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Bioremediation and decolorization of anaerobically digested distillery spent wash

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A facultative anaerobic pure bacterial culture L-2 capable of growth on 12.5% (v/v) diluted digested spent wash supplemented with glucose (10 g/l) was isolated from an Indian distillery. It achieved 31% decolorization and 57% COD reduction after 7 days' incubation. The advantages of using such a culture for digested spent wash bioremediation are apparent in providing a realistic approach for decreasing its pollution potential prior to disposal.

Introduction

Anaerobically digested cane molasses spent wash is a dark-brown-coloured effluent with high chemical oxygen demand (COD), high pollution potential and is bioremediation resistance. Cane molasses are one of the most common raw materials used in ethanol production. Approximately 30 billion litres of spent wash is annually generated in India alone by 254 cane molasses based distilleries (A.I.D.A., 1993). For every one litre of ethanol produced, 10 to 15 litres of spent wash are generated. A typical distillery generates over half a million litres of spent wash daily (Dahiya and Vimal, 1984). This effluent is a highly coloured compound with an extremely high COD load (Sirianuntapiboon *et al.*, 1988) and which is difficult to treat by normal biological processes such as activated sludge or anaerobic lagooning (Singh and Nigam, 1995). Its recalcitrance is due to the presence of brown polymers melanoidins which are formed by the maillard amino-carbonyl reaction (Wedzicha and Kaputo, 1992). These compounds have antioxidant properties which render them toxic to many microorganisms, such as those present in wastewater treatment processes (Kitts *et al.*, 1993).

Spent wash disposal into the environment is hazardous and has high pollution potential. Its high COD ≈90,000 mg/l (FitzGibbon *et al.*, 1995), means that its disposal into natural water bodies results in their eutrophication. Its highly coloured components also lead to the reduction of sunlight penetration in rivers, lakes or lagoons which in turn decreases both photosynthetic activity and dissolved oxygen concentrations causing detriment to aquatic life. on land is equally detrimental, causing a reduction in soil alkalinity and manganese availability, inhibition of seed germination and ruin of vegetation (Kannabiran and Pragasam, 1993; Agrawal and Pandey, 1994).

In India spent wash is usually utilized in an anaerobic digestion step to produce methane and reduce its organic load. Several investigators have suggested the potential uses of microbial systems in the decolorization of spent wash. In this paper we aimed at isolating bacterial cultures capable of both bioremediation and decolorization of anaerobically digested distillery spent wash to further decrease its potential pollutive impact.

Materials and methods

Analysis of raw and digested spent wash

The raw spent wash was collected at Associated Distillery Ltd., Hisar, India and the digested spent wash from its bio-methanation plant. Analyses were carried out to determine their specific gravity, total reducing sugar, total suspended solids, carbon, nitrogen, phosphorus, chemical oxygen demand, electrical conductivity, protein and pH. Total reducing sugars were determined by the 3,5-dinitrosalicylic acid (DNS) method. Total suspended solid was determined by dry weight at 105°C of a pellet obtained by centrifuging a 50 ml sample of digested spent wash (DSW) at 10,000 g for 30 minutes. The specific gravity of the DSW was measured using a hydrometer and all other parameters as described in Singh and Nigam (1995).

Enrichment and isolation

Soil samples were collected at Associated Distillery Ltd., Hisar, India at various sites including one near an anaerobic digester and a lagoon of digested spent wash. Two grams of soil samples were added to test tubes containing 20 ml of 12.5% diluted digested spent wash medium. Three different enrichment media were used: in the first, digested spent wash was the sole carbon and nitrogen source; in the second, spent wash was the sole carbon source and additional nitrogen (as ammonium sulphate) was added and in the third, the digested spent wash was the sole nitrogen source while additional carbon (as glucose) was added. Tubes were incubated at 37°C for 7 days under both aerobic and anaerobic conditions (using anaerobic jars) to enrich for melanoidin-degrading cultures. Test tubes showing some decolorization were subcultured successively several times. The isolation of microbial cultures was carried out using the spread plate and streak plate technique on agar medium containing the following 1% glucose, 0.5% yeast extract, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$ and 2% agar at pH 7.5 value.

Inoculum preparation and sampling

Inoculum was prepared in nutrient broth under anaerobic conditions for 72 h. Two ml were used to inoculate 20 ml media before incubation. Two ml samples were withdrawn at intervals for chemical analyses and decolorization measurements.

Decolorization assay

Decolorization of digested spent wash was measured as a decrease in optical density measurements at 475 nm of the culture media supernatant against uninoculated spent wash medium and expressed as the percentage decrease in absorbance.

Results and discussion

Spent wash is a highly recalcitrant waste product which does not decompose by the usual biological treatment methods. This is because of its content of melanoidin; a polymer formed by the aminocarbonyl reaction in food processing and preservation. This polymer is not easily degraded by microorganisms although it is highly distributed in nature. The chemical components of our raw and digested spent wash are shown in Table 1.

Microbial decolorization of melanoidin has been mainly observed in fungi. Sirianuntapiboon *et al.* (1988) screened for fungi with this capability. Ohmomo (1985) reported that a strain of *Aspergillus* sp. showed high decolorization activity, which was caused by the adsorption of melanoidin to mycelia. Watanabe *et al.* (1982) and Aoshima *et al.* (1985) screened a basidiomycete to decolorize model melanoidin, and speculated that the decolorization process was coupled to a sugar oxidase enzyme.

Table 1 The characteristics of both the undigested and the anaerobically digested spent-wash (SP)

Parameter	Undigested SP	Digested SP
pH	4.4	7.5
Density	1.1	1.0
Electrical conductivity	16.3	14.0
Total solids (g/l)	110.7	29.6
Volatile solids (g/l)	84.5	14.2
Total carbon (g/l)	48.0	8.3
Total nitrogen (g/l)	3.4	1.7
Total phosphorus (g/l)	0.1	0.1
Total sugar (g/l)	25.0	12.5
Crude protein (mg/l)	21.2	10.6
COD (g/l)	128.0	20.6

Several sets of enrichment cultures were initiated before we succeeded in isolating some bacterial strains capable of various degrees of decolorization of digested spent wash. The degree of decolorization varied between 6% and 28% (data not shown). Most of the cultures were obtained using the digested spent wash as a nitrogen source while supplying extra carbon as glucose. Among the cultures obtained isolate L-2, a Gram positive non-motile facultative anaerobic rods belonging to the genus *Lactobacillus*, gave the highest decolorization and COD reduction and therefore was selected for further investigation. This isolate was capable of growth at 45°C and 4%(v/v) NaCl.

The effects of glucose content and the medium's pH value on decolorization are shown in Fig. 1. Supplying a readily available carbon source appears to be necessary for decolorization to occur. Growth and decolorization did not occur without glucose added and increasing decolorization occurred at up to 10 gl^{-1} glucose. Increasing glucose above than 10 gl^{-1} did not significantly increase the percentage decolorization Fig. 1. This indicates that the digested spent wash contained little, if any, readily available carbon although it contains high total sugars content (Table 1). The media pH values also had a marked effect on decolorization with the highest percentage achieved between 7.0 to 7.5 which is the preferred range for bacterial growth.

When both decolorization and COD reduction were monitored as a function of time; the results (Fig. 2) showed increasing percentage decolorization and COD removal between days 1 and 5. This rate was reduced

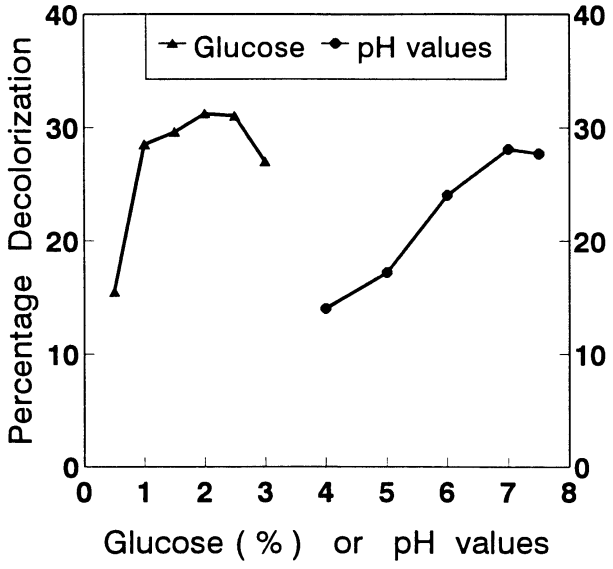

Figure 1 Percentage decolorization at different glucose concentration and pH values.

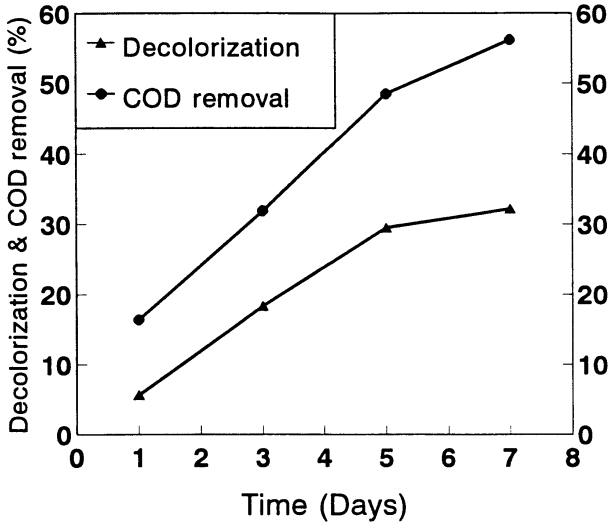

Figure 2 Percentage decolorization and COD reduction at up to 7 days incubation.

between days 5 and 7. The maximum percentage COD removal was 56.2% while maximum percentage colour reduction achieved was 31.2%. As the added glucose would most likely have disappeared rapidly (within the first day of incubation), decolorization therefore seems to occur upon the growth of culture L-2 on this readily available carbon and upon its exhaustion cells appear to degrade the refractile carbon source component of the digested spent wash. This would also indicate that decolorization might occur as a result of a secondary metabolic reaction resulting from a secondary metabolite. Whether it is due to sugar oxidase as speculated by Watanabe *et al.* (1982) and Aoshima *et al.* (1985) remain unconfirmed.

It is concluded that bacterial culture suitable for the degradation and decolorization of digested spent wash may be a realistic approach for the treatment of this highly recalcitrant waste. The role of the added carbon and the need dilute however are two major shortcomings that have to be addressed in future research.

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