BIODEGRADATION OF DISTILLERY EFFLUENT BY FUNGI

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ABSTRACT

In the present work biodegradation ability of *Chaetomium globosum* and *Aspergillus nidulans* have been evaluated. Physical and chemical analysis of effluent collected from Sanjivani distillery industry located at Kopergaon, Dist Ahemadnagar. (M.S) India were carried out to observe the changes in Turbidity, pH, TSS,TDS, COD, Sodium and Magnesium using *Chaetomium globosum* and *Aspergillus nidulans. Chaetomium globosum* and *Aspergillus nidulans were* isolated from soil sample collected from Bhavan's College campus and purified by serial soil dilution method. Both the fungi were found to reduce the pollutant dynamically. The reduction was noticed at 4hrs, 8hrs, 12hrs, and 24 hours of time interval. Quantity of fungal inoculum in terms of discs, showed its quantitative effect for reduction of pollutants. In case of *Chaetomium globosum* with one disc of inoculum showed more significant changes ($P \leq 0.05$) with TSS and COD level, whereas addition of two and three discs of inoculum more significant changes were noticed in pH, turbidity, TSS, COD level and Mg reduction. For *Aspergillus nidulans* when only one disc of inoculum was added only two parameters (TSS, and COD) showed the significant changes. ($P \leq 0.05$) by adding Two discs of inoculum parameters like TSS, TDS and COD showed the significant changes. While with three discs of inoculum, the significant changes were noticed in pH, TSS, and COD.

Keywords: Biodegradation, distillery effluent, *Chaetomium globosum* and *Aspergillus nidulans*

INTRODUCTION

Sugar industry is renowned in Maharashtra for sugar production. The molasses obtained from the industry is promoted for manufacturing of liquor. During this process the highly polluted liquor effluent is generated. Low pH value, high organic load, depletion of oxygen content and bad smell etc. are some of the major pollution problem. These characteristics of effluent do not allow it to discharge into the water body.

The high COD value is responsible for distraction of aquatic life. The physical and chemical treatment is found to be insufficient whereas the biological treatment is most often found to be effective.

Many workers have revealed the role of microbes for degradation of pollutants, among microbes the fungi are very well known for their decomposition property. Fungi have tremendous capacity for treating industrial hazardous waste in support of an environment. Many experiments were carried out with different varieties of fungi, and it was observed that fungi are highly effective against industrial waste (Aassadi *et al.,* 2001).

Some fungi are reported for removal of heavy metals from effluent (Akthar and Mohan, 1995). Biosorbtion of heavy metals by dead fungal cell was reported by Hemambika B. Johncy Rani M. And Rajesh Kannan V (2011).Removal of melanoidin present in distillery effluent by fungi was reported by Agarwal R Lata S, Gupta M, Singh P (2010). Pant Deepak and Adholeya (2010) have developed a novel fungal consortium for the treatment of molasses distillery wastewater. The literature survey reveals that no researcher has incorporated *Chaetomium globosum* and *Aspergillus nidulans*.individually for their degradation capacity as far as distillery industries is concerned.

Di-pietro (1991) noticed role of few isolates of *Chaetomium globosum* against the pytium ulticum which causes the diseases called as dumping –off of sugar beet. Kalra K. L. (1989) has studied the role *Chaetomium globosum* in the bio-conversion of kinnow-mandrin wastes into soil protein along with other fungi such as *Sporotrichum pulverulentum.*

Role of *Aspergillus nidulans* in the industrial effluent is observed by Basile laey (2008) for degradation of cyanide by the action of cyanide hydratase which is a subset of nitrilase.

Thus in the present work these two organisms were selected to study for their degradation capacity relating to liquor effluent with various physical and chemical parameters such as Turbidity, pH, TSS (total suspended solids), TDS (Total dissolved solids), Chemical oxygen demand(COD), Sodium and Magnesium.

It was proposed to determine the course of activity of respected fungi *Chaetomium globosum* and *Aspergillus nidulans* dynamically in the time interval of 4hrs, 8 hrs, 12 hrs, and 24 hours to find out degradation potency in minimum length of time.

MATERIALS AND METHODS

Distillery effluent was collected from M/s Sanjivani sugar industry, located at Kopergaon, Dist. Ahamadnagar, The changes in physical characters of effluent such as turbidity and pH were monitored with the help of NEPHELO-TURBIDITY METER 132 and pH meter, TSS (total suspended solids), TDS (Total dissolved solids) were calculated by filtration method (APHA-1985). Similarly changes in COD level were analyzed by standard methods (APHA-1985).The changes in metallic constituents such as Sodium and Magnesium were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES).

Isolation and identification of fungi:

The fungal culture of *Chaetomium globosum* and *Aspergillus nidulans* obtained and purified from the soil sample of Bhavan's college campus. The culture was re-cultivated periodically on PDA at 28°C. Identification of characters was done by comparing with the description provided by Udaya prakash (2004).

Experimental procedure:

To observe the effect of fungi on the effluent fresh culture of *Chaetomium globosum* and *Aspergillus nidulans,* grown on PDA was used. About 100 ml of effluent was taken into six 250 ml borosil sterilized flasks. First set of three flasks were inoculated with 1, 2, 3, inoculums discs of 8 mm diameters, of *Chaetomium globosum* respectively. In similar manner another set of 3 flasks were inoculated with 1, 2, 3 discs of *Aspergillus nidulans*. One flask each was kept as control for both the organisms. All the flasks were incubated at 28°C and the results were recorded at time interval of 4 hours 8 hours, 12 hours and 24 hours for physical and chemical changes.

RESULTS AND DISCUSSION

The present study shows that the liquor effluent sample was acidic in nature and the pH was found to be 4.5. Table no.1 reveals high turbidity due to high amount of total dissolved solids (TDS) and total suspended solids (TSS). The liquor effluent sample in addition shows high amount of COD value (289.6 mg/L). This specifies that amount of organic matter present in effluent is towards higher side of its limits which may create stress upon biological system if released in water body as it is. From the present data, it is clear that effective treatment is required to reduce the level of pollution. So that if it is release in the water body the natural degradation system will not suffer from stress and the aquatic life will also get least affected. The effluent sample shows traces of sodium and magnesium (Saha *et al.,* 2004).

Table 1: Characteristics of an effluent sample

Turbidity	80 NTU
pH	4.5
TSS(total suspended solids)	0.21 gm/L (for 10 mL of sample)
TDS (total dissolved solids)	0.08 gm/L (for 10 mL of sample)
Chemical oxygen Demand (COD)	289.6 mg/L (in 5mL of sample)
Sodium (Na)	25.587 (mg/L)
Magnesium(Mg)	100.626(mg/L)

Table 2: Effect of *Chaetomium globosum* on the Turbidity of effluent.

Time	*Chaetomium globosum* : (NTU- NEPHELO-TURBIDITY METER 132.)			
	Disc-I	Disc-II	Disc-III	Control flask. (without the fungal disc)
4 hours	161 NTU	287 NTU	416 NTU	215 NTU
8 hours	100 NTU	103 NTU	125 NTU	261 NTU
12 hours	166 NTU	158 NTU	114 NTU	264 NTU
24 hours	195 NTU	142 NTU	170 NTU	330 NTU
	3.0850**	2.0470**	3.0850**	

Level of significance are * = p ≤ 0.1and ** = p≤0.05

Table 3: Effect of *Aspergillus nidulans* on the Turbidity of effluent

Time	***Aspergillus nidulans* (NTU- NEPHELO-TURBIDITY METER 132.)**			
	I disc	II discs	III discs	Control flask without any fungal disc.
4 hours	222 NTU	346 NTU	426 NTU	158 NTU
8 hours	228 NTU	257 NTU	250 NTU	212 NTU
12 hours	147 NTU	133 NTU	100 NTU	234 NTU
24 hours	136 NTU	161 NTU	196 NTU	250 NTU
	0.9611*	0.2048*	0.4136*	

Level of significance are * = p ≤ 0.1and ** = p≤0.05

Table no. 2 and 3 shows the change in the turbidity of effluent sample due to addition of inoculum discs. Addition of fungi in the effluent sample did not give clear results of turbidity within 24 hours of time interval, experimental flasks of both the fungi showed variations in turbidity. In case of *Chaetomium globosum* when all experimental readings were compared with control readings at given time interval, the turbidity was observed to be slightly reduced except at 4 hours of time interval. (Table-2) Reduction in turbidity was observed more clearly in third flask at 8 hours (125 NTU) and 12 hours (114 NTU) of time interval as compare to other experimental flasks and control flask. At 24 hours of time interval all the experimental flasks showed rise in turbidity but when compare to control flask the readings were observed to be reduced.

For *Aspergillus nidulans* (Table- 3) the reduction in turbidity was observed in all experimental flasks at 12 hours of time interval. But at 24 hours of time interval in second (161 NTU) and third (196 NTU) experimental flask slight rise in turbidity was noticed.

Table 4: Effect of *Chaetomium globosum* on the pH of effluent.

Time	I disc	II discs	III discs	Control flask without any fungal disc
4 hours	4.5	4.5	4.5	4.5
8 hours	4.5	4.5	5.0	4.5
12 hours	5.0	5.0	5.0	4.5
24 hours	5.0	5.0	5.5	4.5
	1.7320*	1.7320*	2.449**	

Level of significance are,* = p ≤ 0.1 and ** = p≤0.05

Table 5: Effect of *Aspergillus nidulans* on the pH of effluent

Time	Disc I	Disc II	Disc II	Control flask without any disc.
4 hours	4.5	4.5	4.5	4.3
8 hours	4.5	4.5	4.5	4.5
12 hours	4.5	5.5	5.0	4.5
24 hours	5.0	5.5	5.5	4.5
	1.7320*	1.732*	3.844**	

Level of significance are,* = p $\leq$ 0.1 and ** = p$\leq$0.05

Table no. 4 and 5 shows the change in the pH of effluent sample due to the inoculation of fungal discs. The changes in pH were clearly observed when experimental flasks were compared with control flask.

For *Chaetomium globosum* the changes in pH were noticed from 8 hours onwards. All the experimental flasks showed the changes in pH from 4.5 to 5.5.during 8 to 24 hours of time interval where as in control flask the pH of effluent did not change (Table-4).

In case of *Aspergillus nidulans* changes in experimental reading were noticed from 12 hours onwards especially in second and third flask the pH got changed to 5.0 and 5.5. The first flask showed change in pH at 24 hours pH 5 (Table no.5). From the table 4 and 5, it was observed that these changes did not occur immediately after the inoculation. Experimental flasks of both the fungi revealed that, fungi took time to get acclimatize with environmental conditions provided to them (liquor effluent with acidic pH).

Table 6: Effect of *Chaetomium globosum* on the TSS of effluent.

Time	I disc	II discs	III discs	Control flask without any fungal disc
4 hours	20	21	19	24
8 hours	19	20	16	25
12 hours	18	18	15	21
24 hours	16	22	14	22
	3.7464**	2.2000**	4.949**	

Level of significance are,* = p $\leq$ 0.1 and ** = p$\leq$0.05

Table 7: Effect of *Aspergillus nidulans* on the TSS of effluent

Time	I disc	II discs	III discs	Control flask without any fungal disc
4 hours	15	20	13	24
8 hours	13	18	11	20
12 hours	21	14	16	23
24 hours	18	12	14	25
	3.039**	3.299**	6.333**	

Level of significance are, * = p $\leq$ 0.1 and ** = p$\leq$0.05

Table No. 6 and 7 reveals the change in the TSS of effluent sample due to the inoculation of fungal discs. Addition of fungi in liquor effluent is significantly responsible for changing the TSS level of effluent sample. Since less reduction was noticed within 24 hours of time interval; as the high load of organic and inorganic matter is responsible for slow down the efficiency of fungi (Cujying jia *et al.,* 2007).

Experimental flasks of both *Chaetomium globosum* and *Aspergillus nidulans* showed similar responses as far as TSS is concerned. Table no. 8and 9 indicates the changes in the TDS of effluent sample by the addition of fungal inoculum. The significant reduction was noticed in experimental flask with two discs of *Aspergillus nidulans*.

Table 8: Effect of *Chaetomium globosum* on the TDS of effluent.

Time	I disc	II discs	III discs	Control flask without any fungal disc
4 hours	10	9	6	11
8 hours	6	8	8	14
12 hours	8	11	10	7
24 hours	9	16	13	15
	1.5067*	0.2679*	1.0369*	

Level of significance are, * = p ≤ 0.1and** = p≤0.05

Table 9: **Effect of *Aspergillus nidulans* on TDS of liquor effluent**

Time	I disc	II discs	III discs	Control flask without any fungal disc
4 hours	10	13	8	10
8hours	8	11	9	8
12hours	9	13	7	9
24hours	7	10	9	11
	1.2247*	2.2738**	1.3145*	

Level of significance are,* = p ≤ 0.1and** = p≤0.05

Table 10: Effect of *Chaetomium globosum* on the COD of effluent

Time	I Disc	II discs	III discs	Control flask without any disc
4 hours	235.2	264.0	232.0	289.6
8 hours	232.0	216.0	214.4	283.2
12 hours	179.2	171.2	211.2	264.0
24 hours	148.8	142.4	172.8	254.4
	3.277**	2.673**	4.369**	

Level of significance are, * = p ≤ 0.1and** = ≤0.05

Table 11: Effect of *Aspergillus nidulans* on the COD of effluent

Time	I Disc	II discs	III discs	Control (without addition of fungi) mg/L
4 hours	251.2	259.2	243.2	292.0
8 hours	240.0	216.0	156.8	289.6
12 hours	228.8	184.0	152.0	288.0
24 hours	166.4	177.6	136.0	299.2
	6.423**	4.416**	8.316**	

Level of significance are,* = p ≤ 0.1 and ** = p≤0.05

Table Number 10 and 11 indicates the changes in the COD of effluent sample by the addition of fungal discs. Experimental flasks of both the fungi showed noticeable reduction in COD removal from effluent sample, irrespective of quantity of inoculums.

In case of *Chaetomium globosum,* although all three flasks showed reduction in COD level when compared with control flask but at 24 hours of time interval maximum reduction in COD level was observed in second flask (142.4 mg/L) over the first(148.8 mg/L) and third flask (172.8 mg/L)(Table-10).

For *Aspergillus nidulans* all the three experimental flasks showed the reduction in COD level in 24 hours of time interval than the control flask (Table-11). Experimental flasks of both the fungi showed noticeable reduction in COD removal from effluent sample, the quantitative effect of fungal inoculum has observed in terms of COD reduction.

Table 12: Effect of *Chaetomium globosum* on the sodium of effluent

Time	I-disc	II-discs	III discs	Control without any disc
4 hours	25.587	28.754	29.259	16.476
8 hours	63.633	18.496	19.410	22.947
12 hours	45.563	19.316	21.449	24.645
24 hours	36.717	21.345	18.358	26.159
	0.127*	0.541*	0.289*	

Level of significance are,* = p $\leq$ 0.1 and ** = p$\leq$0.05

Table 13: Effect of *Aspergillus nidulans* on the sodium of effluent

Time	I-disc	II-discs	III-discs	Control without any disc
4 hours	51.640	17.962	19.024	28.124
8 hours	66.770	17.827	19.797	22.155
12 hours	25.587	28.754	29.259	16.476
24 hours	52.520	23.508	19.201	25.890
	0.468*	0.546*	0.514*	

Level of significance are,* = p $\leq$ 0.1 and ** = p$\leq$0.05

Table 14: Effect of *Chaetomium globosum* on the magnesium of effluent

Time	I disc	II discs	III discs	Control without any disc
4 hours	101.471	100.645	96.082	100.559
8 hours	103.347	99.094	100.203	100.886
12 hours	102.249	100.070	100.854	100.834
24 hours	100.626	101.156	101.270	84.319
	1.805*	3.485**	3.792**	

Level of significance are,* = p $\leq$ 0.1 and ** = p$\leq$0.05

Table 15: Effect of *Aspergillus nidulans* on the magnesium of effluent

Time	I disc	II discs	III discs	Control without any disc
4 hours	100.626	101.156	101.270	84.319
8 hours	103.319	91.430	100.427	101.040
12 hours	103.196	100.971	98.254	100.795
24 hours	102.949	95.309	98.032	100.859
	1.259*	1.184*	0.274*	

Level of significance are,* = p $\leq$ 0.1 and** = p$\leq$0.05

Table 12 and 13 as well as 14 and 15 shows the changes in quantity of sodium and magnesium due to the addition of fungal discs in effluent sample. For *Aspergillus nidulans* reduction in sodium quantity was noticed clearly from 8 hours onwards, experimental readings were observed to be less than control reading. (Table -13), but at 4^{th} and 24^{th} hours of time interval slight rise in the quantity was noticed (28.124mg/L).

For magnesium *Aspergillus nidulans* showed similar response like sodium, (table-15) it might be to maintain the ionic balance in protoplasm. (SUSAN ISAAC -1986)

For *Chaetomium globosum* reduction in sodium was noticed at 8 hours and 12 hours of time interval in all three experimental flasks, when compared with control flask (Table-12). Except at 24 hours of time interval where slight rise was noticed (26.159 mg/L).

For magnesium, reduction was noticed more clearly at 12hours in first flask. (96.082mg/L) as compared to second flask (100.203 mg/L) third flask (101.270mg/L).and control flask (101.270mg/L) (Table-14). Thus it could be concluded that within 24 hours of time interval reduction of sodium and magnesium was caused by *Chaetomium globosum* and *Aspergillus nidulans*.

CONCLUSION

In the present study, the biodegradation ability of fungi such as *Chaetomium globosum* and *Aspergillus nidula*ns was evaluated under various experimental conditions. Physicochemical analysis of liquor effluent revealed the level of pollutants. It was observed that all the values of pollutants were complying with the limits as per stated by CPCB (Central Pollution Control Board.)

Initially fungal inoculum in the form of discs was added to the effluent sample to assess whether these fungi are able to degrade the effluent or not, and also to ascertain whether quantity of fungal inoculum plays an accountable role or not. It was noticed that both the fungi required quite more time to get acclimatized in the effluent. This phenomenon was observed while studying the pH of effluent sample. Initially in the time interval of 4-8 hours the pH remained unchanged but later on pH changed gradually from 4.5 to 5.5.

Other characters like turbidity, TSS (Total suspended solids), TDS (Total dissolved solids) did show a little variations during 24 hours. On the other hand both the fungi did show better reduction in COD (Chemical oxygen demand) level in given interval of time.While studying the metallic constituents such as sodium (Na) and magnesium (Mg) both *Chaetomium globosum* and *Aspergillus nidulans* showed poor responses in 24 hours, irrespective of fungal quantity.

The statistical analysis reveals that if more quantity of inoculum is added in the effluent sample then more numbers of parameters get affected. Such as in case of *Chaetomium globosum* when only one disc was added the parameters like Turbidity, TSS, and COD showed more significant result ($P \leq 0.05$) When two discs were added more significant result was observed in Turbidity, TSS, COD, and Mg($P \leq 0.05$). For three discs the significance was noticed in pH, TSS, COD, and Mg ($P \leq 0.05$).

In case of *Aspergillus nidulans* when only one disc was added the parameters like TSS and COD showed more significant result ($P \leq 0.05$). With respect to two discs significant changes were noticed in TSS, TDS, and COD. ($P \leq 0.05$) For three discs of inoculum parameters like pH, TSS and TDS showed the more significant change. ($P \leq 0.05$) Therefore it could be concluded that quantitative addition of fungal inoculum is directly proportional to changes in number of parameters of effluent in given period of time.

Hence from this study it could be concluded that both the fungi namely *Chaetomium globosum* and *Aspergillus nidulans* do possess the biodegradation ability, both fungi are able to reduce the pollutants of effluent sample in 24 hours,

It is expected that, Present hourly based study (dynamic) could lead to much better achievement towards expected development in treatment technology to ascertain minimum time interval required for maximum quantitative reduction of pollutant by providing favorable conditions or altering other physico chemical parameters. Then it might be possible to have a significant reduction. Due to which pollutants from effluent can be reduced effectively in minimum time interval.

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