Decolourisation of molasses spent wash by Phanerochaete chrysosporium

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Phanerochaete chrysosporium decolourised molasses spent wash (MSW) (6.25% v/v) supplemented with glucose (25 g/l) by 85% after 10 days' incubation. Cells immobilised in calcium-alginate decolourised MSW much more rapidly than free cells but there was less overall colour decrease (59%) after 10 days.

Introduction

Molasses is one of the most important raw materials used in the commercial production of ethanol due to its low cost and availability. In a typical ethanol distillery in India, 10/15 litres of molasses spent wash (MSW) are produced for every litre of alcohol with approximately 20,000 litres of spent wash produced per day in each distillery (AIDA, 1994). There are several environmental problems associated with MSW, generated as a byproduct of the molasses fermentation process.

MSW is a highly coloured effluent with the potential to cause eutrophication of waterways due to its high pollution load, with a COD in the order of 90,000 mg/l (FitzGibbon et al., 1995). The coloured nature of MSW is due to the presence of natural polymers called melanoidins, formed by the Maillard amino-carbonyl reaction (Wedzicha & Kaputo, 1992). Melanoidins have been shown to have antioxidant properties and are toxic to many microorganisms typically used in wastewater treatment (Kitts et al., 1993). Due to the recalcitrant nature of melanoidins, conventional wastewater treatment process are unable to remove the colour from the MSW which has then the potential to block out light from contaminated waterways thus preventing oxygenation. In addition to being a water pollutant, MSW also causes manganese deficiency when disposed of in soil, resulting in a loss of soil fertility (Agrawal & Pandey, 1994). In this study we report the use of the white-rot fungus Phanerochaete chrysosporium to decolourise MSW and investigate the effect of media composition on the decolourisation process.

Materials and methods

Production of molasses spent wash

MSW was produced by distillation of a 23% (v/v) molasses solution fermented by Kluyveromyces marxianus (Banat et al., 1992). Molasses fermentation was carried out for 24 hours in 1L conical flasks fitted with air traps, using a reciprocal shaker (100 rev/min) at 45°C. The MSW was stored at 4°C until required.

Media and culture conditions

Phanerochaete chrysosporium ATCC 24725 was grown on the following medium which contained (g/litre): glucose, 25; peptone, 12.5; KH\textsubscript{2}PO\textsubscript{4}, 2.5; MgSO\textsubscript{4}.7H\textsubscript{2}O, 1.25 (pH 5.0) with molasses spent wash, 6.25% (v/v), and was sterilised by autoclaving. Where indicated the concentration of glucose, peptone and MSW was altered. Flasks (100 ml) containing 25 ml medium were inoculated with 0.45 g/l dry weight of macerated fungal biomass, pregrown on the above medium without addition of molasses spent wash, and incubated for 10 days on a rotary shaker (150 rev/min) at 37°C. Samples (1 ml) were routinely taken for analysis and all flasks were set up in duplicate.

Immobilisation of fungal cells

The method used for the immobilisation of fungal cells within Ca-alginate gel was similar to that described by De Rome and Gadd (1991). Macerated fungal biomass, to a final concentration of 0.45 g/l dry weight, was mixed with a 2% (w/v) solution of sodium alginate. This solution was delivered to a cold 0.1M CaCl\textsubscript{2} solution as droplets, the resulting beads were allowed to harden for 2 minutes and then the CaCl\textsubscript{2} solution was replaced.
with a fresh solution. The beads were then stored at 4°C for 2 hours before use. Aseptic conditions were maintained throughout bead preparation.

Analytical methods
Decolourisation of the MSW at 475nm was monitored in culture supernatants using a scanning spectrophotometer as previously described (FitzGibbon et al., 1995). Biomass was determined as dry weight per volume (g/litre). Culture media was filtered using Whatman No. 1 filter paper, the mycelia were recovered, washed thoroughly using distilled H₂O and dried at 80°C overnight and then weighed.

Results
There is a gap in our knowledge concerning the ability of microorganisms to decolourise molasses spent wash, which is a potential pollutant generated by molasses distilleries. A number of studies have reported that Coriolus versicolor is capable of MSW decolourisation (Watanabe et al., 1982; FitzGibbon et al., 1995) but little is known about the potential for other white-rot fungi to remediate this wastewater. In this study we investigated the effect of media composition on the ability of Phanerochaete chrysosporium ATCC 24725 to decolourise MSW (Table 1).

Many of the screening regimens previously employed for identifying microorganisms with melanoidin decolourising ability use media containing both glucose and peptone (FitzGibbon et al., 1995). In this study it can be seen that P. chrysosporium decolourised MSW most effectively in the absence of peptone and in the presence of glucose (85% colour decrease), compared with only 21% decolourisation when both substrates were present. This level of melanoidin decolourisation is comparable with that reported for a strain of Coriolus (80% colour removal) (Watanabe et al., 1982) and is much higher than that previously reported for the bacterium Lactobacillus hilgardii (28% colour removal) (Ohmomo et al., 1988a). In media which contained only basal salts and peptone the colour of the supernatant actually intensified over the incubation period (26% colour increase) (Table 1). A similar phenomenon was previously reported for a number of bacterial strains capable of melanoidin metabolism (Ohmomo et al., 1985). Most surprisingly our study found that P. chrysosporium was also capable of decolourising MSW in the absence of either glucose or peptone (49% colour reduction). This is the first time that a microorganism has been shown to decolourise MSW without the presence of an additional carbon source.

<table>
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<th>Table 1</th>
<th>Effect of media composition on decolourising ability of Phanerochaete chrysosporium</th>
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<tbody>
<tr>
<td>Medium type</td>
<td>Colour removal (%)</td>
</tr>
<tr>
<td>Basal salts</td>
<td>49</td>
</tr>
<tr>
<td>Basal salts + glucose + peptone</td>
<td>21</td>
</tr>
<tr>
<td>Basal salts + glucose</td>
<td>91</td>
</tr>
<tr>
<td>Basal salts + peptone</td>
<td>-26</td>
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The absorbance (475nm) of the media at T₀ was 0.422 and the colour removal was measured after 10 days. Basal salts media contained (g/litre); molasses spent wash, 6.25% (v/v); KH₂PO₄, 2.5; MgSO₄·7H₂O, 1.25 (pH5.0).

<table>
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<tr>
<th>Table 2</th>
<th>Effect of MSW concentration on colour removal by P. chrysosporium</th>
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<tr>
<td>Molasses spent wash (%)</td>
<td>Colour removal (%)</td>
</tr>
<tr>
<td>6.25</td>
<td>85</td>
</tr>
<tr>
<td>12.5</td>
<td>7</td>
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<td>25</td>
<td>3</td>
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The absorbance (475nm) of the media at T₀ was 0.422 at 6.25%, 0.82 at 12.5% and 1.63 at 25% MSW concentration. The colour removal was measured after 10 days.

The medium which gave most decolourisation of MSW and contained only glucose and basal salts was chosen for further investigation. Increasing the MSW concentration in the media to greater than 6.25% (v/v) resulted in a significant decrease in the decolourising ability of P. chrysosporium (Table 2). This may be explained by a decrease in water activity of the media or an increase in the concentration of compounds, such as gallic and vanillic acid, which have been shown to be present in MSW (FitzGibbon et al., 1995) and are potential inhibitors of microbial activity (Buswell and Eriksson, 1994). When glucose concentration was decreased to 12.5 g/litre decolourisation of MSW decreased to 44% (compared to 85% colour reduction at 25 g/litre glucose concentration), whilst an increase in glucose concentration to 50 g/litre resulted in no increase in MSW decolourisation.

The ability of P. chrysosporium to decolourise MSW media under optimal conditions was then investigated using free and immobilised cells (Figs. 1 and 2). It can be seen that in the free-cell experiment maximum biomass production was achieved very rapidly by day 2 (Fig. 1). Decolourisation of the MSW did not, however, commence until the fungus had reached idiophase with maximum colour removal occurring by day 8 (Fig. 1).
These observations suggest that enzymes involved in decolourisation of MSW are induced only when the organism has completed its growth. It was previously reported that manganese peroxidase was responsible for decolourisation of melanoidin by a strain of Coriolus versicolor (Dehorter and Blondeau, 1993). This and other enzymes involved in lignolytic activity are present in P. chrysosporium and are induced in stationary phase. Further work is necessary to investigate the potential role of the lignolytic enzymes of this fungus in decolourisation of melanoidins.

Immobilisation of P. chrysosporium in Ca-alginate beads resulted in a much more rapid decolourisation of MSW than with free-cells. Maximum colour removal occurred between day 0 and day 2 (Fig. 2), however, the level of colour removal was reduced from 85% with free-cells to 59% with immobilised cells after 10 days (cell-free alginate beads were capable of reducing the colour of MSW by a maximum of 25% after 6 days of incubation). Previous studies with Ca-alginate immobilised cells of Coriolus versicolor had also achieved similar levels of colour removal (60% after 2 days) (Ohmomo et al., 1988a) whilst studies with immobilised Lactobacillus hilgardii reported only 40% colour removal after 6 days incubation (Ohmomo et al., 1988a). Further studies are required to optimise conditions for melanoidin colour removal by immobilised P. chrysosporium, however, it is clear that this organism has the potential to be useful in the bioremediation of MSW wastewater.

References
AIDA. (1994). All India Distillers Association and Sugar Technologists Association of India, New Delhi, Annual Report.