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Melanoidin degradation in distillery effluent by *Bacillus cereus*.

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ABSTRACT

Distillery spent wash is a pollution intensive wastewater generated by distilleries. Its dark brown colour is due to recalcitrant melanoidin pigments. The ability of microorganisms to degrade and metabolize a wide variety of compounds has been recognized and exploited in various bio treatment processes. In the present study investigation of the potential of various bacterial cultures isolated from soil at effluent discharge site of distillery was done for decolourization, BOD and COD reduction of effluent. Screening of bacteria having ability to degrade melanoidin pigment and thereby decolorize distillery waste water was followed by estimation of chemical and biochemical oxygen demand of the sample before and after bacterial treatment according to potassium dichromate method and Winkler's iodometric method. *Bacillus cereus* demonstrated greater potential for bioremediation as compared to other bacterial isolates. Under optimum conditions *Bacillus cereus* was able to decolorize the spent wash distillery effluent by 56 % and reduced COD by 63 % after 72 hrs of incubation. Maximum decolourization and COD reduction was found within pH range of 6.5 to 7.0, and temperature range of 25 to 37°C. Microbial decolourization by bacteria is an environment friendly cost-effective alternative to chemical decomposition process before its disposal.

Key words: Decolourisation, melanoidin, distillery effluent.

INTRODUCTION

Industrialization is vital to nation's economy as it serves as a vehicle for development. However, there are associated problems resulting from introduction of industrial waste products in the environment. Distillery spent wash is the unwanted residual liquid waste generated during alcohol production and pollution caused by it is one of the most critical environmental issue. Many of the products are problematic because of their persistence (low biodegradability) and or toxicity (Olukanni, 2006). The wastewater released from distilleries and fermentation industries are a major source of soil and aquatic pollution due to

presence of water soluble recalcitrant colouring compound called melanoidin. (Evershed *et al*.) Melanoidin pigments are formed by the nonenzymatic amino carbonyl reaction i.e. Maillard reaction (Raghukumar *et al*., 2001). Despite the standards imposed on effluent quality, untreated or partially treated effluent very often finds access to water courses. The distillery wastewater with its characteristic unpleasant odour poses a serious threat to the water quality in several regions around the globe. The ever-increasing generation of distillery spent wash on the one hand and stringent legislative regulations of its disposal on the other has stimulated the need for developing new technologies to process this effluent efficiently and economically. A number of clean up technologies have been put into practice and novel bioremediation approaches for treatment of distillery spent wash are being worked out. Potential microbial (anaerobic and aerobic) as well as physicochemical processes as feasible remediation technologies to combat environmental pollution are being explored.

The untreated effluent containing molasses is characterized by dark colour, high temperature, low pH, high ash content and high percentage of dissolved organic and inorganic matter (Beltran *et al.*). Disposal of such effluent would lead to eutrophication of water bodies (FitzGibbon *et al.*1998). Highly colored nature of molasses spent wash can block sunlight from rivers and streams thus reducing oxygenation of water by photosynthesis and thereby become detrimental to aquatic life (Agarwal *et al.* 2010). Conventional biological processes such as activated sludge treatment process is insufficient to treat melanoidin containing wastewater released from distilleries and fermentation industries (Chandra,2008) Only 6-7 % degradation of melanoidin has been achieved in the conventional anaerobic-aerobic effluent treatment processes, hence alternative treatment processes have been explored. (Kalvathi *et al.* 2001) Bioremediation is a popular and attractive technology that utilize metabolic potential of microorganisms to clean up environment (Watanbe, 2001). Some reports are available indicating application of bacteria (Bhargava, 2010), fungi (Pazouki *et al.* 2008) etc for bioremediation of distillery spent wash.

Several researchers have investigated the role of various microorganisms in the degradation of melanoidin in spent wash. *Corriolus hirstus* exhibited ability to decolorize melanoidin by 74% in GPY medium (Miyata *et al.* 2000) A few bacterial strains i.e. *Pseudomonas, Enterobacter, Aeromonas, Acinetobacter* are reported to be capable of degrading some of the recalcitrant compounds in the anaerobically digested distillery spent wash (Ghosh *et al.* ,2002).

In the present study an attempt was made to explore the possibility of isolating efficient indigenous micro flora as biodegraders for use in decolourization and biotreatment of distillery waste water. Focus of the study included isolation and screening of microorganisms for their ability to decolorize and or reduce BOD & COD of distillery waste water.

MATERIALS AND METHODS

Collection of samples:

Distillery spent wash was collected from distillery unit of Natural sugar and allied industries Ltd. Sainagar Tq. Kallam, Dist., Osmanabad. Characterization of effluent for pH, colour, TSS (Total suspended solids), BOD, COD was done according to standard methods. Soil samples were collected from an area near disposal unit of same industry for screening of microorganisms having melanoidin degrading ability.

Isolation and identification of melanoidin degrading microorganism:

Soil samples were purposefully collected from the disposal site of distillery for screening efficient melanoidin degrading microorganisms, since chances of isolating microbes having the ability to degrade melanoidin is high at such places Screening of bacteria having ability to degrade melanoidin pigment and thereby decolorize distillery waste water was done by enrichment and isolation using GYP (Glucose Yeast Peptone) medium (Himedia). Since bacterial isolates were isolated from a soil sample contaminated with distillery effluent there was no need to acclimatize them. Eight bacterial species B1-B8 were screened through enrichment technique. Biochemical characterization for identification of bacterial isolates obtained was done by standard protocols (Harley and Prescott, 1996). Pure cultures of different bacterial isolates B1-B8 were maintained on minimal salt glucose agar medium containing 5% of spent wash.

Decolourization studies (Assay)

For decolourisation studies initial absorbance of the effluent was measured at 475nm using UV Visible Spectrophotometer (Shimadzu UV-1601). A loop full

pure culture of each isolate from their respective media was inoculated in a flask containing minimal medium with 10% spent wash distillery waste water in 250 ml flask and incubated at room temperature to undergo decolourization. Pattern of degradation was studied for every 8-hour interval by using 5ml aliquot for analyzing decolourization status. Uninoculated minimal salt medium and minimal salt medium with 10% spent wash were used as blank and control respectively. The decolourization was measured according to formula

$$Decolourisation\ (\%) = \frac{[Initial\ abs - final\ abs]}{Initial\ absorbance} \times 100$$

Isolates showing better results as compared to others were selected for further investigation.

COD and BOD measurements

Chemical and biochemical oxygen demand of the sample before and after bacterial treatment were determined according to potassium dichromate method and Winkler's iodometric method respectively (APHA, AWW WPCF 19 ed-1995).

Effect of pH on decolourisation

Effect of pH was determined by inoculating bacterial culture in spent wash medium adjusted to pH values within range 4.00 to 9.00 and incubated at room temperature.

Effect of temperature on decolourisation

Effect of temperature on decolourisation was studied by inoculating the isolate in spent wash medium at different temperature within range of 25°C to 45°C.

Effect of incubation period on decolourisation and COD reduction

Effect of incubation period on decolourisation and COD reduction was studied by inoculation of spent wash medium with B8 isolate i.e. *Bacillus cereus* and incubation of the medium for 24, 48, and 72 hours.

RESULTS AND DISCUSSION

Molasses spent wash is a highly recalcitrant waste product because of its melanoidin content. The dark brown colour of distillery waste water is due to presence of a complex biopolymer called melanoidin generated by maillard reaction. Treatment of distillery spent wash by physical and chemical methods are found unsuitable on industrial scale (Shah -et-al, 1989). It is now realized that microbial treatment provides a safer and less expensive alternative to physico-chemical methods for decolourization of spent wash.

Distillery soil isolates were obtained and subjected to routine biochemical tests for identification. Out of all isolates *Bacillus cereus* showing maximum decolourization was selected for further studies. Decolourization was followed spectrophotometrically by measurement of optical density at 475 nm, which is a λmax for melanoidin. Effects of various parameters on decolourization process was also studied. Maximum decolourization and COD reduction was found within pH range of 6.5 to 7.0(fig 1), and temperature ranges of 25 to 37°C (fig 2) the preferred range for growth of the above isolates.

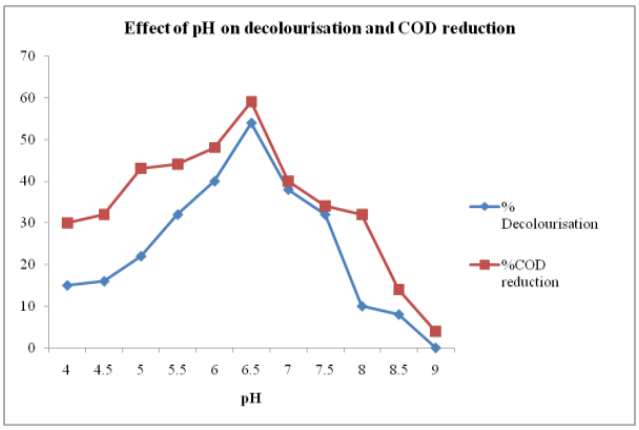

Figure 1.Effect of pH on decolourisation and COD reduction

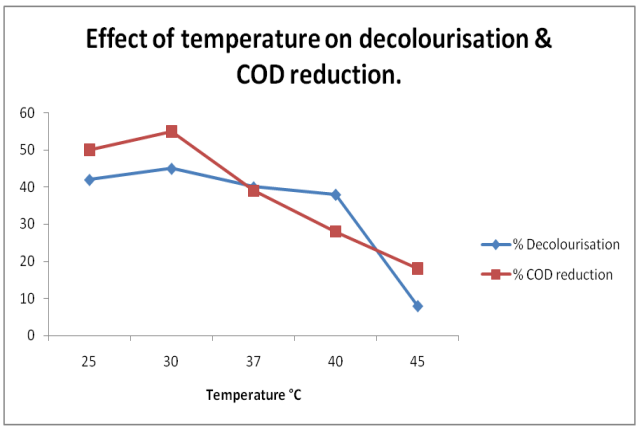

Figure 2.Effect of temperature on decolourisation and COD reduction

Under optimum conditions the isolate *Bacillus cereus* was able to decolorize the spent wash by 56 % and reduced COD by 63 % after 72 hrs of incubation (fig 3).

Decrease in optical density of melanoidin at its λmax and appearance of new peak in the spectra with respect to control was the clue for degradation. Physico-chemical analysis of the spent wash effluent before and after treatment with isolate indicated appreciable reduction in case of most of the parameters was observed, especially colour, BOD, COD, etc. This reduction indicates decrease in toxic effect of discharging spent wash effluent in receiving water bodies (Table 1).

Growth pattern of the isolate with respect to colour removal indicated that within first 24 hrs growth was initiated without any decolourization ; but after 24 hr, gradual increase in growth with decolourization was observed up to 72 hr.(Fig.3)

Table 1-Physicochemical analysis of effluent

Sr. No	Parameter	Before treatment	After treatment
1.	Colour	Dark brown	Pale brown
2.	Odour	Sweet	Odourless
3.	pH	2-4	6.8-7.2
4.	BOD(mg/L)	70,840	30,000
5.	COD(mg/L)	1,46,380	54,160
6.	TDS(mg/L)	8900	1500

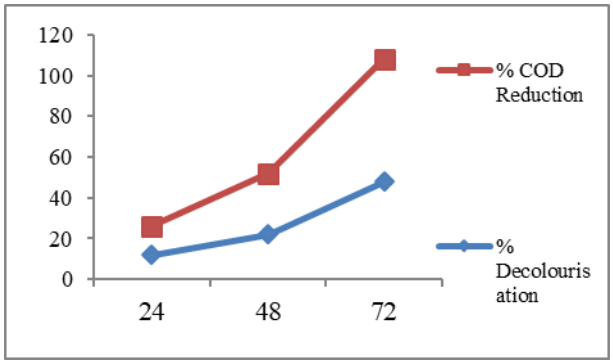

Figure 3: Effect of incubation period on decolourisation and COD reduction

From the above study it is clear that, *Bacillus cereus* a local isolate was found to be very efficient organism in decolorization and reduction of BOD & COD distillery effluent. This approach can be further exploited to develop a cost effective, ecofriendly alternative for treatment of distillery spent wash.

CONFLICT OF INTEREST:

The Authors declare no conflict of interest.

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