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# Bioremediation of Distillery Wastewater by Sequential Anaerobic and Aerobic Treatment in Pilot Scale Bioreactor

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**Abstract—***The world's total production of alcohol from cane molasses distillery is more than 15 million m$^3$ annually. The 312 distilleries in India produce 3.97 billion litres of alcohol and generating 66 billion litres of wastewater annually. This huge quantity of distillery wastewater if disposed untreated can cause considerable stress on the water reservoirs leading to extensive damage to aquatic life.*

*Anaerobic and aerobic treatment in pilot scale bioreactor was conducted for removal of various pollutants present in the distillery effluent. In anaerobic treatment, colour (45 per cent), BOD (60 per cent), COD (26 per cent), TSS (20 per cent) and TDS (27 per cent) were decreased in eight days. The anaerobically treated effluent was separately treated in bioreactor in presence of fungal strain, Aspergillus fumigatus, and bacterial strain, Pseudomonas fluorescens. Data of study showed reduction in colour (76 per cent), TSS (70 per cent), BOD (78 per cent), COD (85 per cent) and TDS(72 per cent) by Aspergillus fumigates whereas Pseudomonas fluorescens indicated removal in colour (56 per cent), BOD (68 per cent), COD (78 per cent) TSS(82 per cent) and TDS (84 per cent) by day third by aerobic microorganisms, when eight days anaerobically treated effluent was further transferred to aerobic Microorganisms, Bioremediation, TDS, Treatment, Decolourisation.*

**Keywords***: Distillery, Effluent, Microorganisms, Bioremidiation, TDS. Treatment, Decolourisation.*

## 1. INTRODUCTION

Ethyl alcohol production in cane sugar molasses based distilleries constitutes a major industry in South America and Asia. The world's total production of alcohol from cane molasses distillery is more than 15 million m$^3$ annually. The 312 distilleries in India produce 3.97 billion litres of alcohol and generating 66 billion litres of wastewater annually.[1] In India, bulk of the alcohol is being produced from sugar cane molasses.

Cane Molasses is a thick viscous by-product of the sugar industry which is acidic in nature, dark brown in colour, rich in salts, and it also consists sugar which could not be crystallized. For producing alcohol, the molasses is diluted with water into a solution containing 15-20% of sugars. This solution is then inoculated with yeast strain and is allowed at room temperature for fermentation. The fermented wash is distilled in a series of distillation columns to produce alcohol of adequate strength and quality. This alcohol is used for potable and industrial purpose. [3]

The distillery wastewater has enormous potential to produce 1500 million cubic meters of biogas. The population equivalent of distillery wastewater based on BOD has been reported to be as high as 6.9 billion which means that contribution of distillery waste in India to organic pollution is approximately eight times more than the entire Indian population.[2]

This huge quantity of distillery wastewater if disposed untreated can cause considerable stress on the water reservoirs leading to extensive damage to aquatic life. Physical and chemical treatment options of the residue have not been very successful until now. Anaerobic biological processes have received high consideration in effluent treatment, owing to high capability to treat slowly degradable substrates at high concentrations, produce very low sludge, require low energy and provide possibility for energy recovery through combustion of methane

Digestion in anaerobic conditions is most typically employed as a primary treatment for distillery effluents. Such solution is favoured by the fact, that during anaerobic degradation about 50% of the COD contained in spent wash can be converted to biogas at only about 10% sludge generation.[4]

The high organic concentration in the effluent can make anaerobic treatment profitable, particularly due to the energy yield in form of methane, combining environmental soundness with economical usefulness due to possible savings in the fuel needs of the distillery.[5]

The present study aims to assist the removal of colour and other pollution parameters of distillery effluent by anaerobic treatment that was subsequently treated by fungus (*Aspergillus Fumigatus)* and bacterial strain (*Pseudomonas fluorescens*) separately in two steps bioreactor

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

The effluent samples of Distillery industry were collected from the Symbhaoli Distillery, Moradabad, India. The effluent was collected in clean plastic containers and brought to the laboratory for immediate refrigation at $4^0$C until utilized for further analysis.

### 2.2 Isolation and identification of microorganisms

Various Fungal strains were isolated from sediment sludge of the distillery situated at outside premises and six morphologically distinct fungal isolates were isolated on potato dextrose agar plates. The fungal strains were identified based on different microscopic and morphological structures as appearance of colour, texture quality, mycelium growth and formation of spore and filaments attachment. Bacterial strains were grown and developed in the chemostat by continuous enrichment consisting mineral salt medium (MSM). pH was adjusted between 7.0 to 7.6 throughout the course of enrichment. The chemostat culture was developed in 2 liter glass vessel which has effective volume 1 liter provided by stirring, 275 rev/min; temperature $25^0$C; pH 9-12; an air flow of 575 ml/min and medium flow rate of 15 ml/h. Samples present in culture were collected under aseptic conditions. The growth of the bacterial community was determined by serial dilution in colony forming unit (cfu). The characterization microbial cells appeared on the nutrient agar plate were performed depending upon morphology of colonies based on various parameters (colour, opacity, diameter, form, elevation, texture spreading nature and margin smoothness). The different colonies appeared on nutrient agar plates, were further streaked on another nutrient agar plates for selective growth. The procedure of streaking was repeated three times to ensure the purity of each isolate. The morphologically distinct isolates were characterized in accordance with Bergy's Manual of Determinative Bacteriology.[6] The bacterial isolates were also characterized and identified by a commercial microplate test (Biolog, Incorporated, Hayward, CA) based on the utilization of 95 carbon sources.[6] $A_{590}$ was determined after 8 and 1 day on a microtitre plate reader. The isolates were identified by using the Microlog software. These tests were repeated five times for statistical significance.

### 2.3 Design of Two step bioreactor

The collected distillery effluent was placed in 100 litre plastic container. In which 10 per cent anaerobic inoculum (from cow dung) was added and purging was done by liquid $N_2$ gas to remove $O_2$ from the container. This container is connected to the column (bioreactor) with the plastic tubes having Set I of the sequential bioreactor consists of a column where fungus was used for the treatment. Set I of glass bioreactor consisted 50L effluent transferred from anaerobic container. Effluent of Set I bioreactor was inoculated with *Aspergillus fumigatus* fungus strain at the rate of 10 percent (w/v) of the effluent and supplemented with carbon (sucrose 0.2 per cent) and nitrogen (urea (0.1 per cent) as carbon and nitrogen source. This column was connected with another reactor below in sequential way (set II) where effluent from set I was further treated with bacterial strain *Pseudomonas fluorescens* (10 per cent inoculum).During this process continuous supply of effluent was maintained. The sample was collected after 0, 1, 3 and 7 days from anaerobic tank, Set I and Set II respectively and change in pollution load was determined.

### 2.4 Aerobic Bioreactor

Sequential aerobic bioreactor was made by fabrication of glass vessel size 100 L, which was filled up to 10 cm, layer by gravel as solid support for immobilization of microbial cells. The column was equipped with stirring and aeration facilities and was connected with another vessel; size 100 L in a sequential way. *Aspergillus fumigatus* (F3) fungal strain was applied for the treatment of distlillery effluent in first set and bacterial strain, *Pseudomonas fluorescens* of the microbial community from the chemostat in second set bioreactor. The effluent was supplemented with sucrose (0.2 per cent) and urea (0.1 per cent). The samples were collected after on day 1, 3, 7 and 15 from the set 1 and set 2, respectively and change in pollution load was determined.

### 2.5 Analysis

The pH of the effluent is measured with the help of using electronic pH meter. Effluent after treatment was analyzed for TSS, BOD,TDS, chemical oxygen demand as described in APHA (1995)[7].The colour content in the effluent was measured as described by Singh et al.[6] In this method sample was centrifuged at 10,000 rpm for 30 min and pH was adjusted to 7.6. Absorbance was measured at 465 nm and was transformed into colour unit.

## 3. RESULTS

Six fungal strains ($F_1$, $F_2$, $F_3$, $F_4$, $F_5$, and $F_6$,) were isolated on potato dextrose agar plate by serial dilution method, and bacterial community developed in the chemostat. The isolates were applied in batch culture for analysis of pollutants of distillery effluent after supplementation of 0.2 and 0.1 per cent glucose and urea. It was observed that F4 identified as *Aspergillus fumigatus* and bacterial strain identified as *Pseudomonas fluorescens* by Biolog test method showed maximum potential to remove colour and other parameters. So these microbial strains were applied in laboratory scale testing of 2 litre sequential bioreactor.

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The bio treatability study of fungal and bacterial strain for the treatment of distillery effluent was performed in anaerobic and aerobic culture conditions. Data recorded on this aspect clearly indicated that the reduction of pollution load by fungal and bacterial strain was more pronounced in immobilized cells in glass column compared to anaerobic set of bioreactor (Fig.1 and 2). The parameters used as indicators of pollution load are COD, BOD, colour, TSS, TDS and pH. Sequential treatment was conducted with anaerobic and aerobic microorganisms. In set I distillery effluent treated by anaerobic microorganisms. After anaerobic treatment data indicated significant reduction in colour (45 per cent), BOD (60 per cent), COD (25 per cent), TSS (20 per cent) and TDS (27 per cent) in 8 days incubation (Fig. 1). Slight increase in biomass and pH (from pH 8 to 6.6) were also observed during treatment but biomass increase was much lower in anaerobic treatment than aerobic treatment. In aerobic condition, treatment with fungal strain (*Aspergillus fumigatus*), showed significant removal in colour (76 per cent), BOD (78 per cent), COD (85 per cent), TSS (70 per cent) and TDS (72 per cent) after 7 days treatment, while bacterial (*Pseudomonas fluorescens*) treatment indicate significant reduction in colour (56 per cent), BOD (68 per cent), COD (78 per cent), TSS (82 per cent) and TDS (84 per cent) after 7 days treatment (Fig. 2). The rate of reduction of COD in all treatments was higher during initial days (i.e. 1, 3, days); with a decline at later stage (Fig. 1 and 2). Significant increase in Biomass (27 per cent) and change in pH (from pH 8 to 5) was also observed with fungal and bacterial treatment.

## 4. DISCUSSION

Six fungal strains isolated from distillery effluent, the decolorization potency of *Aspergillus Fumigatus* was higher on day 1 followed by *Phoma* sp. and *Paecilomycesvarioti,*. Therefore, in present study *Aspergillus fumigatus* was selected for treatment of distillery effluent in 100 litre sequential anaerobic and aerobic bioreactor. Results of anaerobic treatment showed that all the pollution parameters were decreasing slowly during different time intervals. No increase in biomass was observed during the anaerobic treatment. This suggests that anaerobic treatment resulted in slow growth of anaerobic microorganisms and less sludge production. Slight change in pH indicated the slow degradation of toxic compounds present in the effluent. Slow TSS reduction in anaerobic treatment indicated the elimination of toxic compounds from the effluent. The anaerobic treated effluent was separately applied in bioreactor in presence of fungi and bacteria in step second and third of bioreactor for further elimination of pollution parameters. Data of the study indicated that degradation of pollution parameters was comparatively fast in aerobic treatment than anaerobic treatment. It may be due to unique capability of aerobic microorganisms to secrete enzymes that efficiently degraded colour causing compounds and chlorinated phenols from sugarcane molasses spent wash effluent [8, 9,10].

Concept of sequential treatment is very important because in this case both anaerobic, and aerobic fungi and bacteria can be applied for treatment of effluent in different stages. Fungi has better capability to remove colour from the effluent, however, bacteria is more potent for degradation of aromatic compounds.[11] In addition, two or more microorganisms can be tried sequentially, one member of which may carry out the initial catabolic reactions and another may complete the rest of the metabolic pathway to mineralize the organic compounds completely. Such consortia have been developed for mineralisation of bicyclic aromatics such as chlorinated phenyls, chlorinated dibenzofurans and naphthalene sulfonates. [12, 13]

Four white-rot fungal cultures were examined for their ability to decolourize and bioremediate anaerobically digested molasses spent wash (DMSW) generated by biomethanation plants. Two cultures *Coriolusversicolour*and *Phanerochaetechrysospotium* showed an ability to decolourize and reduce the COD of diluted DMSW (125% v/v). Maximum decolourization (71.5 and 53.5%) and COD reduction (90.0 and 73.0%) were achieved in 6.25% (v/v) DMSW medium by *C. versicolour*and *P. chrysospotium*, respectively.. Decolorization and biodegradation of anaerobically digested sugarcane molasses spent wash effluent from biomethanation plants by white-rot fungi. *Phanerochaetechrysosporium* and *Trametes*

*versicolour* are the most widely studied among these. *Phanerochaetechrysosporium* JAG 40 resulted in 80% decolourization of diluted synthetic melanoidin (absorbance unit of 3.5 at 475 nm), as well as with 6.25% anaerobically digested spent wash. Decolorization and biodegradation of anaerobically digested sugarcane molasses spent wash effluent from biomethanation plants by white-rot fungi. Decolorization of synthetic and spent wash melanoidins using the white-rot fungus *Phanerochaete chrysosporium* JAG-40.[14]

*Flavodonflavus* (Klotzsch) *Ryvarden*, a basidiomycete (NIOCC strain 312) isolated from decomposing leaves of a sea grass, decolourized pigments in MSW by 80% after 8 days of incubation, when used at concentrations of 10% and 50%. Decolorization of molasses spent wash by the white rot fungi Flavadonflavus isolated from marine habitat. . *Aspergillus fumigatus* has been found to be effective for decolourization of anaerobically treated distillery wastewater. Application of response surface methodology observed for optimization of important parameters in decolorizing treated distillery wastewater using *Aspergillus fumigatus* UB2.60. Fungal consortium was employed in fluidized film aerobic system (FFAS). [6]The analyzed effluent at the end of FFAS treatment showed a reduction of 70% in BOD and 63% in COD without causing any colour change.[15] The fungi *GeotrichumcandidumromanCoriolusversicolourromanPhanerochaetechrysosporium, and Mycelia sterilia* were screened for their ability to decolourize spent wash and to reduce the COD level. A 10 day pretreatment with *Geotrichumcandidum* at

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30°C resulted in reducing the COD by 53.17% and total phenols by 47.82%, enabling other bioremediating organisms to grow. *Coriolus versicolour* immobilized in a packed-bed reactor reduced the COD of spent wash by a further 50.3%, giving an overall reduction in COD of 77% to 15,780 mg/l .[16]

Bacterial cultures are capable of bioremediation of distillery spent wash. They observed that two aerobic bacterial isolates LA-1 and D-2 brought about maximum decolourization (36.5% and 32.5%) and COD reduction (41% and 39%) under optimized conditions in eight days. The most prominent bacterial species isolated from the reactor liquid belonged to *Pseudomonas*, while *Bacillus* was isolated mostly from colonized carriers. *Pseudomonas fluorescens*,was reducedmelanoidin wastewater (MWW) up to 76% under non-sterile conditions and up to 90% in sterile samples. Decolorization of synthetic and spent wash melanoidins using the white-rot fungus *Phanerochaetechrysosporium* JAG-40.

Acetogenic bacteria are capable of oxidative decomposition of melanoidins. Biodegradation of potato slops from a rural distillery by thermophilic aerobic bacteria. achieved biodegradation of potato slops (distillation residue) by a mixed population of bacteria under thermophilic conditions up to $60^0$C. A COD removal of 77% was achieved under non-optimal conditions.[17]

Colour removal by fungal strain (*Aspergillus fumigatus*) and bacterial strain (*Pseudomonas fluorescens*) was faster in comparison to anaerobic microorganisms in sequential bioreactor. This might be due to action of microbial strains immobilized in the soil on chlorolignin compounds resulting in its mineralization. Chlorinated compounds that present in the effluent were degraded into their metabolites due to action of microbial strains.

A significant reduction in colour, BOD, COD and TDS during the experimental study in the treatment process can be considered as the result of mineralisation of toxic organic compounds in the effluent and also due to the activity to aromatic ring oxidation enzymes. Effluent showed decrease in pH (acidic) during aerobic treatment due to conversion of complex organic compounds in simple inorganic acids. Increase in biomass during aerobic treatment suggested fast growth of microorganisms. Available data of earlier studies indicated that chlorinated phenols are mineralized to chlorine free end products .[17]

Result of the present study indicated that Sequential anaerobic and aerobic treatment is more efficient in removal of colour and organic compounds, because anaerobic microorganisms were able to remove highly chlorinated substances more efficiently than aerobic microorganisms. Aerobic microorganisms removed the last remaining chlorine atom.

Present study concluded that sequential anaerobic and aerobic treatment helps in efficient removal of colour causing compounds and TDS, and also suggested further investigation in direction of purification and characterization of enzymes microorganisms involved in degradation of colour causing compounds.

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## LEGENDS

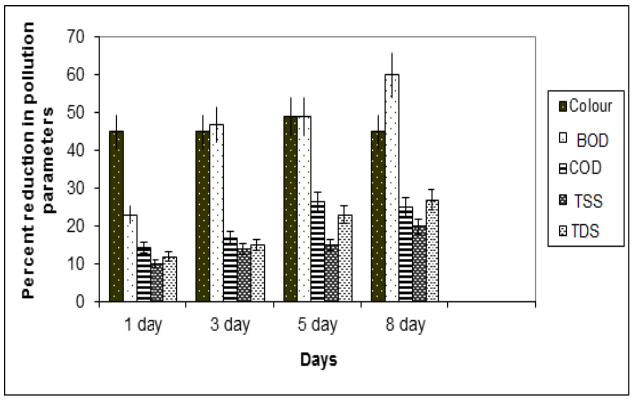


**Figure 1: Changes in TSS ($mgl^{-1}$), colour (CU), BOD ($mgl^{-1}$), COD ($mgl^{-1}$)and TDS ($mgl^{-1}$)in anaerobic treatment of distillery effluent in two step bioreactor (100 litre) during different time intervals.**

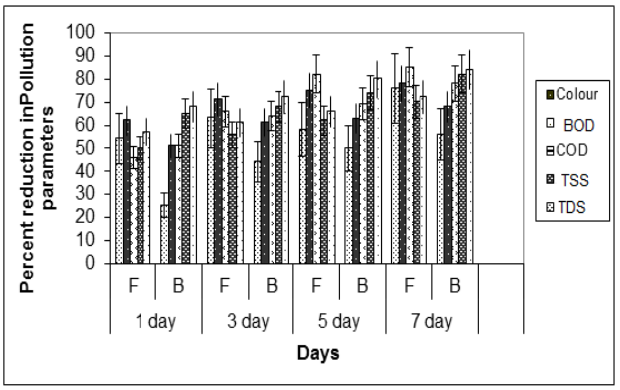


**Figure 2: Changes in TSS ($mgl^{-1}$), colour (CU), BOD ($mgl^{-1}$) COD ($mgl^{-1}$) and TDS($mgl^{-1}$)in aerobic treatment of distillery effluent by fungus F (*Aspergillus fumigatus*) and bacteria B (*Pseudomonas fluorescens*) during different days anaerobically treated effluent in two step bioreactor 100 litre during different time intervals.**

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