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Investigation on Biodegradation of Effluent by Using Different Fungi

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ABSTRACT: Degradation and detoxification of Distillery waste water is very important in today's World due to increase in water pollution. To reduce this problem some micro-organisms like Bacteria Fungi Algae play an important role. Distillery waste water has dark brown color due to presence of high molecular weight organic substance called melanoidin. Aim of study is to investigate the organisms having the capacity of decreasing water pollution by their metabolic activities. Fungi like *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma viridis*, *Fusarium solani*, *Cladosporium* spp are used for this investigation. These fungi showed differential interaction with effluent by using some elements from effluent they show their growth which leads to decrease the colour, pH, TS, TDS, TSS and other parameters of effluent which show adverse effect on growth, development and reproduction of aquatic life.

I. INTRODUCTION

Environmental pollution from human activities is a major problem for the present World which leads to water pollution, air pollution, soil pollution which show adverse effect on the human as well as macro and microorganisms. It is an important challenge to reduce this pollution for normal growth and development of organisms to maintain ecosystem. Industrial waste water is one of the main sources of water pollution i.e., effluent from distillery industries, textile industries, pharmaceutical industries and other industries create water pollution which disturb aquatic life of organisms. This problem ultimately disturbs the food chain which cause harmful effect on ecosystem. Hence to decrease water pollution is very important task to maintain aquatic ecosystem.

Waste water generated from distillery industries contain some organic compounds, heavy metals, dyes, toxic elements etc which cause water pollution. Effluent released from distilleries called a spent wash which contain heavy metals such as potassium, phosphorous, sulphate etc. Effluent has dark brown colour due to presence of melanoidin, high ash, contain high percentage of dissolved organic and inorganic matter, acidic pH and high and C.O.D. Distillery effluent is a major source of water and soil pollution because effluent form layer on water which blocks entry of sunlight into bottom layers of rivers and stream which leads to disturb or stops photosynthesis of aquatic plants and algae. Effluent produces abnoxious smell, undiluted effluent shows toxic effect on fish, algae, fungi and other aquatic life. Soil pollution and acidification is caused due to distillery waste water. Wine distillery waste water contains heavy metals i.e. iron, zinc, metal ions like Ca^{++} , K^{+} , Na^{+} , Sulphate, Nitrate, Phosphate etc after release in water they cause problem for eutrophication and show adverse effect on environment. Dark brown colour of effluent is due to presence of melanoidin (Melanoidins are brown coloured high molecular weight heteropolymers that are formed when sugars and amino acids combine) through Maillard reaction at high temperature and low water activity from acid hydrolysis. Melanoidin is a biopolymer which is hard to decompose by microorganisms. It has antioxidant properties which cause adverse effect on microbes. A typical cane molasses of distillery generate 12-15 L of spent wash per litre of Ethanol production. In India around 212 distilleries produce around 30 billion litre of spent wash annually. Spent wash is strongly acidic, dark brown in colour hydrophobic, viscous liquid waste with strong objectionable odour. Disposal of distillery spent wash on land reduces soil fertility, reduces soil alkalinity, inhibit seed germination. Distillery waste water contain some organic compounds like, gallic acid, p-Coumaric acid, lactic acid (29% V/V), tartaric acid (10% V/V), Succinic acid (26% V/V), acetic acid (10% V/V), malic acid (8% V/V). Several fungi are used for decolourization and degradation of distillery waste water. Many physiochemical methods are available for the degradation and decolourization of spent wash. Advantages of these physiochemical methods in combination with fungal treatment is that it can effectively decolorize undiluted waste water otherwise dilution at field scale will increase the amount of waste water considerably.

II. MATERIALS & METHODS

1) EFFLUENT:

Waste water of distillery industry was collected from cane molasses industry i.e. Radico distillery at Shendra MIDC, Aurangabad (Maharashtra).

Waste water was characterized and analyzed for pH, Total Solid, Total Suspended Solid, Total Dissolved Solid, Turbidity, Colour etc.

2) MICRO-ORGANISMS:

Five fungi are used to treat distillery effluent such as, *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma viridi*, *Fusarium solani*, *Cladosporium* spp. The stock culture of the isolated fungi are made for the assay (parameters).

3) MEDIA:

Fungal cultures of isolated fungi are grown on PDA (potato dextrose agar) PDB (potato dextrose broth) was used to grow fungi with effluent.

4) CHARACTERIZATION OF DISTILLERY WASTE EFFLUENT:

Distillery waste effluent was analysed for pH, TS, TDS, TSS, Absorbance at 425nm.

DETERMINATION OF PH:

pH of the samples are determined by using pH meter which has been initially calibrated by using buffer solutions of known pH before analysis. After incubation of fungi at room temperature with effluent in potato dextrose broth media should different pH.

Table No.1. changes in pH (Before and After Inoculation) are measured shown in the Table

Organism	Initial pH	pH after 7 days
<i>Aspergillus niger</i>	4.48	3.86
<i>Aspergillus flavus</i>	4.48	5.40
<i>Cladosporium</i> spp	4.48	3.01
<i>Fusarium solani</i>	4.48	4.32
<i>Trichoderma viridi</i>	4.48	5.12

DETERMINATION OF TOTAL SOLIDS:

A clean china dish was taken and dried at 103°C-105°C in an oven. 10 mL of effluent sample was accurately pipette out into a dish, weighed and evaporated to dryness on a steam bath. The residue was dried in an oven for about 1 hr at 103°C-105°C and again weighed after cooling at room temperature. The cooling was done until the weight of the dish plus residue was constant. The weight of the dish was subtracted to obtain the weight of the total solids.

By using following formula Total Solids are calculated:

$$TS = \frac{A - B}{V} \times 1000$$

Where,

A = Final weight of dish

B = Initial weight of dish

V = Amount of sample taken

Table No.2.: After seven days of incubation Total Solids measured

Sr No.	Name of fungi	Control	Total solids
1	<i>Aspergillus niger</i>	67 mg/lit	79 mg/lit
2	<i>Aspergillus flavus</i>	67 mg/lit	78 mg/lit
3	<i>Cladosporium spp</i>	67 mg/lit	81 mg/lit
4	<i>Fusarium solani</i>	67 mg/lit	64 mg/lit
5	<i>Trichoderma viridi</i>	67 mg/lit	74 mg/lit

DETERMINATION OF TOTAL DISSOLVED SOLIDS:

10 ml of effluent sample was taken into a conical flask through pipette. It was filtered in funnel with glass fibre filter paper which has been pre-dried at 103°-105°C. The glass fibre was carefully removed from the Gooch and dried to a constant weight at 103°-105°C and the weight of the filter paper is subtracted to obtain the weight of Total dissolved Solids.

Total dissolved solids are calculated by using following formula:

$$A-B \times 1000/V$$

Where,

A=Final weight of filter paper

B=Initial weight of filter paper

V=weight of sample taken

Table 3 :Total dissolved solids Calculated After seven days of incubation

Sr No.	Name of fungi	Control	TDS
1	<i>Aspergillus niger</i>	35 mg/lit	14 mg/lit
2	<i>Aspergillus flavus</i>	35 mg/lit	42 mg/lit
3	<i>Cladosporium spp</i>	35 mg/lit	11 mg/lit
4	<i>Fusarium solani</i>	35 mg/lit	14 mg/lit
5	<i>Trichoderma viridi</i>	35 mg/lit	7 mg/lit

TOTAL SUSPENDED SOLIDS:

Total suspended solids are determined as the difference between the total solids and total dissolved solids. By using the following formula

$$TSS = TS - TDS$$

Table No.4: Total suspended solids pre and post inoculation

Sr No.	Fungi used	TSS	Control
1	<i>Aspergillus niger</i>	65 mg/lit	32 mg/lit
2	<i>Aspergillus flavus</i>	36 mg/lit	32 mg/lit
3	<i>Cladosporium spp</i>	63 mg/lit	32 mg/lit
4	<i>Fusarium solani</i>	67 mg/lit	32 mg/lit
5	<i>Trichoderma viridi</i>	71 mg/lit	32 mg/lit

DETERMINATION OF ABSORBANCE (O.D.):

Firstly O.D of the effluent was observed at 425 nm wavelength which was 3.3956 and after seven days of incubation

Table No.5. Difference in optical density were seen at pre and post inoculation

Sr No.	Fungi used	Control	O.D after seven days
1	<i>Aspergillus niger</i>	3.3956	2.7440
2	<i>Aspergillus flavus</i>	3.3956	2.6961
3	<i>Cladosporium spp</i>	3.3956	2.3614
4	<i>Fusarium solani</i>	3.3956	2.1814
5	<i>Trichoderma viridi</i>	3.3956	2.0186

CHARACTERIZATION OF UNTREATED WASTE EFFLUENT:

Table :6 Characterization of mean of treated waste effluent is shown in the following.

pH	4.48
TS	67 mg/lit
TSS	32 mg/lit
TDS	35 mg/lit
O. D	3.3956

MICROORGANISMS AND INOCULUMS:

Degradation and detoxification of effluent are carried out by using fungal suspension of *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium spp*, *Fusarium solani*, *Trichoderma viridi*.

Pure culture of these fungi was prepared and maintained into the slants. (i.e. Kept in freezer at constant temperature).

FLASK CULTURE:

Optimization studies Are performed in 100 ml sterile conical flask containing 50 ml PDB media plus 25 ml effluent and 1 ml fungal suspension of each fungi in different conical flasks. One flask was kept as control containing only effluent and no fungal suspension for comparison and all flasks are kept at room temperature for seven days.



Fig 1: Flasks containing effluent with fungal culture in PDB after seven days of incubation Showing decolorization of effluent.

III. RESULT

The absorbance of effluent before the inoculation was 3.3956 at 425 nm. After period of seven days of fungal inoculation the absorbance measured and highest absorbance was shown by *Aspergillus niger* i.e. 2.7440 at 425 nm and lowest absorbance was shown by *Fusarium solani* i.e. 2.0186 at 425 nm. pH of effluent before fungal inoculation was acidic i.e. 4.48. After 7 days of inoculation change in pH was observed in all flasks, but *Cladosporium* spp and *Aspergillus niger* showed more acidic pH as compared to effluent i.e. 3.01 and 3.86 respectively. *Trichoderma viridi* and *A. flavus* showed decrease in pH i.e. 5.12 and 5.40 respectively which is important for growth and development of other organisms.

Before inoculation of fungi TS of the effluent was 67 mg/lit and after fungal inoculation value of TS was changed in *Cladosporium* containing flask value of TS was increased i.e. 81, *A. niger* containing flask showed also an increase in TS i.e. 79, but *Fusarium solani* containing flask showed decrease in TS i.e. 64 mg/lit. TSS of effluent before inoculation was 32 mg/lit and after inoculation TSS increased in flask containing *Fusarium solani* i.e. 71 mg/lit and flask containing *Cladosporium* spp showed TSS 67 mg/lit, but *A. flavus* containing flask showed decrease in TSS i.e. 36 mg/lit. TDS of effluent before inoculation was 35 mg/lit, but after seven days of fungal inoculation change in TDS was observed. Increased amount of TDS was observed in flask containing *A. flavus* i.e. 42 mg/lit. *A. niger*, *T. viridi*, *Cladosporium* spp show decrease in TDS i.e. 14 mg/lit, 11 mg/lit, 14 mg/lit respectively. *Fusarium solani* showed drastic change in decreasing TDS i.e. -7 mg/lit.

IV. CONCLUSION

On the basis of the result, it is concluded that biological agents like Fungi play an important role in biodegradation and biodegradation of harmful substances present in the distillery effluent by their metabolic activities. When effluent is discharged into the water bodies like rivers and streams Due to the presence of dark brown melanoidin biopolymer in the distillery effluent weight cannot penetrate well into the water bodies which affects the photosynthesis of aquatic plants and consequently affect other organisms present in that ecosystem, but decolorization of effluent by Fungi help to minimize this problem and maintain the ecosystem in its proper condition. So, the biological method of effluent treatment is cheap, economically feasible and not harmful to other organisms as compared to chemical and physical methods of effluent treatment.

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