propose a schematic pathway of degradation of indigo dye by *Diutina rugosa* (Fig. 7). The NADH-DCIP reductase enzyme releases NADH as electron donor in the reaction medium. The electron so donated facilitated the reductive cleavage of the asymmetric bond of indigo dye to produce 1, 2-dihydro-3H-indol-3-one and cyclopentanone (Fig. 7) [54]. The deamination of the indigo dye is further catalyzed through the oxidative action of lignin peroxidase (Fig. 7).

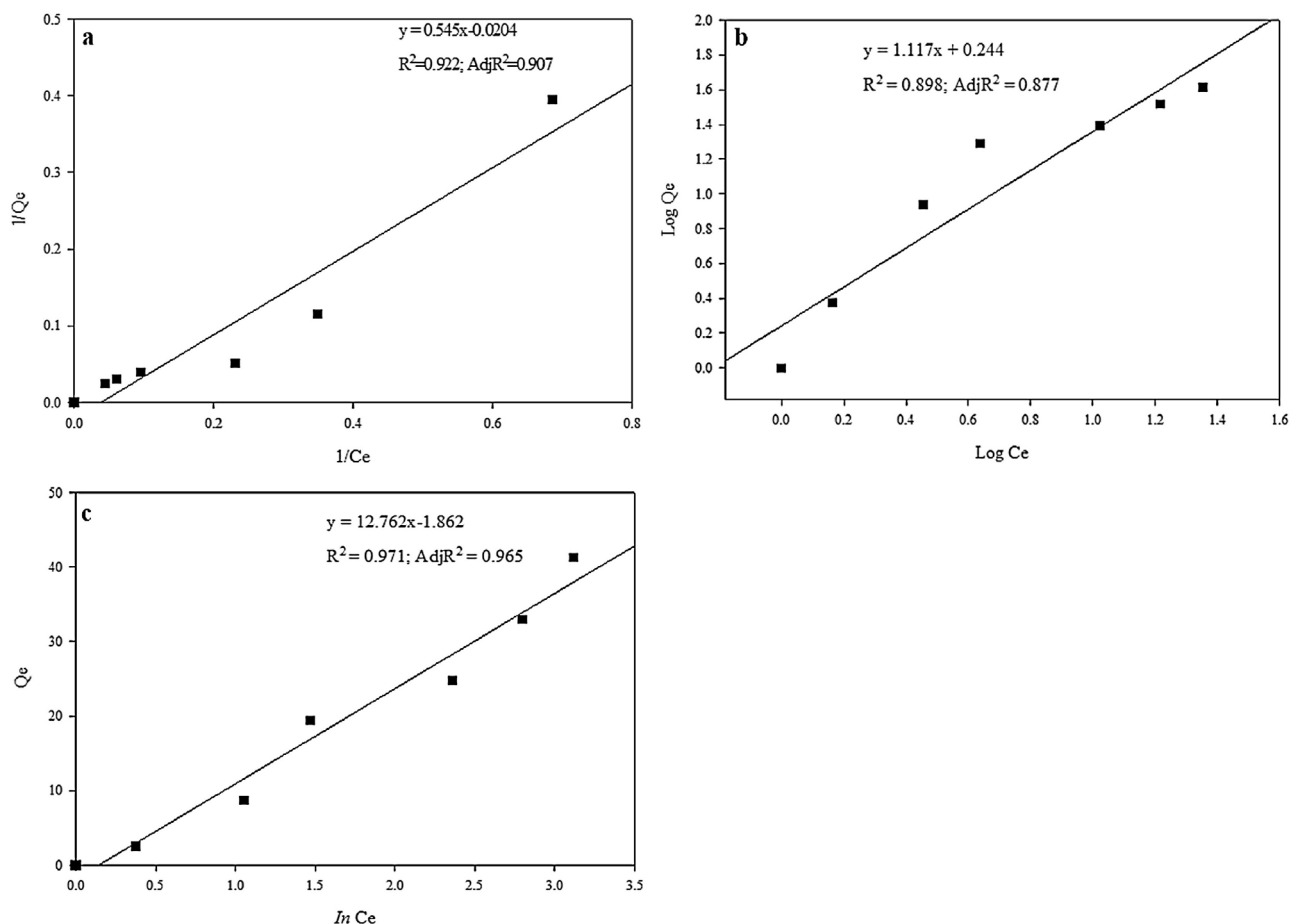


Fig. 8. (a) Langmuir's adsorption isotherm (b) Freundlich's adsorption isotherm (c) The Temkin's adsorption isotherm for indigo dye decolorization by *Diutina rugosa*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. Adsorption isotherms

Despite the fact that the yeast biomass resulted in steeper slopes of their respective decolorization isotherms (Fig. 8a–c) at regions of lower concentration which suggested a high affinity of the cells for the binding indigo dye molecules. The equilibrium data were found to be statistically significant at 1 percent significant level (p -value = 0.001) when analyzed with the Langmuir, Freundlich and Temkin adsorption isotherms. Therefore, the isotherm models were sufficient for this adsorption studies since the p -value is less than 0.01. Equilibrium biosorption isotherms are prerequisite to understanding the relationship and interaction between the adsorbate (indigo dye) with adsorbent (*Diutina rugosa*) cells. Adsorption parameters of Q_e and C_e as a function of the dye concentration (C_0) at the equilibrium plot was fit with the Langmuir, Freundlich and Temkin isotherm equations. It could be seen that the adjusted correlation coefficient of determination ($\text{AdjR}^2 = 0.907$ and $\text{AdjR}^2 = 0.965$) for Langmuir and Temkin isotherms respectively fits better for indigo dye decolorization (Fig. 8a and c) than the Freundlich isotherm ($\text{AdjR}^2 = 0.877$) (Fig. 8b). While Freundlich's isotherm only revealed that the physicochemical parameters (pH, temperature, adsorbent dose-yeast biomass, adsorbate concentration-indigo dye concentration) exhibited 87.7%; 96.5% and 90.7% causality strength were recorded for Temkin's and Langmuir's isotherms. Overall, best correlation and goodness of fit of the equilibrium data obtained was exhibited by the Temkin model result. In a similar vein, the results of correlation coefficient of determination; $R^2 = 0.922$, 0.898 and 0.971 for Langmuir, Freundlich and Temkin's model respectively. However, the Freundlich model where linearization allows also determination of maximal capacity (q_{\max}) showed

seemingly poor goodness of fit for the experimental data. The correlation coefficient of determination ($R^2 = 0.971$) for Temkin adsorption isotherm affirmed the strong influence of both concentration and temperature in the biosorption process. These results were in slight agreement with related work done by Charumathi and Das [19] who reported the monolayer adsorption of *Candida tropicalis* biomass in the degradation of basic violet 3. The results of the adsorption through Langmuir isotherm studies further revealed the unimolecular layer of the indigo dye molecules. The Temkin model results however, confirmed that the yeast cells could equally exhibit both monolayer and heterogeneous adsorption characteristics in the biosorption process of indigo dye. The results further implied, that the prediction of experimental data is quite satisfactory since over 50% of causality strength were recorded for all adsorption isotherms studied.

4. Conclusion

The present study revealed the indigo dye biodegradation potentials of *Diutina rugosa*. The biosorption process of indigo dye by the yeast proved to be dependent largely on the pH of the solution, cell biomass, temperature and concentration of the dye. The study reported two major intermediate metabolites after degradation of indigo dye through an enzyme-mediated system deployed by the yeast. Langmuir's and Temkin's isotherm studies revealed both monolayer and heterogeneous biosorption nature of *Diutina rugosa*, which depends largely on both concentrations of the indigo dye and temperature of the reaction medium. The Freundlich isotherm studies, however, confirmed that the heterogeneous biosorption behavior of *Diutina rugosa* cannot be attributed only to the concentration of the indigo dye. Conclusively,

Diutina rugosa has proven to be a cheap, economic, effective, efficient and eco-friendly alternative in the bioremoval of vat dyes (indigoid dyes) from polluted environment.

Conflict of interest

The authors hereby declare no conflict of interest.

Funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

The authors hereby acknowledge the technical inputs and contributions of technologists at the Department of Botany, University of Lagos, Lagos State, Nigeria and Department of Chemical Sciences, Redeemers University, Ede, Osun State, Nigeria

References

- D.S.C. Balan, R.T.R. Monterio, Decolorization of textile dye indigo by lignolytic fungi, *J. Biotechnol.* 89 (2001) 141–145.
- K. Harazono, K. Nakamura, Decolorization of mixtures of different reactive textile dyes by the white-rot basidiomycete *Phanerochaete sordaria* and inhibitory effect of polyvinyl alcohol, *Chemosphere* 59 (2005) 63–68.
- D. Cardon, P.C.M. Jansen, *Phlebotomera cyanescens* (Schumacher & Thonn.), Dyes and Tannins/Colourants and Tannins, PROTA, Wageningen, 2005 88pp.
- M.S. Mahmoud, Decolorization of certain reactive dye from aqueous solution using Baker's Yeast (*Saccharomyces cerevisiae*) strain, *HBRC J.* 12 (2014) 88–98.
- D. Oliveira, P. Carneiro, M. Sakagami, V. Zanon, G. Umbuzeiro, Chemical characterization of a dye processing plant effluent—identification of the mutagenic components, *Mutat. Res.* 626 (2007) 135–142.
- B. Manu, S. Chaudhari, Decolorization of indigo and azo dye in semicontinuous reactors with long hydraulic retention time, *Process Biochem.* 38 (2003) 1213–1221.
- A. Mario, S. Esplugas, G. Saum, How and why combine chemical and biological processes for wastewater treatment, *Water Sci. Technol.* 35 (4) (1997) 321–327.
- S. Chang, C. Chou, S. Chen, Decolorization of azo dyes with immobilized *Pseudomonas luteola*, *Process Biochem.* 36 (8–9) (2001) 757–763.
- R. Campos, R. Kandelbauer, K.H. Robra, A. Cavaco-Paulo, G.M. Gubit, Indigo degradation with purified laccases from *Trametes hirsuta* and *Sclerotium rolfsii*, *J. Biotechnol.* 89 (2–3) (2001) 131–139.
- C. Pinedo-Rivilla, J. Aleu, I.G. Collado, Pollutants biodegradation by fungi, *Curr. Org. Chem.* 13 (2009) 1194–1214.
- K. Selvam, K. Swaminathan, K.S. Chae, Decolorization of azo dyes and a dye industry effluent by white rot fungus *Thelphora* sp., *Bioresour. Technol.* 88 (2003) 115–119.
- P. Giardina, V. Faraco, C. Pezzella, A. Piscitelli, S. Vanhulle, G. Sannia, Laccases: a never-ending story, *Cell Mol. Life Sci.* 67 (2010) 369–385.
- B. Perlati, M.F.G.F. Da Silva, J.B. Fernandes, M.R. Forim, Validation and application of HPLC-ESI-MS/MS method for the quantification of RBBR decolorization a model for highly toxic molecules, using several fungi strains, *Bioresour. Technol.* 124 (2012) 37–44.
- M. Asgher, S. Kausar, H.N. Bhatti, S.A.H. Shah, M. Ali, Optimization of medium for decolorization of Solar golden yellow R direct textile dye by *Schizophyllum commune* IBL-06, *Int. Biodeterior. Biodegradation* 61 (2008) 189–193.
- Z. Yu, X. Wen, Screening and identification of yeasts for decolorizing of synthetic dyes in industrial wastewater, *Int. Biodeterior. Biodegradation* 56 (2005) 109–114.
- Q. Yang, L. Yang, L. Hei, S. Zheng, Using ammonium tolerant yeast isolates: *Candida halophila* and *Rhodotorula glutinis* to treat high strength fermentative wastewater, *Environ. Technol.* 24 (2003) 383–390.
- K. Kumari, T.E. Abraham, Biosorption of anionic textile dyes by nonviable biomass of fungi and yeast, *Bioresour. Technol.* 98 (2007) 1704–1710.
- J.P. Jadhav, S.P. Govindwar, Biotransformation of malachite green by *Saccharomyces cerevisiae* MTCC 463, *Yeast* 23 (2006) 315–323.
- D. Charumathi, N. Das, Removal of synthetic dye Basic violet 3 by immobilised *Candida tropicalis* grown on sugar bagasse extract medium, *Int. J. Eng. Sci. Technol.* 2 (2010) 4325–4335.
- Z. Aksu, G. Donmez, A comparative study on the biosorption characteristics of some yeasts for Remazol Blue reactive dye, *Chemosphere* 50 (2003) 1075–1083.
- K. Kwasniewska, Biodegradation of crystal violet (hexamethyl-p-rosaniline chloride) by oxidation of red yeasts, *Bull. Environ. Contam. Toxicol.* 34 (1985) 323–330.
- M. Bustard, G. McMullan, A.P. McHales, Biosorption of textile dyes by biomass derived from *Kluyveromyces marxianus* IMB 3, *Bioprocess. Eng.* 19 (1998) 427–430.
- C. Meehan, I.M. Banat, G. McMullan, P. Nigam, F. Smyth, R. Marchant, Decolorization of Remazol Black-B using a thermotolerant yeast, *Kluyveromyces marxianus* IMB3, *Environ. Int.* 26 (1,2) (2000) 75–79.
- P.O. Bankole, A.A. Adekunle, O.F. Obidi, Mycodecolorization of Reactive Red HE7B dye by *Achaetomium strumarium* and *Aspergillus flavus* and shelf life determination, *Cogent Environ. Sci.* 9 (1) (2017) 1–14.
- N. Saitou, M. Nei, The neighbour-joining method: a new method for reconstructing phylogenetic trees, *Mol. Biol. Evol.* 4 (1987) 406–425.
- S. Kumar, G. Stecher, K. Tamura, MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, *Mol. Biol. Evol.* 33 (2016) 1870–1874.
- S.M. Patil, V.V. Chandanshive, N.R. Rane, R.V. Khandare, A.D. Watharkar, S.P. Govindwar, Bioreactor with *Ipomoea hederifolia* adventitious roots and its endophyte *Cladosporium cladosporioides* for textile dye degradation, *Environ. Res.* 146 (2016) 340–349.
- O. Lowry, N. Rosebrough, A. Farr, R. Randall, Protein measurement with the folin phenol reagent, *J. Biol. Chem.* 193 (1951) 265–275.
- N. Hatvani, I. Mees, Production of laccase and manganese peroxidase by *Lentinus edodes* on malt containing by product of the brewing process, *Process Biochem.* 37 (2001) 491–496.
- M.D. Salokhe, S.P. Govindwar, Effect of carbon source on the biotransformation enzymes in *Serratia marcescens*, *World J. Microbiol. Biotechnol.* 15 (1999) 229–232.
- D.C. Kalyani, A.A. Telke, V.V. Dhanve, J.P. Jadhav, Ecofriendly biodegradation and detoxification of Reactive Red 2 textile dye by newly isolated *Pseudomonas* sp. SUK1, *J. Hazard. Mater.* 163 (2009) 735–742.
- A.V. Patil, J.P. Jadhav, Evaluation of phytoremediation potential of *Tagetes patula* L. for the degradation of textile dye Reactive Blue 160 and assessment of the toxicity of degraded metabolites by cytogenotoxicity, *Chemosphere* 92 (2013) 225–232.
- APHA, Standard Methods for the Examination of Water and Wastewater, 21st ed., American Public Health Association, Washington, DC, 2005.
- I. Langmuir, The adsorption of gases on plane surfaces of glass, mica and platinum, *J. Am. Chem. Soc.* 40 (1918) 1362–1403.
- H. Freundlich, Adsorption in solution, *Phys. Chem. Soc.* 40 (1906) 1361–1368.
- M.I. Temkin, V. Pyzhev, Kinetics of ammonia synthesis on promoted iron catalyst, *Acta Phys. Chim. Sin.* 12 (1940) 327–356.
- H.F. Pajot, J.I. Farina, F.L.I. Catellanos, Evidence on manganese peroxidase and tyrosinase expression during decolorization of textile industry dyes by *Trichosporon akyioshidainum*, *Int. Biodeterior. Biodegradation* 65 (2011) 1199–1207.
- C. Tian, R. Tian, Y. Zhou, Q. Chen, H. Cheng, Decolorization of indigo dye and indigo dye-containing textile effluent by *Ganoderma weberianum*, *Afr. J. Microbiol. Res.* 7 (11) (2013) 941–947.
- P. Kaushik, A. Malik, Fungal dye decolorization: recent advances and future potential review article, *Environ. Int.* 35 (2009) 127–141.
- Y. Wu, T. Li, L. Yang, Mechanisms of removing pollutants from aqueous solutions by microorganisms and their aggregates: a review, *Bioresour. Technol.* 107 (2012) 10–18.
- C.F. Iscen, I. Kiran, Biosorption of reactive black 5 dye by penicillium restrictum: penicillium restrictum: the kinetic study, *J. Hazard. Mater.* 143 (2007) 335–340.
- M. Iqbal, A. Saeed, Biosorption of reactive dye by loofa sponge-immobilized fungal biomass of *Phanerochaete chrysosporium*, *Process Biochem.* 42 (2007) 1160–1164.
- M. Asgher, N. Azim, H.N. Bhatti, Decolorization of practical textile industry effluents by white rot fungus *Coriolus versicolor* IBL-04, *Biochem. Eng. J.* 47 (2009) 61–65.
- S.H. Chen, A.S.Y. Ting, Biodecolorization and biodegradation potential of recalcitrant triphenylmethane dyes by *Corioliopsis* sp. isolated from compost, *J. Environ. Manage.* 150 (2015) 274–280.
- R.M.A. Abedin, Decolorization and biodegradation of Crystal Violet and Malachite Green by *Fusarium solani* (Maritus) Saccardo. A comparative study on the biosorption of dyes by the dead fungal biomass, *American-Eurasian J. Bot.* 1 (2008) 17–31.
- A. Geethakarthy, B.R. Phanikumar, Adsorption of reactive dyes from aqueous solutions by tannery sludge developed activated carbon: kinetic and equilibrium studies, *Int. J. Environ. Sci. Technol.* 8 (3) (2011) 561–570.
- M. Solís, A. Solís, H.I. Pérez, N. Manjarrez, M. Flores, Microbial decoloration of azo dyes: a review, *Process Biochem.* 47 (12) (2012) 1723–1748.
- A.S. Yargic, R.Z. Yarbay Sahin, N. Ozbay, E. Onal, The effect of different operating conditions on removal of reactive dye by green carbon adsorption, *JOSUNAS* (2013) 498–510.
- S. Renganathan, W.R. Thilagaraj, L.R. Miranda, P. Gautam, M. Velan, Accumulation of acid orange 7 acid red 18 and reactive black 5 by growing *Schizophyllum commune*, *Bioresour. Technol.* 97 (2006) 2189–2193.
- U. Shedbalkar, R. Dhanve, J. Jadhav, Biodegradation of triphenylmethane dye cotton blue by *Penicillium ochrochloron* MTCC 517, *J. Hazard. Mater.* 157 (2008) 472–479.
- H.Y. Yu, G.M. Zeng, G.H. Huang, D.L. Huang, Y.N. Chen, Lignin degradation by *Penicillium simplicissimum*, *Huan Jing Ke Xue* 26 (2005) 167–171.
- S.B. Pointing, I.I.P. Vrijmoed, Decolorization of azo and triphenylmethane dyes by *Pycnoporus sanguineus* producing as the sole phenoloxidase, *World J. Microbiol. Biotechnol.* 16 (2000) 317.
- H. Kelewou, M. Merzouki, A. Lhassani, Biosorption of textile dyes basic yellow 2 (BY2) and basic green 4 (BG4) by the live yeast *S. cerevisiae*, *J. Mater. Environ. Sci.* 5 (2) (2014) 633–640.
- A. Moutaouakki, Y. Zeroula, F.Z. Dzayri, M. Talbi, K. Lee, M. Balgane, Purification and partial characterization of azoreductase from *Enterobacter agglomerans*, *Arch. Biochem. Biophys.* 413 (2003) 139–146.