Role of Fungal Species in Bioremediation of Sugar-Mill Effluent

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Abstract : Control of water pollution by Natural processes such as bioremediation has been found to be ecofriendly and economic. Such processes convert hazardous compounds into innocuous products in a most effective way. Suitable microbes initiate various physical and chemical reactions in polluted water resulting in degradation and removal of pollutants. Sugar-industry consume large quantities of water for various processes and discharge equally large volumes of waste waters containing variety of pollutants and colouring matter. In the present study, attempt has been made to bring about bioremediation of sugar mill effluent collected from "Braj Kishore Singh Champaran Kisan SSK Ltd., Chorma, Pakridayal, East Champaran" sugar mill. The samples of effluent water were tested for the individual bioremedial efficiency of native fungi present. Three fungi namely, *Aspergillus niger*, *Penicillium sp* and *Fusarium sp* were identified and selected for this bioremedial study. All the three were known to bring about bioremediation which had been confirmed by measuring the % of reduction potential in pH, EC, TDS, OD, BOD, COD and increase in fungal growth. Maximum degrading potential has been observed in the case of *Aspergillus niger*.

Index Terms **- Bioremediation, fungi, Sugar-mill effluent.**

I. INTRODUCTION

Waste water discharge by the industries is one of the major causes of environment pollution particularly in the developing countries. These industrial effluents are constantly contaminating our environment. Indian distilleries have initiate some steps to minimize their water consumption and recycle the treated waste water.[1-5]

Microorganisms due to their inherent capacity to metabolize a variety of complex compounds have been utilized since long back for biodegradation of complex toxic compounds present in various industries wastes for environmental safety.[6-7]

Therefore, in the present study an attempt has been made to bring out the capabilities of fungi for bioremediation of sugar mill effluent and the efficiency of bioremediation was finally validated with the three fungi isolated as native organism based on the values obtained for the parameters such as pH, EC, TDS, OD, BOD, COD and fungal growth.

II. MATERIALS AND METHOD

The sugar mill effluent were collected from the "Braj Kishore Singh, Champaran Kisan SSK Ltd." situated at Chorma, Pakridayal, East Champaran. Effluents cans that have been prewashed with 10% nitric acid and thoroughly rinsed with demonized water.

One ml of the sample was cultured in a sterile Petri plate which contains Malt Extract Agar Medium (MEA) by pour plate method and incubated at room temperature for 4 to 5 days. Fungal species developed on the medium were observed periodically. The fungal colonies were grown on MEA and sub-cultured on Potato Dextrose Agar (PDA)[11]. For enumeration of fungi potato dextrose agar containing potato (200g), dextrose (20g) agar (15g), distilled water (1000ml) at pH 5-6 was used. To obtained pure culture, the cultures were repeatedly streaked with nutrient agar medium and incubated at 37^0C for two hours. Isolated fungal cultures were identified by colony morphology, gram staining, microscopic observation and conformation test. Immediately after the collection of sugar mill effluent and inculation of fungal strains, biological parameters such as DO, BOD, COD and viable counts were measured. Then on the seventh day the same parameters were measured to evaluate and compare their individual bioremediation efficiency. Measurements were done with control also. DO was measured using the modified Winker's method. BOD was measured with the five day incubation method. COD was carried out using the $KMnO_4$ method. Data were tabulated and results were derived based on the values obtained. The treatment efficiency was validated by calculating the % reduction of physico-chemical parameter and biological parameters. The fungi were stained with lacto phenol cottle blue and identified using manual of Onions et.al.[12].

Three potential fungal species were identified such as Aspergillus niger, Pencillium sp and Fusarium sp and selected for biodegration study.

Effluent collected from the sugar mill was autclaved at 121^0C for 15 minutes to make them sterile before inoculating the selected fungal strains. Initially physicochemical parameters and biological parameters were measured.[13]

106 cells/ml of the uniform suspension of each strain was inoculated into 2 liters of sterile sample taken in conical flasks. Then it was covered with cotton and aeration was supplied for 10 minutes twice a day and various parameters were measured regularly. Experiments were conducted in duplicates and repeated three times. Experiments was carried over a period of seven days under laboratory condition and everyday measurements for pH, OD, EC and TDS were carried over for a regular interval of 2 hours using standard procedure.[14]

III. Results and Discussion

The treatment efficiency was validated by calculating the percentage reduction of all the parameters measured. With the sample number 1 where there was inoculation of *Aspergillus niger* with the sugar mill effluent, there was a decrease in pH from 6.10 to 4.4, EC decreased from 805 micro mhos/cm to 654 micro mhos/cm, TDS decreased from 539 mg/litre to 438 mg/litre and OD declined from 0.30 to 0.15. The decrease noted in these parameters in terms of percentage was -38.63, 18.76, 18.74 and 50 respectively.

Table 1

Variation in pH, Electrical conductivity (EC) and Total dissolved solid (TDS) in the effluent from sugar mill industry treated with various fungus.

Days	Control			Effluent treated with various fungus								
				Aspergillus niger			Penicillium sp			Fusarium sp		
	pH	EC	TDS	pH	EC	TDS	pH	EC	TDS	pH	EC	TDS
1	6.0	713	477.7	6.10	805	539	5.9	777	521	5.6	790	529
2	5.8	695	466	5.2	798	535	5.3	755	506	4.7	752	504
3	5.6	670	449	4.9	778	521	5.0	726	486	4.7	736	493
4	5.3	660	442	4.8	750	503	4.7	709	475	4.6	714	478
5	5.1	652	437	4.7	732	490	4.6	683	458	4.4	692	464
6	5.0	644	431	4.4	654	438	4.5	657	440	4.1	665	446
%	-20.0	9.677	9.643	-38.63	18.76	18.74	-31.11	15.44	15.54	-36.59	15.82	15.68

*EC (unit : micro mohs/cm) *TDS (unit : mg/liter)

Table 2

Variation in effluent, Biological oxygen demand (BOD) and chemical oxygen demand (COD) in the effluent from sugar mill industry treated with various fungus

S.No.	Samples	Parameters								
		Fungal growth in Cfu/ml×10^6			BOD in g/l			COD in g/l		
		Initial	Final	Growth Rate %	Initial	Final	% of reduction	Initial	Final	% of reduction
1	*Control*	68	76	11.76	14.2	10.4	26.76	16.8	12.2	27.38
2	*Aspergillus sp*	130	202	55.38	16.8	8.8	47.62	18.8	10.4	44.68
3	*Penicillium sp*	120	176	46.67	16.2	9.6	40.74	17.4	10.2	41.38
4	*Fusarium sp*	128	170	32.81	16.0	10.4	35.00	16.4	11.2	31.70

These values were shown in Table 1 and Table 2. The same parameters were measured when the sugar mill effluent was inoculated with the fungus *Penicillium sp*. The value pH decreased from 5.9 to 4.5, EC from 777 micro mhos/cm to 657 micro mhos/cm, TDS from 521 mg/litre to 440 mg/litre and OD from 0.28 to 0.22. The percentage reduction potential of all these parameters was -31.11, 15.44, 15.54 and 21.43 respectively. When the sugar mill effluent was treated with the fungus *Fusarium sp*, pH decreased from 5.6 to 4.1, EC from 790 micro mhos/cm to 665 micro mhos/cm, TDS from 529 mg/litre to 446 mg/litre and OD from 0.27 to 0.15. The percentage of reduction potential for above parameters were -36.59, 15.82, 15.68 and 44.44 respectively.

Variation in BOD and COD during bioremediation of sugar mill effluent treated with various fungal isolates :

The process of bioremediation is generally understood with higher percentage of reduction accompanied with BOD and COD. These values were noted in control as well as for samples of sugar mill effluent inoculated with three different fungus species namely *Aspergillus sp*, *Penicillium sp* and *Fusarium sp*. Significant reduction in BOD and COD values were noted i.e. 47.62% and 44.68% with the samples inoculated with *Aspergillus niger* for the purpose of bioremediation. The percentage of reduction in BOD and COD were 40.74 and 41.38 in *Penicillum sp*. The fungal species *Fusarium sp* brought a reduction in BOD and COD to extend of 35.00% and 31.70% with the sugar mill effluent. The percentages of reduction for all these parameters are collectively shown in Table-4.

Growth rate of various fungal isolates with the sugar mill effluent during fungal bioremediation :

Growth rate of various fungal species inoculated with the sugar mill effluent were calculated by measuring their viable counts on the 1st day and 7th day. The increase in their viable counts was uniform throughout study independent upon the nature of fungus. The percentage of increase in their growth rate was 55.38, 46.67 and 32.81 with respect to *Aspergillus niger*, *Penicillium sp* and *Fusarium sp* respectively. The percentage of growth rate with control was only 11.76. All the experiments were carried out in duplicate and the average of these two were taken for tabulation. These values were tabulated in table-1, table-2 and table-3. The percentage reduction potential for these parameters has been grouped in table 4 for comparison.

Table 3
Variation of OD with respect to fungal growth in the effluent from sugar mill industry.

Days	Control	Effluent treated with various fungus		
		Aspergillus niger	Penicillium sp	Fusarium sp
1	0.26	0.30	0.28	0.27
2	0.24	0.28	0.27	0.25
3	0.24	0.27	0.25	0.22
4	0.25	0.24	0.25	0.17
5	0.23	0.18	0.23	0.16
6	0.22	0.15	0.22	0.15
%	15.38	50	21.43	44.44

Table 4.
Variation of physico-chemical parameters in terms of percentage

S.No.	Samples	% of pH reduction	% of EC reduction	% of TDS reduction	% of OD reduction	% of BOD reduction	% of COD reduction	% of viable counts
1	Control	-20.0	9.677	9.643	15.38	26.76	27.38	11.76
2	Effluent+ Aspergillus niger	-38.63	18.76	18.74	50.00	47.62	44.68	55.38
3	Effluent+ Penicillium sp	-31.11	15.44	15.54	21.43	40.74	41.38	46.67
4	Effluent+ Fusarium sp	-36.59	15.82	15.68	44.44	35.00	31.70	32.81

IV. CONCLUSION

Sugar mill effluent is generated during the processing of sugarcane in different units such as mill house, boiler house and filters wash. Other than this, improper handling of molasses and this leakage and over flow from storage tanks also contribute to high pollution load (Rao and Dutta 1987). Generally sugar mill effluent is slightly blackish ash in colour with disagreeable odour, high value of BOD, COD and total suspended solids. According to Singh et. al., (1998) disagreeable colour and odour of the sugar mill effluent could be due to the decomposition of organic matter or presence of various aromatic and volatile organic compounds. With the inoculation of *Aspergillus niger* with the sugar mill effluent, there was a decrease in pH, EC, TDS and OD.

The decrease noted in these parameters in terms of percentage was -38.63, 18.76, 18.74 and 50 respectively. The same parameters were measured when the sugar mill effluent was inoculated with the fungus *Penicillium sp*. The percentage of reduction noted with pH, EC, TDS and OD were -31.11, 15.44, 15.54 and 21.43 respectively. When the sugar mill effluent was treated with the fungus *Fusarium sp*, the percentage of reduction potential for above parameters above parameters were -36.59, 15.82, 15.68 and 44.44 respectively. Significant reduction in BOD and COD values were noted i.e. 47.62% and 44.68% with the samples inoculated with *Aspergillus niger* for the purpose of bioremediation compared to the other two species.

The three potential fungal species *Aspergillus niger*, *Pencillium sp* and *Fusarium sp* identified and isolated from the sugar mill effluent. To select the fungal isolate with higher bioremediation potential, out of the three species isolated from the sugar mill effluent the percentage of reduction potential produced in different parameters were compared. It was understood that there was a higher reduction percentage of EC, TDS, BOD and COD when the sugar mill effluent was inoculated with *Aspergillus niger* fungal species when compared to other fungal species and hence *Aspergillus niger* was proved to possess higher bioremediation potential.

REFERENCES

[1] E. Booth, Methods in Microbiology, Academic Press, New York, 1971.

[2] A.H.S. Onions, D. Allsopp and H.O.W. Egginns, Smith's Introduction in Industrial Mycology, 7th Edn., Edward Arnold Publishers Ltd., U.S.A. 1981.

[3] S.B.Kim, F.Hayase and H.Kato, Agric, Biol. Chem., 1985, 39, 785.

[4] I.M. Banat et. al., Enu. Hith., 1996, 23, 547.

[5] V.Kumar et. al., Microbios, 1997, 89, 81.

[6] R.P.Evershed et. al. Sci, 1997, 278, 432.

[7] R.Chandra and H. Singh, Ind. J. Environ. Prot, 1999, 19, 833.

[8] O.U.Ezeronye and P.O.Okerentugba, World J. Microbial, Biotechno., 1999, 15, 515.

[9] R.Gupta and K.G.Mukerji, Bioremediation : Past, Present and Future, A.P.H. Pub. Co. New Delhi, 2001.

[10] APHA, STD Methods for examination of water and wastewater, 20th Edn, American Public Health Association, Washington DC, 2005.

[11] P.Kumar and R.Chandra, Baccilus. Spp. Biores. Technol., 2006, 7, 2096.

[12] Solomon, S.K. : Enviromental pollution and its Management in Sugar Industry in India : An appraisal. Sugar Tech, (2008) 7(1) : pp 7781.

[13] S.Buvneswari, S.Damodar Kumar and M. Murugesan, Int. J. Curr. Microbial. App. Sci. 2013, 2, 50.

[14] A.D.Kshirsagar and V.R.Gunale : Pollution status of river Mula (Pune city), Maharashtra, India, J.Ecophysio and Occupati Hlth..., (2011), 11:80-90.

[15] H.J.Porwal, A.V.Mane and S.G.Velhal, Biodegradation of Dairy effluent by using microbial isolates obtained from activated sludge, Water Resource and Industry; Elsevier, 2015, Vol.9, p.1-15