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Research Paper

Characterization of Molasses Spent Wash and its decolorization using Mushroom Cultivation

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Abstract: *Molasses spent wash is a by-product of sugar cane, sugar beet and citrus industries. Molasses contains mixture of uncrystallizable sugar and non-sugar components. It is one of the most difficult waste products to dispose due to its acidic pH, dark brown colour, high ash content, high percentage of dissolved organic and inorganic matter.Molasses spent wash is one of the major components of growth media used in many industrial processes but presence of melanoidin, a recalcitrant compound causes several toxic effects on the living system. This is an attempt to study the Physico-chemical characteristics, microbial screening of molasses spent wash and use of mushroom in the decolorization of molasses spent wash. It was seen that mushroom spawn showed better growth on medium prepared in100% molasses spent wash as compared to 75%, 50% and 25% and also reduced its dark brown colorization.*

Keywords: Isolation, melanoidin decolorization, microbial diversity, molasses spent wash, mushroom cultivation.

Introduction

India is a largest sugar producing country. It has the second largest network of molasses based distilleries in Asia producing of about 2300 million litres of alcohol annually [1]. Molasses spent wash is the waste product produced after the distillation of alcohol from molasses. For every one litre production of ethanol about 10-15 litres of molasses spent wash is generated[2]. Molasses spent wash which is a by-product of sugar cane, sugar beet and citrus industries contains mixture of uncrystallizable sugar and non-sugar components. It has dark brown coloration, high temperature, very low pH and high concentration of dissolved inorganic and organic matter[1,3]. Dark brown coloration of spent wash is mostly due to presence of melanoidin. Melanoidin is an organic compound formed by the reaction between amino acids and carbohydrates called as Maillard Reaction[4]. These effluents are environmentally hazardous due to their high biochemical and chemical oxygen demands.

When released in water bodies, its dark colour hinders sunlight penetration which affects the photosynthetic activity and dissolved oxygen concentration in water leading to harmful effects on aquatic life forms, also its release in soil reduce soil alkalinity, manganese availability, inhibit seed germination and affects vegetation[5,6]. Melanoidin has antioxidant property and is highly toxic to the living system. Due to recalcitrant property of melanoidin, it is very necessary to treat it before disposal in water or soil[2]. A number of physical and chemical methods have been used for the removal of melanoidin but still they are not useful as it requires high reagent dosages and generates large amount of sludge [1,7] and treatment with biological method for decolorization or degradation of spent wash can serve to be a good alternative.

Mushrooms have been used for the treatment and found to be effective. They have the capacity to

produce lignocellulolytic enzyme complex which includes oxidative enzymes laccase and manganese peroxidase involved in lignin degradation and the hydrolytic enzymes xylanase and cellulase involved in hemicellulose and cellulose degradation respectively[8]. *Pleurotus sp.* has been used in the bioconversion, bioremediation and biodegradation of various xenobiotic compounds and industrial effluents[9]. *Pleurotus sp.* are known to be easiest and less expensive mushrooms to grow and in conversion of substrate mass into mushrooms[10].

Material and Methods

Physico-chemical characteristics of molasses spent wash

Molasses spent wash sample was collected in sterilized polythene bags from Sugarcane industry, Pravaranagar. The collected sample was assessed for parameters as pH, temperature, colour, odour, total solids, COD and BOD. Temperature and pH of the sample was measured at the time of collection[11]. These parameters were measured using standard methods[12].

Microbial Screening

Isolation of Bacteria

Molasses spent wash sample was mixed withsteriledistilled water in 1:9 ratio in a conical flask. The flask was placed on an electric shaker to get a homogenous suspension. Serial dilutions of molasses were prepared and one ml of 10^{-5} dilution was plated in petridishes containing potato dextrose agar and nutrient agar medium. The plates were incubated at 30ºC for one or two days and bacteria appearing overthe medium were further used for morphological and biochemical characterization [13].

Morphological and biochemical characteristics

Morphological characters such as size, shape, colour, elevation, margin, opacity, bacterium shape and gram staining were performed for identification of the bacteria. Various biochemical tests were performed as given by Bergey's Manual of Determinative Bacteriology[14]. Isolated bacteria were studied for their ability to produce enzymes viz. lipase, amylase, carboxymethyl cellulase and protease enzymes.

Isolation of fungi

Molasses spent wash sample was mixed with sterile distilled water in 1:9 ratio in a conical flask. The flask was placed on an electric shaker to get a homogenous suspension. Serial dilutions of molasses were prepared and one ml of 10^{-5} dilution was plated on potato dextrose agar containing streptomycin sulphate to prevent bacterial growth. The plates were incubated at 25±2°C for 5 days and fungi grown on medium were observed under microscope by cytoplasmic staining using lactophenol cotton blue [13].

Cultivation of Mushroom (*Pleurotus*sp.) on Molasses spent wash and decolorization of molasses

Modified Media was prepared by dissolving Potato Dextrose Agar in molasses spent wash as a substitute of distilled water to make final graded concentration 10%, 25%, 50%, 75% and 100%.All the other conditions were kept same as per the standard media preparation technique [13]. Sterile plates were poured with different concentration of medium and spawns of *Pleurotus sp.* were inoculated in each plate. The plates were incubated at 25ºC for optimal mycelial growth. Potato dextrose agar was used as control for cultivation of mushroom. Also plates containing crystal violet dye were prepared and studied for decolorization by mushroom.

Results and Discussion

Physico-chemical analysis

The physio-chemical characteristics of molasses spent wash produced showed that it is acidic in nature, dark brown in colour, has a jaggery like smell and comprise high composition of BOD, COD andtotal solids (Table 1).

Table 1: Physico-chemical analysis of Molasses spent wash

S. No.	Characteristics	Observation
1	Odour	Jaggery
2	Colour	Dark brown
3	pH	4.3 – 4.6 ± 0.3
4	Temperature	50-60 ºC
5	COD (mg/l)	140000 ± 0.1
6	BOD (mg/l)	70000 ± 0.3
7	Total solids (mg/l)	180000 ± 0.3

Isolation of Bacteria

Different bacterial species were isolated on Nutrient Agar Medium. These were further studied for their morphological and biochemical characteristics using Bergey's Manual[14]and identified to be *P. putida, E. coli, S. aureus, B. subtilis*and *Micrococcus sp.*(Table 2 & 3).Similar results are observed by Pillai[13]. *Bacillus sp.* are found to be effective in reducing COD and colour under optimal conditions of pH, carbon and nitrogen concentrations[2,4]. *Pseudomonas sp*. are also known to have ability to degrade pollutants present in the waste water[7]. Also Bacterial sp. isolated form molasses spent wash produced various enzymes such as lipase, amylase, carboxymethylcellulase and protease enzymes which might be showing degradative effect. Bacteria are easy to culture and so can be used for decolorization and reducing BOD and COD levels in molasses spent wash.

Morphological, biochemical and cultural characteristics

Table 2: Morphological characteristics

Characters	*P. putida*	*E. coli*	*S. aureus*	*B. subtilis*	*Micrococcus spp.*
Size	4mm	1mm	3mm	3mm	2mm
Shape	Irregular	Rod shaped	Round	Round	Irregular
Colour	Creamish white	Reddish	Cream	Transparent	Yellow
Margin	Irregular	Circular	Circular	Circular	Irregular
Elevation	Convex	Convex	Convex	Flat	Convex
Consistency	Regular	Irregular	Regular	Irregular	Irregular
Opacity	Trans parent	Trans parent	Trans parent	Opaque	Opaque
Gram nature	Negative	Positive	Positive	Positive	Negative
Opacity	Transparent	Transparent	Transparent	Opaque	Opaque
Gram nature	Negative	Positive	Positive	Positive	Negative

Table 3: Biochemical characteristics

S. No.	Biochemical test	*P. putida*	*E. coli*	*S. aureus*	*B. subtilis*	*Micrococcus spp.*
1	MacConkey agar test	+ve	-ve	-ve	-ve	-ve
2	Indole test	-ve	+ve	-ve	+ve	-ve
3	Methyl red test	+ve	+ve	+ve	+ve	+ve
4	Catalase test	-ve	+ve	-ve	+ve	-ve
5	Cytochrome oxidase	+ve	+ve	-ve	+ve	-ve
6	Starch hydrolysis test	+ve	-ve	+ve	-ve	+ve
7	Nitrate reduction test	-ve	+ve	+ve	+ve	-ve
8	Urea hydrolysis test	-ve	-ve	-ve	-ve	-ve
9	Vogusproskauer test	+ve	-ve	+ve	-ve	-ve
10	H2S production test	+ve	-ve	+ve	-ve	+ve
11	Citrate utilization test	-ve	-ve	-ve	-ve	-ve

Isolation of fungi

Different fungal species were isolated from molasses spent wash namely *Aspergillus niger, Trichoderma viridae, Fusarium sp*. and *Candida albicans* (Figure.1). Out of the 4 isolated species *A. niger* was found to be abundant, which is similar to the result obtained by Pillai[13]. *Candida sp*. also has been known in decolorization of recalcitrant compound melanoidin found in molasses spent wash[4]. Various fungal species are known in bioremediation due to production of degradative enzymes. Fungal species such as *Flavadon flavus* has been reported to decolorize Molasses spent wash by Raghukumar[15].

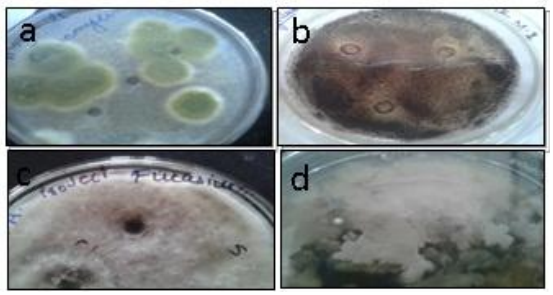

Figure 1: Fungal flora in molasses a. *Trichodermaviridae* b. *Aspergillusniger* c. *Fusarium sp.* d. *Candida albicans*

Cultivation of Mushroom spawns (*Pleurotus*sp.) on Molasses spent wash and decolorization of molasses

Mushroom spawns were grown on media containing graded concentration of molasses spent wash (10%, 25%, 50%, 75% and 100%). Of the concentrations used, highest growth of mycelia was seen in plate containing 100% molasses. Also reduction in dark brown colour was observed (Figure. 2). These results correspond to the result obtained by Espindola[9] on using *Pleurotus florida*. Similarly, *Pleurotus sp.* spawns grown on plates containing crystal violet dye also showed growth of mycelia and helped in decolorization of dye to some extent (Figure.3). This degradation may be due to the enzymes produced by mushroom.

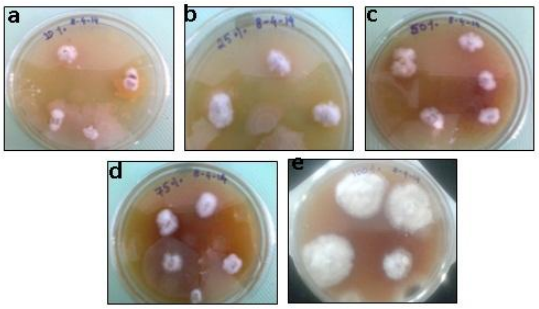

Figure 2: Growth of *Pleurotus sp*. on media containing molasses spent wash a. 10% MSW b. 25% MSW c. 50% MSW d. 75% MSW e. 100% MSW

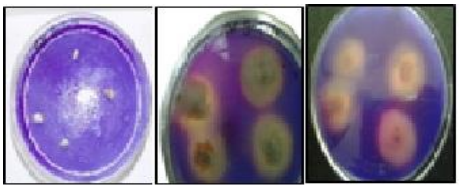

Figure 3: Growth of *Pleurotus sp*. on media containing crystal violet dye

Conclusion

Molasses spent wash generated from the sugarcane industries or distilleries cause various adverse effects in water as well as in soil due to its acidic nature, high BOD and COD levels and dark colour present due to melanoidin. In the present study, an attempt has been done to screen the microbial and fungal flora from molasses spent wash. It has been observed that various bacterial species (*Pseudomonas sp., Bacillus sp.*) and fungal species (*A. niger, Candida sp.*) are known to have degrading ability. Also growth of *Pleurotus sp*. spawns on molasses spent wash media has been effective and reduced colour of molasses to some extent. This result may be due to the capacity of mushrooms to produce lignocellulolytic enzymes in degradation of lignin, cellulose and hemicellulose. Use of mushroom in decolorization has been tried at petriplate level but can be scaled up and used for decolorization at large levels. Also enzymes can be isolated from the mushrooms and used directly to treat the coloured and recalcitrant spent wash. Our study concludes that biological treatment can be an effective way to reduce colour and pollution load from the distillery effluent.

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