Evaluation of molasses-melanoidin decolourisation by potential bacterial consortium discharged in distillery effluent

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Abstract

The extracted sugarcane molasses-melanoidins showed the presence of Mn (8.20), Cr (2.97), Zn (16.61), Cu (2.55), Fe (373.95), Pb (2.59), and Ni (4.18 mg L⁻¹) along with mixture of other organic compounds which have endocrine-disrupting chemicals (EDCs) properties. A consortium of aerobic bacteria comprising *Klebsiella pneumoniae* (KU321273), *Salmonella enteric* (KU726954), *Enterobacter aerogenes* (KU726955), and *Enterobacter cloacae* (KU726957) showed the optimum decolourisation of molasses-melanoidins up to 81% through co-metabolism in the presence of glucose (1.0%) and peptone (0.2%) as a carbon and nitrogen source, respectively. The absorption spectrum scanning by UV-visible spectrophotometer between 200 and 700 nm revealed reductions of absorption spectrum of organic compounds present in bacterial degraded sample of melanoidins in range of 200–450 nm compared to control. The degradation and decolourisation of melanoidins by bacterial consortium was noted by induction of manganese peroxidase and laccase activities in sample supernatant. Furthermore, the TLC and HPLC analysis of bacterial decolourised melanoidins also showed degradation and reduction of absorption peak at (295 nm), respectively. Furthermore, FT-IR and GC-MS analysis also showed the change of functional group and disappearance of ion peaks. This indicated the degradation and depolymerisation of melanoidins and cleavage of C=C, C=O and C≡N conjugated bonds which resulted in reduction of colour. The metabolic analysis also showed the disappearance of some organic compounds and generation of new metabolites. Furthermore, the seed germination test using *Phaseolus mungo* L. showed toxicity reduction in decolourized effluent.

Keywords Melanoidins · Metabolites · PMDE decolourisation · Endocrine-disrupting chemicals · Toxicity reduction

Introduction

Melanoidins are negatively charged, complex, heterogeneous non-linear polymers produced through non-enzymatic Maillard reaction (MR) between amino compounds and carbohydrates, and are highly resistant to biological and chemical degradation (Wedzicha and Kaputo 1992; Wang et al. 2011). It is a major colouring constituents of dark-brown effluent released from sugarcane molasses-based ethanol

producing distillery industries. During the distillation process, an average of 12–15 L of effluent is generated per litre of ethanol production (Chandra and Kumar 2017a). Dark coloured effluent from the distilleries is one of the most obvious indicators of water pollution. Apart from the colour, the discharged distillery effluent also contains high amount of total dissolved solids (TDS) and recalcitrant pollutants in form of complex polymer containing heterocyclic nitrogenous compounds of aldehyde-amines, various heavy metals, phenolic compounds and plant derived resins and fatty acids which behave as endocrine-disrupting chemicals (EDCs) (Chandra and Kumar 2017a). The major metallic constituents in distillery effluent is reported as iron (Fe), copper (Cu), manganese (Mn), lead (Pb), zinc (Zn), cadmium (Cd), and nickel (Ni). Furthermore, it has also been reported that melanoidins have net negative charges; therefore, various heavy metals strongly bind with melanoidins to form large organo-metallic complex molecules (Migo et al. 1997). Therefore, discharged effluent after anaerobic

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treatment causes environmental problems in aquatic and soil ecosystem which ultimately affect to aquatic flora and fauna. Therefore, adequate treatment of the distillery effluent is warranted prior to its discharge into environment (Chandra et al. 2008; Satyawali and Balakrishnan 2008).

The conventional aerobic–anaerobic secondary treatment approaches such as activated sludge, anaerobic digestion, and anaerobic lagoons processes have been found ineffective to remove the colour of distillery effluent due to the recalcitrant nature of melanoidins and presence of other complex co-pollutants. Moreover, the effluent after anaerobic treatment (methanogenesis) retain dark-brown colour due to complexation of organic and inorganic pollutants present in spent wash (Wolfson et al. 1953). In addition, the various physico-chemical methods, i.e., flocculation, nanofiltration, coagulation, electro-flotation, adsorption, catalytic thermolysis, oxidation, and ozonation have also been applied for elimination of melanoidins from distillery effluent (Liu et al. 2013; Liang et al. 2009; Chaudhari et al. 2005, 2007; Onyango et al. 2011). However, these methods were found not applicable at large scale due to high operation cost, high consumption of chemicals and generation of huge amount of toxic sludge, and other secondary pollutants. Therefore, researchers are still searching the biological treatment methods as the best alternative. In recent year, natural treatment with microbial decolourisation process of effluent is drawing attention of workers world over, because they are eco-sustainable and cost-effective alternative to chemical methods (Bharagava et al. 2009). The growing acceptance of microorganisms for decolourisation and detoxification of melanoidins containing distillery effluent is due to fact that some microorganisms have a specific extracellular ligninolytic enzymatic system known as manganese peroxidase (MnP), laccase, and lignin peroxidase (LiP) capable of breaking a large number of C=C, C=O, and C≡N bonds present in melanoidins (Miyata et al. 2000). Though, some works have been reported with fungal treatment, but due to growth limitation of fungus in aquatic conditions at low pH restricted its large-scale application for development of effluent treatment process. In contrast, the bacterial consortium has been reported more promising due to their immense environmental adaptability and versatile metabolic pathway for catabolism of complex recalcitrant compounds.

Some workers have reported the bacterial decolourisation and degradation of sugarcane molasses-melanoidins under optimized conditions (Bharagava et al. 2009; Kumar and Chandra 2006; Yadav and Chandra 2012). However, most of decolourisation and degradation of molasses-melanoidins is reported at 475 nm only as purified melanoidins through dialysis process with specific molecular weight, while the molasses-melanoidins contains mixture of Maillard reaction products (MRPs) (i.e., initial, intermediate, and advanced stages with variable molecular weight). However, due to

presence of a mixture of different molecular weight (5 to 40 KDa) MRPs in molasses-melanoidins it shows variable absorption spectrum at different wavelength. But, absorption and biodegradation pattern of different peaks in molasses-melanoidins is not reported so far. Therefore, it is essential to reveal the reduction pattern of specific peak of molasses-melanoidins in degradation and generation of its metabolic products during decolourisation process. Moreover, no study has been conducted for assessment of molasses-melanoidin degradation present in its original form for the feasibility of any potential bacterial consortium.

Hence, the prime objectives of this study were to optimize the environmental conditions for the decolourisation and degradation of molasses-melanoidins extracted from post-methanated distillery effluent (PMDE). Furthermore, the analysis of absorption spectrum between 200 and 700 nm by UV–Vis spectrophotometer has been correlated for their structural changes and reduction in colour by investigation through thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), fourier transform-infrared spectroscopy (FT-IR), and gas chromatography–mass spectrometry (GC–MS) analysis. Furthermore, the stability of developed potential bacterial consortium at variable nutritional and environmental conditions was evaluated for its effective degradation and degradation of molasses-melanoidins containing distillery effluent for the development of feasible technology. The toxicity evaluation of molasses-melanoidins prior and after bacterial degradation was also done using seed germination test of *Phaseolus mungo* L.

Materials and methods

Chemicals and reagents

All the chemicals and reagents used in this study were of analytical grade. The standard chemical reagents for BOD (Biological oxygen demand) and COD (Chemical oxygen demand) measurement, and organic solvents namely ethyl acetate and isopropanol for the extraction organic compounds from molasses-melanoidins, were procured from Merck (Merck KGaA, Darmstadt, Germany). The derivatizing reagents such as pyridine, BSTFA (*N,O*-bis (trimethylsilyl) trifluoroacetamide), and TMCS (trimethylchlorosilane) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Collection of distillery effluent and extraction of molasses-melanoidins

PMDE after anaerobic treatment (methanogenesis) was collected aseptically in pre-sterilized plastic container (capacity 25 L; Tarsons Products Private Limited, USA) from the

effluent collection tank of M/s Unnao Distilleries and Breweries Limited Unnao, Uttar Pradesh (26°32'0" N, 80°30'0" E), India. All the collected samples were transported to laboratory and kept at 4 °C for further analysis. Molasses-melanoidins were extracted from PMDE by following the isopropanol method described earlier by Kalavathi et al. (2001). The resulted melanoidins was dried in a hot air oven at 80 °C, powdered, and used in this study.

Medium to study decolourisation

The medium used for melanoidins decolourisation study containing (g L⁻¹) glucose 1.0%, peptone 0.5%, MgSO₄·7H₂O 0.05%, and K₂HPO₄ 0.1% w/v). This medium was supplemented with different concentration of molasses-melanoidins, viz., 1000–3500 mg L⁻¹ (w/v) to make relationship between the decolourisation and concentration of molasses-melanoidins. The final pH of medium was adjusted to 7.0 using 0.1 N NaOH or 0.1 N HCl.

Preparation of bacterial culture

The aerobic bacterial consortium consisting with four bacterial strains *Klebsiella pneumoniae* (KU726953), *Salmonella enterica* (KU726954), *Enterobacter aerogenes* (KU726955), and *Enterobacter cloacae* (KU726957) isolated from distillery sludge sample in our laboratory to degrade synthetic Maillard reaction products (MRPs) was used in this study (Kumar and Chandra 2018). The seed culture was prepared for degradation by inoculating the pure bacterial colonies in broth medium and incubated for 24 h in continuous shaking condition (120 rpm) in 250 mL Erlenmeyer flask containing sterilized 100 mL mineral salt medium supplemented with K₂HPO₄ (0.1%), MgSO₄·7H₂O (0.05), and molasses-melanoidins (1000 mg L⁻¹) and incubated at 35 ± 1 °C and 130 rpm.

Physico-chemical analysis of molasses-melanoidins

The physico-chemical properties such as colour, pH, BOD, COD, TDS, total solid (TS), volatile solids (VS), chloride, total nitrogen, ammoniacal nitrogen, phenol, sulphate, phosphate, and heavy metals of molasses-melanoidins before and after bacterial decolourisation were carried out using the standard methods (APHA 2012).

Measurement of decolourisation efficiency

The decolourisation experiment was carried out in Erlenmeyer flasks (250 mL) containing 100 mL of previously prepared sterile melanoidins containing medium. The flasks were inoculated with overnight grown fresh culture of four identified bacterial strains, i.e., *K. pneumoniae* (KU726953),

S. enterica (KU726954), *E. aerogenes* (KU726955), and *E. cloacae* (KU726957). The each bacterial culture was inoculated for their exponential phase with highest optical density (O.D.) such as *K. pneumoniae* (OD₆₂₀ 0.24), *S. enterica* (OD₆₂₀ 0.24), *E. aerogenes* (OD₆₂₀ 0.24), and *E. cloacae* (OD₆₂₀ 0.24). The different bacteria were inoculated in flask in equal ratio, i.e., 1:1:1:1 and incubated at 37 ± 1 °C under shaking flask condition (120 rpm) for 192 h. The sample were collected at every 24 h interval during incubation and centrifuged at 10,000×g for 10 min at 4 °C for measurement of decolourisation efficiency. Melanoidins decolourisation efficiency was monitored by measuring the change in absorbance maxima of the melanoidins at 295 nm after scanning of absorbance maxima using a UV–Vis spectrophotometer (Evolution-201, Thermo Scientific, USA). Simultaneously, the control flasks without any bacterial inoculation were also incubated in shaker with the same temperature and shaking speed. Finally, the bacterial cell in flask was found in ratio of 2:1:2:2. This indicated the optimum ratio of different bacteria in consortium for maximum decolourisation of molasses-melanoidins. Therefore, the ratio of different bacteria in consortium was selected in ratio of 2:1:2:2 for optimisation of decolourisation process at different nutritional and environmental parameters. The decolourisation efficiency was expressed as percent (%) of decolourisation:

$$\text{Decolourisation (\%)} = \frac{(A_i) - (A_f)}{(A_i)} \times 100,$$

where A_i is the initial absorbance and A_f is the final absorbance of medium after decolourisation at the λ_{max} 295 nm.

Optimisation of culture conditions for decolourisation and degradation of melanoidins

Effect of carbon and nitrogen source

To find the most appropriate carbon source, viz., sucrose, fructose, glucose, xylose, lactose, and starch, at 1.0% (w/v) were evaluated for the melanoidins decolourisation and degradation. In another experiment, different organic and inorganic nitrogen sources, viz., peptone, beef extract, yeast extract, sodium sulphate, ammonium chloride, and urea, were added into the GPM medium at 0.5% (w/v) concentration to evaluate the best nitrogen source for melanoidins decolourisation and bacterial growth.

Effect of environmental parameters

To evaluate the effect of different environmental parameters on melanoidins decolourisation, the same experiment was

carried out at varying temperatures (25–50 °C), pH (4–12), and shaking speed (100–220 rpm).

Evaluation of bacterial growth, biomass, and SEM observation of bacterial consortium

During decolourisation experiment, bacterial cell growth was monitored by measuring the optical density (O.D.) at 620 nm at 12 h periodic interval, while the bacteria grown sample for biomass determination and SEM micrograph was collected from the flask after 144 h of incubation. For bacterial biomass determination, the bacterial cells were centrifuged (6500×g) for 10 min at 4 °C, the pellets were washed thrice with distilled water to remove the attached medium contents and then dried in an hot air oven at 80 °C until getting a constant dried weight reported in the form of dry cell mass (g L⁻¹). For SEM micrograph, the bacterial cells were fixed with 1% (w/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 h and washed again with distilled water. The fixed samples were then dehydrated through 25, 50, 75, 95, and 100% ethanol solutions in increasing concentration for 5 min, at each step. The samples were then dried in critical point drier and coated with a thin conductive film of gold in a sputtering coater and observed under SEM (FEI Quanta 450, Hillsboro, USA).

Enzyme assay

To measure the MnP and laccase activity during melanoidins degradation, the bacterial degraded supernatant was obtained by centrifugation at 6500×g for 10 min at 4 °C. The MnP and laccase activity was determined using the phenol red (Lobachemie, Mumbai) and guaiacol (HiMedia, Mumbai), respectively, as reported earlier (Arora et al. 2002). One international unit (IU) of enzyme activity was defined as 1.0 µM product formed per minute under assay conditions.

Decolourisation and degradation analysis

UV–Vis and FT-IR spectroscopic analysis

Melanoidins decolourisation was quantitatively analysed using a UV–Vis spectrophotometer in the wavelength range between 200 and 700 nm at 25 °C, whereas biodegradation was monitored by FT-IR analysis of the sample was performed in range of 400–4000 cm⁻¹ using a spectrophotometer (Nicolet™ 6700, Thermo Scientific, USA) to explore the chemical nature of the melanoidins. The separated sample of melanoidins was mixed with KBr (potassium bromide) to prepare the pellet for FT-IR analysis.

Detection of EDCs and other organic compounds

The EDCs and other organic compounds present in treated and untreated melanoidins containing samples were extracted by ethyl acetate under acidic condition (pH < 2.0) as previously described by Chandra and Kumar (2017a). The organic solvent phase was dried over anhydrous Na₂SO₄, and the solvent was evaporated to dryness using a stream of nitrogen gas at room temperature. The residues were dissolved in 2.0 mL methanol. The sample in methanol was filtered through 0.22-µm syringe filters and used for TLC, HPLC, and GC–MS analysis.

TLC and HPLC analysis

The concentrated sample resolved on a pre-coated silica-gel TLC plate (silica G 20×20 cm, 0.25 mm thickness). This plate was developed in an organic solvent system toluene: chloroform: methanol: water (10:10:9:9 v/v). After development, the bands of decolourisation were observed under UV light through gel documentation system (Uvtech Cambridge, 1320012; GeNei, Bangalore, India). HPLC analysis was performed on 515 HPLC instrument equipped with a diode array detector system (1100 series, Agilent Technologies, USA) and reverse phase C₁₈ column (250×4.6 mm, 5 µm particle size) by using the gradient of solvent A (Milli-Q water) and solvent B (acetonitrile with 0.1% TFA) (Merck KGaA, Darmstadt, Germany) at a flow rate 0.4 mL min⁻¹ for 60 s. The detection was monitored at wavelength 295 nm (absorption maxima) to assess the decolourisation and degradation of melanoidins.

GC–MS analysis

Identification of EDCs and other organic compounds before and after bacterial treatment was carried out by GC–MS analysis. In the process of GC–MS analysis, the extracted samples from untreated and bacterial treated were derivatized with trimethylsilyl (TMS) as described earlier by Chandra and Kumar (2017a). Where an aliquot (2.0 µL) of derivatized sample was injected in GC–MS instrument (Trace GC Ultra Gas Chromatograph; Thermo Scientific, FL, USA) equipped with a TriPlus auto sampler coupled to TSQ Quantum XLS triple quadrupole mass spectrometer (Thermo Scientific, FL, USA). Furthermore, the separation of organic compounds occurred in DB-5MS capillary column. The temperature of GC oven was programmed; it was started from 65 °C (hold for 2.0 min), increased to 230 °C at a rate of 6 °C min⁻¹, and finally reached to 290 °C (hold for 20 min) at the rate of 10 °C min⁻¹ increased. Helium used as carrier gas at a flow rate of 1.1 mL min⁻¹. Mass spectrum (MS) was operated in the positive electron ionisation (+EI) mode at 70 eV. The detected organic compounds extracted from

untreated and treated samples were identified by matching with the MS library NIST version 1.0.0.12 available with instrument.

Phytotoxicity assessment

The toxicity assessment of untreated and bacterial treated melanoidins was studied with *Phaseolus mungo* L. seed germination using the Petri dish method as reported earlier (Chandra and Kumar 2017a, b). Prior to conducting the test experiment, the seed surface was sterilized with 0.1% mercury chloride (HgCl_2) solution for 2 min to avoid any microbial contamination. Furthermore, seeds were subjected to repeated washings with sterilized distilled water to remove the residual HgCl_2 . Subsequently, ten seeds of *P. mungo* L. were placed separately in sterilized glass Petri dishes lined with three filter paper discs (Whatman® Qualitative Filter Paper No. 1). These filter discs were then wetted with 10 mL of tap water for control and with the same volume of treated and untreated samples separately followed by incubation at room temperature (25 °C) for a period of 3 consecutive days. The seed germination parameters like germination percent, germination index (GI), relative toxicity percent, phytotoxicity percent, and stress tolerance index (STI) were calculated using the formula described earlier (Chandra and Kumar 2017a, b).

Statistical analysis

To avoid any experimental errors, each experiment was performed in triplicate. The standard deviation (SD) was calculated using Microsoft Excel (ver. 2007, Microsoft®, USA) and results presented as mean $\pm$ SD value. The mean value of various physico-chemical parameters of untreated and treated melanoidins was assessed Student's 't' test using SPSS software package (version 22.0; SPSS Inc., Chicago, IL, USA).

Results and discussion

Physico-chemical analysis

The physico-chemical properties of control and bacterial decolourised melanoidins containing samples are presented in Table 1. The value of colour intensity and pH was higher in the control sample. In addition, the concentration of BOD, COD, TS, TDS, VS, chloride, total nitrogen, ammoniacal nitrogen, phenol, sulphate, phosphate, and various heavy metals such as Mn, Cr, Zn, Ni, Cu, Fe, Pb, and Cd was found higher, as shown in Table 1. The BOD/COD ratio of untreated melanoidins sample was found 0.39. The high

pH of control sample might be due to the binding of high concentrations of soluble salts and heavy metals with melanoidins (Chandra and Kumar 2017b; Chandra et al. 2018). The high BOD and COD values of the prepared melanoidins solution suggested the presence of organic and inorganic compounds in higher quantities. The low BOD/COD ratio indicated that melanoidins containing sample have some toxic compounds, since some caramelized sugar might also be generated during the MR. Therefore, high TDS of solution is found in this study. In addition, the high content of heavy metals in distillery effluent could be due to the corrosive effect of sugarcane juice during the sugar manufacturing process (Chandra et al. 2018; Chandra and Kumar 2017b). Besides, metals might also be added during the fermentation and distillation processes of fermented sugarcane molasses in distilleries, which is finally discharged as PMDE with alkaline pH. Our findings also corroborated well with earlier observation (Chandra and Kumar 2017a). Phenols are fragmentary product of Amadori compound, which play a key role during the melanoidins formation. The high levels of organic and inorganic parameters in distillery effluent are also corroborated with the previous observations (Yadav and Chandra 2012; Chandra and Kumar 2017a).

In contrast to above, the bacterial decolourised sample obtained after 168 h incubation showed reduction in all physico-chemical parameters, as shown in Table 1. This indicated the biodegradation and biotransformation of various organic and inorganic contents by potential bacterial consortium. Initially, during degradation of melanoidins, the pH of medium was decreased to 4.47, but after 168 h of bacterial growth, the pH was gradually enhanced. The reduction of pH at the initial stage of bacterial growth might be due to production of organic acid such as ethanedioic acid, acetic acid, phosphoric acid, and octadecenoic acid (Davidek et al. 2006). The increased pH of media might be due to depolymerisation of MRPs and degradation of organic compounds by enzymatic activity of bacterial consortium during degradation of melanoidins. This induced the solubility and utilisation of melanoidins which favored the mineralisation and co-metabolism of melanoidins (Bharagava et al. 2009). However, the evaluation of BOD and COD values at various time intervals showed that there was gradual decrease in BOD and COD values with increase in percent decolourisation (76%), as shown in Fig. 1. BOD/COD ratio is typically a measurement used to describe the organic composition of wastewater. This BOD/COD ratio has been commonly used as an indicator for biodegradability index (Metcalf and Eddy 2003). In our study, the BOD/COD ratio of bacterial decolourised melanoidins was found 0.46. This indicates the utilisation of organic and inorganic chemical compounds present in melanoidins containing sample by bacterial consortium. It

Table 1 Physico-chemical characteristics of molasses-melanoidins before and after bacterial decolourisation

S. no.	Parameter	Control	Bacterial decolourised		% reduction after 168 h
			After 72 h	After 168 h	
1.	Colour intensity	150,000 ± 2.64	90,002 ± 2.64	15,244 ± 3.60 ^a	89.83
2.	pH	8.53 ± 0.01	4.47 ± 0.00	8.76 ± 0.02 ^a	−2.69
3.	BOD	2487.66 ± 110.12	2235 ± 1.00	1352.33 ± 1.52 ^a	25.51
4.	COD	6296.66 ± 6.65	4252.66 ± 1.52	2932 ± 51.15 ^a	53.43
5.	TS	6940 ± 5.29	5525 ± 2.64	4772 ± 3.60 ^a	45.6
6.	TDS	10,527.66 ± 6.65	6424 ± 3.51	4534.33 ± 5.50 ^a	56.92
7.	VS	1059.33 ± 2.08	753.01 ± 2.64	242 ± 5.13 ^a	77.15
8.	Chloride	2746 ± 4.35	1525.66 ± 1.52	805 ± 8.38 ^a	70.68
9.	Total nitrogen	195.33 ± 4.06	184.12 ± 1.48	164.66 ± 2.08 ^a	15.70
10.	Ammoniacal nitrogen	131.21 ± 1.24	102.74 ± 3.78	65.23 ± 4.21 ^a	50.12
11.	Phenol	6892 ± 7.54	4520.53 ± 3.27	1997 ± 1.00 ^a	71.02
12.	Sulphate	13,463.33 ± 3.78	5247.19 ± 0.83	3756.66 ± 6.80 ^a	72.09
13.	Phosphate	41.52 ± 1.60	31.60 ± 0.47	17.28 ± 0.06 ^a	58.38
14.	Trace elements				
(a)	Mn	8.20 ± 0.09	6.21 ± 0.01	4.26 ± 0.01 ^a	48.04
(b)	Cr	2.97 ± 0.026	2.02 ± 0.02	1.06 ± 0.02 ^a	64.30
(c)	Zn	16.61 ± 0.00	8.52 ± 0.00	3.89 ± 0.01 ^a	76.58
(d)	Cu	2.55 ± 0.01	1.232 ± 0.00	0.22 ± 0.02 ^a	91.37
(e)	Fe	373.95 ± 0.36	150.725 ± 0.66	17.06 ± 0.02 ^a	95.43
(f)	Pb	2.59 ± 0.00	1.62 ± 0.00	0.65 ± 0.02 ^b	74.90
(g)	Cd	BDL	BDL	BDL	—
(h)	Ni	4.18 ± 0.00	1.25 ± 0.01	1.05 ± 0.00 ^a	74.88

All values are mean ($n=3$) ± SD in mg L^{−1} except colour intensity (Pt–Co unit), pH, and temperature (°C). Student's *t* test [two tailed as compared to untreated sample (after 168 h)]

BOD biological oxygen demand, COD chemical oxygen demand, TS total solid, TDS total dissolved solid, VS volatile solid

^aHighly significant at $p < 0.001$

^bLess significant at $p < 0.05$

Fig. 1 Decolourisation of molasses-melanoidins, BOD and COD reduction at various time intervals

appears that bacterial consortium carried out biodegradation of some organic components of MRPs. Statistical analyses revealed that there was statistically significant difference

($p < 0.001$) between all physic-chemical parameters of control and bacterial decolourised melanoidins except pH.

Effect of different carbon and nitrogen sources

Melanoidins decolourisation was evaluated using different carbon sources at 1% (w/v) concentration for 168 h of bacterial incubation. The results showed that the efficiency of consortium increase with increase in glucose concentration from 0.1 to 1% and achieved maximum decolourisation (76%) at 1.0% concentration of glucose (Fig. 2a). This indicated that decolourisation of melanoidins was result of co-metabolism of MRPs polymer in the presence of glucose, while further increase in glucose concentration does not support the bacterial growth and decolourisation of melanoidins. However, sucrose, starch, lactose, xylose, and fructose were found less effective compared to glucose, which showed decolourisation of melanoidins up to 42–58%. This indicated that carbon bounds with melanoidins are not bio-available to growing bacterial consortium. Therefore, the

Fig. 2 Effect of different nutritional and environmental parameters on degradation and decolourisation of molasses-melanoidins **a** effect of carbon sources, **b** effect of nitrogen sources, and **c** effect of pH and shaking speed (effect of temperature)

decolourisation of melanoidins without supplementation of carbon source is not observed. However, the presence of glucose in media was readily available to bacterial consortium for their growth. Carbon source provided energy for survival and growth of microorganisms, which are essential for the cleavage of conjugated C=C, C=O and C≡N bonds of melanoidins by electron donation from hydrogen peroxide (H_2O_2) generated by glucose by the action of bacterial glucose oxidase in growth media (Miyata et al. 1998, 2000; Kalavathi et al. 2001). Similar observation has also been reported by the previous workers during bacterial treatment of anaerobically treated distillery spent wash (Santal et al. 2011; Ghosh et al. 2004). This indicated that our bacterial consortium was more effective for decolourisation and degradation of melanoidins containing effluent. This might be due to the presence of high amount of enzyme which facilitates the decolourisation and degradation processes of complex MRPs (Kumar and Chandra 2006).

The effect of different organic and inorganic nitrogen sources at a concentration of 1.0% (w/v) along with glucose (1.0% w/v) showed that peptone at 0.2% (w/v) concentration was found most effective organic nitrogen source

which enhanced decolourisation of melanoidins up to 78% (Fig. 2b). Furthermore, increase in concentration of peptone resulted in gradual decline in degradation capability of the developed bacterial consortium inhibited the decolourisation process. This might be due to allosteric inhibition of bacterial enzymes. However, the presence of other organic nitrogen sources, i.e., beef extract, yeast extract, and urea, could show the melanoidin decolourisation up to 34–78% only, whereas inorganic nitrogen sources such as sodium sulphate and ammonium chloride could show the decolourisation up to 21 and 25%, respectively. This finding showed the inhibitory effect of sodium sulphate and ammonium chloride on bacterial growth. Kumar and Chandra (2006) and Miyata et al. (2000) have also studied the inhibitory effect of inorganic nitrogen sources for melanoidins decolourisation. Similarly, various nitrogen sources were optimized by various workers for decolourisation of melanoidins, but peptone was reported most effective for colour reduction (Sirianuntapi-boon and Phothilangka 2004; Tiwari et al. 2013; Ravikumar et al. 2011). Kirk et al. (1978) reported that in the presence of peptone, microorganisms catalyse enzymatic degradation of lignin and lignin-like compounds during the secondary

phase of metabolic growth. Induction and secretion of MnP are triggered by nutrient limitations such as carbon and nitrogen sources (Wong 2009; Chandra et al. 2017). At high concentration, there was no significant melanoidins decolourisation which was noted due to surplus supplementation of nitrogen which inhibited the bacterial growth. Hence, bacterial consortium utilized tiny amount of peptone for highest decolourisation, BOD, and COD reduction.

Effect of pH, temperature and shaking speed

The pH and temperature are important factors for the optimal decolourisation of melanoidins and physiological performance of bacterial culture. The maximum decolourisation (79%) of melanoidins by bacterial consortium was found at pH 8.1. This showed the optimum pH of growing bacterial consortium for decolourisation of melanoidins. At alkaline pH (8.1), the higher decolourisation of melanoidins might be due to combined action of bacterial enzyme and loosen of conjugated C=C, C=O, and C≡N bonds of melanoidins. Furthermore, increase in pH inhibited the decolourisation process of melanoidins (Fig. 2c). This might be due to either toxicity of variable pH or low bio-availability of melanoidins. At high pH decrease in colour was due to depolymerisation of melanoidins by bacterial consortium. Our results are supported by the previous observation of Hayase et al. (1984), who achieve maximum decolourisation of melanoidins by H₂O₂ treatment at alkaline pH. The fluctuation in the optimum pH (8.1), melanoidins decolourisation was reduced might be due to inhibition of the enzyme activities responsible for decolourisation of melanoidins, or loss of cell viability. Because, all enzymes are made up of proteins, and hence, some proteins are denatured at high or low pH range. Similarly, it was also observed that increase in temperature (25–37 °C) enhanced decolourisation of melanoidins from 35 to 79% (Fig. 2d). The developed bacterial consortium also showed maximum decolourisation (81%) of melanoidins at 37 °C, while, furthermore, the increase in temperature up to 45 °C adversely affected the growth and decolourisation ability of the bacterial consortium. Similar pattern of melanoidins decolourisation at variable temperature is also reported by other researchers (Santal et al. 2011; Tiwari et al. 2013; Mohana et al. 2007). Furthermore, the effect of shaking speed showed that the optimum decolourisation of melanoidins by potential bacterial consortium was observed at 180 rpm, as shown in Fig. 2c. However, increase in shaking speed resulted in decrease in decolourisation process of melanoidins. This might be due to breakage of bacterial cell wall at a higher shaking speed. In addition, bacterial cells might be stressed and could not grow well at very high shaking speeds. These results were in accordance with those of previous studies (Yadav and Chandra 2012; Tiwari et al. 2014). The decolourisation and degradation of

melanoidins was greatly influenced by concentration of melanoidins. The decolourisation rate of melanoidins by bacterial strains was decreased with an increase concentration of melanoidins. Maximum decolourisation (79%) of melanoidins was observed at 3200 mg L⁻¹ concentration. Further increase in melanoidins concentration (3200–3500 mg L⁻¹) in medium inhibited the melanoidins decolourisation process of molasses-melanoidins.

Bacterial growth and biomass

The periodic monitoring of bacterial growth curve and biomass showed maximum OD₆₂₀ (2.49) and biomass (5.22 g L⁻¹; CFU/mL 27.5 × 10⁶) at 144 h incubation period during decolourisation process (Supplementary Figs. S1 and S2). This indicated the most optimum condition for highest bacterial growth and biomass production to achieve maximum decolourisation (79%) of melanoidins. In addition, the SEM analysis of growing bacterial biomass showed dense bacterial cell. This indicated rapid bacterial growth in melanoidins containing medium; this resulted for degradation and decolourisation of melanoidins. Furthermore, incubation of bacterial consortium did not increase the rate of melanoidins decolourisation. The direct correlation of OD₆₂₀ with high bacterial biomass also indicated the direct involvement of bacterial consortium in decolourisation and degradation process of melanoidins.

Enzymatic mechanism of melanoidins degradation

Ligninolytic enzymes play a key role in decolourisation and degradation of coloured effluents and support the growth of microorganisms for the utilisation of complex recalcitrant compounds (Bonugli-Santos et al. 2012; Pant and Adholeya 2007). During melanoidin degradation, the MnP was found to be the dominating enzyme at the initial stage of melanoidins degradation. The activity of MnP reached maximum at 168 h (3.8 U mL⁻¹ min⁻¹), while the laccase activity was

Fig. 3 Manganese peroxidase (MnP) and laccase activity during decolourisation and degradation of molasses-melanoidins

found maximum ($2.39 \text{ U mL}^{-1} \text{ min}^{-1}$) at 144 h of incubation (Fig. 3), and showed maximum decolourisation (79%) of melanoidins. This might be due to direct involvement of MnP for the cleavage of conjugated C=C, C=O, and C $\equiv$ N bonds of melanoidins for degradation, while further incubation due to generation of phenolic compounds induces the laccase production from bacterial cells. Therefore, laccase was noted higher concentration at the later stage of growing bacterial consortium during the decolourisation evaluation. The MnP and laccase have a broad substrate oxidising enzymes capable for breaking a large quantity of different chemical bonds present in phenolic and non-phenolic recalcitrant compounds (Bonugli-Santos et al. 2012; Wong 2009). However, after 168 and 144 h incubation, it showed gradual decrease of MnP and laccase enzyme activities of potential bacterial consortium, respectively. Miyata et al. (1998) verified that synthetic melanoidins are decolourized by the participation of MnP and manganese independent peroxidase (MIP) of *Trametes hirsutus* pellets, and the extracellular H_2O_2 produced by glucose oxidase along with partial participation of laccase. The presence of melanoidins and other similar compounds can induce the expression of MnP and laccase genes, both described as having synergistically action in the degradation and depolymerisation of melanoidins and other industrial pollutants (González et al. 2008; Miyata et al. 2000). The depolymerisation of melanoidins can be ascribed to the action of MnP and laccase activity. The findings of our study are well supported by earlier observations reported by various researchers (Pant and Adholeya 2007; Bharagava et al. 2009; Yadav and Chandra 2012).

UV-Vis and FT-IR spectroscopy

UV-Vis spectrophotometric analysis is the most commonly used methods of MR (Martins and Van Boekel 2003; Echavarría et al. 2014). The result revealed that melanoidins showed different absorption peaks in the UV region and their maximum absorbance was noted at λ_{max} 295 nm. In addition, melanoidins also showed different stable peaks in a range of between 200 and 400 nm (Fig. 4). The comparative UV-Vis absorption spectrum of control and bacterial decolourised melanoidins sample showed the reduction of absorption spectrum in bacterial degraded sample at the end of 168 h of incubation, as shown in Fig. 4. Several organic compounds such as hydroxymethyl furfural, reductones, and furanones formed during the early stages of the MR are related to absorbance in UV region (Echavarría et al. 2013a, b). The expansion of the MR is generally monitored by the enhanced in absorbance of either 280 nm, 320–350 nm, or 420–450 nm corresponding to the early MR products for pyrazine compounds (at 280 nm) (Gu et al. 2010), advanced stage for soluble pre-melanoidins formation (at 320–350 nm), and final MRPs (at 420–450 nm) corresponding to the development of the medium and formation of specific compounds (Billaud et al. 2004). On the basis of our findings, it can be stated that the formation of various peaks in UV and visible region indicated the presence of complex mixture of early, advanced, and final MRPs. Our findings are well corroborated with the earlier results reported by Echavarría et al. (2013a, b). UV-visible spectroscopic analysis indicates loss of melanoidins peaks and a significant spectral shift indicating biotransformation of melanoidins to unrelated metabolites. The shifting of peaks indicated the

Fig. 4 UV-Vis spectral analysis of the untreated and bacterial decolourised molasses-melanoidins at various time intervals

biotransformation of melanoidins molecule to some other metabolites. Furthermore, the FT-IR spectra analysis also revealed significant absorbance intensities between control and bacterial decolourised sample as shown in Supplementary Fig. S3. The absorption band and their tentative assignment are given in Supplementary Table 1. The appearance and disappearance of some peaks as well as shifting in peaks is indicated the conversion of complex toxic compounds into simple metabolites.

TLC and HPLC analysis

The TLC chromatogram under UV light showed dark spot in control, while decolourised sample after 168 h bacterial incubation had three bands corresponding to retardation factor (R_f) value of 0.64, 0.71, and 0.78, as shown in Fig. 5a. This clearly indicated the degradation of melanoidins into intermediate metabolic products with R_f value 0.64, 0.71, and 0.78. Furthermore, the HPLC analysis of control sample also showed a single major peak at retention time (RT) 2.0 min, while, in 72 h, bacterial decolourised sample was less compared to untreated sample. This indicated the capability of the bacterial consortium to decolourize and degrade melanoidins by their enzymatic action. Furthermore, after 168 h treatment of melanoidins, the HPLC chromatogram also showed shifting of peak, decrease in peak area height (Fig. 5b). The differences in the peak pattern confirm biodegradation of melanoidins. This study revealed that potential bacterial consortium effectively reduced pollutants level

of melanoidins containing medium. These findings are well supported by earlier results reported by various workers (Tiwari et al. 2012; Bharagava et al. 2009; Yadav and Chandra 2012).

GC–MS analysis

The GC–MS chromatogram of organic compounds extracted with ethyl acetate from untreated and treated sample is shown in Fig. 6 and organic compounds have been identified in detail at various RT based upon mass to charge ratios (m/z). The major peaks detected in the control samples at different RT were identified as silanol, trimethyl and trimethyl ester with boric acid (RT:7.06), D-(-)lactic acid, and trimethyl ether, TMS ester (RT:9.08), butyldimethyl(2-styryl[1,3] dithian-2-yl) silane (RT:10.88), propane, 1,2,3-tris[(tert-butyl dimethylsilyl)oxy] (RT:12.85), acetic acid, [bis[(TMS)oxy]phosphinyl]-TMS ester (RT:13.71), hexadecanoic acid, 2-[(TMS)oxy]-1,3-propenediyl ester (RT:27.99), 9,12-octadecanoic acid(Z,Z)-, TMS ester (29.98), octadecanoic acid, ethyl ester (RT:30.84), *cis*-10-nonadecanoic acid (RT:31.88), hexadecanoic acid (RT:34.50), and octadecanoic acid, ethyl ester (RT:36.11). Several minor peaks were also noted at RT: 7.06; 7.76; 10.51; 11.07; 12.35; 12.85; 17.35; 19.09; 21.25; 23.08; 23.70; 29.95; 29.98; 30.84; 33.49; 34.50; 35.11; 35.50; 35.75; 35.82; 35.83; 36.10; 37.43; 39.17; and 39.40. This corresponded to silanol, trimethyl-trimethyl ester with boric acid; bis(dimethyl-t-butylsilyl)oxalate; D-lactic acid; bis(dimethyl-t-butylsilyl)oxalate; ethanedioic

Fig. 5 Chromatograph of molasses-melanoidins before and after bacterial decolourisation during **a** TLC and **b** HPLC analysis. **C** control (untreated), **BD** bacterial decolourised sample after 168 h incubation

Fig. 6 GC–MS chromatogram of TMS derivatized organic compounds extracted from molasses-melanoidins **a** control, **b** bacterial decolourised sample after 72 h, and **c** 168 h incubation

acid; butyldimethyl(2-styryl[1,3]dithian-2-yl)silane; silanol, trimethyl-,benzoate; propane, 1,2,3-tris[(tert-butyldimethylsilyl)oxy]; pyrrolozine 1-one,7-propyl; undecenoic acid; dodecanoic acid; dotriacontate; pyrrolo(1,2-a)pyrazine-1,4-dione; 11-cis-octadecenoic acid, TMS ester; 9,12-octadecanoic acid(Z,Z)-, TMS ester; octadecenoic acid; 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl)ester; hexadecanoic acid, 2,3-bis[(TMS)oxy]propyl ester; β -sitosterol; querecetin 7, 3',4' trimethoxy; 1,7-pentatriacontene; 2-monostearin; 1-monolinoleoylglycerol TMS ester; octadecanoic acid, 2,3 bis[(TMS)oxy]propyl ester; stigmasta-5, 22-dien-3-ol (3 β ,22E), stigmasterol; and silane[[(3 β)-cholesta-5-en-3-yl]

trimethyl, respectively. Moreover, some minor peaks were observed at different RT, but these peaks could not be identified, because they were not available in the NIST library.

D(-)lactic acid, trimethyl ether, TMS ester and acetic acid, and [bis[(TMS)oxy]phosphinyl]-TMS ester are the major volatile alkaline degradation products of hexose (ADPH) sugar and discharged in effluent after anaerobic treatment. These detected compounds have been listed as EDCs by the USEPA (2012). The octadecanoic and hexadecanoic acid were also detected in our study; they are plant origin fatty acid which have been reported to be anti-quorum sensing molecules of bacterial product (Singh

et al. 2013). Moreover, in some studies, octadecanoic and hexadecanoic acids have also been reported to be a toxic compound in aquatic systems and DNA fragmentation inducer in a human melanoma cell line, respectively (Kamaya et al. 2003; de Sousa Andrade et al. 2005). However, the toxicity of octadecanoic and hexadecanoic acid is well known on metabolic activity of bacteria (Koster and Cramert 1987). However, these compounds are also listed under EDCs as per USEPA (2012). Similar compounds have been reported by various researchers (Gonzalez et al. 2000; Quinn et al. 2007), and so our data support results reported previously (Chandra and Kumar 2017a, b). Similarly, ethanedioic acid, pyrrolizidine, and benzenedicarboxylic acid have been detected in control sample. Ethanedioic acid is a toxic colourless crystalline organic acid found in some plants, while pyrrolizidine and benzenedicarboxylic acid are the fragmentary ADPH. These compounds might occur as a residual fraction of the distillation process during ethanol production and remain after anaerobic treatment of spent wash. However, dotriacontane, phyhydrocarbons, have also been identified in untreated sample. These compounds have been as key constituents of environmental pollutants responsible for dermal irritation (Muhammad et al. 2005). Apart from this, other compounds were also identified as stigmasta-5, 22-dien-3-ol (3 β ,22E), stigmasterol, and β -sitosterol. These are the major phytosterols with a chemical structure similar to cholesterol, soluble in water at all pH values, and previously reported to be present in *Saccharum officinarum* L. and effluent discharged from sugarcane molasses-based distilleries. These compounds are screened under the EDCs list of USEPA (2012). In a previous study, it has been reported that in environment, aerobic and/or anaerobic microorganisms may transform β -sitosterol and other sterols into androgenic hormones, i.e., 5- β -androstan 3,17-dione and androstan 4-en-3,17-dion (Taylor et al. 1981). Such androstane derivatives of sterols may ultimately interfere with endocrine system of fish and produce hermaphroditism or other morphological defects. A role of these compounds in masculinising the fish population and reducing fish numbers has been suggested (Jenkins et al. 2003). We have also detected several other organic compounds in control and bacterial decolourised sample. These organic compounds constituent the main components of spent wash and discharge in PMDE from yeast or original sugarcane molasses, which remains after secondary treatment of spent wash. The presence of organic acids, phenolics, and long-chain aliphatic compounds in distillery effluent was reported previously by various workers (Gonzalez et al. 2000; Yadav and Chandra 2012). The maximum numbers of organic compounds present in untreated sample are well-known toxicants; complex and

high-molecular-weight compounds pose an environmental risk to plant and animal including human.

However, the major peaks correspond to toxic organic compounds detected in the control sample diminished after bacterial treatment and appearance of few new peak indicated the generation of new metabolic products, as shown in Fig. 6 and Table 2. This suggested that complex and high-molecular-weight organic compounds were degraded by bacterial consortiums with the help of extracellular ligninolytic enzymes utilize its as sole source of carbon and nitrogen and play a major role in the decolourisation and degradation of melanoidins. The new metabolic products extracted with ethyl acetate from bacterial decolourised sample were identified as bis(dimethyl-t-butylsilyl)oxalate (RT: 7.77), butane, 2,3-bis(trimethylsiloxy) (RT:7.87), 3-benyl-1,4-diaza-2,5-dioxobicyclo (RT:4.3.0), nonane (RT:31.87); 2-amino-1-phenylethanol, TMS ether (RT:35.24), and 1-monolinoleoylglycerol TMS ether (RT:40.66).

Phytotoxicity evaluation of untreated and bacterial treated sample

The phytotoxicity assessment using higher plants is a fundamental and reliable tool for toxicity evaluation of any environmental sample (OECD 2003). The seed germination test of green gram (*P. mungo* L.) showed inhibitory effects of untreated and bacterial decolourised melanoidins sample at different concentrations in terms of seed germination and growth parameters of seedlings (Table 3 and supplementary Fig. S4). Seeds germinated at rate of 98% with 2% (v/v) concentration of melanoidins containing solution, as the concentration increased the percent germination decrease, with 80% at 5% concentration. There was no seed germination at a concentration 10% after 24 h. However, in bacterial treated sample, 80% germination was recorded in up to a 10% (v/v) concentration, which was higher than with untreated sample. According to Bharagava and Chandra (2010) and Chandra and Kumar (2017a, b), suppression of seed germination at high concentration of melanoidins might be due to the occurrence of toxic organic compounds and dissolve solid which absorbed by the seed prior to germination and affecting different biochemical and physiological process of seed germination. Increased in percent germination in bacterial decolourised melanoidins might be due to lower presence of organic compounds that has created favorable environmental conditions for seed germination and utilisation of nutrient present in melanoidins. With respect to the seedling growth (radical length), the radical length of seeds exposed to untreated sample varies from 18.12 to 2.21 cm at different concentration of melanoidins containing wastewater. When the seeds had been exposed to 10% (v/v) untreated sample, they showed no radical development, but after bacterial treatment, the seeds showed development of

Table 2 Organic compounds identified by GC–MS analysis extracted with ethyl acetate under acidic condition (pH 2.0) from control and bacterial decolourised molasses-melanoidins

S. no.	RT	Name of compound	C	BD	
				3 days	7 days
1.	6.63	Propanoic acid, 2-methyl-2-[(TMS)oxy], TMS ether	–	–	+
2.	7.06	Silanol, trimethyl-, trimester with boric acid	+	–	–
3.	7.28	L-Lactic acid	+	–	–
4.	7.76	Bis(dimethyl-t-butylsilyl)oxalate	+	–	–
5.	7.77	2-Methyl-1,3-propanediol 2TMS	–	–	+
6.	7.87	Cyclohexanol, 4-[(TMS)oxy]- <i>cis</i>	+	–	–
7.	7.95	Butane, 2,3-bis(trimethylsiloxy)	–	–	+
8.	9.08	D-(–)Lactic acid, trimethyl ether, TMS ester	+	–	–
9.	10.51	Ethanedioic acid	+	+	–
10.	10.88	t-Butyldimethyl(2-styryl[1,3]dithian-2-yl) silane	+	+	+
11.	11.07	Butane-1,3-diol, 1-methylene-3-methyl, bis(TMS)ether	+	+	+
12.	12.35	Silanol, trimethyl-, benzoate	+	–	–
13.	12.85	Propane, 1,2,3-tris[(tert-butyl dimethylsilyl)oxy]	+	–	–
14.	13.71	Acetic acid, [bis[(TMS)oxy]phosphinyl]-TMS ester	+	+	–
15.	17.27	5-Methyl-1,3-diazaadamantan-6-one	–	+	–
16.	17.53	Pyrrolozine 1-one, 7-propyl	+	–	–
17.	19.09	Undecenoic acid	+	–	–
18.	20.49	Acetic acid, [[bis[(TMS)oxy]phosphinyl]oxy]-TMS ester	–	+	–
19.	21.88	Silanol, trimethyl phosphate	–	+	–
20.	21.25	Dodecanoic acid	+	–	–
21.	23.08	Dotriacontate	+	–	–
22.	23.70	Pyrrolo(1,2-a)pyrazine-1,4-dione,	+	–	–
23.	23.73	Phosphoric acid, bis(TMS)2,3-bis[(TMS)oxy]propyl ester	–	+	–
24.	27.98	Hexadecanoic acid	+	+	–
25.	29.28	<i>cis</i> -10-Heptadecenoic acid	–	+	–
26.	29.95	11- <i>cis</i> -Octadecenoic acid, TMS ester	+	+	–
27.	29.98	9,12-Octadecanoic acid(Z,Z)-, TMS ester	+	–	–
28.	30.59	11- <i>trans</i> -Octadecenoic acid	–	+	–
29.	30.84	Octadecanoic acid	+	+	–
30.	31.88	1-Eicosanol	+	–	–
31.	31.88	<i>cis</i> -10-Nonadecenoic acid	–	+	–
32.	31.97	3-Benyl-1,4-diaza-2,5-dioxobicyclo(4.3.0)nonane	–	–	+
33.	32.77	β-D-galactopyranoside, methyl 2,3-bis- <i>O</i> -(TMS), cyclic methylboronate	–	+	–
34.	33.49	1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl)ester	+	–	–
35.	34.05	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl)ester	+	+	–
36.	34.50	Hexadecanoic acid	+	–	–
37.	34.55	Hexadecanoic acid, 2,3-bis[(TMS)oxy]propyl ester	+	+	–
38.	34.63	<i>cis</i> -13-Docosanoic acid	–	+	–
39.	35.11	1,7-Pentatriacontene	+	–	–
40.	35.24	2-Amino-1-phenylethanol, TMS ether	–	–	+
41.	35.50	β-sitosterol	+	–	–
42.	35.75	Quercetin 7, 3',4' trimethoxy	+	–	–
43.	35.82	2-Monostearin	+	+	–
44.	35.83	1-Monolinoleoylglycerol TMS ester	+	–	–
45.	36.10	Octadecanoic acid, 2,3 bis[(TMS)oxy]propyl ester	+	+	–
46.	36.11	Octadecanoic acid, ethyl ester	+	–	–
47.	37.43	Stigmasta-5, 22-dien-3-ol (3 β,22E)	+	–	–
48.	39.17	Stigmasterol	–	–	–
49.	39.40	Silane[[[(3β)-cholesta-5-en-3-yl]trimethyl	+	–	–
50.	40.66	1-Monolinoleoylglycerol TMS ether	–	–	+

RT retention time (in minutes), C control, BD bacterial decolourised, + present, – absent, TMS trimethylsilyl

Table 3 Effect of different concentration of melanoidins (treated and untreated) on seed germination and seedling growth of *Phaseolus mungo* L.

Sample	No. of seed plated	Concentration (%)	Germination (%)	Germination index	Reduction in germination (%)	Radical length (cm)	Phytotoxicity (%)	Stress tolerance index
Control	10	0	100±0.00	00±0.00	00±0.00	18.12±0.82	00±0.00	00±0.00
Untreated	10	02	90±0.00	0.17±0.01	10±0.00	03.50±0.17	80.68±3.64	0.19±0.00
	10	05	80±0.00	0.09±0.00	20±0.00	02.21±0.07	87.80±3.99	0.12±0.00
	10	10	NG	—	—	—	—	—
Bacterial decolourised	10	02	90±0.00	0.84±0.04	10±0.00	17.10±0.77	05.62±0.23	0.94±0.04
	10	05	85±0.00	0.71±0.02	15±0.00	15.21±0.65	16.05±0.64	0.83±0.04
	10	10	80±0.00	0.66±0.00	20±0.00	15.11±0.54	16.61±0.52	0.83±0.01

All values are mean of three replicate ± SD

NG no germination, control tap water

a radical (15.11 cm) at 10% concentration of melanoidins. The various parameters like germination index, % phytotoxicity, and stress tolerance index were also observed in untreated and bacterial decolourised melanoidins, as shown in Table 3. These results clearly indicated that the toxicity of melanoidins before bacterial treatment was very high which inhibited the growth of seeds, but after bacterial treatment, the toxicity of melanoidins decreases.

Conclusion

The study revealed that the waste discharged from sugar-cane molasses-based distilleries have EDCs like containing organo-metallic complex which can be degraded by potential bacterial consortium with supplementary source of glucose (1.0%) and peptone (0.2%) at optimum temperature (35 °C), pH (8.1), and shaking speed (180 rpm) through MnP and laccase enzyme activities as bio-tools for degradation and decolourisation of industrial effluent. The metabolic products analysis by TLC, HPLC, and GC–MS analysis confirmed the biodegradation of melanoidins by potential bacterial consortium consisting of *K. pneumoniae* (KU726953), *S. enterica* (KU726954), *E. aerogenes* (KU726955), and *E. cloacae* (KU726957). The toxicity reduction after bacterial decolourisation confirmed the reduction of toxic compounds from the molasses-melanoidin organo-metallic complex.

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest of any type.

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