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Comparitive Study on Potentiality of Bacteria and Fungi in Bioremediation and Decolorization of Molasses Spent Wash

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Effluent originating from the distilleries known as spent wash leads to extensive soil and water pollution. The aim of this study is to isolate and identify bacterial consortium and fungal species which are capable of using recalcitrant compounds of molasses spent wash as a sole carbon source from the soils of abandoned sites of distillery effluent discharge and characterize their ability of reducing the COD, BOD, TSS, TDS and decolorization of spent wash. Between bacterial consortium and fungal species, *Aspergillus* species showed the highest decolorization of about (84.3) and show effective reduction in BOD and COD. This work presents a review of the existing status and advances in biological and physico-chemical methods applied to the treatment of molasses based distillery wastewater. Further, the studies also deal with the roles of microbial enzymes in the decolourisation process to develop a better understanding of the phenomenon.

Key words: Bioremediation, Molasses spent wash, Decolorization, *Bacillus* sp., *Aspergillus.*

Across the world 125 -130 million tons of sugars are produced every year. About 2/3 rd of this is produced from sugarcane and 1/3 rd from sugar beet. In India, all the ethanol produced is by a way of fermentation of sugarcane molasses and its subsequent distillation. There are 285 distilleries in India alone generating 40 liters of wastewater annually.

Spent wash is pollution intensive wastewater generated by distilleries. The effluent is characterized by high chemical oxygen demand (COD) of 80,000 to 1,00,000 mg/l and biological oxygen demand (BOD) of 40,000 to 50,000 mg/l, low pH 4 -5 and strong odour and dark brown colour (Gupta *et al*., 2007). Molasses wastewater is one of the most difficult waste products to dispose. This recalcitrance nature is due to the presence of melanoidin, which are formed in the maillard amino – carbonyl reaction (Wedzicha *et al*., 1992).

The MSW is a potential water pollutant in two ways. First, the highly colored nature of MSW can block out sunlight from rivers and streams, thus reducing oxygenation of the water by photosynthesis and hence becomes detrimental to aquatic life. Secondly, it has a high pollution load, which would result in eutrophication of contaminated watercourses (Sibley *et al.,* 1997). Spent wash also leads to significant levels of soil pollution and acidification in the cases of inappropriate land discharge. It is reported to inhibit seed germination, reduce soil alkalinity and cause soil manganese deficiency and damage

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agricultural crops. However, effect of distillery effluent on seed germination is governed by its concentration and is crop-specific (Sirianuntapiboon *et al.,* 1998).

Many chemical and physical treatment processes were introduced to remove the color substances from the wastewater. However, these processes still have the disadvantage due to high cost, unstable removal efficiency and production of solid waste. In the present study, keeping the complexity of the distillery spent wash in mind; the efforts were directed towards the use of microorganisms for the treatment of effluent. Bioremediation is environmental friendly and cost competitive alternative to chemical decomposition processes. This approach could be used to develop a cost-effective, eco-friendly biotechnology package for the bioremediation of spent wash before its disposal.

Microbial treatments employing pure bacterial cultures have been reported frequently in past and recent years. *Bacillus megaterium, Bacillus cereus, Bacillus smithi, Pseudomonas fluorescens,* are some of the bacteria used for the treatment of molasses wastewater. Among bacteria *Pseudomonas fluorescens*, *Bacillus cereus* and *Bacillus megaterium* showed the highest decolorisation (Murata *et al.,* 1992).

The *Aspergillus fumigatus* G-2-6 strain decolorized 75% of molasses melanoidin on a glycerol-peptone medium at 45$^{æ\%}$C for three days (Ohmomo *et al.,* 1987).

MATERIAL AND METHODS

Collection of distillery spent wash

Distillery spent wash was collected from Thiruarroran sugar limited, Tirumandangudi. The color of the spent wash dark brown and the pH was 3.85, and the other physio-chemical characteristics of spent wash were determined as described in the standard methods for examination of water and wastewater. (APHA 1995).

Sample site background and collection of soil sample

It was expected that bacterial and fungal strains having the ability to degrade the recalcitrant carbon compounds of distillery spent wash might be present in those sites/soils, which were used for the dumping of the treated spent wash. The soil samples were aseptically collected from a site of Thiruarroran distilleries located in Tirumandangudi, in India.

Enrichment and isolation of microorganism

2 gm of soil sample were added to the test tube containing 20ml of 12.5% diluted digested spent wash medium. All the various supplements of carbon source and nitrogen source were given for the microorganism. All the test tube were incubated in incubator shaker for 48 hours at 37°C. the isolation of microbial culture were carried out using 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 % $MgSO_4.7H_2O$ and 2 % agar at pH 7.5 value (Ghosh *et al.,* 2004).

Bacteria and fungal identification methods

The bacterial isolates were identified based on the colony morphology and various biochemical tests. Among the six bacterial isolates, the catalase positive isolates were identified up to species level (Norris *et al*., 1981). The fungal isolates were identified based on macroscopic and microscopic observation and confirmed through Lacto phenol cotton blue mounting method.

Maintainance of pure culture

The isolates were purified in nutrient agar and potato dextrose agar and stored in nutrient agar slants and potato dextrose agar slants respectively at 4°C.

Inoculum preparation and sampling

Bacterial inoculum was prepared in nutrient broth under aerobic conditions for 72 hours and fungal inoculum was prepared in potatoes dextrose broth. From each, 2ml were used to inoculate on 20ml media before incubation. 2ml samples were with drawn at intervals for physio-chemical analysis and decolorization measurements.

Analysis of digested spent wash

Analyses were carried out to determine their colour, pH, Biological oxygen demand, Chemical oxygen demand, total dissolved solid, total suspended solid and total volatile solid.

Decolourisation assay (Sirianuntapiboon *et al.,* 2004): The sample was diluted with 0.1 M acetate buffer solution (pH 6.0) after centrifugation at 6000 rpm for 15 min and the color intensity of the diluted solution was

measured at 475 nm with a spectrophotometer. Decolourisation activity was determined as a decrease of optical density in the absorbance against the original solution. The decolourisation yield was expressed as the degree of decrease in the absorbance at 475 nm against the initial absorbance at the same wavelength.

$$\text{Decolourisation (\%)} \frac{\text{(Initial absorbance - Final absorbance)}}{\text{Initial absorbance}} \times 100$$

RESULTS AND DISCUSSION

The present study was carried out to determine the potentiality of molasses spent wash degraders and to perform physico-chemical analysis of molasses spent wash. *Bacillus* species and *Aspergillus* species were isolated and identified as potential degraders of molasses spent wash.

After a week of good growth on the enrichment medium, serial dilution was performed and spread plate technique was used to obtain the molasses spent degraders in nutrient agar and Rose Bengal chloramphenicol agar plates. Altogether 6 bacteria and 2 fungal isolates were obtained. These isolates were named as isolate 1, 2, 3, 4, 5 and 6 for bacteria and 7 and 8 for fungi.

Identification of the pure culture was done using staining, motility and biochemical tests. The isolates 1, 5 and 6 were identified as members of genera *Bacillus* species, isolates 2, 3 and 4 were identified up to species level as *Bacillus circulans, Bacillus megaterium and Bacillus firmus*, isolate 7 and 8 as *Aspergillus fumigatus and Aspergillus terreus respectively.* (Table 1).

The colour of the spent wash obtained was observed as dark- brown in colour and the pH of the molasses spent wash was found to be 3.85. The pH is more towards the acidic range. The amount of TSS, TDS, TVA, COD and BOD were determined and was found to be very high such as 38400 ppm, 105200 ppm, 100,000 ppm and 40,000 ppm respectively (Table 2).

Decolorisation activity was determined as a decrease in optical density in the absorbance against the original solution. The decolorisation yield was expressed as the degree of decrease in the absorbance at 475 nm against the initial absorbance at the same wavelength. The decolorisation assay in raw spent wash was found to be only 50 % (Table 3).

Table 1. Isolation and identification of Molasses Spent Wash Degraders

S.no	Isolate number	Organism name
1.	Isolate 1	*Bacillus* species
2.	Isolate 2	*Bacillus circulans*
3.	Isolate 3	*Bacillus megaterium*
4.	Isolate 4	*Bacillus firmus*
5.	Isolate 5	*Bacillus* species
6.	Isolate 6	*Bacillus* species
7.	Isolate 7	*Aspergillus fumigatus*
8.	Isolate 8	*Aspergillus terreus*

Bacteria and fungi carried out Bioremediation of the molasses spent wash, which has the ability to recalcitrant pollutants. Bioremediation of molasses spent wash was carried in medium supplemented with glucose, yeast extract, KH_2PO_4 and $MgSO_4.7H_20$ and inoculated with bacteria and fungi and kept for incubation for 15 days. Decolorisation assay was determined every 3 days interval for all the eight organisms and the other physico - chemical characteristics were determined after 15 days of incubation. (Fig. 1).

Decolorisation of molasses spent wash was observed for each isolate and *Aspergillus fumigatus* showed good decolourisation of molasses spent wash. Of all the 8 isolates obtained decolorisation was highest in molasses spent wash inoculated with *Aspergillus fumigatus* which showed the highest decolorisation of 84.3 after 15 days of incubation followed by *Aspergillus terreus* of 80% than bacteria.

The physico-chemical characteristics of the treated molasses spent were determined after 15 days. In all microbial inoculated spent wash, pH was increased to 8 from acidic pH among which *Aspergillus fumigatus* showed the maximum increase of pH to 8.92.The other physico-chemical characteristics like BOD, COD, TSS, TDS and TVA was decreased to maximum level in all the microorganisms.

One of the most important environmental problems faced by the world is

Table 2. Analysis of various physico-chemical parameters in Molasses spent wash

S. No.	Physico-chemical parameters	Untreated	Treated						
			Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8
1.	Colour	Brown	Slightly light brown	Slightly light brown	Slightly light brown	Brown	Brown	Brown	Light brown	Light Brown
2.	pH	3.85	8.5	8.64	8.86	8.8	8.53	8.4	8.92	8.87
3.	Biological Oxygen Demand (BOD)(ppm)	40,000	2,100	1,900	1,800	2,320	1,900	2,200	1,100	1,600
4.	Chemical Oxygen Demand (COD)(ppm)	1,00,000	21,000	19,200	19,100	21,100	20,000	20,800	16,000	16,000
5.	Total dissolved solids (TDS) (ppm)	38,400	33,000	32,000	32,000	36,000	32,000	32,000	31,000	33,000
6.	Total suspended solids (TSS) (ppm)	1,05,200	27,600	27,200	26,800	27,400	28,000	27,000	24,800	26,000
7.	Total Volatile acid (ppm)	2,800	6,771	4,500	6,857	6,171	6,942	6,514	6,771	5,785

Table 3. Bioremediation of molasses spent wash

S. No	Organism name	% of colour removal at 3 days interval				
		3rd day	6th day	9th day	12th day	15th day
1.	*Bacillus* species	71.9	73.48	74.24	74.24	76
2.	*Bacillus circulans*	68	69.69	75.75	75.75	75.9
3.	*Bacillus megaterium*	66	66.06	73.48	74.24	74.5
4.	*Bacillus firmus*	58	61.36	63.63	68.9	69.5
5.	*Bacillus* species	70.58	71	73.96	73.4	74.4
6.	*Bacillus* species	71.96	72.72	73	73.4	73.0
7.	*Aspergillus fumigatus*	79.5	81.06	83.32	83.0	84.3
8.	Aspergillus terreus	73.4	78.78	79.5	78.0	80.1

management of wastes. Industrial processes create a variety of wastewater pollutants; which are difficult and costly to treat. Wastewater characteristics and levels of pollutants vary significantly from industry to industry. Now-a-days emphasis is laid on waste minimization and revenue generation through byproduct recovery. The environment has an impact on every aspect of human life and every human activity affects the environment with social, technological and economic development. Abnormal consumption of various natural resources has increased the level of pollutants such as air pollution, chemical pollution, thermal pollution, radioactive pollution etc., which is increasing at an exponential rate, thus endangering our country's rich ecology and environment.

Rapid industrialization and urbanization in different parts of the country is leading to generation of untreated wastewater, which is disposed of as such in irrigation water bodies. Dye industries, distilleries, chemical-manufacturing industries are increasingly causing problems of soil and water pollution affecting the structure and function of the ecosystem.

Distilleries are among the most highly polluting industries with reference to water and soil pollution .The quantity of wastewater

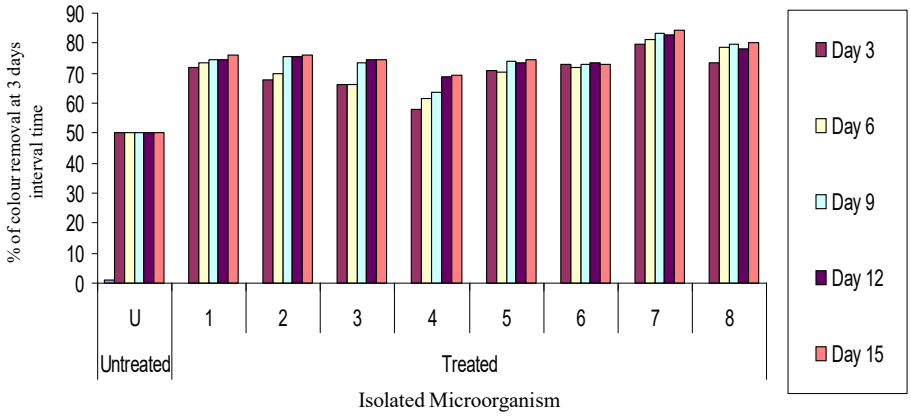

Fig. 1. Decolorization assay at 3 days interval

1. *Bacillus* species; 2. *B. circulans;* 3. *B. megaterium*
4. *B. firmus;* 5. *Bacillus* species 6. *Bacillus* species
7. *Aspergillus fumigatus;* 8. *A. terreus*

generated from distilleries is large and is characterized by a high pollution load. The wastewater also contains high concentration of dissolved solids, high ash content, high temperature and low pH.

The paramount of pollution in our environment is a dire consequence of continually expanding population along with an exponential development in the industrial field. Microbes are ubiquitous in nature and are being exposed to the continuous release of more and more recalcitrant xenobiotic compounds into the environment. No wonder, these microbes, inhabiting polluted environments, are armed with various resistance and catabolic potentials. The catalytic potential of microbes in nature is enormous and this is advantageous to mankind for a cleaner and healthier environment through bioremediation.

Hence the present study was undertaken to compare the potentiality of bacteria and fungi in the bioremediation of molasses spent wash. For the present investigation soil sample and molasses spent wash was collected from sugar industry .the bacterial and fungal species were isolated and identified form the soil sample were recorded.

Biological treatment of molasses spent washes using Bacillus *species* have been successfully achieved and thus can be applied as bioremediation technique (Murata *et al*., 1992). Totally 2 species of fungi belonging to the genus *Aspergillus* was recorded. Between the two fungi, *Aspergillus fumigatus* was recorded as having the highest potential in degrading molasses spent wash. Similar result was reported by using the thermophilic strain, *Aspergillus fumigatus* G-2-6, which decolorizes about 75% of molasses melanoidin within three days (Ohmomo *et al*., 1987).

Many reviews regarding molasses spent wash degradation by the fungi *Aspergillus fumigatus* have been cited, but a very few little work has been reported concerning molasses spent wash degradation by *Aspergillus terreus*. Our present study isolated *Aspergillus terreus* capable of decolorizing molasses spent wash up to 80% after 15 days period of incubation .It has also the potential of reducing BOD, COD, TSS, TDS and TVA to maximum level. Thus it is possible that Aspergillus *terreus* has the potential of degrading molasses spent wash and thus can be used for bioremediation of recalcitrant compounds.

Amylases and proteases in bacteria and peroxidases, manganese independent peroxides, manganese dependent peroxidases, sugar oxidases in fungi have been reported to play an important role in various waste treatment applications. (Preeti Sangave and Aniruddha Pandit, 2006). Although the enzymatic system related with decolorisation of melanoidin is yet to be completely understood, it seems greatly connected with fungal ligninolytic mechanisms .It was suggested that melanoidins were decolorized by the active oxygen (O_2 :H_2O_2) produced by the reaction with sugar oxidases (Deepak Pant and Alok Adholeya , 2007).

Aspergillus fumigatus and *Aspergillus terreus* used in our study showed the highest decolorisation activity within 4 days of incubation, supplemented with glucose, peptone, $MgSO_4$ and KH_2PO_4.This might de due to the adsorption of melanoidin to mycelia (Ohmomo *et al*., 1985) or by the action of sugar oxidase enzyme. (Aoshima *et al*., 1985). Decolorisation activity of fungi was much higher when compared to all the bacteria used in our study.

Further, good results were obtained by the *Aspergillus* species in reducing the other physico-chemical parameters like BOD, COD, TDS, TSS and TVA. *Aspergillus fumigatus* reduced the COD from 100,000 ppm to 16000 ppm followed by *Aspergillus terreus* of 18000 ppm where as the *Bacillus* species reduced the COD from 100,000 ppm to 20, 000 approximately.

The fungal technology is very different from other well-established methods of bioremediation. The differences are primarily due to the unusual mechanisms which are nature has provided them with and several advantages for pollutant degradation. One distinct advantage for pollutant degradation of these fungi over bacterial system is that they do not require preconditioning to the particular pollutant. Bacteria usually must be pre-exposed to a pollutant to allow the enzyme that degrades the pollutant to induced. The pollutant also must be in a significant concentration otherwise the induction of enzyme synthesis cannot occur. But in fungi, the induction of the degrading enzyme is not dependent on the pollutant, so the pollutant can be degraded to a

near non-detectable level than bacteria.

Of all the technologies investigated in waste cleaning, bioremediation has emerged the most desirable approach for cleaning up many environmental pollutants. Bioremediation is a pollution control technology that uses biological system to catalyze the degradation of or transformation of various toxic chemicals to less harmful forms. The ability of microorganisms to transform a variety of microorganisms has led to their use in bioremediation process.

It is clear that decolorisation activity of fungi is high when compared to bacteria Also, our strain could be used not only for decolorisation of molasses spent wash but also for reduction of organic matters such as BOD, COD, TSS, TDS and TVA within few days. Similar report was achieved by using a strain No.BP103 of acetogenic bacteria (Suntud Sirianuntapiboon *et al*., 2004) It was believed that, the bioremediation potential of fungi for the treatment of pollutants is more effective than bacteria.

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