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Biodegradation of Basic Violet 3 by *Candida krusei* isolated from textile wastewater

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Abstract Basic Violet 3 (BV) belongs to the most important group of synthetic colorants and is used extensively in textile industries. It is considered as xenobiotic compound which is recalcitrant to biodegradation. As *Candida krusei* could not use BV as sole carbon source, experiments were conducted to study the effect of cosubstrates on decolorization of BV in semi synthetic medium using glucose, sucrose, lactose, maltose, yeast extract, peptone, urea and ammonium sulphate. Maximum decolorization (74%) was observed in media supplemented with sucrose. Use of sugarcane bagasse extract as sole nutrient source showed 100% decolorization of BV within 24 h under optimized condition. UV–visible, FTIR spectral analysis and HPLC analysis confirmed the biodegradation of BV. Six degradation products were isolated and identified. We propose the biodegradation pathway for BV which occurs via stepwise reduction and demethylation process to yield mono-, di-, tri-, tetra-, penta- and hexa-demethylated BV species which was degraded completely. The study of the enzymes responsible for decolorization showed the activities of lignin peroxidase, lacasse, tyrosinase, NADH-DCIP reductase, MG reductase and azoreductase in cells before and after decolorization. A significant increase in activities of NADH-DCIP reductase and laccase was observed in the cells after decolorization. The yeast *C. krusei* could show the ability to decolorize the textile dye BV using inexpensive source like sugarcane bagasse extract for decolorization.

Keywords Biodegradation · Basic Violet 3 (BV) · *Candida krusei* · Biodegradation pathway · NADH-DCIP reductase · Laccase

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Introduction

Basic Violet 3 (BV) is a triphenylmethane dye extensively used in human and veterinary medicine as a biological stain and in various commercial textile processes (Bumpus and Brock 1988). BV has been identified as a recalcitrant molecule and imparts a violet colour to water, even at low concentrations. Hence the effluents arising from its manufacturing or utilizing units are violet in colour and can not be discharged into the environment for aesthetic reasons. An additional worrying factor is that BV is a mutagen, a mitotic poison and a potent clastogen, possibly responsible for promoting tumor growth in some species of fish (Au et al. 1978; Cho et al. 2003). It is reported that 10–20% of dyes are lost to wastewater as a result of inefficiency in the dying process (Zollinger 1987). Conventional biological wastewater treatment systems are often incapable of effectively removing BV from the wastewater, resulting in its dispersal into the environment.

Many alternative processes have been investigated towards removal of BV from wastewater including adsorption, chemical oxidation and reduction, physical precipitation and flocculation, photolysis, electrochemical treatment, advanced oxidation, reverse osmosis and chemical degradation (Azmi et al. 1998). Microbial decolorization and degradation of dyes has been of considerable interest due to their inexpensive and eco-friendly nature as well as the property of producing a less amount of sludge (Verma and Madamwar 2003). Currently, an extensive research is focused to find out the potent microbial biomass which is as cheap as possible for removal of dyes from large volumes of polluted water (Jadhav and Govindwar 2006).

There are reports of BV biodegradation by bacteria (Sani and Banerjee 1999; Chen et al. 2008a, b; Jang et al. 2005; Ayed et al. 2009) and Fungi (Bumpus and Brock 1988; Azmi et al. 1998). Information is scanty regarding the use of yeast as potential degrader of BV. Yeasts have many advantages as compared to bacteria and filamentous fungi. Yeasts are an inexpensive, readily available source of biomass. Yeasts can adapt and grow under various extreme conditions of pH, temperature and nutrient availability as well as high pollutant concentrations. They not only grow rapidly like bacteria, but like filamentous fungi they also have the ability to resist unfavourable environment (Pajot et al. 2007). Therefore, the aim of the present investigation was to study the biodegradation of BV by a yeast *Candida krusei* isolated from textile wastewater. Moreover, the use of sugarcane bagasse extract as sole nutrient source during the process of biodegradation of BV is one of the attractive features of the study.

Materials and methods

Dyes and chemicals

The triphenyl methane dye BV commonly called Crystal Violet was a generous gift from Manisha textile dyeing works, Kanchipuram, Tamil Nadu, India. NADH, DCIP and ABTS were purchased from Himedia Ltd, Mumbai. Tartaric acid, n-propanol, Hydrogen peroxide solution, methyl red, malachite green, were obtained from SRL chemicals, India. All chemicals used were of the highest purity available and of an analytical grade.

Microorganism and growth medium

Candida krusei was isolated from the sludge sample collected from effluent reservoir in Manisha textile dyeing works, Kanchipuram, Tamil Nadu, India. The yeast was identified to the species level by Vitek 2 Compact Yeast card reader with the software version V2C 1.01 from Council for Food Research and Development (CFRD), Kerala, India. The isolate was maintained in Yeast Extract Peptone Dextrose (YEPD) agar slants. The primary inoculum of the yeast was grown in the mineral media composed of glucose—10 g, KH_2PO_4—1 g, $(NH_4)_2SO_4$—1 g, $MgSO_4{\cdot}7H_2O$—500 mg, Yeast Extract—200 mg, distilled water 1 litre. Semi synthetic medium composed of Basic Violet 3—0.01 g l^{-1}, $(NH_4)_2SO_4$—0.28 g l^{-1}, NH_4Cl—0.23 g l^{-1}, KH_2PO_4—0.067 g l^{-1}, $MgSO_4{\cdot}7H_20$—0.04 g l^{-1}, $CaCl_2{\cdot}2H_2O$—0.022 g l^{-1}, $FeCl_3{\cdot}6H_2O$—0.005 g l^{-1}, yeast extract—0.2 g l^{-1}, NaCl—0.15 g l^{-1}, $NaHCO_3$—1.0 g l^{-1}, 1 ml of a trace element solution containing $ZnSO_4{\cdot}7H_2O$—0.01 g l^{-1}, $MnCl_2{\cdot}4H_2O$—0.1 g l^{-1}, $CuSO_4{\cdot}5H_2O$—0.392 g l^{-1}, $CoCl_2{\cdot}6H_2O$—0.248 g l^{-1}, $NaB_4O_7{\cdot}10H_2O$—0.177 g l^{-1}, and $NiCl_2{\cdot}6H_2O$—0.02 g l^{-1} was prepared and the pH of the medium was adjusted to 6.5 with 0.1 N HCl and 0.1 N sodium hydroxide solutions. The dye solution was autoclaved separately and mixed with the sterilized medium. To prepare the sugarcane bagasse extract (10%), desired amount of sugarcane bagasse was boiled in 100 ml of distilled water. The total sugar content of the extract was analysed as 8 g l^{-1} using anthrone method (Pons et al. 1981).

Decolorization experiments

A loopful of microbial culture of *C.krusei* was inoculated in 250 ml Erlenmeyer flask containing 100 ml of mineral medium under optimal growth conditions (pH-6.5; 28°C). Cells from exponential phase were inoculated into mineral medium containing BV at a concentration of 10 mg l^{-1} and incubated at 28°C under static and shaking condition (120 rpm). An aliquot (3 ml) of culture media was withdrawn at the different time intervals and centrifuged at 10,000 rpm for 15 min to separate the cell mass. The supernatant was analyzed for residual dye concentration by reading the absorbance at 584 nm using UV–Visible spectrophotometer and the pellet

was dried at 40°C to a constant weight for biomass estimation. All the experiments were carried out in triplicates. Abiotic controls (without organisms) were also included. The decolorization percentage was calculated as follows:

$$\text{Decolorization } \% = \frac{C_0 - C_f}{C_0} \times 100 \quad (1)$$

where, C_0 initial concentration of dye (mg l^{-1}), C_f final concentration of dye (mg l^{-1}).

Effect of cosubstrates on semi synthetic medium during decolorization of BV

Candida krusei did not decolorize BV in semi-synthetic medium (pH 6.5) indicating that the dye could not be used as the sole carbon and nitrogen source. Hence, the effect of additional cosubstrates (carbon and nitrogen sources) on co-metabolism of BV was evaluated using glucose, sucrose, maltose, lactose, peptone, yeast extract, ammonium sulphate and urea. To study the effect of carbon and nitrogen sources on decolorization of BV, semi-synthetic medium having following composition was used: BV—0.10 g l^{-1}, $(NH_4)_2$ SO_4—0.28 g l^{-1}, NH_4Cl—0.23 g l^{-1}, KH_2PO_4—0.067 g l^{-1}, $MgSO_4{\cdot}7H_2O$—0.04 g l^{-1}, $CaCl_2{\cdot}2H_2O$—0.022 g l^{-1}, $FeCl_3{\cdot}6H_2O$—0.005 g l^{-1}, yeast extract—0.2 g l^{-1}, NaCl—0.15 g l^{-1}, $NaHCO_3$—1.0 g l^{-1}, 1 ml of a trace element solution containing $ZnSO_4{\cdot}7H_2O$—0.01 g l^{-1}, $MnCl_2{\cdot}4H_2O$—0.1 g l^{-1}, $CuSO_4{\cdot}5H_2O$—0.392 g l^{-1}, $CoCl_2{\cdot}6H_2O$—0.248 g l^{-1}, $NaB_4O_7{\cdot}10H_2O$—0.177 g l^{-1}, and $NiCl_2{\cdot}6H_2O$—0.02 g l^{-1}. The semi-synthetic medium was supplemented with different carbon and nitrogen sources such as glucose, sucrose, lactose, maltose, urea, ammonium sulphate, yeast extract and peptone (1% each). All the experiments were conducted at pH 6.5 and temperature 28°C.

Use of sugarcane bagasse extract as sole nutrient source during decolorization of BV

The decolorization studies using sugarcane bagasse extract (10%) was carried out at pH (3, 5, 7, 9) by adjusting the pH of the medium containing 10 mg l^{-1} using 0.1 N HCl and 0.1 N NaOH. The yeast was grown in sugarcane bagasse extract medium at respective pH for 48 h at 28°C. Similarly, decolorization was carried out at different temperatures 28, 37 and 45°C under optimum pH. The flasks containing sugarcane bagasse extract medium were incubated at respective temperatures for 30 min before addition of the dye (10 mg l^{-1}). The decolorization of BV was tested at different concentrations of dye (10, 25, 50,100 mg l^{-1}). The yeast was grown in sugarcane bagasse extract medium at respective dye concentrations under optimum pH at 28°C

Preparation of enzyme samples and enzyme assays

Candida krusei cells grown in medium were harvested before and after decolorization by centrifugation at 10,000 rpm for 15 min. The supernatant was used for the study of extracellular enzyme activities. The cell pellet was suspended in potassium phosphate buffer (50 mM, pH 7.4) for sonication, keeping sonifier output at 40 A and giving eight strokes each of 40 s with a 2 min interval at 4°C. This extract was used for the study of intracellular enzyme activities. All enzyme assays were performed at 30°C where reference blanks contained all components except the enzyme. All assays were run in triplicate and average rates were calculated and one unit of enzyme activity was defined as a change in absorbance unit per min per ml of enzyme.

Oxidative enzymes

Laccase activity was determined in a reaction of 2 ml containing ABTS (10%) in 0.1 M acetate buffer (pH 4.9) and measured an increase in optical density at 420 nm (Hatvani and Mecs 2001). Tyrosinase activity was determined in a reaction mixture of 2 ml containing catechol (0.01%) in 0.1 M phosphate buffer (pH 7.4) at 410 nm (Zhang and Flurkey 1997). Lignin peroxidase activity was determined by monitoring the formed proponaldehyde at 300 nm in a reaction mixture of 2.5 ml containing 100 mM n-propanol, 250 mM tartaric acid, and 10 mM H_2O_2 (Telke et al. 2008).

Reductase enzymes

NADH-DCIP reductase activity was determined in an assay mixture containing 50 μM DCIP, 28.57 mM NADH in 50 mm potassium phosphate buffer (pH 7.4) and 0.1 ml of enzyme solution in total volume of

5 ml. The DCIP reduction was calculated using the extinction coefficient of 19 mM/cm (Salokhe and Govindwar 1999). MG reductase activity was determined in reaction mixture containing 323 μM Malachite green, 28.57 mM NADH in 50 mM potassium phosphate buffer (pH 7.4) and 0.1 ml enzyme solution in a total volume of 5.0 ml. Absorbance of remaining malachite green was calculated using the extinction coefficient of 8.4×10^{-3} mM/cm (Jadhav and Govindwar 2006). Azo reductase activity was determined by monitoring the decrease in the methyl red concentration at 440 nm in a reaction mixture of 2.2 ml containing 152 μM methyl red, 50 mM sodium phosphate buffer (pH5.5) and 20 μM NADH (Saratale et al. 2009).

Extraction and analysis of decolorization products

Decolorization was monitored by UV visible spectroscopic analysis, whereas biodegradation was monitored by high performance liquid chromatography (HPLC) and Fourier transform infrared spectroscopy (FTIR). Identification of metabolites was carried out by GC–MS. The metabolites produced during biodegradation of BV were extracted with equal volume of ethyl acetate from clear supernatant. The extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The crystals obtained were dissolved in a small volume of HPLC-grade methanol and used for analysis.

HPLC analysis was carried out on a Waters instrument equipped with a dual λUV–VIS detector and a C18 column; the mobile phase used was methanol at a flow rate of 0.5 ml/min for 10 min. FTIR analysis was done in the mid IR region of 400–4000 cm^{-1} with 16 scan speed. The samples were mixed with spectroscopically pure KBr in the ratio 5:95. The pellets were fixed in a sample holder for analyses. The GC/MS analysis of metabolites were carried out using Agilent 6890 GC equipped with Agilent 5973 N mass selective detector. The mass spectrometer was operated in the electron impact mode with an electron current of 70 eV. Aliquots of 1 μl were injected automatically with an auto sampler (AUC20i) in splitless mode via a GC inlet (injector temperature 250°C). A HP-5 MS capillary column (30m × 0.25 mm ID, 0.25 μm film thickness) was connected directly to the ion source of the mass spectrometer. The oven temperature was kept isothermal for 1 min at 50°C, was then increased to 270°C at a rate of 10°C min^{-1}. The injector, MS source and MS quad temperature were 250, 230 and 150°C, respectively. The GC/MS system was operated in full scan (m/z 50–500). The biodegradation products were identified by comparison of retention time and fragmentation pattern, as well as with mass spectra in the NIST spectral library.

Treatment of textile wastewater using *C. krusei*

The textile wastewater was collected from Manisha textile dyeing works, Kanchipuram, Tamil Nadu, India. The treatment of textile wastewater using *C. krusei* was conducted in batch process. The textile wastewater was supplemented with sugarcane bagasse extract containing 24 g l^{-1} of total sugars and adjusted to pH 7. *C.krusei* cells from exponential growth phase were harvested and transferred to textile wastewater containing BV. The decolorization of textile wastewater was monitored at regular time intervals using UV–Visible Spectrophotometer.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparisons test. Readings were considered significant when P was ≤ 0.05.

Results and discussion

Effect of static and shaking conditions on decolorization

The culture of *C. krusei* under agitation condition showed a better growth (1.5 g l^{-1}) than that under static condition (0.5 g l^{-1}). As the cell growth was higher at agitated condition, the decolorization was also observed maximum (70%) than that under static condition (15%). The result suggested that oxygen was required for complete decolorization of dye. Therefore, shaking conditions were adopted to investigate decolorization in the following experiments. Similar results were reported on biodegradation of azo dyes by yeast *Issatchenkia occidentalis* where maximum decolorization was observed under aerobic conditions (Ramalho et al. 2004). They reported that

under anoxic condition decolorization was lesser due to absence of metabolic activity.

Effect of cosubstrates on decolorization

Table 1 showed that the decolorization of BV was greatly affected by the addition of various carbon and nitrogen sources. The culture showed maximum decolorization in presence of glucose (70%) and sucrose (74%) whereas the addition of maltose and lactose showed no decolorization. The supplementation of peptone and yeast extract in the semi-synthetic medium showed less decolorization (15 and 20%, respectively) whereas there was no decolorization in presence of ammonium sulphate and urea. Therefore, the result suggested that the decolorization of BV by *C. krusei* was dependent on carbohydrate metabolism. However, Saratale et al. (2009) reported the inhibition of the decolorizing activity of *Trichosporon beigeii* due to addition of glucose. They also reported that the decolorization performance could be enhanced by addition of yeast extract.

Effect of the use of sugarcane bagasse extract on decolorization

As the decolorization of BV was found to be maximum (100%) using sugarcane bagasse extract as sole nutrient source, effect of physicochemical parameters viz. pH, temperature and initial dye concentrations on decolorization were also tested. The optimum pH, temperature and dye concentration were found to be 7, 28°Cand 10 mg l^{-1} for effective decolorization of BV by *C. krusei* grown in sugarcane bagasse extract medium. It was observed that the decolorization percentage decreased to 40% with increasing dye concentration up to 100 mg l^{-1}. Complete inhibition of yeast growth causing no decolorization was noted beyond 100 mg l^{-1} of BV (Table 2). Saratale et al. (2009) also reported that biodegradation of Navy blue HER by *T. beigeii* was greatly affected by initial dye concentration beyond 100 mg l^{-1}.

Table 1 Decolorization of Basic Violet 3 by *C. krusei* grown in semi synthetic media supplemented with cosubstrates

Media	Decolorization %
SM	ND
SM + glucose	70
SM + sucrose	74
SM + lactose	ND
SM + maltose	ND
SM + yeast extract	20
SM + peptone	15
SM + urea	ND
SM + ammonium sulphate	ND
Sugarcane bagasse Extract	100

SM semi-synthetic medium, *ND* no decolorization

Enzyme activities during decolorization of BV

Several reports have shown that the degradation of dyes by fungi are mediated by several lignin modifying enzymes *viz.* laccase, manganese dependent peroxidase, lignin peroxidase and to some extent by aminopyrine N-demethylase (Raghukumar et al. 1996). The relative contributions of these enzymes in decolorization of dyes may be different for each microorganism. In case of fungi, Laccase in *Cyathus bulleri* (Vasdev et al. 1995) and *T. versicolor* (Wong and Yu 1999), lignin peroxidase in *P. chrysosporium* (Pasti-Grigsby et al. 1992), ligninase-catalysed oxidation in *T. versicolor* (Young and Yu 1997), manganese peroxidase in an unidentified white-rot fungus (Zhang et al. 1999) and cytochrome P450 system mediated both reduction and the *N* methylation reactions in *Cunninghamella elegans* (Cha et al. 2001) are reported to mediate the degradation of synthetic dyes.

Very few reports are available with enzymes involved in biodegradation of synthetic dyes by yeast. NADH-DCIP reductase and MG reductases activity was reported in *Saccharomyces cerevisiae* MTCC 463 in biodegradation of Malachite green (Jadhav and Govindwar 2006). Similarly, NADH-DCIP reductase activity was explored in *T. beigelii* NCIM-3326 during decolorization of textile dye Navy blue HER (Saratale et al. 2009). Oxidative and reductive enzymes activities were recorded during the time course of decolorization. The data shown in Table 3 represents the enzymatic activities present in the cells and supernatants before and after decolorization. Lignin peroxidase, laccase, tyrosinase, NADH-DCIP reductase, MG reductase and azo reductase activities were observed in cells before and after decolorization. We observed the significant

Table 2 Decolorization of Basic Violet 3 by *C. krusei* grown in sugarcane bagasse extract at different pH, temperature and dye concentration

	Parameters										
	pH				Temperature (°C)			Dye concentration (mg l^{-1})			
	3	5	7	9	28	37	45	10	25	50	100
Decolorization %	75	88	100	90	100	77	54	100	80	65	40
Time required for decolorization (h)	30	28	24	28	24	24	24	24	32	45	53

Table 3 Enzyme activities during decolourization of Basic Violet 3

Enzymes	Enzyme activity			
	Cell biomass		Supernatant	
	Before Decolorization	After Decolorization	Before Decolorization	After Decolorization
Oxidative enzymes				
Lignin peroxidase[a]	0.12 ± 0.005	0.19 ± 0.012	0.005 ± 0.001	0.012 ± 0.007
Laccase[a]	15.57 ± 0.02	112.4 ± 0.025*	0.001 ± 0.0002	0.005 ± 0.001
Tyrosinase[a]	0.19 ± 0.05	0.23 ± 0.064	0.002 ± 0.001	0.004 ± 0.0005
Reductive enzymes				
NADH-DCIP reductase[b]	32.30 ± 2.5	340.00 ± 3.46*	NA	NA
MG reductase[c]	0.32 ± 0.002	1.23 ± 0.03*	NA	NA
Azoreductase[d]	0.54 ± 010	3.23 ± 1.03*	NA	NA

Values are mean of the experiments ± SEM, Data was analyzed by one-way ANOVA Test. Enzyme activities in cell biomass after decolorization of Basic Violet 3 are significantly different from the enzyme activities in cell biomass before decolorization when compared by Tukey–Kramer multiple comparison Test * $P < 0.05$; *NA* no activity

[a] Enzyme activity—units ml^{-1} min^{-1}

[b] μg DCIP reduced ml^{-1} min^{-1}

[c] μg Malachite green reduced ml^{-1} min^{-1}

[d] μg Methyl red reduced ml^{-1} min^{-1}

induction in the activities of NADH-DCIP reductase and laccase in the cells obtained after decolorization compared to the control cells. A significant increase in the enzyme activity of NADH-DCIP reductase was observed over a period of BV decolorization as compared to laccase after complete decolorization. Similar inductive pattern of reductase enzymes were observed during decolorization of methyl red by *S. cerevisiae* MTCC 463 (Jadhav et al. 2006) and *Galactomyces geotrichum* MTCC 1360 (Jadhav et al. 2008) and *T. beigeilii* NCIM-3326 (Saratale et al. 2009). From these results we conclude that the isolated yeast *C. krusei* could degrade triphenyl methane dye BV via enzymatic mechanism.

Analysis of decolorization products

To understand the possible mechanism of the dye decolorization, the products of decolorization were analysed by UV–Visible spectral analysis, FTIR-spectral analysis and HPLC analysis. The decolorization products were identified by GC–MS. UV–visible spectral scan (200–800 nm) of supernatants at different time intervals showed decolorization and decrease in dye concentration from batch culture (Fig. 1). BV displayed one peak at λmax 584 nm in the UV–Vis spectra. The intensity of this peak decreased remarkably after addition of *C. krusei* cells due to decolorization. The peak at 584 nm

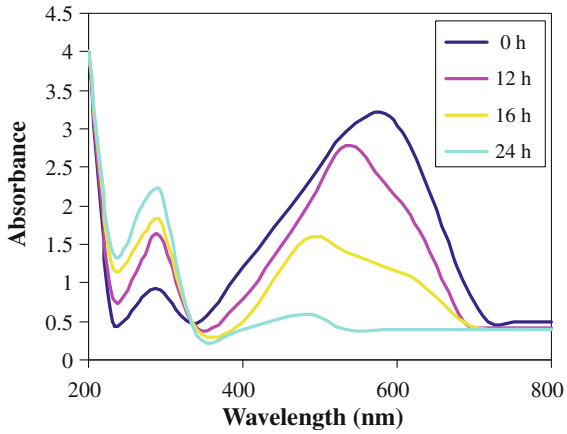

Fig. 1 UV–Visible spectra of Basic Violet 3 (10 mg l^{-1}) biodegraded by *C. krusei* at different time. pH-6.5; temperature 28°C

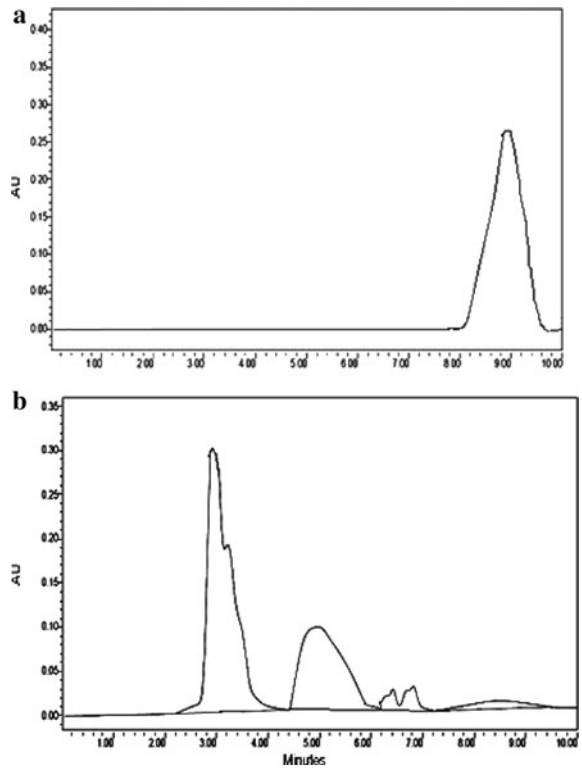

Fig. 2 HPLC analysis of control dye Basic Violet 3 (**a**) and its degradation products 16 h (**b**)

was decreased with shifts towards lower wavelength (584–480 nm) until complete decolorization of dye was achieved. The absorbance at λmax 584 nm became virtually zero after 24 h and an increase in absorbance towards UV region was observed suggesting that new byproducts were produced in the process of dye decolorization.

HPLC analysis of dye sample collected at 0 h of decolorization showed only one major peak with retention time 9.00 min (Fig. 2a). Compared to control dye, HPLC analysis of decolorization sample (16 h) showed additional peaks at different retention time 8.6, 6.9, 6.5, 5.0, 3.4, and 3.0 min (Fig. 2b). HPLC analysis of samples collected after 24 h showed no peaks in the chromatographs indicating complete degradation of BV (data not shown).

FTIR spectra of control BV (Fig. 3a) showed the specific peaks in fingerprint region (1500–500 cm^{-1}) for the monosubstituted and para disubstituted benzene rings. The peaks at 1585 cm^{-1} supports for C=C stretching vibrations in benzene ring. The peak at 1167 cm^{-1} corresponds to aromatic C–N stretching vibrations. The peak at 1474 cm^{-1} indicates aromatic C–C stretch. The peak at 2917 cm^{-1} corresponds to C–H asymmetric stretching vibrations. The absorption bands below 900 cm^{-1} indicates the aromatic nature of the compounds. The peaks at 828.87, 715.87, 610.87, 563 cm^{-1} indicate the presence of benzene ring. FTIR spectra of degradation products (Fig. 3b) showed peaks at 3409 cm^{-1} for the presence of N–H stretching, 1660 cm^{-1} for C=O stretching, 1405 cm^{-1} for C–H stretching of alkane, 1135 cm^{-1} for C–O stretching. Absence of peaks at 828.87, 715.87, 610.87, 563 cm^{-1} indicated the loss of benzene ring. The FTIR spectra data supported the degradation of BV by *C. krusei*.

Table 4 showed the GC–MS data of the control dye BV and its degradation products at different time. GC–MS data of control dye showed retention time 16.82 min (Fig. 4a) with molecular weight 373.21. Gas chromatogram of the degradation products obtained at 16 h showed the existence of six intermediates (Fig. 4b). The six intermediates with retention time 15.14, 14.56, 13.56, 11.75, 9.63, 7.25 min were identified as *N*,*N*-dimethyl-*N*′,*N*′-dimethyl-*N*″-methyl pararosaniline with molecular weight 355.1175, *N*,*N*-dimethyl-*N*′,*N*′-dimethyl pararosaniline with a molecular weight 342.035, *N*,*N*-dimethyl-*N*′methyl pararosaniline with a molecular weight 331.4142, *N*,*N*-dimethyl pararosaniline with a molecular weight 316. 6547, *N*-methyl pararosaniline with a molecular weight 302.1579, Pararosaniline with a

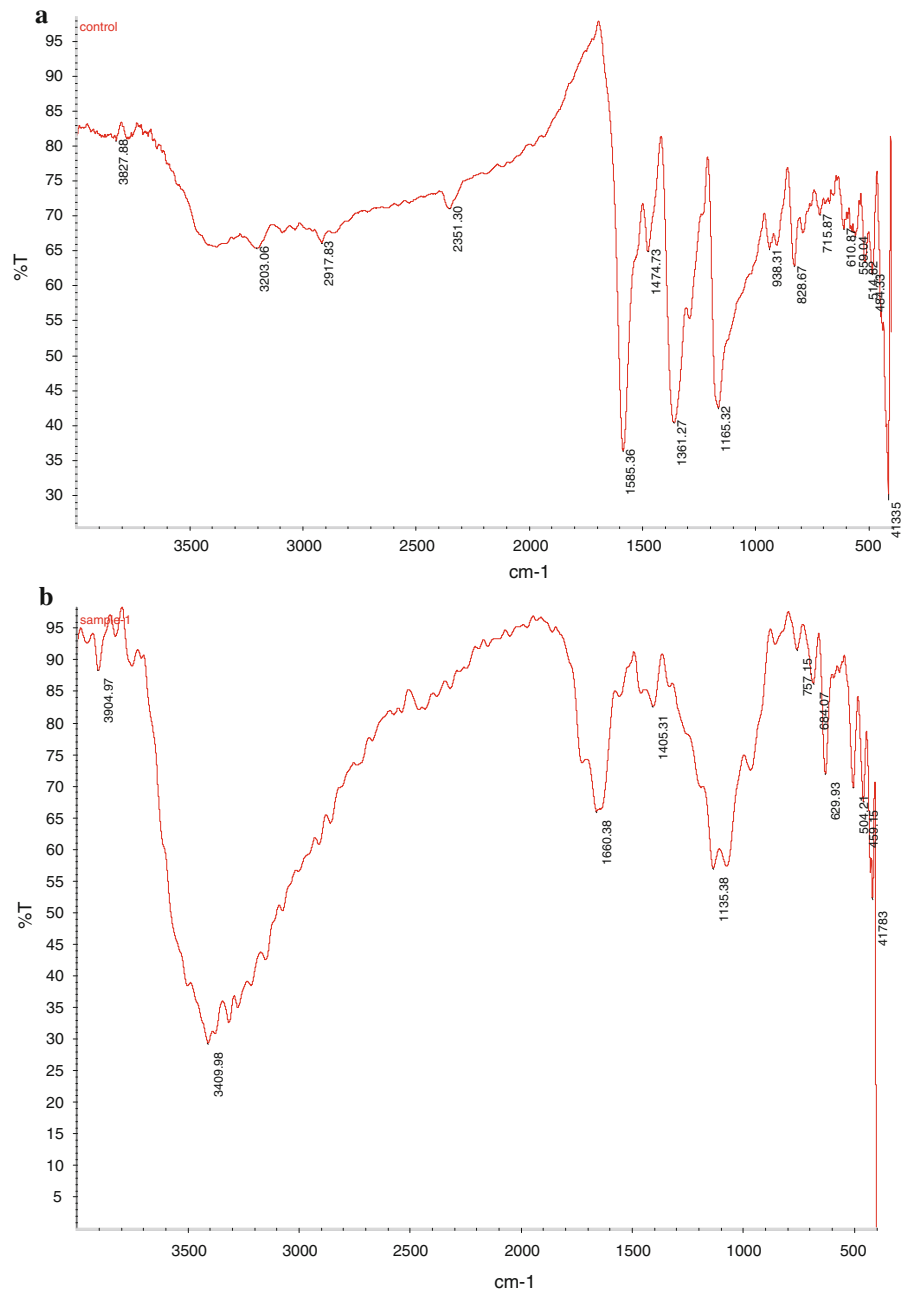

Fig. 3 FTIR spectral analysis of control dye Basic Violet 3 (**a**) and its degradation products (**b**)

molecular weight 288.4370, respectively. Gas chromatogram (Fig. 4c) of degradation products after 24 h did not show the existence of any compound which confirmed the complete degradation of BV by *C. krusei*.

Studies have shown that BV is converted to its colorless leuco derivatives by intestinal microflora and several anerobic bacteria (McDonald and Cerniglia 1984; Henderson et al. 1997). Yatome et al. (1991) reported that major degradation product of BV by growing cells of *Bacillus subtilis* IFO 13719 was Michler′s ketone. The same degradation product was again detected by Yatome et al. (1993) with growing cells of *Nocardia coralline*. However, other workers have also identified compounds such as *N*,*N*,*N*′, *N*′,*N*′′-pentamethyl pararosaniline, *N*,*N*,*N*′,*N*′′-tetramethyl pararosaniline, *N*,*N*′,*N*′′-trimethylpararosaniline during degradation of BV by white rot-fungi

Table 4 GC-MS data of degradation products of Basic Violet 3 by *C. krusei* at different time intervals

Time of decolorization (h)	Retention time	Molecular weight	Compound
0	16.82	373.21	*N*,*N*,*N*′,*N*′,*N*′′,*N*′′-hexamethyl pararosaniline
16	15.17	357.111	*N*,*N*-dimethyl-*N*′,*N*′-dimethyl-*N*′′-methyl pararosaniline
	14.56	342.035	*N*,*N*-dimethyl-*N*′,*N*′-dimethyl pararosaniline
	13.56	331.4142	*N*,*N*-dimethyl-*N*′methyl pararosaniline
	11.75	316.6547	*N*,*N*-dimethyl pararosaniline
	9.63	302.1579	*N*-methyl pararosaniline
	7.25	288.4370	Pararosaniline
24	No peak	–	–

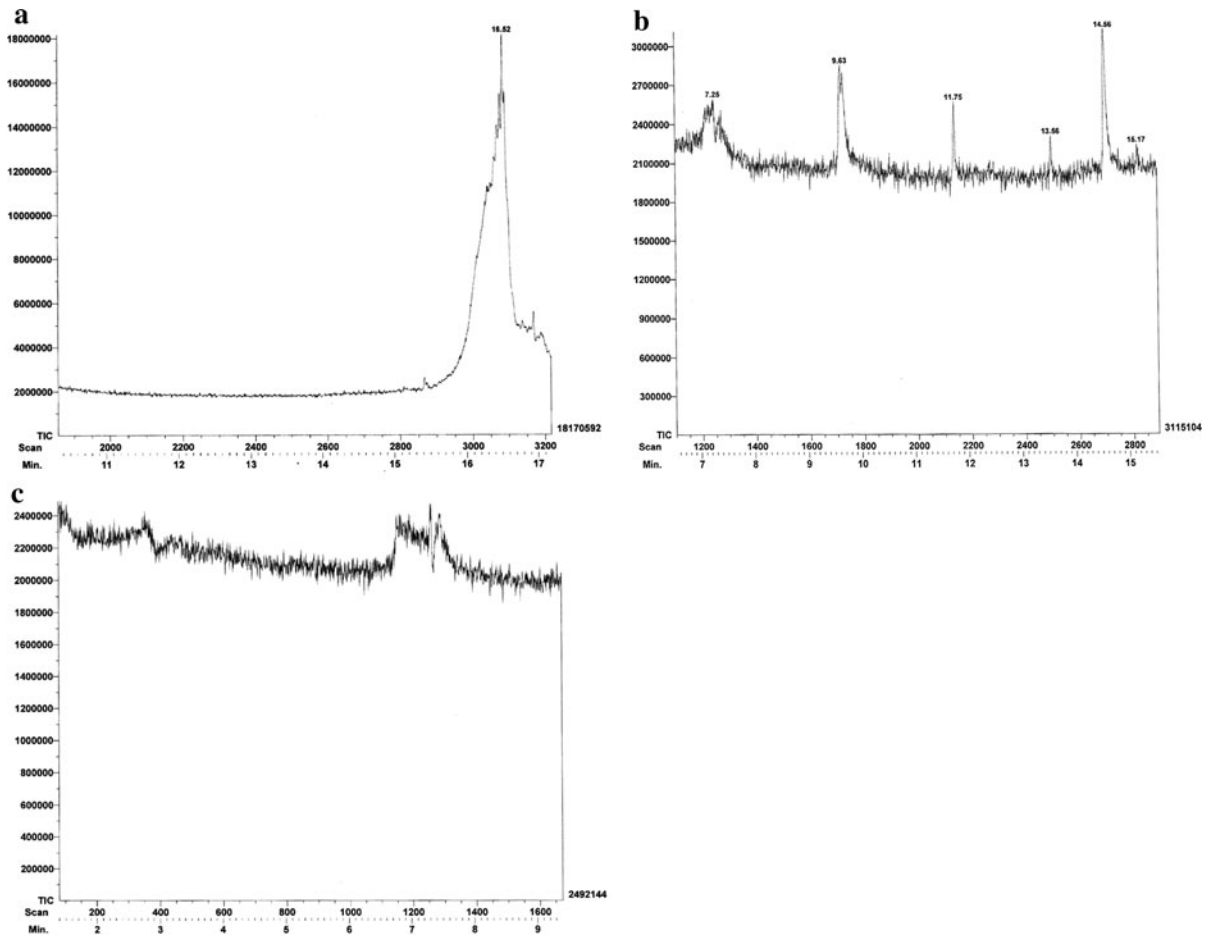

Fig. 4 Gas chromatogram of control dye Basic Violet 3 (**a**) and its degradation products at 16 h (**b**), 24 h (**c**)

under ligninolytic conditions (Ollikka et al. 1993; Bumpus and Brock 1988). Cha et al. (2001) reported that BV could be transformed by the filamentous fungus *Cunninghamella elegans* ATCC 36112 through sequential *N*-demethylation to *N*,*N*′,*N*′-trimethylpararosaniline. Chen et al. (2008b) reported formation of Michler's ketone and *N*,*N*-dimethylaminophenol during biodegradation of BV by

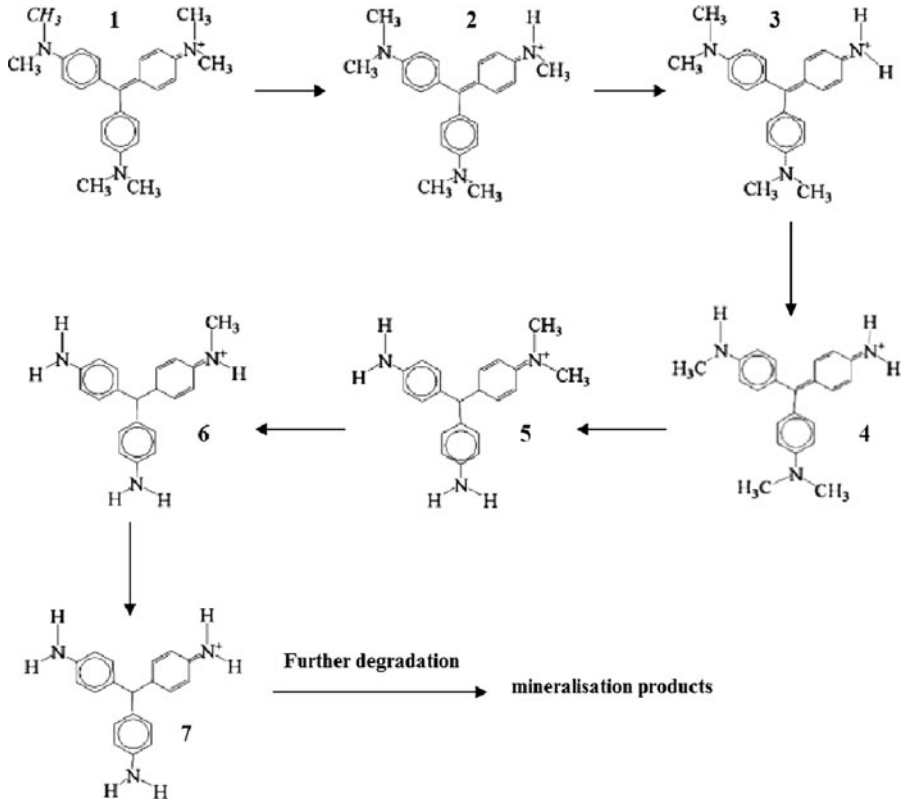

Fig. 5 Proposed biodegradation pathway of Basic Violet 3 by *C. krusei* under shaking condition. (*1*) *N*,*N*-dimethyl-*N*′,*N*′-dimethyl-*N*″,*N*″-dimethyl pararosaniline, (*2*) *N*,*N*-dimethyl-*N*′,*N*′-dimethyl-*N*″-methyl pararosaniline, (*3*) *N*,*N*-dimethyl-*N*′,*N*′-dimethyl pararosaniline, (*4*) *N*,*N*-dimethyl-*N*′methyl pararosaniline, (*5*) *N*,*N*-dimethyl pararosaniline, (*6*) *N*-methyl pararosaniline, (*7*) pararosaniline

Shewanella sp. NTOU1. They also reported further degradation of Michler's ketone to *N*,*N*-dimethylaminobenzaldehyde and *N*,*N*-dimethylaminophenol by the same strain. In addition, same group of workers also reported the demethylation of Michler's ketone to [*N*,*N*-dimethylaminophenyl] [*N*-methylaminophenyl] benzophenone and degradation of *N*,*N*-dimethylaminobenzaldehyde to 4-hydroxybenzaldehyde and other compounds.

Based on our results of analysis of intermediate products, we propose the degradation pathway of BV by *C. krusei* as shown in Fig. 5. According to our proposal, degradation of *N*,*N*-dimethyl-*N*′,*N*′-dimethyl-*N*″,*N*″-dimethyl parasoaniline (BV) occurs via stepwise reduction and demethylation processes. The sequence of products formed during reduction is as follows: *N*,*N*-dimethyl-*N*′,*N*′-dimethyl-*N*″,*N*″-methyl parasoaniline, *N*,*N*-dimethyl-*N*′,*N*′-dimethyl-parasoaniline, *N*,*N*-dimethyl-*N*′,*N*′-methyl parasoaniline, *N*,*N*-dimethyl-parasoaniline, *N*-methyl parasoaniline and parasoaniline. The compound pararosaniline showed complete degradation. Therefore, the present results show that *C. krusei* could degrade BV (10 mg l^{-1}) completely after 24 h.

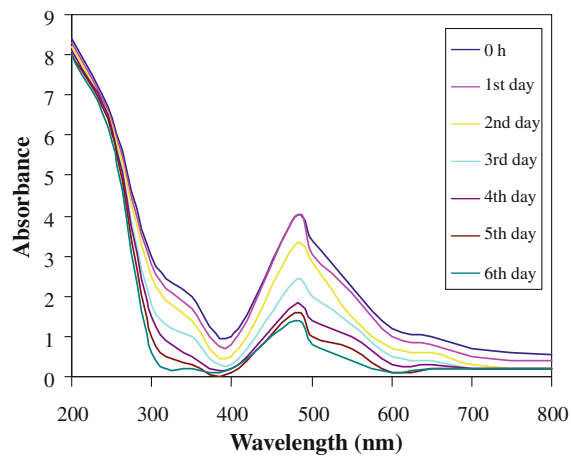

Fig. 6 Decolorization of textile wastewater using *C. krusei*. pH-7; temperature 28°C

Treatment of textile wastewater using *C. krusei*

Figure 6 shows the gradual decrease in absorbance of textile wastewater at λ_{max} 490 nm when treated with *C. krusei* cells. The treatment of textile wastewater in

batch mode showed that *C. krusei* could decolorize textile wastewater only up to 65% in 6 days. Complete decolorization of textile wastewater was not achieved due to presence of other toxic textile dyes and pollutants. Therefore, further research is needed for complete decolorization of textile wastewater along with the development of bioreactor system using *C. krusei.*

Conclusion

The obtained results indicate that the isolated yeast *C. krusei* was capable of degrading BV completely using sugarcane bagasse extract as sole nutrient source in a batch system. Use of sugarcane bagasse extract, an inexpensive waste, as nutrient source is one of the attractive features of this study. Based on the degradation products detected by GC–MS, it is proposed that degradation of BV followed stepwise reduction and de-methylation to pararosaniline which was completely degraded. Enhancement in the activities of enzymes viz. NADH-DCIP reductase and Lacasse during degradation indicated the involvement of these enzymes for the degradation of BV. The present study indicates the decolorization of BV in synthetic solutions as well as in textile wastewater using *C. krusei*. Based on the results, it can be concluded that *C. krusei* can serve as promising microorganism for the treatment of textile wastewater containing BV.

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