Biodegradation (2010) 21:703–711
DOI 10.1007/s10532-010-9336-1

ORIGINAL PAPER

Biodegradation of the major color containing compounds in distillery wastewater by an aerobic bacterial culture and characterization of their metabolites

Ram Naresh Bharagava · Ram Chandra

Received: 22 July 2009 / Accepted: 22 January 2010 / Published online: 10 February 2010

Abstract This study deals the biodegradation of the major color containing compounds extracted from distillery wastewater (DWW) by an aerobic bacterial consortium comprising *Bacillus licheniformis* (DQ79010), *Bacillus* sp. (DQ779011) and *Alcaligenes* sp. (DQ779012) and characterization of metabolic products. The degradation of color containing compounds by bacteria was studied by using the different carbon and nitrogen sources at different environmental conditions. Results revealed that the bacterial consortium was efficient for 70% color removal in presence of glucose (1.0%) and peptone (0.1%) at pH 7.0 and temperature 37°C. The HPLC analysis of control and bacterial degraded samples has shown the reduction in peak area as well as shifting of peaks compared to control indicating the bacterial degradation as well as transformation of color containing compounds from DWW. The comparative LC–MS–MS and other spectrophotometric analysis has shown the presence of dihydroxyconiferyl alcohol, 2, 2′-bifuran-5-carboxylic acid, 2-nitroacetophenone, p-chloroanisol, 2, 3-dimethylpyrazine, 2-methylhexane, methylbenzene, 2, 3-dihydro-5-methylfuran, 3-pyrroline, and acetic acid in control samples that were biodegraded and biotransformed into 2-nitroacetophenone, p-chloroanisol, 2, 2′-bifuran, indole, 2-methylhexane, and 2, 3-dihydro-5-methylfuran by bacterial consortium. In this study, it was observed that most of the compounds detected in control samples were diminished from the bacterial degraded samples and compounds 2, 2′-bifuran and indole with molecular weight 134 and 117 were produced as new metabolites during the bacterial degradation of color containing compounds from DWW.

Keywords Distillery wastewater · Melanoidins · Biodegradation · Aerobic bacteria · Metabolites

R. N. Bharagava · R. Chandra (✉)
Environmental Microbiology Section, Indian Institute of Toxicology Research (CSIR), Post Box 80, M.G. Marg, Lucknow, Uttar Pradesh 226001, India
e-mail: rc_microitrc@yahoo.co.in; ramchandra_env@indiatimes.com

Introduction

The dark brown color of distillery wastewater is not only due to the presence of a complex biopolymer called melanoidins, which are generated by the Maillard reaction (Ohmomo et al. 1985; Chandra et al. 2008), but also to the caramel colorants, which are generated during the processing of sugarcane juice in sugar industries, as well as during the distillation of sugarcane molasses (Chandra et al. 2008). The chemical structure of melanoidins is still not completely understood, but it is assumed that it does not have a definite structure as its elemental composition and chemical structures largely depend

on the nature and molar concentration of parent reacting compounds and reaction conditions including pH, temperature, heating time and solvent system used (Yaylayan and Kaminsky 1998; Cammerer and Kroh 1995). Melanoidins are very recalcitrant in nature, hard to degrade by microbes and behave as anionic hydrophilic polymers, which can form stable complexes with metal cations (Plavsic et al. 2006; Migo et al. 1997).

Distillery wastewater is the major source of soil and water pollution due to high BOD (23,000), COD (47,400), TDS (10,480), phenolics (510), sulfate (3,786), and phosphate (739.0 mg l^{-1}) (Bharagava et al. 2008). These values are much higher than the permissible limit 40, 120, 2,100, 0.5, 750, and 1.0 mg l^{-1}, respectively (USEPA 2000; CPCB 2003). In soil, it inhibits seed germination, reduce soil alkalinity as well as manganese availability whereas in aquatic system, it causes reduction in penetration of sunlight, reducing the oxygenation of water by photosynthesis (Bharagava et al. 2008; Kumar and Chandra 2006). Mahimaraja and Bolan (2004) has estimated the LC_{50} value for distillery wastewater, 0.5% by using a bio-toxicity test on fresh water fish *Cyprinus carpio* var. *communis.* Later, Ramakritinan et al. (2005) have reported that respiratory process in *Cyprinus carpio* under distillery wastewater stress get affected resulting in a shift towards anaerobiosis at organ level during the sublethal intoxication. Hence, it requires pre-treatment for its safe disposal into the environment.

However, the treatment of distillery wastewater by physical or chemical methods such as ozonation (Kim et al. 1985), chemical methods (Chandra and Singh 1999), flocculation treatment (Migo et al. 1997) and activated carbon adsorption (Chandra and Pandey 2001) was found not feasible due to high cost and generation of secondary pollutants. It is also difficult to treat it by conventional biological treatment methods because melanoidins have antioxidant properties that render them toxic to aquatic macro and microorganisms (Chandra et al. 2008; Kumar et al. 1997; Sirianuntapiboon et al. 2004). Various authors have reported the microbial decolorization of melanoidins and distillery wastewater by using various fungal strains such as *Coriolus*, *Aspergillus*, *Phanerochaete* and some bacterial strains such as *Pseudomonas*, *Bacillus*, *Alkaligenes*, and *Lactobacillus* (Mohana et al. 2007; Kumar and Chandra 2006; Kumar et al. 1997; Ohmomo et al. 1985). Unfortunately, detailed information regarding the chemical nature of color contributing compounds (melanoidins, caramel, and phenolics), and their metabolic products is not available, which is very essential for the effective decolorization of distillery wastewater for environmental safety.

However, the structural characterization of color containing compounds/melanoidins and their metabolic products has proved to be rather challenging due to extreme complexity of Maillard reaction products (MRPs) that are known to range from small molecules to extremely large polymers. In addition, the molecular weight, structure as well as elemental composition of MRPs is strongly influenced by the ratio and type of reactants as well as reaction conditions such as temperature, reaction time, pH control and solvent used (Cammerer and Kroh 1995; Yaylayan and Kaminsky 1998). Recently, the methods such as electrospray ionization-mass spectrometry (ESI–MS), IR, ^{1}H NMR and liquid chromatography-mass spectrometry (LC–MS/MS) has emerged as novel techniques to provide a means of structurally characterizing the MRPs/melanoidins. Hence, the objectives of this study were to optimize the degradation of the major color containing compounds (melanoidins) extracted from distillery wastewater by an aerobic bacterial consortium and characterize the metabolic products produced during the bacterial degradation process.

Materials and methods

Collection of distillery wastewater and extraction of the major color containing compounds

Distillery wastewater was collected from *M/s* Unnao distillery and brewery, Ltd. Unnao (UP), India. From distillery wastewater, the color containing compounds (i.e. melanoidins) were extracted following the isopropanol method (Kalavathi et al. 2001). Equal volume (150 ml) of distillery wastewater and isopropanol was taken in a separating funnel (500 ml), mixed well and left for separation of two layers. After separation of layers, the isopropanol layer containing major color containing compounds was taken and dried in a hot air oven at 50°C, powdered and used in further studies.

Media composition

The modified GPYM medium containing glucose (1%), peptone (0.1%), K_2HPO_4 (0.1%) and $MgSO_4 \cdot 7H_2O$ (0.05%) in double distilled water was used through out this study. To this medium, the color containing compounds extracted from distillery wastewater were added (3,000 mg l^{-1}) to obtain the optical density 3.5 at 475 nm and pH was adjusted at 7.3 ± 0.1.

Bacterial culture and culture conditions

An aerobic bacterial consortium comprising the three bacterial strains *Bacillus licheniformis* (DQ779010), *Bacillus* sp. (DQ779011) and *Alcaligenes* sp. (DQ779012) isolated previously for their capability to degrade synthetic and natural melanoidins from distillery wastewater (Bharagava et al. 2009) was used in this study. These bacterial cultures were used to inoculate the GPYM agar plates amended with color containing compounds (3,000 mg l^{-1}) and agar 1.3% at pH 7.3 ± 0.1. After 48 h of growth in solid GPYM medium, the bacterial colonies were inoculated into 150 ml Erlenmeyer flasks containing 50 ml of autoclaved GPYM broth amended with color containing compounds (optical density 3.5 at 475 nm) and incubated at 35°C for 48 h under shaking flasks condition (125 rpm, Innova 4230 Refrigerated Incubator shaker, New Brunswick, USA). This 48 h grown bacterial culture in GPYM broth amended with color containing compounds was used as inocula in degradation study.

Optimization of nutritional and environmental parameters

The degradation of color containing compounds was optimized by supplementing the GPYM broth with different carbon sources including glucose, galactose, sucrose, fructose, ribose, and mannose at 1.0% (w/v) and organic and inorganic nitrogen such as yeast extracts, beef extract, peptone, sodium nitrate, ammonium nitrate, and urea at 0.1% (w/v) concentration, respectively. In addition, various environmental parameters including pH (5–10), temperature (30–45°C), shaking speed (50–175 rpm), and inoculum size (1–10%) were also optimized to achieve the optimum degradation of color containing compounds from DWW (Mohana et al. 2007; Kumar and Chandra 2006).

Biodegradation experiment

The degradation experiments were carried out in triplicate in 250 ml Erlenmeyer flasks containing 100 ml of sterile modified GPYM broth supplemented with color containing compounds (3,000 mg l^{-1}). The flasks were inoculated with 4% (v/v), 48 h grown mixed bacterial culture (4.4×10^6 CFU ml^{-1}) and incubated at 37°C under shaking flask condition (125 rpm, Innova 4230 Refrigerated Incubator shaker, New Brunswick, USA) for six consecutive days. The degradation of color containing compounds was monitored spectrophotometrically (Techcomp, UV-2300 spectrophotometer, Korea) in terms of bacterial growth, and reduction in color density (absorbance) at 620 and 475 nm, respectively and was expressed as decrease in absorbance at 475 nm against the initial absorbance at the same wavelength (Kumar and Chandra 2006; Bharagava et al. 2009).

Metabolite characterization

Isolation and purification of metabolites

A portion (200 ml) of both biotic (degraded) and abiotic (control) samples was centrifuged at 5,000 rpm for 10 min at 4°C to separate the bacterial biomass and other suspended particles from biotic and abiotic samples, respectively. The supernatant was vaccum evaporated at 40°C to concentrate metabolites and reduce the volume up to 50%. Each 100 ml aliquot of the concentrated biotic and abiotic samples was extracted thrice with equal volume of ethyl acetate (Shen et al. 2007; Yaylayan and Kaminsky 1998). The ethyl acetate extract from both biotic and abiotic samples were pooled, dehydrated with anhydrous sodium sulfate and vacuum dried at 40°C. The dry residue obtained was dissolved in HPLC grade methanol and used for metabolites analysis.

HPLC analysis

The degradation of color containing compounds (i.e. melanoidins) by mixed bacterial culture and

metabolites were analyzed using a Waters 515 HPLC system equipped with reverse phase column C-18 (250 mm × 4.6, particle size 5 μm) at 27°C and 2487 UV/VIS detector via millennium software. The methanol eluent (20 μl) was injected into the HPLC and monitored at wavelength 290 nm to assess the degradation of color containing compounds (Yaylayan and Kaminsky 1998). The mobile phase was consisted of methanol and water in volume ratio of 70:30 and set at the flow rate of 1.0 ml min^{-1}.

Spectroscopic analysis

The electrospray ionization-mass spectrum (ESI–MS) of biotic and abiotic samples was recorded with micromass Quattro II triple quadruple mass spectrometer. The samples were injected into the ESI source by a syringe at the flow rate of 5 μl min^{-1}. The ionization source and desolvation gas temperature were set at 130 and 400°C, respectively; whereas the cone gas and desolvation gas flow rates were set at 850 and 150 l h^{-1}, respectively. The capillary and cone voltage were set at 3.60 kV and 35 V, respectively while argon gas was used in collision cell. The collision energies for 84.00 and 279.20 fragment ions were 40 and 20 eV, respectively.

Infrared (IR) spectra were recorded on a Pye Unicam SP3-200 instrument as thin KBr discs and values were expressed in cm^{-1}. The samples for protonic nuclear magnetic resonance (H^1 NMR) spectrum were exchanged three times with D_2O under nitrogen atmosphere, dissolved in 400 μl of D_2O and transferred to a 5-mm NMR tube. The samples were scanned for ^1H NMR spectrum at 25°C with a Bruker, advance DRX-200 NMR spectrometer and the chemical shifts were recorded in ppm (δ) with TMS at 0.00 as an internal standard.

Liquid chromatography-MASS spectrometry (LC–MS/MS) analysis was performed using 2690 separation module with Quattro Ultima triple quadropole mass spectrometry detector (Waters, Micromass, Manchester, UK) equipped with an Atlantis® dC-18 column (2.1 × 50 mm, 3 μm; Waters, Milford, MA, USA). The mobile phase was consisted of 0.1% trifluoroacetic acid (TFA) in water and the flow rate was set at 0.2 ml min^{-1} and the column re-equilibration was performed between every injection.

Results and discussion

Optimized nutritional and environmental parameters

The degradation of major color containing compounds of distillery wastewater studied by using different carbon sources including glucose, galactose, sucrose fructose, ribose, and mannose at 1.0% (w/v) concentration has revealed that out of carbon sources used, glucose was the most adequate carbon source allowing 70% degradation of color containing compounds at 144 h incubation period (Fig. 1). It was also observed that the degradation of color containing compounds increases with increase in glucose concentration from 0.5 to 1.0% (w/v) and further, increase in glucose concentration did not improve degradation but did improve bacterial growth and biomass production. However, sucrose, fructose, and galactose were also found to be fairly good carbon sources allowing 57–68% degradation of color containing compounds, but mannose was relatively poor carbon source allowing 51% degradation only.

Results also revealed that out of organic and inorganic N sources used, peptone was most adequate nitrogen source allowing 68% degradation of color containing compounds at 0.1% (w/v) concentration (Fig. 2) and further, increase in peptone content resulted in a continuous decrease in degradation

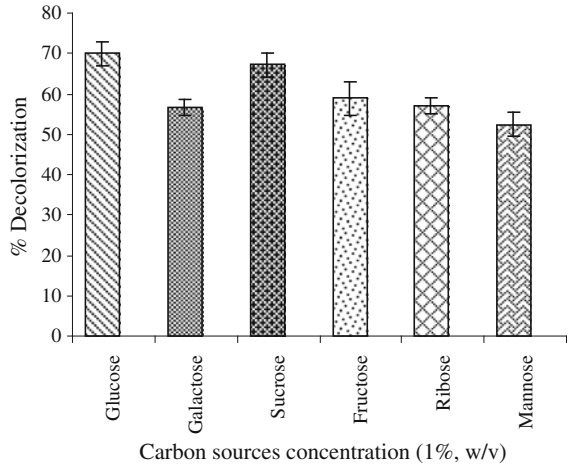


Fig. 1 Decolorization of color containing compounds by mixed bacterial culture in presence of different carbon sources

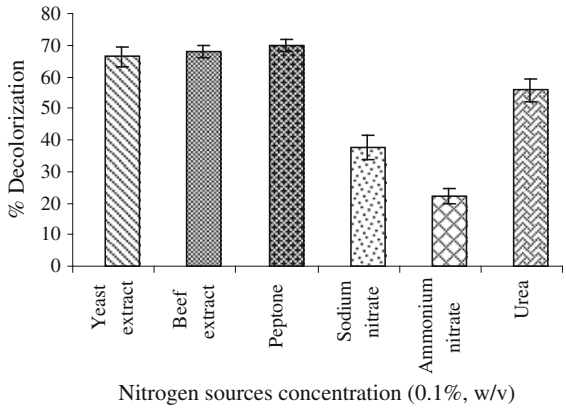


Fig. 2 Decolorization of color containing compounds by mixed bacterial culture in presence of different organic and inorganic nitrogen sources

capability of the mixed bacterial culture. Our findings are well corroborated with the earlier results reported by Mohana et al. (2007) and Sirianuntapiboon et al. (2004) who reported the optimum color removal from anaerobically treated distillery spent wash by bacteria and yeast belonging to *Citeromyces* sp. in presence of 1–3% glucose in 8 days. It indicated that the degradation and decolorization of color containing compounds present in distillery wastewater involve similar degradation mechanisms.

In addition, the maximum degradation of color containing compounds was recorded at pH 7.0 ± 0.1, and pH higher or lower than 7.0 showed reduction in growth and degradation capability of the mixed bacterial culture. The solubility of the major color containing compounds (i.e. melanoidins) is largely depend on the medium pH as it is less soluble in acidic than in alkaline pH (Mohana et al. 2007). It was also observed that increase in temperature from 30 to 37°C was accompanied with increased degradation of color containing compounds and further, increase in temperature (>37°C) adversely affected the degradation of color containing compounds possibly due to the loss of enzyme activity at higher temperature (Boer et al. 2006).

Further, the mixed bacterial culture has shown the optimum degradation of color containing compounds when incubated at 125 rpm and further increase in shaking speed decreased the degradation capability of mixed bacterial culture possibly due to the mechanical injury of bacterial cells at a higher shaking speed (>125 rpm). It was also observed that degradation of color containing compounds increases with increase in inoculum size and reached optimum at 4% (v/v) inoculum size ($\sim 4.4 \times 10^6$ CFU ml^{-1}) and further increase in inoculum size resulted higher biomass production only.

Biodegradation of color containing compounds

Results indicated that the mixed bacterial culture was capable for 70% degradation of color containing compounds (Fig. 3) at optimized nutritional and environmental conditions. During the degradation process, a marked increase in optical density (OD) for bacterial growth at 620 nm was observed indicating the fast growth of bacterial consortium and attained optimum growth at 120 h of incubation period. However, there was less decrease in color intensity at initial 24 h incubation period, possibly due to the utilization of glucose by bacteria and subsequently utilization of color containing compounds as sole carbon, nitrogen, and energy source. Thereafter, a sharp decrease in color intensity was observed up to 120 h of incubation period and this reduction in color intensity might be largely attributed to the bacterial degradation of color containing compounds.

Characteristics of the major color containing compounds and their metabolites

The HPLC analysis of bacterial degraded samples has shown the reduction in peak areas compared to control sample (Fig. 4) indicating that decrease in color intensity might be largely attributed to the bacterial degradation of color containing compounds.

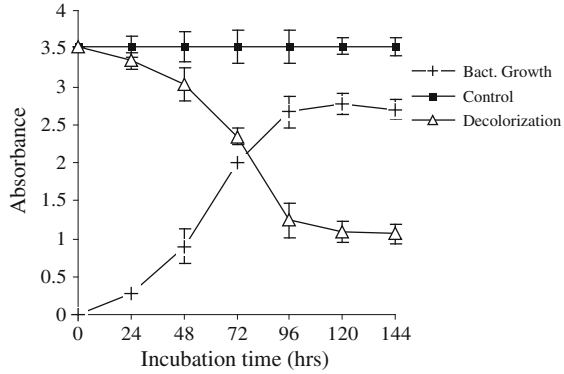


Fig. 3 Bacterial growth and decolorization of color containing compounds by mixed bacterial culture

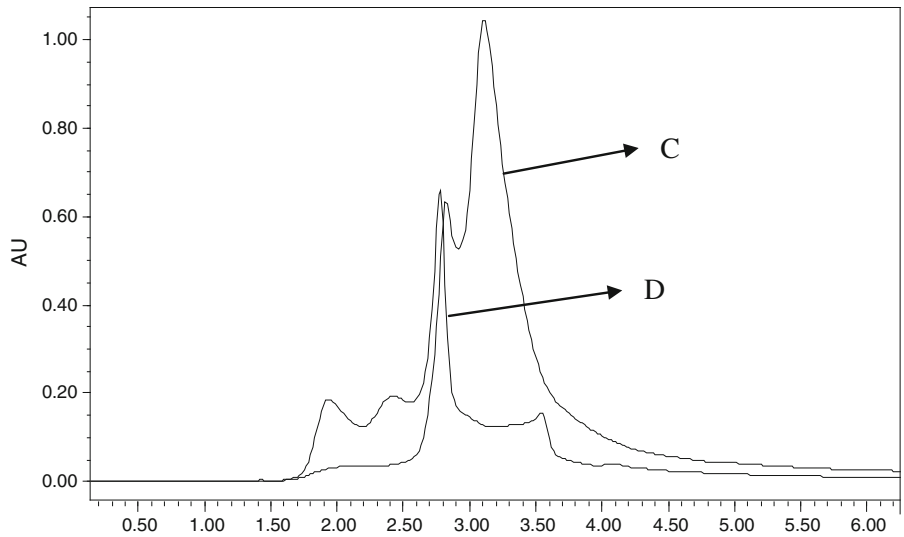


Fig. 4 Comparative HPLC chromatogram of color containing compounds before (*C*) and after bacterial decolorization (*D*)

In addition, the bacterial degraded samples also showed shifting of peaks compared to control samples indicating the biodegradation as well as biotransformation of color containing compounds by bacteria.

The structural characterization of MRPs/melanoidins has proved to be rather challenging due to extreme complexity of MRPs that are known to range from small molecules to extremely large polymers. Various authors have reported that MRPs/melanoidins are complex polymers consisting of repeating units of furans and/or pyrroles, which are produced during the advanced stages of Maillard reaction and remain linked by polycondensation reactions (Tressl and Wondrak 1998). In addition, Hofmann (1998) also reported that MRPs are low molecular weight colored compounds, which crosslink proteins via €-amino groups of lysine or arginine and polymerized through aldol-type condensation reactions to produce high molecular weight colored MRPs/melanoidins. The chemical structure of these MRPs/melanoidins is mainly built of sugar degradation products, which largely depend on the starting materials as well as on reaction conditions (Cammerer and Kroh 1995).

The electrospray ionization-mass spectrum (ESI–MS) of control sample has shown three major molecular ion peaks at (*m/e*) 453, 381, and 317 with molecular mass (*m/z*) 452, 380, and 316, respectively. The MS–MS fragmentation pattern of these molecular ion peaks has indicated the generation of several product ion peaks at (*m/e*) 183, 133, 115, 101, 87, 85, 69, and 60 with molecular mass (*m/z*) 182, 134, 115, 100, 96, 84, 69, and 60, respectively. The ESI–MS of bacterial degraded sample has shown only two major peaks at (*m/e*) 449, and 413 with molecular mass (*m/z*) 449, and 413, respectively. The MS–MS fragmentation pattern of these peaks indicated the generation of several product ion peaks at (*m/e*) 181, 179, 165, 91, 85, and 69, respectively.

For the structural characterization, the control and bacterial degraded samples were also analyzed through infrared (IR) spectroscopy, which has shown the stretching frequencies (v) at 3,426, 2,940, 1,721, 1,658, 1,641, 1,566 and 1,408 cm^{-1} corresponding to

Table 1 Infrared (IR) spectroscopic properties of color containing compounds and their metabolites isolated from control and bacterial decolorized samples, respectively

Sample	Stretching frequencies (λ max)	Assignments
Control	3,326, 2,940,1,721, 1,658, 1,641,1,566, and 1,408 cm^{-1}	–OH, –C–H, =C=O,–CHO, –COOH, –C=C–, and –NO_2 groups, respectively
Decolorized	3,426, 2,940, 1,721, and 1,408 cm^{-1}	–OH, –C–H, =C=O, and NO_2 groups, respectively

the presence of an alcoholic (–OH), –C–H stretching, ketonic (=C=O), aldehydic (–CHO), carboxylic (–COOH), carbon carbon double bond (–C=C–) and an asymmetric $–NO_2$ group, respectively (Table 1). Simultaneously, the ^{1}H NMR spectrum of control and degraded samples recorded in up and down fields has shown signals at δ 4.88 ppm for the presence of methoxy group while signals at δ 4.83 and δ 2.20 ppm has indicated the presence of multiplet of six protons responsible for two protons each for olefinic, enolic, alcoholic and aliphatic groups, respectively. In down field region, two multiplet of protons were observed, one between δ 7.52–7.55 ppm indicating the presence of four aromatic protons and other between δ 7.71–7.74 ppm for the presence of four other aromatic protons. The signals at 8.21 ppm have clearly indicated the presence of an aldehyde carbon, which confirms a –CHO group as side chain.

The LC–MS–MS analysis of control sample has shown three major molecular ion peaks at (*m/z*) 181, 179, and 165 with molecular mass (*m/z*) 182, 178, and 165, respectively (Fig. 5C). The MS–MS fragmentation pattern of these molecular ion peaks has indicated the generation of several small product ion peaks at (*m/e*) 141, 107, 101, 91, 85, 69, and 60 with molecular mass (*m/z*) 142, 108, 100, 92, 84, 69, and 60, respectively. On the basis of IR and ^{1}H NMR results indicating the presence of different functional groups and protons, the compounds detected in control samples were characterized as dihydroxyconiferyl alcohol ($C_{10}H_{14}O_3$), 2, 2′-bifuran-5-carboxylic acid ($C_9H_6O_4$), 2-nitroacetophenone ($C_8H_7NO_3$), p-chloroanisol (C_7H_7ClO), 2, 3-dimethyl-pyrazine ($C_6H_8N_2$), 2-methylhexane (C_7H_{16}), methylbenzene (C_7H_8), 2, 3-dihydro-5-methylfuran (C_5H_8O), 3-pyrroline (C_4H_7N), and acetic acid ($C_2H_4O_2$), respectively.

The LC–MS–MS analysis of bacterial degraded sample has shown only one major molecular ion peak at (*m/e*) 165 with molecular mass (*m/z*) 165 (Fig. 5D). The MS–MS fragmentation of this molecular ion peak has indicated the generation of five small products ion peaks at (*m/e*) 143, 133, 117, 101, and 85 with molecular mass (*m/z*) 142, 134, 117, 100, and 84, respectively. Further, on the basis of IR and ^{1}H NMR results, these fragmented product ions were characterized as 2-nitroacetophenone ($C_8H_7NO_3$), p-chloroanisol (C_7H_7ClO), 2, 2′-bifuran ($C_8H_6O_2$), indole (C_8H_7N), 2-methylhexane (C_7H_{16}), and 2, 3-dihydro-5-methylfuran (C_5H_8O), respectively. It was also observed that most of the compounds detected in control samples were diminished from the bacterial degraded samples (Table 2). The peak area of compound 2-nitroacetophenone showed no change in bacterial degraded samples whereas compound 2, 2′-bifuran and indole with molecular weight 134 and 117 was produced as new metabolites during the bacterial degradation of color containing compounds from distillery wastewater.

Conclusion

This study revealed that the developed bacterial consortium was capable of 70% degradation of color containing compounds in presence of glucose and peptone as carbon and nitrogen source at 1.0 and 0.1% (w/v) concentration, respectively at pH 7.0 and temperature 37°C. The comparative LC–MS–MS

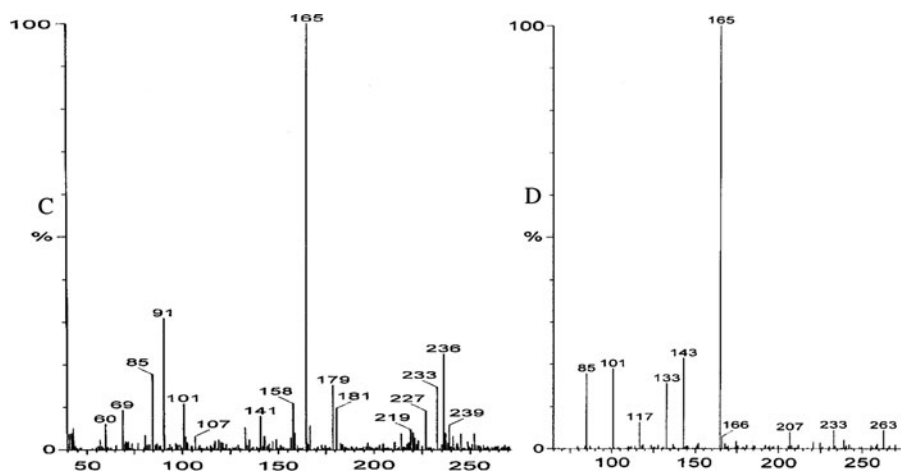
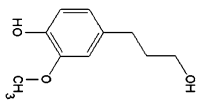
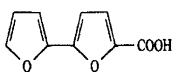
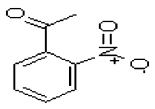
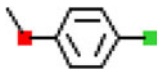
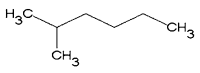
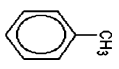
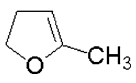
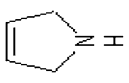
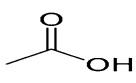
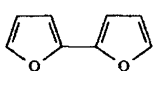
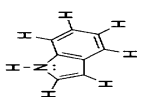


Fig. 5 LC-ESI/MS/MS chromatogram of control (*C*) and bacterial decolorized sample (*D*)

Table 2 Color containing compounds and their metabolites identified in control and bacterial decolorized samples, respectively (pH 7.0; temperature 37°C; shaking speed 125 rpm, and inoculum size 4.4×10^6 CFU/ml^{-1})

Sl. no.	Identified color containing compounds			Sample	
	Structure	*m/z* value	Chemical name	Control	Decolorized
1.		182	*Dihydroxyconiferyl alcohol ($C_{10}H_{14}O_3$)*	+	−
2		178	*2, 2′-bifuran-5-carboxylic acid ($C_9H_6O_4$)*	+	−
3.		165	*2-nitroacetophenone ($C_8H_7NO_3$)*	+	+
4.		142	*p-chloroanisol (C_7H_7ClO)*	+	+
5.		108	*2, 3-dimethyl-pyrazine ($C_6H_8N_2$)*	+	−
6.		100	*2-methylhexane (C_7H_{16})*	+	+
7.		92	*Methyl benzene (C_6H_8)*	+	−
8.		84	*2, 3-dihydro-5-methylfuran (C_5H_8O)*	+	+
9.		69	*3-pyrroline (C_4H_7N)*	+	−
10.		60	*Acetic acid ($C_2H_4O_2$)*	+	−
11.		134	*2, 2′-bifuran ($C_8H_6O_2$)*	−	+
12.		117	*Indole (C_8H_7N)*	−	+

+ present, − absent

and other spectrophotometric analysis of control and bacterial degraded sample have shown that most of the color containing compounds detected in control sample were diminished from the bacterial degraded samples. The disappearance of color containing compounds from bacterial degraded samples could be related with the color removal associated with the degradation of color containing compounds. In addition, compounds 2, 2′-bifuran and indole with molecular weight 134 and 117 were produced as new metabolites during the bacterial degradation of color containing compounds. All these observations have revealed that the developed bacterial consortium was capable for the effective degradation and decolorization of color containing compounds and could be useful for the decolorization and detoxification of distillery wastewater for environmental safety.

Acknowledgments The financial assistance received by Mr. R.N. Bharagava, SRF from University Grants Commission and Council of Scientific and Industrial Research (CSIR Network project NWP-19), New Delhi is highly acknowledged.

References

Bharagava RN, Chandra R, Rai V (2008) Phytoextraction of trace elements and physiological changes in Indian mustard plants (*Brassica nigra* L.) grown in post methanated distillery effluent (PMDE) irrigated soil. Biores Technol 99:8316–8324

Bharagava RN, Chandra R, Rai V (2009) Isolation and characterization of aerobic bacteria capable of the degradation of synthetic and natural melanoidins from distillery wastewater. World J Microbiol Biotechnol 25:737–744

Boer CG, Obici L, D'Souza CGM, Peralta RM (2006) Purification and some properties of Mn peroxidase from *Lentinula edodes*. Process Biochem 41:1203–1207

Cammerer B, Kroh LW (1995) Investigation of the influence of reaction conditions on the elementary composition of melanoidins. Food Chem 53:55–59

Chandra R, Pandey PK (2001) Decolorization of anaerobically treated distillery wastewater by activated charcoal adsorption method. Ind J Environ Prot 2:132–134

Chandra R, Singh H (1999) Chemical decolorization of anaerobically treated distillery wastewater. Ind J Environ Prot 19(11):833–837

Chandra R, Bharagava RN, Rai V (2008) Melanoidins as major colorant in sugarcane molasses based distillery wastewater and its degradation. Biores Technol 99:4648–4660

CPCB (2003) Charter on corporate responsibility for environmental protection. Workshop organized at Mumbai by MPCB on 03.01.2003. http://cpcb.nic.in//Charter/charter5.htm

Hofmann T (1998) Studies on melanoidin-type colorants generated from the Maillard reaction of protein-bound lysine and furan-2-carboxaldehyde-chemical characterization of a red colored domain. Z Lebensm Unters Forsch 206: 251–258

Kalavathi DF, Uma L, Subramanian G (2001) Degradation and metabolization of the pigment-melanoidin in distillery wastewater by the marine cyanobacterium *Oscillatoria boryana* BDU 92181. Enzyme Microbiol Technol 29:246–251

Kim SB, Hayase F, Kato H (1985) Decolorization and degradation products of melanoidins on ozonolysis. Agric Biol Chem 49:785–792

Kumar P, Chandra R (2006) Decolorization and detoxification of synthetic molasses melanoidins by individual and mixed cultures of *Bacillus* sp. Biores Technol 7:2096–2102

Kumar V, Wati L, Nigam P, Banat IM, MacMullan G, Singh D, Marchant R (1997) Microbial decolorization and bioremediation of anaerobically digested molasses spent wash wastewater by aerobic bacterial culture. Microbios 89: 81–90

Mahimaraja S, Bolan NS (2004) Problems and prospects of agricultural use of distillery spent wash in India. 3rd Australian New Zealand Soils Conference. 5–9 December'2004. University of Sydney, Australia

Migo VP, Del Rosario EJ, Matsumura M (1997) Flocculation of melanoidins induced by inorganic ions. J Ferment Bioeng 83:287–291

Mohana S, Desai C, Madamwar D (2007) Biodegradation and decolorization of anaerobically treated distillery spentwash by a novel bacterial consortium. Biores Technol 98:333–339

Ohmomo S, Itoh N, Wantanabe Y, Kaneko Y, Tozawa Y, Udea K (1985) Continuous decolorization of molasses wastewater with mycelia of *Coriolus versicolor* Ps4a. Agric Biol Chem 49:2551–2555

Plavsic M, Cosovic B, Lee C (2006) Copper complexing properties of melanoidins and marine humic material. Sci Total Environ 366:310–319

Ramakritinan CM, Kumaraguru AK, Balasubramanian MP (2005) Impact of distillery effluent on carbohydrate metabolism of freshwater fish *Cyprinus carpio*. Ecotoxicol 14:693–707

Shen SC, Tseng KC, Wu JSB (2007) An analysis of Maillard reaction products in ethanolic glucose-glycine solution. Food Chem 102:281–287

Sirianuntapiboon S, Phothilangka P, Ohmomo S (2004) Decolorization of molasses wastewater by a strain No.BP103 of acetogenic bacteria. Biores Technol 92:31–39

Tressl R, Wondrak GT (1998) New melanoidin-like Maillard polymer from 2-deoxypentoses. J Agric Food Chem 46:104–110

USEPA (2000) National primary drinking water standards. <http://www.epa.gov/safewater/contaminants/index.html#primary>

Yaylayan VA, Kaminsky E (1998) Isolation and structural analysis of Maillard polymers: caramel and melanoidins formation in glycine/glucose model system. Food Chem 63:25–31