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ASPERGILLUS FLAVUS AND PHANEROCHAETE CHRYSOSPORIUM AS POTENTIAL DELIGNIFYING MYCOFLORA OF DUMP YARD: A CASE STUDY

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ABSTRACT

The increasing urbanization, industrialization has direct impact on urban waste. Solid waste management is an important factor of environmental hygiene and needs to be integrated with total environmental planning. Biodegradation is termed as natural process of recycling. Fungi play an important role in biodegradation as they are more active in carbon assimilation than bacteria and actinomycetes. The results of physico-chemical parameters of selected soil samples at different depths showed an increase in organic carbon content than surface soils and sub surface soils. The pH of all samples was neutral to slightly alkaline, shows the favorable condition for the growth of fungi.

The scope of the present work attempts to search for an effective method of delignification by using lignolytic soil fungi. The cellulose and lignin degradation has been effectively done by Phanerochaete Chrysosporium (80%) and Aspergillus flavus (75%). Where Phanerochaete Chrysosporium shown the maximum release of carbon dioxide during biodegradation. Pre and post degradation studies were carried where there is a decrease in pH of the soils were observed due to the degradation by fungal species.

Key Words: Bio-degradation, Solid waste, Lignin, Cellulose, Phanerochaete chrysosporium, Aspergillus flavus.

INTRODUCTION

In developed countries, the problem of municipal solid wastes has been tackled very effectively and converted into opportunities to generate energy or organic manure. The multifarious benefits of aerobic composting, anaerobic digestion and vermicomposting have drifted the attention of scientists to eco friendly technology, which employs microorganisms for waste biodegradation studies. Fungi are excellent decomposers because they can break down and utilize a wide variety of complex compounds. The saprophytic fungi are important in the decomposition of sugar, cellulose and lignin. The lignolytic fungi produce exoenzymes that break down large polymers to smaller molecules then absorb these molecules into thin cells by endoenzymes. The decomposition of lignocellulose is rated as the most important degradative event in the carbon cycle[3]. Lignin is a complex polymer of phenyl propane units, which are cross linked to each other with a variety of different chemical bonds. Fungi have developed the necessary enzymes to break lignin apart. The initial reactions are mediated by extra cellular lignin and manganese peroxidases, primarily and aerobic process, and in an anaerobic environment lignin can persist for very long periods[18]. The effect of lignin on the bio-availability of other cell wall components is thought to be largely a physical restriction with lignin molecules reducing the surface area available to enzymatic penetration and activity[11]. With large amount of lignin present some of the lignin would be overlapping other lignin molecules rather than cellulose so the incremental effect will be smaller[6]. Adding small amounts of nitrogen to woody materials can increase lignin degradation rates. Over a two week incubation with a white-rot fungus at 39-40^{o}C (the optimum temperature for the growth of *Phanerachaete Chrysosporium*) adding only 0.12% nitrogen (dry weight basis), lignin degradation in order of pulp

increased from 5.2% to 29.8%[19]. The brown rot fungus has the capacity to transform lignin to some extent. Primarily it utilizes the carbohydrates of lingocellulosic complex, leaving residue of modified lignin which is brown in colour. These fungi do modify lignin by demelthylation, hydroxylation and side chain oxidants but are not able to completion. The most appropriate microbial species for lignin degradation and lignin removal from waste with a wide ability to degrade cellulose and *Phanerocheate chrysosporium*, *P.magnoia. Aspergillus sps and Pencillium sps*. The use of white rot fungus for eco-friendly disposal of organic waste and the use of *Phanerochaete chrysosporium* degrade major components of lingo-cellulosic materials. It is also able to synthesize lignocellulytic enzymes.[17] Lignin can also be degraded by using *Phanerochaete ostreatus*. *Phanerochaete chrysosporium* has been studied extensively for remediation of soils contaminated with organic compounds.[14]

The role of fungi in the degradation of complex carbon compounds such as starch, cellulose, pectin lignin insulin, xylan, araban etc, is well known. Biodegradation waste includes kitchen, garden waste and unsold spoiled discarded vegetable from market, food waste and paper. This waste is rich in cellulose and lignin products. The scope of the present work attempts to search for an effective method of delignification by using lignolytic soil fungi. In this study an attempt was made to evaluate the biodegradation studies of lignin and cellulose by using lignolytic fungi.

EXPERIMENTAL

The study was carried out at Kapulauppada a dumping yard in the outskirts of Visakhapatnam to identify and characterize the isolated lingo-cellulolytic fungi. The collected soil samples were collected to screening for lingo-cellulolytic fungi with all necessary precautions. The soil samples were made serial dilutions with sterile distilled water and these dilutions were inoculated in the Czapeck's-Sucrose-Nitrate-Agar-Medium[4] to which necessary antibiotics were added to control bacterial growth in laminar air flow and then incubated at 28±1 ^{o}C . The pure cultures of the (*Aspergillus flavus*, hink *ex Gray (Moniliacear) Phanerochaete chrysosporium MTCC-787*) were obtained from MTCC Chandigarh for biodegradation studies. Similarly the pure cultures of *Phanerochaete chrysosporium* and *Aspergillus flavus* were inoculated with lignocellulosic rich soils and carbon dioxide release was estimated. The collected soil samples were subjected to bio-degradation experiments and they were analyzed for physico-chemical parameters including both lignin and cellulose, for pre and post bio-degradation assays. The experiment were carried out by adapting standard procedures from manual of American Public Health Association[1]. Cellulose and lignin were estimated by adapting standard procedures given by[17] [16] respectively.

In present study the rate of biodegradation was estimated in terms of carbon dioxide released. The seven-day-old fungal cultures, was added to the culture bottle containing soil samples. Small rest tubes containing 0.1 N Sodium hydroxide were suspended with the help of a thread. The culture bottles were closed with stopper and sealed to ensure air tight conditions and incubated at 28±1 ^{o}C the carbon dioxide release during biodegradation was absorbed by sodium hydroxide in the vials. During estimation the content of vials were quantitatively transferred to a conical flask followed by the addition of 5ml of saturated solution of Barium Chloride to precipitate the Carbon Dioxide and Barium Carbonate, two or three drops of Phenolphthalein indicator were added. The residual amount of sodium hydroxide in the flask was measured by titrating it against 0.1 N Hydrochloric acid. The end point was the disappearance of pink colour[9].

RESULTS AND DISCUSSIONS

The results of the physico chemical parameters of soil samples are given in table – 1 which elucidates that in soil samples which were collected at a depth of 15 cm. Organic matter & organic carbon increased when compared to control and surface soil samples. The pH of all the samples was neutral to slightly alkaline, shows the favorable conditions of the growth of fungi. It was observed that both cellulose and lignin degradation had been effectively done by *Phanerochaete chrysosporium* showed the maximum (Fig 2 and Fig 3). Total nitrogen content in unsterlised soil sample was 15.4% and 15cm deep soil it was 20.0% where as in case of sterlised soil the nitrogen content was 14.26% and 15cm deep soil was 19.8% where the control of unsterlised sample was 13.06% and sterlised was 11.06%. The measurement of carbon dioxide release during the bio-degradation may be used as an index of cellulose decomposition[15].

Table 2 summarizes the Carbon dioxide released during bio degradation in a comparative account for *Phanerochaete chrysosporium* and *Aspergillus flavus*. On the 5th day of degradation Carbon dioxide was released by *Phanerochaete chrysosporium* was observed to be 7.3mg/gm where as it was 6.3mg/gm for *Aspergillus flavus*. On the 9th day of degradation Carbon dioxide evolved by *Phanerochaete chrysosporium* was maximum of 14.74mg/gm and that of *Aspergillus flavus* 13.64mg/gm. On the 15th day of degradation the Carbon dioxide release was observed to be 8.32mg/gm by *Phanerochaete chrysosporium* and 7.04mg/gm by *Aspergillus flavus* (Figure 1).

The competition among the species was studied by the antagonism studies where the growth of *Phanerochaete chrysosporium* in competition with *Aspergillus flavus* was observed to be equally scattered in the petriplate which was same with *Aspergillus flavus* in competition with *Phanerochaete chrysosporium*. This was also proved from the Carbon dioxide release and biodegradation of cellulose in soil samples where in *Phanerochaete chrysosporium* was able to degrade effectively in competition with the indigenous sps which does not contain any antagonistic characters for *Phanerochaete chrysosporium*,[14].

Kent[13] stated that shaking condition is detrimental to growth and lignin degradation, however, static conditions do not disrupt fungal mat and allow proper penetrations of raw materials, similarly low nitrogen supplement induces lignin degradation. Sugar supplement in low concentration has been reported by[7] to induce lignolytic system by providing initial metabolizable substrate and also repress cellulolytic system.

The various levels of biodegradation are due to the activities of fungal enzymes. These fungi are capable of producing enzymes such as α- endogluconase, and β-glucosidase in their system[8] . Cellulose enzyme is capable of degrading crystalline forms of cellulose of endo β- 1, 4 glucanases and exo β-1, 4 glucanases (cellulobiohydrolases) and β- glucosidases (cellulobioses). The net effect of these three enzymes is to rapidly decrease the polymer strength with a slow increase in reducing group[5,10] .

The biodegradation studies revealed that both the *Phanerochaete chrysosporium* and *Aspergillus flavus* were effectively reduced the concentrations of lignin and cellulose in test soils (Figure-2 and 3). A comparative account was given in table-3. The result elucidated that the degradation was shown maximum by soils having *Phanerochaete chrysosporium* and *Aspergillus flavus* than the control. Physico-chemical characteristics of soil of pre & post degradation studies and from these results were presented in Table-4. The degradation in terms of decrease in pH was also observed[2] . This study also revealed that after completion of biodegrading studies, organic carbon decreased when compared to control. The decrease in organic carbon content in all the treatments may be due to loss of carbon dioxide during the process of microbial decomposition[12].

CONCULSION

One of the thrust areas of biotechnology research involving lignocelluloses has been the need to isolate and identify microorganisms, which are hyper producers of lignocellulolytic enzymes. Interest has been focused on not only finding enzymes which could break down lignocelluloses compounds much more rapidly, but also enzymes that would withstand wide range of pH, temperature and inhibitory agents. A constant search for new potential lignocellulolytic fungi is thus taking place.

In the present study, species of *Phanerochaete chrysosporium* and *Aspergillus flavus* isolated from dump yard soil were found to be potential biodegraders of lignin and cellulose. These fungi produced cellulose and decomposed cellulose at a very fast rate. These Fungi can thus be utilized effectively as agents of biodegradation in waste recycling process.

Microbial degradation has an additional advantage over chemical delignification of operating at low temperature. In the light of increased interest in the utilization of lingo-celluloses for the production of ethanol.

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Table-1:Physico- chemical characteristics of soil samples collected from Kapulauppada.

		Surface soil	15 cm deep soil	Control	Surface soil	15 cm deep soil	Control
S. No.	Parameter	Unsterilised sample	Unsterilised sample	Unsterilised sample	Sterilized sample	Sterilized sample	Sterilized sample
1	pH	8.06	8.15	7.84	8.06	8.23	7.83
2	Conductivity (Milli mhos)	0.38	0.58	0.22	0.231	0.294	0.366
3	Moisture content (%)	16.139	18.243	9.229	NIL	NIL	NIL
4	Bulk Density($g/m^{3)}$	25.288	25.520	30.133	28.631	25.885	31.045
5	Organic carbon (%)	1.98	2.22	0.42	1.66	1.99	0.24
6	Chlorides (mg/g)	0.375	0.649	0.098	0.349	0.549	0.047
7	Nitrates (mg/g)	16.875	20.625	8.75	11.875	10.256	2.25
8	Sulphates (mg/g)	25.15	45.0	7.5	17.5	23.5	2.5
9	Phosphates (mg/g)	0.87	1.18	0.625	0.75	1.0	0.58
10	Total nitrogen (%)	15.4	20.0	13.06	14.26	19.8	11.06
11	Cellulose (μ g)	0.35	0.6	0.15	0.3	0.51	0.09
12	Lignin (μ g)	4.5	3.5	3.0	2.5	2.0	1.0
13	C/N ratio (%)	0.12	0.11	0.03	0.11	0.10	0.02

Table-2: Showing comparative study of carbon dioxide release during degradation by *Phnerochaete species & Aspergillus species*.

Name of the species	Time of degradation									
	1st day	3rd day	5th day	7th day	9th day	11th day	13th day	15th day	17th day	19th day
Aspergillus flavus	0.42	3.08	6.38	9.9	13.6	12.98	9.02	7.04	2.86	1.02
Phanerochaete sp Chrysosposium sp	0.6	4.2	7.3	10.3	14.7	13.68	10.04	8.32	4.03	2.01

Table-3: Cellulose and Lignin degradation in soil samples by Fungi in micrograms (µ g):

S.No.	Time period	*Aspergillus species*		*Phanerochaete species*	
		Cellulose (µ g)	Lignin (µ g)	Cellulose (µ g)	Lignin (µ g)
1	Initial	0.6	6.4	0.4	7.5
2	3rd day	0.35(44.8%)	6.0(6.25%)	0.3(37.5%)	6.25(16.6%)
3	5th day	0.21(65.0%)	5.81(9.2%)	0.25(37.5%)	5.75(23.3%)
4	7th day	0.05(91%)	5.50(14.06%)	0.05(87.5%)	5.50(26.6%)
5	9th day	0.02(96.6%)	5.00(21.87%)	0.02(95%)	5.12(31.73%)
6	11th day	Nil	4.81(24.84%)	Nil	4.9(34.66%)
7	13th day	Nil	4.75(25.78%)	Nil	4.9(34.66%)
8	15th day	Nil	3.2(50%)	Nil	4.03(46.2%)
9	17th day	Nil	1.6(75%)	Nil	3.5(53.3%)
10	19th day	Nil	Nil	Nil	1.5(80%)

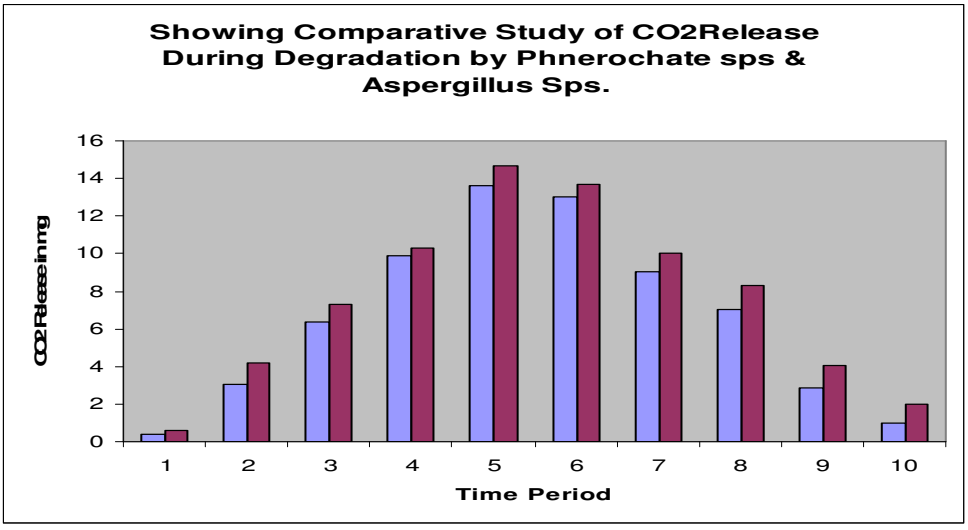

Fig.-1: Graph showing carbon dioxide released (mg) during lignin and cellulose biodegradation.

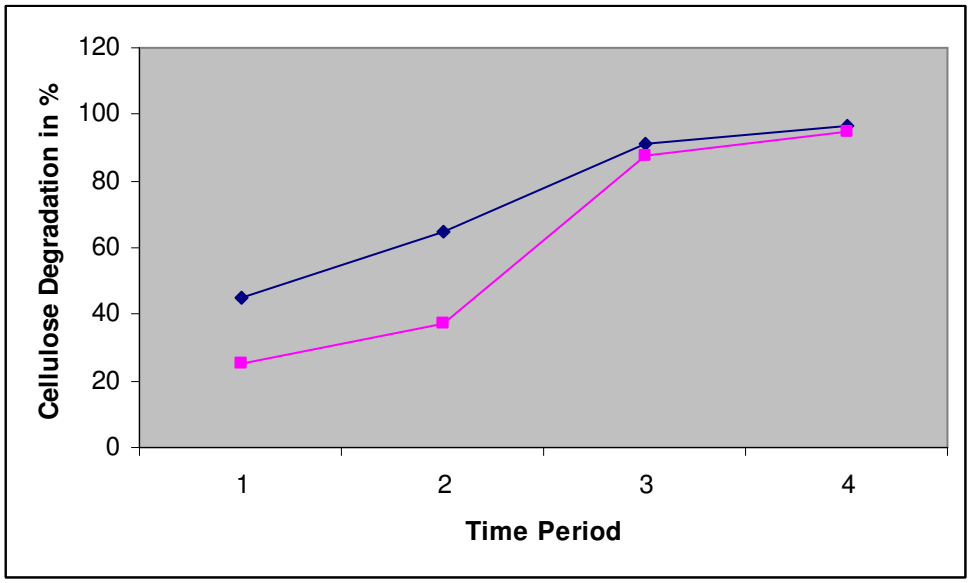

Fig.-2: Cellulose degradation (%) by *Aspergillus sp* and *Phanerocheate sp*

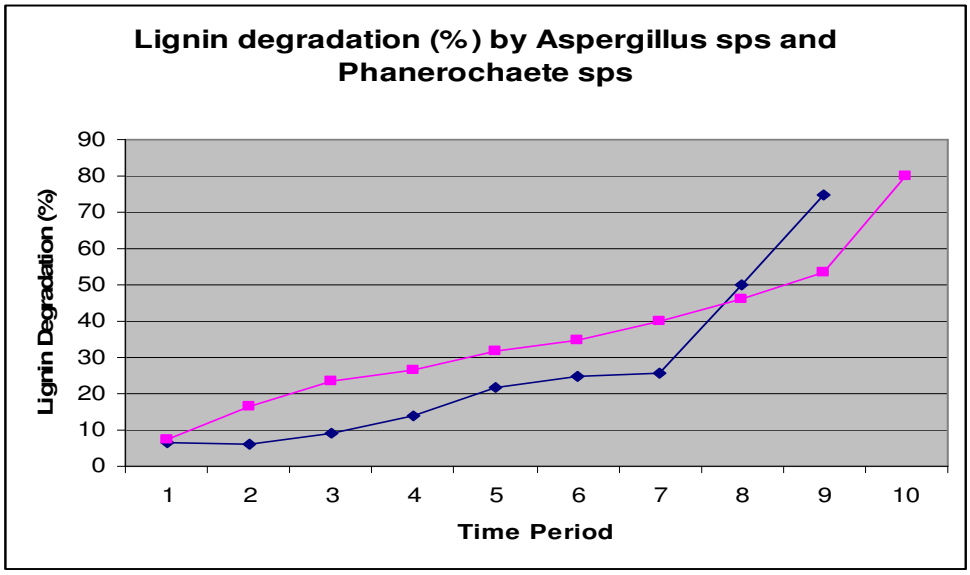

Fig.-3:Lignin degradation (%) by *Aspergillus sps* and *Phanerochaete sps*.

Table-4: Physico-chemical characteristics of soil sample after biodegradation study.

Name of the species	1st day	3rd day	5th day	7th day	9th day	11th day	13th day	15th day	17th day	19th day
pH(C)	7.02	7.0	6.3	6.0	5.9	5.7	5.6	5.3	5.2	5.0
pH(As.sp)	7.94	7.63	7.20	6.93	6.54	6.03	5.83	5.46	5.04	4.94
pH(Ph.sp)	8.0	7.96	7.82	7.62	7.02	6.56	5.9	5.84	5.52	5.03
Organic carbon(C)	1.84	1.5	1.2	1.1	0.8	0.7	0.3	0.1	Nil	Nil
Organic carbon(As.sp)	1.9	1.72	1.72	1.56	1.32	1.16	0.93	0.65	0.3	0.1

Organic carbon(Ph.sp)	2.1	1.9	1.56	1.32	1.15	0.97	0.86	0.56	0.2	Nil
Nitrates (C) (mg/g)	13.4	11.4	8.76	5.42	3.62	2.01	1.1	0.3	Nil	Nil
Nitrates (As.sp) (mg/g)	15.28	12.62	9.48	6.26	4.72	3.62	1.42	0.64	0.2	Nil
Nitrates (Ph.sp) (mg/g)	16.4	13.3	10.42	8.28	5.62	3.23	2.4	0.3	0.1	Nil
Sulphate (C) (mg/g)	20.6	19.8	17.2	13.0	12.2	10.6	9.6	8.6	6.6	0.2
Sulphate (As.sp) (mg/g)	22.7	19.4	16.4	12.9	12.1	10.2	9.3	7.6	5.3	3.2
Sulphate (Ph.sp) (mg/g)	23.6	20.2	19.2	16.2	13.6	12.2	10.6	8.9	7.2	4.2
Phosphates (C) (mg/g)	0.9	0.76	0.52	0.33	0.20	0.097	0.03	0.004	Nil	Nil
Phosphates (As.sp) (mg/g)	1.0	0.86	0.72	0.56	0.41	0.29	0.18	0.10	0.05	Nil
Phosphates (Ph.sp) (mg/g)	1.2	0.96	0.82	0.64	0.56	0.32	0.22	0.08	0.02	Nil
Total Nitrogen (C) (mg/g)	15.0	13.0	11.0	8.3	7.3	5.6	4.1	2.1	0.6	Nil
Total Nitrogen (As.sp) (mg/g)	17.6	14.0	12.0	9.9	8.5	7.0	5.0	3.0	1.6	0.8
Total Nitrogen (Ph.sp) (mg/g)	19.0	17.6	14.3	12.6	9.7	8.6	7.2	4.3	2.0	0.4
C/N Ratio (C) (%)	0.12	0.11	0.10	0.13	0.10	0.12	0.07	0.5	Nil	Nil
C/N Ratio (As.sp) (%)	0.10	0.12	0.13	0.14	0.13	0.13	0.17	0.18	0.12	Nil
C/N Ratio (Ph.sp) (%)	0.11	0.10	0.12	0.12	0.126	0.13	0.13	0.15	0.15	0.25

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