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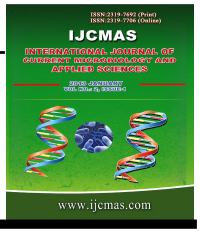

Original Research Article

Bioremediation studies on sugar-mill effluent by selected fungal species

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Keywords

Sugar mill Effluent; Bioremediation; Fungi

A B S T R A C T

Bioremediation has been recognized as a environment friendly and less expensive method which involves the natural processes resulting in the efficient conversion of hazardous compounds into innocuous products. This technique involves suitable microbes undergoing various physical and chemical reactions in the polluted water system and during the microbial metabolism, the pollutants are degraded and removed. Recently microbial bioremediation has emerged as an alternative technique to such traditional chemical treatments. Sugar industries consume large quantities of water for various processes and discharge equally large volumes of waste waters containing variety of pollutants and coloring matter. In the present study attempt has been made to bring about the bioremediation of sugar mill effluent collected from the Cheyyiar Cooperative Sugar mill Ltd. Thiruvannamalai District and 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 studies. 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 i.e. pH(-38.63%), EC(18.76%), TDS(18.74%), BOD(47.62%) and COD(44.68%) among all.

Introduction

Wastewater discharge by the industries is one of the major causes of environmental pollution particularly in the developing countries. These industrial effluents when constantly are contaminating our environment pose serious threat to human life. Paper and pulp mills, molasses based alcohol distilleries, tanneries, dye-making units and textiles are some of the major industries that produce and discharge highly coloured effluent. Each of these industrial effluents creates some specific problem besides producing aesthetically unacceptable intense coloring of soil and water bodies. These block the passage of

light to the lower depths of the aquatic system resulting in the cessation of photosynthesis, leading to anaerobic conditions which in turn result in death of aquatic life causing foul smelling toxic waters.

These are several initiates being followed by Indian distilleries to minimize their water consumption and recycle the treated waste water. However in addition to this research to address existing gaps is also necessary to provide a comprehensive and cost effective solution to enable the industries to become lower water consuming and zero discharge units. The wastewater released from distilleries and fermentation industries are the major source of soil and aquatic pollution due to presence of water-soluble recalcitrant coloring compounds called melanoidins (Evershed, *et al*., 1997) which are highly resistant to microbial attach and conventional biological processes such as activated sludge treatment process which are insufficient to treat these melanoidins containing wastewater released from distilleries and fermentation industries. These wastewaters released from industries require pretreatment before its disposal into the environment (Mohana, *et al*., 2007; Kumar and Chandra 2006). The conventional aerobic wastewater treatment method would not be appropriate because of the large land space requirement, as well as high capital costs (mechanical or diffused aeration systems) and operational costs. Degradation and decolorisation of these wastewaters by chemical methods (Chandra and Singh 1999), flocculation treatment and physico-chemical treatment such as ozonation(Kim *et al*., 1985) and activated carbon absorption have been accomplished, but these methods are not economically feasible on large scale due to cost limitation, whereas biological decolorisation by using fungi such as Coriolus Aspergillus, Phanerochaete and certain bacterial species as Bacillus, Alkaligenes and Lactobacillus (Kumar, *et al*., 1997; Ohmomo, *et al*., 1985b; 1987; Kumar and Chandra, 2006,) have been successfully achieved and thus can be applied as a bioremediation techniques.

Microorganism due to their inherent capacity to metabolize a variety of complex compounds have been utilized since long back for biodegradation of complex toxic and recalcitrant compounds present in various industrial wastes for environmental safety. Microorganisms have been used to remove organic matter and toxic chemicals from domestic and industrial wastes discharged for many years (Gupta and Mukarjee, 2001). Microbial treatment systems have advantage of being simple in design and low in cost (Banat, *et al*., 1996). Fungi their biology, economic value and pathogenic capabilities are not new to human society. They have been used from fermentation of foods to production of pharmaceuticals. Fungi thrive well in inhospitable habitats with environmental extremes because of their enzyme systems (Cooke, 1979). Fungi are involved in the biodegradation of undesirable materials or compounds and convert them into harmless tolerable or useful products. Fungi are recognized for their superior aptitudes to produce a large variety of extracellular proteins, organic acids and other metabolities and for their capacities to adapt to severe environmental constraints (Lilly and Barnett, 1958). Fungal systems appear to be most appropriate in the treatment of colored and metallic effluent (Ezeronyl, 1999). Fungi not only produce various metabolities like citric acid, homogeneous proteins, heterogenous proteins, peroxides but have

shown their effectiveness for removal, reduction and detoxification of industrial effluent ingredients.

Therefore in this 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.

Materials and Methods

Collection and processing of sugar mill effluent

The sugar mill effluent were collected from the Cheyyar Cooperative Sugar Mills Ltd. Thiruvannamalai district in clean polythene 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 grown on MEA and the fungal colonies were subcultured on Potato Dextrose Agar (PDA) slants (Booth 1971). For enumeration of fungi Potato dextrose agar containing potato (200gm), dextrose (20gm), agar (15gm), distilled water (1000ml) at pH 5-6 was used. To obtain pure culture the cultures were repeatedly streaked with nutrient agar medium and incubated at 37°C for 2 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 inoculation 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. Dissolved oxygen (DO) was measured using the modified winklers method and biochemical oxygen demand (BOD) with the five day incubation method. Chemical oxygen demand was carried out using the potassium permanganate 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* (1981). Three potential fungal species were identified such as *Aspergillus niger*, *Penicillium* sp and *Fusarium* sp and selected for biodegradation study.

Effluent collected from the sugar mill was autoclaved at 121°C for 15 minutes to make them sterile before inoculating the selected fungal strains. Initially physico-chemical parameters and biological parameters were measured (APHA, 2005). 10^6 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. Experiment was carried over for 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. pH was measured using Orabeco, portable pH meter model 64. EC and TDS were determined by the standard procedure (Richard, 1954).

Results

Trace out efficient fungal species based on the values such as pH, EC, TDS and OD

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 mhos/cm) *TDS (unit: mg/liter)

Table.2 Variation in cfu, 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 X 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 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 percentages 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 *Penicillium* 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, 2 and 3 (Fig.1 to 5). The percentage reduction potential for these parameters have been grouped in table 4 for comparison.

Discussion

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,

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* sp**	***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	-20.00	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

Fig. 1 pH Vs DAYS

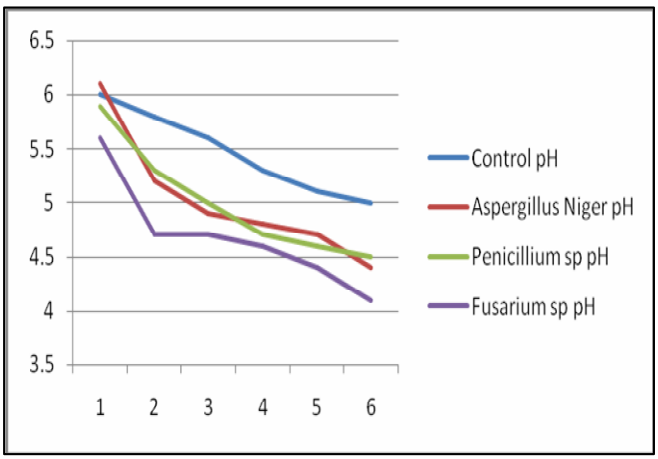


Fig. 2 EC Vs DAYS

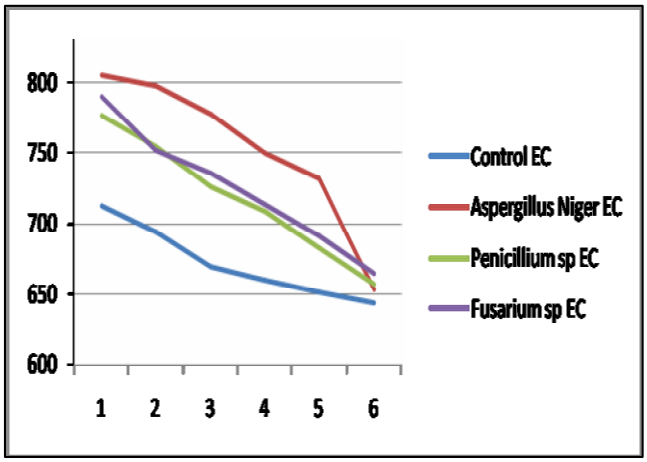


Fig. 3 TDS Vs DAYS

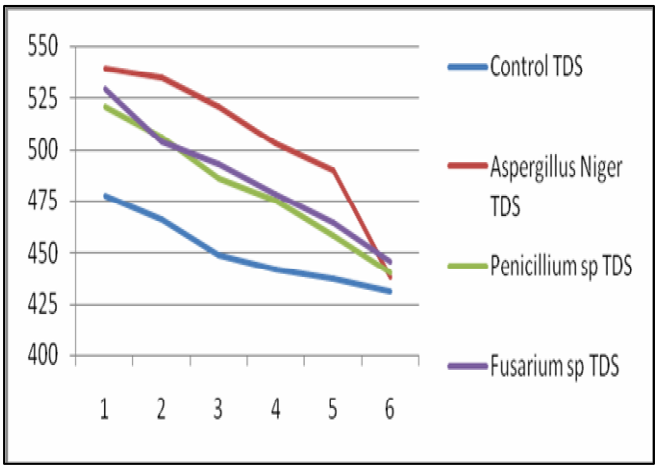


Fig. 4 OD Vs DAYS

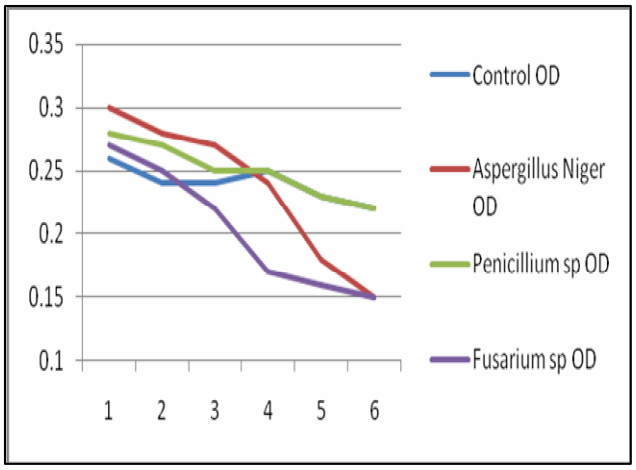
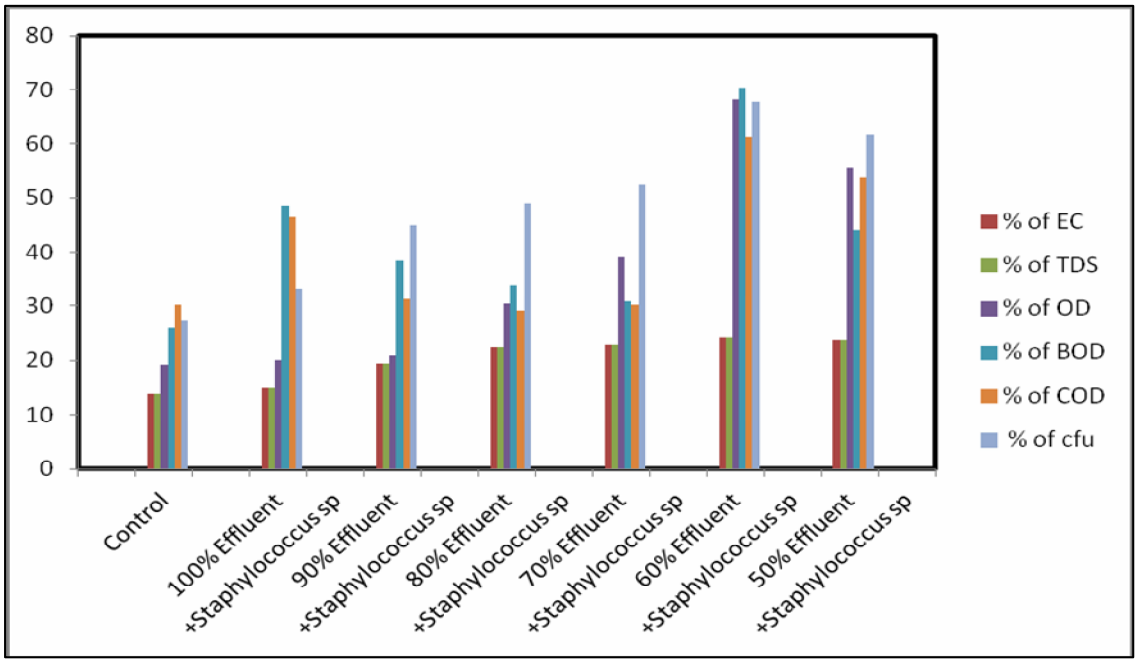


Fig. 5 Variation of parameters with different samples



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, Penicillium 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

APHA. 2005. STD methods for examination of water and wastewater 20th edition. American Public Health Association. Washington D.C.

Banat, I.M., P.Nigam, G.Mcmullan, R.Marchant and Singh, D. 1996. The isolation of thermophilic bacterial cultures capable of textile dyes decolourisation, Env. Hlth. 23(4): 547-51.

Booth, E. 1971. Methods in microbiology, Academic Press. New York.

Chandra, R., and Singh. H. 1999. Chemical decolourisation of anaerobically treated distillery effluent. Ind. J. Environ. Prot. 19 (11): 833-837.

Cooke, W.B. 1979. The Ecology of Fungi. CRE Press Inc. Boca Raton, Florida.

Evershed, R. P., H.A. Bland, P.F.Van Bergen, J.F. Carter, M.C.Horton and Rowley conwy, P.A. 1997. Volatile compounds in archaeological plant remains and the Maillard reaction during decay of organic matter. Sci. 278(5337): 432-433.

Ezeronye, O.U., and Okerentugba, P.O. 1999. Performance and efficiency of a yeast biofiller for the treatment of a Nigerian fertilizer plant effluent. World J Microbiol Biotechno. 15: 515-6.

Gupta, R., and Mukerji, K.G. 2001. Bioremediation : Past, Present and Future. In Role of microbes in the management of envtal pollution. Tewari. R. Mukerfi. KG. Gupta JK. Gupta LK.(eds) A.P.H. Publishing Corp. NewDelhi. 73-81.

Kim, S.B., F.Hayase, and Kato, H. 1985. Decolourisation and degradation products of melanoidins on ozonolysis. *Agric. Biol. Chem.* 39: 785-792.

Kumar, P., and Chandra, R. 2006. Decolorisation and detoxification of synthetic molasses melanoidins by individual and mixed cultures *Bacillus* spp. Biores. Technol. 7, 2096-2102.

Kumar, P., and Chandra, R. 2006. Decolorisation and detoxification

synthetic molasses melanoidins by individual and mixed cultures *Bacillus* Spp. Biores. Technol. 7: 2096-2102.

Kumar, V., L.Wati, P.Nigam, M.Banat, G.MacMullan, H.D.Sing, and Marchant, R. 1997. Microbial decolourisation and bioremediation of an aerobically digested molasses spent wash effluent by aerobic bacterial culture. Microbios. 89. 81-90.

Lilly, V.M., and Basnett, H.L.1958. Physiology of the Fungi, Mc Graw-Hill booki Co, 1st Ed. New York.

Mohana, S., C.Desai, and Madamwar, D. 2007. Biodegradation decolorisation of an aerobically treated distillery spent wash by a novel bacterial consortium. Biores. Technol. 98:333-339.

Ohmomo, S., I.Aoshima, Y.Tozawa, N.Sakurada and K.Veda. 1985. Purification and some properties of melanoidin decolourizing enzumes p.3. an dp.4 from mycelia of coriolus very colours Ps.4a. Agric,Biol. Chem. 49: 2047-2053.

Ohmomo, S., M.Kainuma, K.Kamimura, S.Sirianuntapiboon, I.Oshima, and Atthasumpunna, P. 1988. Adsorption of melanoidin to the mycelia of *Aspergillus oryzae*. Agric. Biol. Chem. 52:381-386.

Onions, A.H.S., D.Allsopp, and Egginns, H.O.W. 1981. Smiths' Introduction in Industrial Mycology (7th ed). Edward Arnold Publishers Ltd.

Rao, M.N., and Dutta, A.K. 1987. Waste water treatment Second edition, Oxford and publishing company Pvt. Ltd.

Singh, D.K., D.Kumar and Singh, V.P. 1985. Studies on pollutional effects of sugar mill and distillery effluent on seed germination and seedling growth of three varieties of rice. J. Environ. Bio. 1. 6 (1):31 – 35.