Environmental Health Hazards of Post Methanated Distillery Waste and Development of New Technology on Decolourisation and Detoxification of Post Methanated Distillery Effluent for its Recycling and Re-use

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Alcohol distilleries are among the 17 most polluting industries in India, the second largest producer of ethanol in Asia. Sugarcane molasses is a basic raw material for the manufacture of ethanol in the country.

In India there are more than 397 distilleries producing $3.25 \times 10^9$ L of alcohol and generating $40.40 \times 10^{10}$ L of wastewater annually. An average molasses based distillery generates $15$ L of spent wash per lit of alcohol produced.

Spent wash after anaerobic digestion in sugarcane molasses based distilleries known as PMDE. PMDE retains high BOD ($18,000–22,000$ mg/l), COD ($32,400–35,000$ mg/l), colour ($1,50,000–1,80,000$ Co-Pt), sulphate ($3,100–5,760$ mg/l), phenol ($4.0–4.2$ mg/l), total suspended solids (TSS; $11,920–25,308$ mg/l), total dissolved solids (TDS; $10,480–77,776$ mg/l) and having heavy metals.
Fig. 1: The process of alcohol manufacture and effluent generation.

1. Spentwash
2. Neutralised
3. Cooled
4. Methane reactor (Hydrolysis, acidogenesis, methanogenesis)
5. Post methanated distillery effluent (PMDE)

C1
C2
The Maillard reaction

The major colournat is synthesized with result of Maillard reaction by reacting the sucrose and mixture of amino acids (aspartic acid, glycine, lysine, histidine, arginine and tryptophan) present in sugarcane juice at high temperature during concentration of sugar juice.

I. Initial Stage: Produce colourless, without absorption in the UV (About 280 nm)
   - Reaction A: Sugar-amine condensation
   - Reaction B: Amadori rearrangement

II. Intermediate Stage: Products colourless or yellow, with strong absorption in the UV
   - Reaction C: Sugar dehydration
   - Reaction D: Sugar fragmentation
   - Reaction E: Amino acid degradation (Strecker degradation)

III. Final Stage: Products highly coloured
   - Reaction F: Aldol condensation
   - Reaction G: Aldehyde-amine condensation and formation of heterocyclic nitrogen compounds
The dark colour of post methanated distillery effluent is mainly due the presence of an amino-carbonyl complex compound known as melanoidin, which is generated by non-enzymatic browning maillard reactions.

- The molecular weight of melanoidin is ranges from 5- 40 KDa.
- The melanoidin is largely soluble in alkaline than acidic medium.
**Table 1: Organic compound identified by GC-MS analysis extracted with ethyl acetate extract from untreated post methanated distillery effluent**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>RT</th>
<th>Name of compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>7.06</td>
<td>Silanol, trimethyl-,trimester with boric acid</td>
</tr>
<tr>
<td>2.</td>
<td>7.28</td>
<td>L-Lactic acid</td>
</tr>
<tr>
<td>3.</td>
<td>7.76</td>
<td>Bis(dimethyl-t-butylsilyl)oxalate</td>
</tr>
<tr>
<td>4.</td>
<td>7.87</td>
<td>Cyclohexanol, 4-[(TMS)oxy]-cis</td>
</tr>
<tr>
<td>5.</td>
<td>8.35</td>
<td>D-(--)Lactic acid, trimethyl ether, TMS ester</td>
</tr>
<tr>
<td>6.</td>
<td>10.51</td>
<td>Ethanedioic acid</td>
</tr>
<tr>
<td>7.</td>
<td>10.90</td>
<td>t-Butyldimethyl(2-styryl[1,3]dithian-2-yl) silane</td>
</tr>
<tr>
<td>8.</td>
<td>11.07</td>
<td>Butane-1,3-diol, 1-methylene-3-methyl, bis(TMS)ether</td>
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<td>9.</td>
<td>12.35</td>
<td>Silanol, trimethyl-,benzoate</td>
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<td>10.</td>
<td>12.90</td>
<td>Propane, 1,2,3-tris[(tert-butyldimethylsilyl)oxy]</td>
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<tr>
<td>11.</td>
<td>13.57</td>
<td>Acetic acid, [bis[(TMS)oxy]phosphinyl]-TMS ester</td>
</tr>
<tr>
<td>12.</td>
<td>27.98</td>
<td>Hexadecanoic acid</td>
</tr>
<tr>
<td>13.</td>
<td>28.01</td>
<td>1-phenyl 1-propanol</td>
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<tr>
<td>14.</td>
<td>28.54</td>
<td>n-Pentadecanoic acid</td>
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<tr>
<td>15.</td>
<td>29.88</td>
<td>9,12-Octadecanoci acid(Z,Z)-, TMS ester</td>
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<tr>
<td>16.</td>
<td>30.84</td>
<td>Octadecanoic acid</td>
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<tr>
<td>17.</td>
<td>31.31</td>
<td>1-Eicosanol</td>
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<tr>
<td>18.</td>
<td>32.13</td>
<td>Methyl 19-methyl-eicosanoate</td>
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<tr>
<td>19.</td>
<td>33.49</td>
<td>1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl)ester</td>
</tr>
<tr>
<td>20.</td>
<td>33.82</td>
<td>Hexadecanoic acid, 2-[(TMS)oxy]-1,3-propenediy ester</td>
</tr>
<tr>
<td>21.</td>
<td>35.11</td>
<td>1,7-Pentatriacontene</td>
</tr>
<tr>
<td>22.</td>
<td>35.82</td>
<td>2-Monostearin</td>
</tr>
<tr>
<td>23.</td>
<td>35.72</td>
<td>Octadecanoic acid, ethyl ester</td>
</tr>
<tr>
<td>24.</td>
<td>35.75</td>
<td>Quercetin 7, 3’,4’ Trimethoxy</td>
</tr>
<tr>
<td>25.</td>
<td>38.55</td>
<td>1-Monolinoleylglycerol TMS ester</td>
</tr>
<tr>
<td>26.</td>
<td>39.74</td>
<td>2-isoropyl-5-methyl-1-heptanol</td>
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<tr>
<td>27.</td>
<td>41.12</td>
<td>Propanoic acid</td>
</tr>
<tr>
<td>28.</td>
<td>42.01</td>
<td>Tetradecanoic acid</td>
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</table>
# Table 1: Health Hazards of Heavy Metals

<table>
<thead>
<tr>
<th>Heavy Metal</th>
<th>Toxicities</th>
<th>Maximum Cont. Level (MCL) mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>Skin manifestations, visceral cancers, vascular disease</td>
<td>0.050</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Kidney damage, renal disorder, human carcinogen</td>
<td>0.01</td>
</tr>
<tr>
<td>Chromium</td>
<td>Headache, diarrhea, nausea, vomiting, carcinogenic</td>
<td>0.05</td>
</tr>
<tr>
<td>Copper</td>
<td>Liver damage, Wilson disease, insomnia</td>
<td>0.25</td>
</tr>
<tr>
<td>Nickel</td>
<td>Dermatitis, nausea, chronic asthma, coughing, human carcinogen</td>
<td>0.20</td>
</tr>
<tr>
<td>Zinc</td>
<td>Depression, lethargy, neurological signs and increased thirst</td>
<td>0.80</td>
</tr>
<tr>
<td>Lead</td>
<td>Damage the fetal brain, diseases of the kidneys, circulatory system, and nervous system</td>
<td>0.006</td>
</tr>
<tr>
<td>Mercury</td>
<td>Rheumatoid arthritis, and diseases of the kidneys, circulatory system, and nervous system</td>
<td>0.00003</td>
</tr>
<tr>
<td>Iron</td>
<td>Deterioration of the gut-lining, abdominal pain, in children 3 g can cause death</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Health Hazards of Heavy Metals

- Liver Damage
- Kidney Damage
- Skin Manifestation
- Drying patches on buffalo
- Vomiting
- Headache
- Rheumatoid arthritis
• The dark color effluent hinders photosynthesis by blocking sunlight and is therefore deleterious to aquatic life. Hence, the indiscriminate discharge of PMDE from distilleries is a major source of soil and water pollution which directly or indirectly affects the animals and human beings.

PMDE showing soil pollution and health hazards

PMDE discharge from M/S Kedia distillery Ltd (c) and PMDE prior to mixing in the river.
Besides, these wastewaters are being used by farmers to irrigate crops plants without considering its adverse effects, soil fertility as well as on groundwater quality.

The metals present in effluents may accumulate in crop plants in excessive quantities quite enough to cause the clinical problems in animals and human beings consuming these metal rich crop plants products.

It also causes inhibition of seed germination and depletion of vegetation by reducing the soil alkalinity and manganese availability in agricultural land receiving distillery effluent.

Fig. Agricultural soil pollution by distillery effluent released from distillery industry.
There is no set methodology for sludge disposal. The common disposal process for sewage includes land filling, land application and incineration.

Land filling is the first choice for sewage disposal. However, the future of sludge disposal through land filling is not very bright due to the fact that a large volume of soil is required to cover the waste in order to prevent the leaching of potentially toxic compounds including metals and phenols. Landfill sites are not easy to locate and strictly regulated to prevent odor emission. Leaching of various toxic compounds also contaminated the ground and surface waters.

Metals at higher concentration in the growth media can function as stressors causing physiological constraints that decrease plant vigour and affect plant growth, development and yields. This will cause negative impact on root weight, number of leaves and shoots weight of plants been reported that at high concentrations, nodule/plant and nodule/root reduced due to the higher concentrations of heavy metals leached from sewage sludge.
The generation of reactive oxygen species is also stimulated in the presence of metals, which can seriously disturb normal metabolism through oxidative damage of cellular compartments. To counteract this damage, highly efficient antioxidant defense mechanism in plant cell can deactivate metals stress generated by reactive oxygen radical.

Sludge generated from various sector have also been utilized in various agricultural uses due to the fact that significant amount of nitrogen, phosphorus and other organic matters are available in the industrial and domestic sludge. In view of this, distillery sludge having high organic content may be a good alternative to be used as a fertilizer for soil amendments.

The optimization of distillery sludge concentrations with garden soil for different commonly growing crops is required. This may affect the physico-chemical properties and microbiological processes. In addition, the presence of heavy metals, phenolic and sulfur compounds in distillery sludge are ambiguous for plant growth and their effect on environment is not very well known.
Furthermore, the vegetative growth parameters of *P. mungo* at all tested concentrations showed inhibitory effect (except 10% sludge amended soil) versus the control. The root length and number of leaves of *P. mungo* at 10% (w/w) sludge concentration increased by 3.45 and 16.66%, respectively compared to control. At 80% (w/w) sludge-amended soil (after 60 days of study), 48.94 and 21.66% reduction in shoot length and root length was observed.

Effect of distillery sludge root length. Shoot length and leaves number and their respective biomass of *P. mungo*

<table>
<thead>
<tr>
<th>Sludge concentration (%)</th>
<th>Root length (cm)</th>
<th>Biomass of root (g/plant)</th>
<th>Shoot length (cm)</th>
<th>Biomass of Shoot (g/plant)</th>
<th>No. of leaves/plant</th>
<th>Biomass of leaves (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.73 ± 0.48</td>
<td>0.221</td>
<td>19.67 ± 0.75</td>
<td>0.525</td>
<td>12.00 ± 0.45</td>
<td>0.354</td>
</tr>
<tr>
<td>10</td>
<td>13.17 ± 0.53</td>
<td>0.268</td>
<td>21.17 ± 0.7</td>
<td>0.569</td>
<td>14.00 ± 0.55</td>
<td>0.390</td>
</tr>
<tr>
<td>20</td>
<td>9.17 ± 0.32</td>
<td>0.198</td>
<td>9.00 ± 0.65</td>
<td>0.612</td>
<td>9.67 ± 0.42</td>
<td>0.234</td>
</tr>
<tr>
<td>40</td>
<td>8.17 ± 0.35</td>
<td>0.122</td>
<td>8.57 ± 0.31</td>
<td>0.312</td>
<td>9.40 ± 0.40</td>
<td>0.186</td>
</tr>
<tr>
<td>60</td>
<td>7.83 ± 0.26</td>
<td>0.098</td>
<td>8.50 ± 0.31</td>
<td>0.102</td>
<td>8.33 ± 0.35</td>
<td>0.102</td>
</tr>
<tr>
<td>80</td>
<td>6.50 ± 0.22</td>
<td>0.018</td>
<td>2.50 ± 0.08</td>
<td>0.069</td>
<td>8.00 ± 0.33</td>
<td>0.026</td>
</tr>
</tbody>
</table>
Our earlier study revealed that during the irrigation with post methanated distillery effluent (PMDE), there is heavy metal accumulation in Indian mustard (*Brassica compestris*) and wheat (*Triticum aestivum*). This indicated the health hazards to human without proper treatment (Chandra et. al., 2009).
Effect of distillery sludge on seed germination and growth parameters of green gram (Phaseolus mungo L.)

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Abstract

Experiments were carried out to study the effect of distillery sludge amendments with garden soil (10, 20, 40, 60, 80 and 100%) on seed germination and growth parameters of Phaseolus mungo L. Germination percentage and index values decreased with rise in sludge concentration. Soil amended with 10% (w/w) sludge showed favourable growth while >10% was inhibitory for plant growth. Soil amended with 10% (w/w) distillery sludge induced the growth in root length, shoot length, number of leaves, biomass, photosynthetic pigment, protein and starch with 20% (w/w) sludge amended soil had variable effects on the root, shoot, leaves and nodules of P. mungo L. At concentrations >10% reduced all the growth parameters, viz., root length, shoot length, number of leaves, biomass, photosynthetic pigment, protein and starch of P. mungo. Malondialdehyde (MDA) product of lipid peroxidation was also enhanced in both root and leaves of sludge amended soil grown P. mungo at all the sludge amendments and exposure periods. A coordinated increase in cytokine, non-protein thiol and ascorbic acid antioxidants was up to 40 days of growth. After this period a decrease was observed. The N, P, K and Mg accumulation followed the order shoot > root > leaf. Calcium accumulation was highest in the upper part of the plants (including shoot and leaves). Furthermore, heavy metals content were also increased in different parts of Phaseolus mungo grown on increasing concentration of sludge amended garden soil with time. Zinc and copper accumulation was maximum versus other heavy metals. Based on these studies, sludge having concentrations ≤10% (w/w) can be applied as a fertilizer.

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Keywords: Distillery sludge; Antioxidants; Growth parameters; Phaseolus mungo L.; Heavy metals; Soil amendments

I. Introduction

The disposal of industrial wastes is a worldwide problem. Among various industries, sugarcane molasses-based (distilleries) industries occupy a prominent place in Indian economy. There are approximately 300 distilleries in India, releasing 3.5 x 109 L effluent annually [1]. The distillery effluent is characterized by high biochemical oxygen demand (BOD) (40,000–50,000 mg/L), chemical oxygen demand (COD) (90,000–100,000 mg/L) [2–5], coniformal compounds, sulphate and heavy metal load [5, 6]. When discharged into water bodies, it damages the aquatic ecosystem by reducing photosynthetic activities and dissolved oxygen [6]. In addition, distilleries produce approximately 1500 tonnes of sludge per day during anaerobic digestion of spent wash [7] which is characterized by high organic matter (OM), total dissolved solid (TDS), phenol, sulphate and heavy metals concentrations [2]. There is no set methodology for sludge disposal. The common disposal process for sewage includes land filling, land application and incineration. Among these, land filling is the first choice for sewage disposal. However, the future of sludge disposal through land filling is not very bright due to the fact that a large volume of soil is required to cover the waste in order to prevent the leaching of potentially toxic compounds including metals and phenols. Landfill sites are not easy to locate and strictly regulated to prevent odor emission. Leaching of various toxic compounds also contaminated the ground and surface waters [8]. Metals at supraoptimal concentration in the growth media can function as stressors causing physiological constraints that decrease plant vigour and affect plant growth, development and yields [9]. This will cause negative impact on root weight, number of leaves and shoots weight of plants [10]. It has also

Phytoextraction of trace elements and physiological changes in Indian mustard plants (Brassica nigra L.) grown in post methanated distillery effluent (PMDE) irrigated soil

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ABSTRACT

The metal accumulation potential and its physiological effects in Indian mustard plants (Brassica nigra L.) grown in soil irrigated with post methanated distillery effluent (PMDE) were studied after 30, 60 and 90 days of assay. An increase in the chlorophyll and protein contents was recorded at the lower concentrations of post methanated distillary effluent (PMDE) at initial exposure periods followed by a decrease at higher concentrations of PMDE compared to their respective controls. An enhanced lipid peroxidation in treated plants was observed, which was evidenced by the increased malondialdehyde content in shoot, leaves and seeds at all the concentrations of PMDE and exposure periods compared to their respective controls. This study revealed that Indian mustard plants (B. nigra L.) are well

Accumulation and distribution of toxic metals in wheat (Triticum aestivum L.) and Indian mustard (Brassica campestris L.) irrigated with distillery and tannery effluents

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ABSTRACT

In the present study, accumulation and distribution of toxic metals (Cu, Cd, Zn, Fe, Ni, Mn, and Pb) and their biochemical effect on wheat and mustard plants irrigated with mixed distillery and tannery effluents are reported. Analyses of effluents and soil samples have shown high metal content than the permissible limit except Pb. Further, analyses of plant samples have indicated the maximum accumulation of Fe (100 mg/kg) in wheat root and 500 mg/kg in mustard leaves followed by Zn in root > shoot > leaves > seeds. Minimum increase in photosynthetic pigment was observed between 30 and 60 days while protein content was found maximum between 60 and 90 days of growth period in both plants. An increase in malondialdehyde, cytochrome and ascorbic acid antioxidants content was also observed in root and leaves of treated plants. Up to 60 and 90 days of growth, herb, wheat and mustard plants irrigated with effluents without adequate treatment are health hazards for environment, humans and animals.

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Bacterial degradation of synthetic and natural melanoidins and characterization of their metabolites

• The bacterial consortium comprising *Bacillus licheniformis*, DQ779010; *Bacillus sp.*, DQ779011 and *Alcaligenes sp.*, DQ779012 showed capability for decolourisation of sucrose glutamic acid (SGA) Maillard product and natural melanoidin upto 73.79 and 69.83% respectively in 144 h of incubation due to manganese peroxidase (MnP) enzyme activity.
The Manganese peroxidase (MnP) activity was noted in phenol red (0.1%) containing modified GPYM agar media (Fig 7,8) by isolated potential bacterial strains.

Showing MnP activity and bacterial morphology
Characterization of potential MnP producing bacteria and its metabolic products during decolourisation of synthetic melanoidins due to biostimulatory effect of D-xylose at stationary phase

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**Abstract**

Sucrose-aspartic acid Maillard product (SAA-MP) is the major colourant of distillery effluent as environmental pollutant due to its recalcitrant nature. Three potential manganese peroxidase (MnP) producing bacteria were screened for higher SAA-MP tolerance (3200 mg/l) and characterized as *Bacillus* species (IITRM7, FJ581030), *Raoultella planticola* (IITRM15, GU329705) and *Enterobacter sakazakii* (IITRM16, FJ581031). The consortium of these bacteria showed maximum decolourisation (60%) of SAA-MP (2400 mg/l) in modified GPY medium at optimized nutrient, pH (7.0 ± 0.2), shaking speed (180 rpm) and temperature (35 ± 2°C) after 144 h incubation. The addition of D-xylose enhanced the decolourisation of SAA-MP from 60 to 75% along with reduction of BOD and COD. The electrospray ionization-mass spectrum (ESI-MS) analysis showed removal of various compounds after bacterial growth and decolourisation of SAA-MP. Formation of new products showed metabolism of SAA-MP. Thus this consortium might be useful for decolourisation of industrial wastewater containing high concentration of melanoidins. D-xylose could be used as biostimulator for this consortium during decolourisation of SAA-MP.
The maximum MnP activity was observed in 96h incubation in shaking flask condition (Fig 10A). The separated MnP on SDS-PAGE electrophoresis yield a single band with molecular weight (M. Wt) of 43 KDa (Fig 10B). The MnP was first time detected and separated from bacterial cell for melanoidin decolourisation.

**Fig 10 (A, B):** MnP and MIP activity shown by bacteria during the degradation of melanoidin, SDS-PAGE of bacterial MnP. Lane L: Protien ladder (16-97.4 KDa); Lane M: Marker protein (Horseradish peroxidase, 43 KDa); Lane 1: Bacterium RNBS1; Lane 3: Bacterium RNBS3 and Lane 4: Bacterium RNBS4
Comparative HPLC chromatogram of control (uninoculated) and degraded (inoculated) samples showing degradation of synthetic melanoidins by strain, RNBS-1 (A), RNBS-3 (B), RNBS-4 (C) and mixed strains (D) after 144 hrs incubation period.

Bharagava & Chandra, 2009; World J. Microbiol Biotechnol
Chandra et. al., 2009 (Bioresource Technology)
LC-ESI/MS/MS chromatogram of control sucrose-glutamic acid Maillard products (A); Expanded mass scale to show the distribution of compounds at m/z 85, 97, 110 and 127 (B) and LC-ESI/MS/MS chromatogram of bacterial degraded sucrose-glutamic acid Maillard products (C).
<table>
<thead>
<tr>
<th>Sl.</th>
<th>CONTROL SAMPLE</th>
<th>DEGRADED SAMPLE</th>
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<tbody>
<tr>
<td>No.</td>
<td>Compounds Detected</td>
<td>RT</td>
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<tr>
<td>1.</td>
<td>Palmitic acid ((C_{16}H_{32}O_2))</td>
<td>256</td>
</tr>
<tr>
<td>2.</td>
<td>2-Nitroacetophenone ((C_8H_7NO_3))</td>
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<tr>
<td>3.</td>
<td>2, 2’-Bifuran ((C_8H_6O_2))</td>
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</tr>
<tr>
<td>4.</td>
<td>Methylindol ((C_9H_9N))</td>
<td>130</td>
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<tr>
<td>5.</td>
<td>Hydroxymethyl-2-furancarboxaldehyde ((C_6H_6O_3))</td>
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<td>6.</td>
<td>5-methyl-2-furancarboxaldehyde ((C_6H_6O_2))</td>
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<td>7.</td>
<td>2-Methylhexane ((C_7H_16))</td>
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<tr>
<td>8.</td>
<td>Furan-2-carboxaldehyde ((C_5H_4O_2))</td>
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<td>9.</td>
<td>2,3-Dihydro-5-methylfuran ((C_5H_8O))</td>
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Isolation and characterization of aerobic bacteria capable of the degradation of synthetic and natural melanoids from distillery effluent

Ram Naresh Bharagava · Ram Chandra · Vibhuti Rai

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Abstract Melanoids, complex biopolymer of amino-carbonyl compounds are the major coloring and polluting constituents of distillery wastewaters. In this study, three aerobic melanoid-degrading bacteria (RBN81, RBN83 and RBN84) were isolated from soil contaminated with distillery effluent and characterized as Bacillus licheniformis (RBN81), Bacillus sp. (RBN83) and Alcaligenes sp. (RBN84) by biochemical tests and 16S rRNA gene sequence analysis. The degradation of synthetic and natural melanoids was studied by using the axenic and mixed bacterial consortium. Results have revealed that the mixed consortium was more effective compared to axenic culture decolorizing 73.79 and 69.83% synthetic and natural melanoids whereas axenic cultures RBN81, RBN83 and RBN84 decolorized 65.88, 62.56 and 60.10% synthetic and 52.69, 48.52 and 56.94% natural melanoids, respectively. The HPLC analysis of degraded samples has shown reduction in peak areas compared to controls, suggesting that decrease in color intensity might be largely attributed to the degradation of melanoids by isolated bacteria.

Keywords Bio degradation · Synthetic and natural · Melanoid · Bacillus licheniformis · Alcaligenes faeulis · Peroxidase

Introduction
Melanoids are natural, brown to black complex biopolymers of amino carbonyl compounds produced by non-enzymatic browning Maillard reactions (Chandra et al. 2008; Reynolds 1968). They are nitrogenous polymers consisting of repeating units of furans and/or pyroles, formed during the advanced stages of Maillard reaction and linked by polycondensation reactions. The melanoid skeleton is mainly composed of sugar degradation products formed in the early stages of Maillard reaction polymerized through aldol-type condensation and possibly linked by amino compounds. Starting reactions as well reaction conditions have a strong influence on the elemental composition as well as structure of melanoids (Cannaner and Kroh 1995). These mixtures of low and high molecular weight components abundantly exist in wastewaters released from distilleries utilizing sugarcane molasses for alcohol production. Melanoids are major coloring and polluting constituents in distillery wastewaters, which retains a dark black color even after anaerobic treatment, and hardly changes in extended aeration tanks, due to its recalcitrant nature (Chandra et al. 2008).

In India there are more than 319 distilleries producing 3.25 × 106 l of alcohol and generating 40.4 × 106 l of wastewater annually (Pant and Adholeya 2007). Melanoid-containing distillery effluents require pretreatment before safe disposal into the environment because direct disposal causes serious soil and water pollution by inducing coloration and eutrophication problems in aquatic environments, reduction of sunlight penetration in rivers, lakes, and lagoons, and this in turn decreases both photosynthetic activity and dissolved oxygen concentration, affecting the normal life cycle of aquatic fauna and flora. On land it causes reduction in soil alkalinity, inhibition of seed

1. Introduction
Maillard reactions are common, non-enzymatic browning reactions taking place between sugars and amino acids during the thermal processing of food materials, sugarcane juice in sugar industries and molasses in distilleries and produced a dark brown to black complex polymer known as melanoids (Chandra et al. 2008), which is the major source of environmental pollution. The molecular weight, structure and elemental composition of melanoids is strongly influenced by the ratio and type of reactants as well as reaction conditions such as temperature, reaction time, pH and solvent used (Yaylayan and Kaminsky, 1998).

Large volume of dark colored distillery effluent released into the environment causes coloration and reduction of sunlight penetration in aquatic environment as well as reduction in soil alkalinity, inhibition of seed germination and damage of vegetation (Chandra et al. 2008). Hence, it requires adequate pre-treatment prior to its discharge into the environment. Bacteria seem to be more effective for the bioremediation of environmental pollutants due to their immense environmental adaptability and biochemical versatility (Ghosh et al., 2002). Some workers have reported the microbial degradation of model as well as molasses melanoids (Miyata et al., 2000; Ghosh et al., 2002). But detailed information regarding the melanoids degrading enzymes and the nature of metabolic products of melanoids degradation has not reported so far. Hence, the objectives of this study were to isolate and characterize the potential aerobic bacteria and enzymes capable to degrade SGMPs and metabolites produced during the bacterial degradation of SGMPs.

2. Methods
2.1 Preparation of sucrose-glutamic acid Maillard products
Sucrose-glutamic acid Maillard products (SGMPs) was prepared as reported earlier (Bharagava et al. 2009).

2.2 Isolation, screening and characterization of SGMPs degrading bacteria
The potential aerobic bacteria capable for SGMPs degradation were isolated from distillery waste contaminated soil collected from MJ's Urmeea distillery and brewery Ltd., Urmeea (UP), India by enrichment technique and screened on the basis of growth and peroxidase activity on GPV agar plates amended with SGMPs solution, 1.3% agar and 0.1% phenol red (w/v) (BDH Ltd.) (Bharagava et al., 2009). Further, the isolated bacteria were characterized following the standard procedures (Barrow and Feltham,

Short Communication
Characterization of sucrose–glutamic acid Maillard products (SGMPs) degrading bacteria and their metabolites

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b School of Studies in Life Sciences, Pt. Ravishankar Shukla University, Raipur 492010, India
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ABSTRACT

Two aerobic bacteria RBN81 and RBN83 capable to degrade and utilise sucrose–glutamic acid Maillard products (SGMPs) as carbon, nitrogen and energy source were isolated and characterized as Alcaligenes faeulis (DQ659815) and Bacillus cereus (DQ569820) respectively by 16S rRNA gene sequence analysis. In present study, mixed bacterial culture was found more effective compared to axenic culture RBN81 and RBN83 decolorizing 73.79%, 66.80% and 62.54% SGMPs, respectively. The SGMPs catalyzing enzyme was characterized as manganese dependent peroxidase (MnP) by SDS-PAGE yielding a single band of 43 KDa. Further, the LC-MS-MS and other spectroscopic analysis have revealed that most of the SGMPs detected in control were diminished from bacteria treated samples. The disappearance of SGMPs from bacteria treated samples could be related with the degradation of SGMPs. © 2009 Elsevier Ltd. All rights reserved.

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Fig. 4 UV-Vis spectrophotometric analysis of (a) Synthetic distillery effluent (b) natural distillery effluent (b)
Fig. 5 UV-Vis spectrophotometric and HPLC analysis of treated and untreated distillery effluent
Bacterial Degradation of Molasses Melanoidin
• Initial 24 hrs incubation showed less decolourisation, possibly due to the utilization of glucose by bacterial consortium and subsequently utilization of color containing compounds as sole carbon, nitrogen, and energy source. This resulted bacterial decolourisation of melanoidin.

• In HPLC analysis, reduction and formation of new peak showed degradation as well as biotransformation.

• Further, the LC-MS/MS confirmed the degradation of six compound i.e. Dihydroxyconiferyl alcohol ($C_{10}H_{14}O_3$), 2, 2’-bifuran-5-carboxylic acid ($C_9H_6O_4$), 2, 3-dimethyl-pyrazine ($C_6H_8N_2$), Methylbenzene ($C_7H_8$), 3-pyrroline ($C_4H_7N$), Acetic acid ($C_2H_4O_2$)] and four compounds i.e. 2-nitroacetophenone ($C_8H_7NO_3$), p-chloroanisol ($C_7H_7ClO$), 2-methylhexane ($C_7H_{16}$), 2, 3-dihydro-5-methylfuran ($C_5H_8O$) have been found unchanged (as recalcitrant) while 2, 2’-bifuran ($C_8H_6O_2$) and Indole ($C_8H_7N$) were found as new compounds (Table 1).
LC-ESI/MS/MS chromatogram of control (C) and bacterial decolorized sample (D)

*Chandra et al., 2010; Biodegradation*
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Compounds Identified</th>
<th>m/z value</th>
<th>Samples</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>Degraded</td>
</tr>
<tr>
<td>1.</td>
<td>Dihydroxyconiferyl alcohol (C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;14&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>182</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>2, 2’-bifuran-5-carboxylic acid (C&lt;sub&gt;9&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>178</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>2-nitroacetophenone (C&lt;sub&gt;8&lt;/sub&gt;H&lt;sub&gt;7&lt;/sub&gt;NO&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>165</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>p-chloroanisol (C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;7&lt;/sub&gt;ClO)</td>
<td>142</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>2, 3-dimethyl-pyrazine (C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;8&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>108</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>2-methylhexane (C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;)</td>
<td>100</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Methylbenzene (C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;8&lt;/sub&gt;)</td>
<td>92</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>2, 3-dihydro-5-methylfuran (C&lt;sub&gt;5&lt;/sub&gt;H&lt;sub&gt;8&lt;/sub&gt;O)</td>
<td>84</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>3-pyrroline (C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;7&lt;/sub&gt;N)</td>
<td>69</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Acetic acid (C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>60</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>2, 2’-bifuran (C&lt;sub&gt;8&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>134</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Indole (C&lt;sub&gt;8&lt;/sub&gt;H&lt;sub&gt;7&lt;/sub&gt;N)</td>
<td>117</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

+: Compound Present; -: Compound Absent

*Chandra et al., 2010; Biodegradation*
Bacterial decolourisation of Post Methanated Distillery Effluent
Growth and decolourisation pattern of PMDE by bacterial consortium in GPYM media

Decolourisation was compared with Manganese peroxidase and laccase activity during the degradation of natural melanoidins by bacterial consortium1&2.
Comparative changes in pH and biomass during degradation of PMDE by bacterial consortium.

HPLC analysis of PMDE before and after bacterial treatment.
1. Formic acid
2. 3-ethyl pyridine
3. di-N-octyl phthalate
4. 1,2 benzenedicarboxilic acid
5. N-hexadecanoic acid
6. Isoxazolecarboperoxoic acid
7. Pentafluoropropionic acid
8. 2,5-pentadecadien-1-ol
9. 2-dimethyl (trimethylsilyl) silyloxytetradecane
10. Benzyl butyl phthalate
11. Pentafluoropropionic acid
12. 2-propyl oxetane
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>RT</th>
<th>Compounds</th>
<th>Control</th>
<th>Sample Degraded</th>
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<td></td>
<td></td>
<td></td>
<td>6 d</td>
<td>12d</td>
</tr>
<tr>
<td>1.</td>
<td>15.51</td>
<td>Formic acid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>16.82</td>
<td>3-ethyl pyridine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>21.92</td>
<td>di-N-octyl phthalate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>22.24</td>
<td>1,2 benzenedicarboxilic acid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>25.09</td>
<td>N-hexadecanoic acid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>27.46</td>
<td>Isoxazolecarboperoxoic acid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>27.92</td>
<td>Pentafluoropropionic acid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>27.99</td>
<td>2,5-pentadecadien-1-ol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>28.25</td>
<td>2-dimethyl (trimethylsilyl) silyloxytetradecane</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>31.31</td>
<td>Benzyl butyl phthalate</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>11.</td>
<td>31.95</td>
<td>Pentafluoropropionic acid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12.</td>
<td>30.24</td>
<td>2-propyl oxetane</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>13.</td>
<td>11.72</td>
<td>1-(1,3-dioxolan-2-yl)-2-propanone</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>14.</td>
<td>13.39</td>
<td>3-amino-1-pyrin-3-y1-pyrrolidine-2,5 dione</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>15.</td>
<td>18.99</td>
<td>3,7-dimethyl-1-octanol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>16.</td>
<td>19.06</td>
<td>1-iodotetradecane</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>17.</td>
<td>21.45</td>
<td>Dibutyl phthalate</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>18.</td>
<td>21.71</td>
<td>1-hexadecanol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>19.</td>
<td>24.69</td>
<td>10-methyl-9-nonadecene</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>20.</td>
<td>28.70</td>
<td>Heptafluororobutyric acid</td>
<td>-</td>
<td>+</td>
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<tr>
<td>21.</td>
<td>11.72</td>
<td>1-(1,3-dioxolan-2-yl)-2-propanone</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>22.</td>
<td>15.09</td>
<td>2,2-diphenyl-carbonic dihydrazine</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>23.</td>
<td>28.67</td>
<td>1-Eicosanol</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Biodegradation of organic compounds of molasses melanoidin (MM) from biomethanated distillery spent wash (BMDS) during the decolourisation by a potential bacterial consortium

Sangeeta Yadav · Ram Chandra

Abstract
Molasses melanoidin (MM) is a major pollutant in biomethanated distillery spent wash (BMDS) due to its recalcitrant properties. The 75% colour and 71% COD of MM (1,000 ppm) were reduced with developed bacterial consortium comprising Proteus mirabilis (IITRM5; FJ381028), Bacillus sp. (IITRM7; FJ581030), Ralstonia planticola (IITRM15; GU529705) and Enterobacter sakazakii (IITRM16, FJ581031) in the ratio of 4:3:2:1 within 10 days at optimized nutrient. Bacterial consortium showed manganese peroxidase and laccase activity during MM decolourisation. The dominant growth of R. planticola and E. sakazakii was noted in consortium during MM decolourisation. The comparative GC-MS analysis of extracted compounds of control and degraded samples showed that most of the compounds present in control were completely utilized by bacterial consortium along with production of some metabolites. The developed bacterial consortium could be a tool for the decolourisation and degradation of melanoidin containing BMDS.

Keywords Decolourisation · GC-MS analysis · Manganese Peroxidase · Molasses melanoidin · Laccase · Phenolics

Introduction
Molasses melanoidin (MM) is dark brown to black natural condensation polymer of sugars and amino acids; they are produced by the non-enzymatic browning reactions known as Maillard reactions (Plavsic et al. 2006). MM is widely discharged in huge amount as environmental pollutant by various agro-based industries especially from distilleries and fermentation industries (Kumar and Chandra 2006). Melanoidin remains about 2% in molasses based distillery effluent as major carbon recalcitrant pollutant together with metals and phenolics (Mani sankar et al. 2004; Chandra et al. 2008). It is hardly degraded by microbes and behaves as anionic hydrophilic polymers, which has high binding tendency with metal cations result into more complex nature of effluent (Migo et al. 1997; Plavsic et al. 2006). Moreover, phenolics present in effluent makes more complex compounds with heavy metals (Chandra et al. 2008).

Melanoidin containing sugarcane molasses based distillery wastewater is the major source of soil and water pollution even after anaerobic digestion due to high biological oxygen demand (BOD, 23000), chemical oxygen demand (COD, 47000), total dissolved 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 BMDS.

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

Introduction
The dark brown color of distillery wastewater is not only due to the presence of a complex biopolymer called melanoids, which are generated by the Maillard reaction (Ohnomo 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 melanoids 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...
Effect of different concentrations of post methanated distillery effluent (PMDE) on seedling growth in *Phaseolus mungo* L. before (A) and after bacterial treatment (B). C: Control/Tap water; 1, 2, 3, 4, 5, 6 and 7 represented 5, 10, 20, 40, 60, 80 and 100% (v/v) concentration of PMDE.
The reduction in amylase activity at higher (>10 and 20%) concentration of untreated and treated PMDE might be due to the high salt load and metals content, affecting various physiological and biochemical process of seed germination.

*Phaseolus* seeds treated with 60 and 80% (v/v) concentration of untreated PMDE have shown reduction in α-amylase activity and no α-amylase activity was observed in seeds treated with 100% (v/v) untreated PMDE.
• Phytoremediation of heavy metals contamination is emerging out as a cost effective green technology. Based on phytoextraction through uptake and accumulation of metal in plant, root and shoot which subsequently can be harvested and remove from polluted site through metal recovery.

• Growth of several potential wetland plants on distillery sludge contaminated site indicated the phytoremediation potential of these plants as natural hyper-accumulator but the detail knowledge regarding the magnitude and pattern of different plants is still unknown.
Fig. Morphological effect of metals, phenol at variable concentration of melanoidin (Set II, ST8–ST13 from left to right) on T. angustifolia during metal accumulation at 20 (a), 40 (b), and 60 days (c) incubation.

Fig. Light micrograph of T. angustifolia root shows metal deposition (dark staining) and disruption of cortex cell (b vs. a; *) and TEM micrograph shows intercellular space ( ) and nucleus size reduction (d) in ST11 as compared to control (c) during metal accumulation in 60 days. Cortex (Ct), phloem (Ph), and xylem (X).
Potential of Typha angustifolia for phytoremediation of heavy metals from aqueous solution of phenol and melanoidin

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ABSTRACT

Typha angustifolia was evaluated for various heavy metals (Cu, Pb, Ni, Fe, Mn, and Zn) biomedia-
tion potential from aqueous solution containing variable concentrations of phenol (100–800 mg L⁻¹) and melanoidin (2500–8500 Cu-CoP) at 20, 40, and 60 days. The concentration of phenol (200–400 mg L⁻¹) along with melanoidin (500–8500 Cu-CoP) showed toxic effect on T. angustifolia along with phenol. Phenol and melanoidin showed adverse effect on T. angustifolia of up to 20 days incubation, but this leads to induction of peroxidase and ascorbic acid activity to cope with adverse conditions. Subsequently, the pollutants were decreased along with plant growth, peroxidase and ascorbic acid also declined. However, with reduction of peroxidase, catalase level was increased. The Cu, Zn, and Ni were accumulated at maximum in all tested conditions. The TEM observations of T. angustifolia showed slight deposition of metals in root and root tip, breakdown of spongyl and palisade parenchyma of leaves at higher concentration of phenol (1000 mg L⁻¹) and melanoidin (5000 Cu-CoP). Thus, this study concluded that T. angustifolia could be a potential phytoremediator for heavy metals from metal, melanoidin, and phenol containing industrial wastewater at optimized conditions.

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Phytoremediation of CD, CR, CU, MN, FE, NI, PB and ZN from Aqueous Solution Using Phragmites Communis, Typha Angustifolia and Cyperus Esculentus

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a Environmental Microbiology Section, Indian Institute of Toxicology Research (CSIR), Lucknow, India
Available online: 18 Mar 2011

Bacterial pretreatment enhances removal of heavy metals during treatment of post-methanated distillery effluent by Typha angustiflora L.

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Received 20 April 2006; revised version accepted 19 March 2007; accepted 8 May 2007
Available online: 21 June 2007

Abstract

A combination of bacterial pretreatment followed by free water surface flow through wetland plants was investigated to determine its effect on removal of heavy metals in biomedia-
tion of post-methanated distillery effluent (PMDE). The bacterial pretreatment was intended to transform the metal complexes and organic pollutants into simpler, biologically assimilable molecules. The 10% and 30% of concentration of PMDE favored luxation; bacterial growth; the 50% concentration suppressed less growth, whereas the undiluted effluent (i.e., 100%) supported very little bacterial growth. The use of bacterial pretreatment combined with the constructed wetland system greatly increased the overall biocompensation of all heavy metals by the plants compared with the control treatment. However, the integration of bacterial pretreatment of PMDE with the Typha angustiflora reduced in enhanced removal of Cd (34.02–61.56%), Cr (31.9–57.46%), Cu (32.8–54.22%), Fe (32.50–61.26%), Mn (35.9–52.89%), Ni (35.8–59.24%), Pb (33.45–59.51%) and Zn (3.55–53.70%) (increase) compared with a control that lacked this pretreatment. In addition to the biocompensation of these heavy metals, several physico-chemical parameters also improved at the 30% effluent concentration: color, BOD, COD, phenol and total nitrogen decreased by 88.35%, 94.85%, 96.30%, 93.75% and 8.29%, respectively, after 7 days of free water surface flow treatment. The results suggest that bacterial pretreatment of PMDE, integrated with phytoremediation will improve the treatment process of PMDE and promote safer disposal of this waste.

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Keywords: Post-methanated distillery effluent; Bacterial pretreatment; Heavy metal; Phytoremediation; Typha angustiflora L.
Characterization of *Phragmites communis* rhizosphere bacterial communities and metabolic products during the two stage sequential treatment of post methanated distillery effluent by bacteria and wetland plants

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b Environmental Genomics Unit, National Environmental Engineering Research Institute (CSIR), Nehru Marg, Nagpur- 440 020, Maharashtra, India

**ABSTRACT**

This study deals with the characterization of rhizosphere bacterial communities and metabolic products produced during the two stage sequential treatment of post methanated distillery effluent by bacteria and constructed wetland plants. Results showed that bacterial treatment followed by wetland plants (*Phragmites communis*) resulted 94.5% and 96.0% reduction in BOD and COD values, respectively. The PCR-RFLP analysis showed the presence of *Stenotrophomonas, Enterobacter, Pantoea, Acinetobacter* and *Klebsiella* sp., as dominant rhizosphere bacterial communities which play an important role in degradation and decolorization of PMDE in wetland treatment system. Further, the LC-MS-MS and other spectrophotometric analysis have shown that most of the pollutants detected in untreated PMDE were diminished from bacteria and wetland plant treated PMDE indicating that bacteria and wetland plant rhizosphere microbes utilized them as carbon, nitrogen and energy source. While, methylbenzene, furfuryl alcohol, and 4-vinyl-2-methoxyphenol were detected as metabolites in bacteria and hexadecanol in wetland plant rhizosphere treated PMDE.
Methanococcus vaniielli strain SB [NR 025718]

Bacillus odyssaeistrain 34hs1 [NR 025258]

Agrobacterium larrymoorei strain AF3.10 [NR 026519]

Thauera selenatis strain AX39 [NR 025212]

Uncultured Acinetobacter sp. clone IITR RCP27 [FJ268988]

Uncultured Acinetobacter sp. clone IITR RCP24 [FJ268985]

Uncultured Acinetobacter sp. clone IITR RCP21 [FJ268982]

Acinetobacter sp. ATCC 31012 [AF542963]

Uncultured Acinetobacter sp. clone IITR RCP33 [FJ268994]

Uncultured Acinetobacter sp. clone IITR RCP37 [FJ268998]

Uncultured Enterobacter sp. clone IITR RCP19 [FJ268980]

Uncultured Klebsiella sp. clone IITR RCP25 [FJ268986]

Klebsiella pneumoniae subsp. pneumoniae [AF228918]

Uncultured Pantoea sp. clone IITR RCP34 [FJ268995]

Enterobacter aerogenes [AB099402]

Uncultured Pantoea sp. clone IITR RCP26 [FJ268987]

Uncultured Klebsiella sp. clone IITR RCP28 [FJ268989]

Uncultured Enterobacter sp. clone IITR RCP32 [FJ268993]

Pantoea ananatis strain 1846 [NR 026045]

Uncultured Stenotrophomonas sp. clone IITR RCP36 [FJ268997]

Uncultured Stenotrophomonas sp. clone IITR RCP23 [FJ268984]

Stenotrophomonas acidaminiphila strain AMX 19 [NR_025104]

Stenotrophomonas acidaminiphila [AF273080]

Uncultured Stenotrophomonas sp. clone IITR RCP22 [FJ268983]

Uncultured Stenotrophomonas sp. clone IITR RCP20 [FJ268998]

Uncultured Stenotrophomonas sp. clone IITR RCP35 [FJ268998]

Uncultured Stenotrophomonas sp. clone IITR RCP29 [FJ268998]

Uncultured Stenotrophomonas sp. clone IITR RCP30 [FJ268991]

Uncultured Stenotrophomonas sp. clone IITR RCP31 [FJ268998]
Detection of *Bacillus* and *Stenotrophomonas* species growing in an organic acid and endocrine-disrupting chemical-rich environment of distillery spent wash and its phytotoxicity

Ram Chandra - Vineet Kumar

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Abstract Sugarcane molasses-based distillery spent wash (DSW) is well known for its toxicity and complex mixture of various recalcitrant organic pollutants with acidic pH, but the chemical nature of these pollutants is unknown. This study revealed the presence of toxic organic acids (butanedioic acid bis(TMS)ester; 2-hydroxyoctanoic acid; benzenepropanoic acid, α-[TMS]oxy], TMS ester; vanillylpropionic acid, bis(TMS)), and other recalcitrant organic pollutants (2-furancarboxylic acid, 5-[[TMS]oxy]methyl], TMS ester; benzoic acid 3-methoxy-4-[[TMS]oxy], TMS ester; and tricarballylic acid 3TMS), which are listed as endocrine-disrupting chemicals. In addition, several major heavy metals were detected, including Fe (163.947), Mn (4.556), Zn (2.487), and Ni (1.175 mg L⁻¹). Bacterial community analysis by restriction fragment length polymorphism revealed that *Bacillus* and *Stenotrophomonas* were dominant autochthonous bacterial communities belonging to the phylum *Firmicutes* and γ-*Proteobacteria*, respectively. The presence of *Bacillus* and *Stenotrophomonas* species in highly acidic environments indicated its broad range adaptation. These findings indicated that these autochthonous bacterial communities were pioneer taxa for in situ remediation of this hazardous waste during ecological succession. Further, phytoxicity assay of DSW with *Phaseolus mungo* L. and *Triticum aestivum* revealed that *T. aestivum* was more sensitive than *P. mungo* L. in the seed germination test. The results of this study may be useful for monitoring and toxicity assessment of sugarcane molasses-based distillery waste at disposal sites.
Role of native plants in phytoremediation and eco-restoration
Phytoremediation of heavy metals by hyperaccumulator

Fig. TEM images of native plants root after phytoextraction of heavy metals (a-c) *Parthenium hysterophorous* (d-f) *Cannabis sativa* (g-i) *Solanum nigrum* (j-l)
Phytoextraction of heavy metals by potential native plants and their microscopic observation of root growing on stabilised distillery sludge as a prospective tool for in situ phytoremediation of industrial waste

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Abstract The safe disposal of post-methanated distillery sludge (PMDS) in the environment is challenging due to high concentrations of heavy metals along with other complex organic pollutants. The study has revealed that PMDS contained high amounts of Fe (2403), Zn (210), Mn (126), Cu (73.62), Cr (21.825), Pb (16.33) and Ni (13.425 mg kg⁻¹) along with melanoids and other co-pollutants. The phytoextraction pattern in 15 potential native plants growing on sludge showed complexation of heavy metals with organic pollutants. This gives a strong evidence of hyperaccumulation for the tested metals from complex distillery waste. Furthermore, the TEM observations of root of P. hysterophorus, C. sativa, Solanum nigrum and R. communis showed formation of multi-nucleolus, multi-vacuoles and deposition of metal granules in cellular component of roots as a plant adaptation mechanism for phytoextraction of heavy metal-rich polluted site. Hence,
Field scale demonstration of PMDE for decolourisation and detoxification process by using bacteria and constructed wetlands plant
Fig. Effect of different time dilution by using coagulant (FeCl₃) on post methanated distillery effluent (PMDE)
Fig : Proposed advanced biological technique for post methanated distillery effluent (PMDE) treatment for safe disposal and re-use

Advantage:
- This will develop a innovative and advanced technique for treatment as per recommended prescribed standard.
- This will also lead to develop a reuse technique for the decolourised and detoxified PMDE for economic growth.
- Generated sludge and biomass of constructed wetland plant treatment system will be used for production of organic manure.
- This technique will be helpful for recharge of ground water level
- This technique will be useful for the monitoring of organic pollutants and bacterial communities which are health hazards
- This technique will be helpful for development of zero pollution discharge technique.

Special Features:
- Comparative simple, economical feasible
- Effective to meet out the recommended standrad for sewage disposal
- This will develop a sustainable technology in Indian scenario

Integration of bacterial treated effluent to constructed wetland plant treatment system
Vertical subsurface flow constructed wetland plant treatment with flow gradient
Ponds for aquaculture

Reduction of TDS
Bioreactor
Composting
Composting
Waste sludge to composting
Generation of sludge
Chopped biomass
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