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Fractionation of sugarcane molasses distillery wastewater and evaluation of antioxidant and antimicrobial characteristics

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ABSTRACT

Sugarcane molasses distillery wastewater (DWW) was fractionated by ultrafiltration (UF) using 100 kDa and 10 kDa membranes. The isolated fractions were characterized for antioxidant activity and content of melanoidins, total phenols, proteins and carbohydrates were measured. Chemical composition of the fractions was determined using GC–MS analysis and antimicrobial property was tested against pure microbial cultures. DWW has high antioxidant activity with the melanoidins content around 2×10^3 times the total phenols content. Diafiltration leads to loss in antioxidant activity. Removing the bound melanoidins compounds from the pure melanoidins core does not affect the antioxidant activity. Among all fractions, the highest antioxidant activity is shown by the > 100 kDa fraction without diafiltration. Ethanol fractionation of > 100 kDa fraction further separates phenols and melanoidins rich fractions from its structure. Ethanol soluble fraction shows high phenols content while the precipitate or aqueous fraction has high melanoidins and carbohydrate content. Antimicrobial potency is mainly observed in arginine rich > 100 kDa fraction against *Bacillus licheniformis*.

1. Introduction

Sugarcane molasses based alcohol distilleries constitute a key agro-based industry in India, with around 356 units having an installed capacity of 4230 million litres per annum (GoI, 2014). These units generate up to 10–15 kL wastewater/kL alcohol that is characterized by dark brown colour attributed to compounds like melanoidins, polyphenols and caramels. Melanoidins, constituting nearly 2% of the distillery wastewater, are complex Maillard reaction products formed by reaction between carbonyl groups of reducing sugars and compounds with free amino groups (Martins and Van Boekel, 2005). Owing to their structural complexity and antimicrobial property, melanoidins are difficult to degrade through conventional biological routes like anaerobic digestion thereby posing challenges in disposal of distillery wastewater (Arimi et al., 2014).

It is established that melanoidins possess potential physiological benefits like antioxidant, antimicrobial and antihypertensive activity (Rufián-Henares and Morales, 2007). Melanoidins are formed during the heat processing of foods like coffee, bread, malt and beef (Nunes and Coimbra, 2010). Antioxidant effect of melanoidins derived from coffee brews (Ludwig et al., 2012; Delgado-Andrade et al., 2005), honey (Brudzynski and Miotto, 2011) as well as synthetic melanoidins prepared from different amino acid-glucose model systems (Rufián-Henares and Morales, 2007) has been studied. Similarly, it is known that polyphenols with antioxidant effect are present in various agro-industrial effluents like olive mill wastewater (Agalias et al., 2007) and artichoke wastewaters (Conidi et al., 2014).

Extensive work has been reported on the treatment of sugarcane molasses distillery wastewater targeting the removal of coloured recalcitrant compounds (Nataraj et al., 2006; Sreethawong and Chavadej, 2008; Thakur et al., 2009; Mohanakrishna et al., 2010; Basu et al., 2015, 2016). Detailed analysis of the wastewater has also been done to better understand melanoidins degradation (Chandra et al., 2008; Bharagava and Chandra, 2010). However, antioxidant and antimicrobial properties of molasses distillery wastewater fractions have not been specifically investigated.

This work aims at understanding (i) the distribution of antioxidant compounds among the high and low molecular weight fractions of sugarcane molasses distillery wastewater, (ii) the content of melanoidins, total phenols, proteins and carbohydrates in each fraction, and (iii) antimicrobial property of the separated fractions. The findings of this work are expected to help in designing suitable treatment schemes that would recover valuable antioxidant/antimicrobial components from distillery wastewater and contribute to improving the conventional biological treatment process used in distilleries.

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2. Materials and methods

2.1. Materials

Distillery wastewater (DWW) was collected from Brajnathpur distillery unit of Simbhaoli Sugars Limited, Uttar Pradesh, India and used as-received. Ultrafiltration (UF) membranes of molecular weight cutoff 100 kDa and 10 kDa were procured from Sterlitech, Mumbai (Table 1).

Chemicals namely 2,2′-Azobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulphate, N,N-Dimethylformamide (DMF), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, D-glucose and glycine were procured from Sigma-Aldrich, New Delhi. Absolute ethanol was obtained from Merck, New Delhi. Agar and Luria-Bertani (LB) broth were supplied by HiMedia, New Delhi. Pure cultures of *Bacillus licheniformis* and *Pseudomonas putida* were obtained from Microbial Type Culture Collection and gene bank (MTCC), Chandigarh. Dextran standards (1 kDa–500 kDa) were procured from Fluka Chemicals, USA.

2.2. Fractionation of DWW

DWW (pH 4.2, chemical oxygen demand (COD) 130 g/L, and suspended solids content 156 g/L) was centrifuged at 8000 rpm for 20 min. UF was done in Sepa ST filtration cell (Osmonics, USA) with an effective filtration area of 16.9 cm^2. Previous studies on coffee melanoidins (Gniechwitz et al., 2008) and synthetic melanoidins (Rufián-Henares and Morales, 2007) has shown predominance of > 100 kDa and < 10 kDa melanoidins components; thus, 100 kDa and 10 kDa membranes were chosen for this work.

Two fractionation schemes were used (Fig. 1).

Table 1
Characteristics of UF membranes.

	100 kDa	10 kDa
Manufacturer	Tri Sep	GE Osmonics
Material	Polyethersulfone	Polyethersulfone
Flux (GFD)/psi	100/20	85/30
pH range	2–11	1–11

- Fractionation Scheme 1 (FS1) (UF through 10 kDa membrane): 100 mL of centrifuged DWW sample was concentrated 10-fold through 10 kDa membrane at 2 bar. The retentate was diafiltered (volume made up to 100 mL with reverse osmosis (RO) water after 10-fold concentration) thrice before isolating pure and bound melanoidins compounds.
- Fractionation Scheme 2 (FS2) (UF through 100 kDa followed by 10 kDa membrane): 100 mL centrifuged DWW was concentrated 10-fold through 100 kDa membrane at 2 bar. Retentate (> 100 kDa) was diafiltered thrice with RO water. The permeate (< 100 kDa) was further concentrated 10-fold through 10 kDa membrane at 4 bar and diafiltered thrice.

Pure melanoidins (melanoidins core without the ionically attached low molecular weight compounds) was isolated from the 10 kDa retentate fraction (obtained from FS1) and 100 kDa retentate, 100 kDa permeate and 10 kDa retentate fractions (obtained from FS2). Samples were incubated overnight in 2 M NaCl (Rufián-Henares and Morales, 2007), followed by UF through 10 kDa membrane. The retentate contained pure melanoidins (PM) while permeate contained low molecular weight bound melanoidins compounds (BMC).

2.3. Characterization of DWW fractions

2.3.1. Molecular weight

High and low molecular weight compounds present in DWW was determined by gel permeation chromatography (GPC) using Waters-1515 isocratic pump, 717 plus autosampler and 2414 refractive index detector, in Ultrahydrogel column (7.8 × 300 mm) preconditioned with HPLC grade water. Sample injection volume was 50 µL and HPLC grade water at flow rate of 0.8 mL/min was used as mobile phase. Calibration curve was prepared with dextran standards of known molecular weight between 1 kDa to 500 kDa.

2.3.2. Antioxidant capacity

Antioxidant capacity of the fractions was measured in terms of radical scavenging activity in two different reaction media viz. by ABTS assay (Delgado-Andrade et al., 2005) in aqueous medium and by DPPH assay (Xu and Chang, 2007) in methanolic medium. $ABTS^+$ was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in dark at 25 °C for 12–16 h

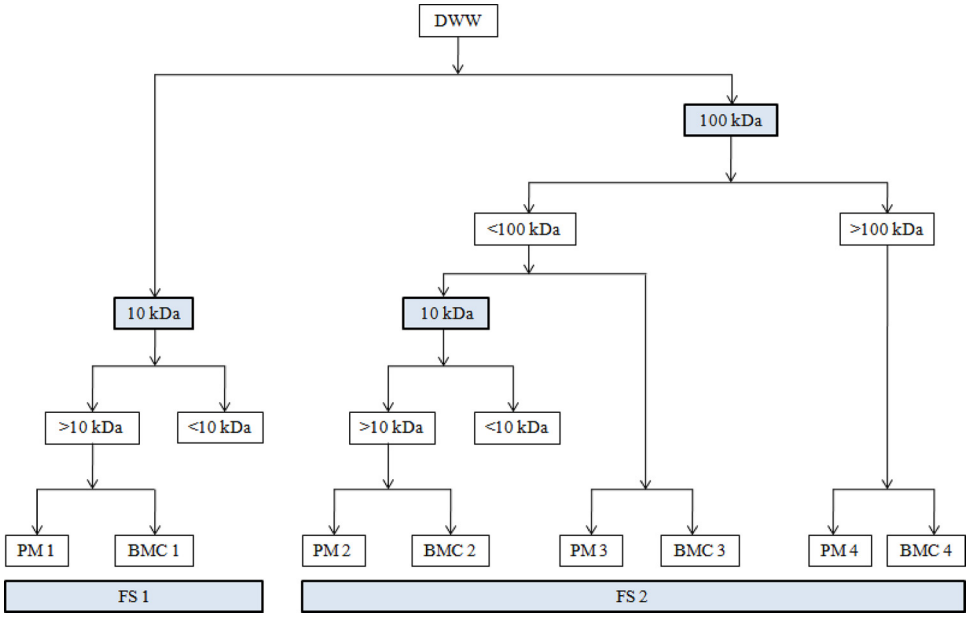

Fig. 1. DWW fractionation schemes (FS).