SOIL FUNGI: POTENTIAL MYCOREMEDIATORS OF LIGNOCELLULOSIC WASTE

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The continual expansion of urbanization and industrial activity has led to the accumulation of a large quantity of lignocellulosic residues throughout the world. In particular, large quantities of paper and bagasse are largely produced in Visakhapatnam. In this work we present the study of the degradability of these substrates with fungi. Three cultures of soil fungi were screened for their ability to degrade cellulose. *Aspergillus flavus* degraded the most, as shown by the highest CO_2 release. Further, *Aspergillus flavus* was tested with the standard fungus *Phanerochaete chrysosporium* for cellulose degradation, which showed nearly equivalent potential.

Keywords: Biodegradation; Lignocellulolytic fungi; Dumping yard; CO_2 release method

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INTRODUCTION

Biodegradation is the general term used for all biologically mediated breakdown processes for chemical compounds, and complete biodegradation leads to mineralization (Bennett 1988). The process of decomposition of organic materials is chiefly microbial, and fungi play an important role, as they are more active in carbon assimilation than bacteria and actinomycetes.

Lignocelluloses waste (LCW) refers to discarded plant biomass composed of cellulose, hemicellulose, and lignin. LCWs may be grouped into different categories such as wood residues (including sawdust and paper mill discards), grasses, waste paper, agricultural residues (including straw, stover, peelings, cobs, stalks, nutshells, non food seeds, bagasse), domestic wastes (lignocellulose garbage and sewage), food industry residues, municipal solid wastes, and the like (Qi et al. 2005; Roig et al. 2006; Rodríguez et al. 2008; Godliving 2009). The cellulose content of paper is degraded by fungal cellulase, a cellulolytic enzyme. It is capable of degrading crystalline forms of cellulose. The enzyme is composed of three enzyme species: endo-β 1, 4 glucanase, exo-β-1, 4-glucanase (cellobiohydrolase), and β-glucosidase (cellobiase). The three enzyme groups work synergistically to hydrolyze crystalline cellulose. The net effect is the release of carbon dioxide, and this has been used as an index to find out decomposition of cellulose (Paul 1992). Biodegradation of lignocellulose by fungi is of interest both because of the need for decay prevention in wood and wood products and because of the potential utility of degradative processes in biotechnological applications (Goodell et.al. 1997).

Due to an increase in the population of Visakhapatnam city, India, the accumulation of paper or wood waste is increasing, and the other reason is the establishment of many small scale industries whose raw materials, byproducts, and wastes are primarily comprising wood or paper or cellulosic waste. The increase in this kind of waste leads to heavy pollution, releasing obnoxious odours, which may also spread diseases. In order to overcome these kinds of problems associated with the cellulosic wastes, their degradation is to be well studied and understood to apply this knowledge in pollution control, for which cellulose-degrading fungi play an important role. The present study is undertaken to meet the respective need.

MATERIALS AND METHODS

Procedure for Sample Collection

The soil samples for screening of lignocellulolytic fungi were collected with a sterile plugged test tube, which was opened in the field to collect a sample and plugged immediately after collection of samples. Simultaneously, the same soil was collected separately to carry out analyses of certain physico-chemical parameters of the soil. This test tube was taken into the laboratory and opened in laminar airflow.

Isolation of Lignocelluolytic Fungi from Soil Samples

The prepared serial dilutions were inoculated in the Czapeck's Sucrose-Nitrate-Agar Medium (Booth 1971) under the laminar air flow in Petri dishes and then incubated at $28^{o} \pm 1^{o}C$. Further sub cultures were made and pure cultures were preserved in the laboratory for further studies.

Biodegradation Studies

The pure cultures of the *Aspergillus flavus*, Link ex Gray (Moniliaceae), *Penicillium chrysogenum* (Moniliaceae), *Thom* and *Rhizopus nigricans* Ehrenberg (Mucoraceae) were tested for biodegradation studies. From the pure culture, a loopful of fungal spores along with mycelia were inoculated in 100ml culture bottles under sterile conditions in 25ml Czapeck's Sucrose-Nitrate- Agar Medium in laminar air flow. After inoculation the culture bottles were incubated at $280 \pm 1^{o}C$. The growth of fungus appeared after 48 hours of inoculation.

Determination of CO_2 Release during Biodegradation

When biodegradation is complete, the end products are mostly carbon dioxide and water. Hence in the present study the rate of biodegradation was estimated in terms of Carbon dioxide released. To the culture bottles with five – day old fungal culture, 500mg of the substrate paper was added in pieces. Small test tubes containing 5ml of 0.1N sodium hydroxide were suspended with the help of a thread. The culture bottles were closed with stopper and sealed to ensure airtight condition and incubated at $28^{o} \pm 10\ ^{o}C$. The CO_2 release during biodegradation was absorbed by sodium hydroxide in the vials. During estimation, the content of vials was quantitatively transferred to a flask, followed by the addition of 5ml of saturated solution of barium chloride to precipitate the CO_2 as

barium carbonate. Two or three drops of phenolphthalein were added. The residual amount of sodium hydroxide in the flask was measured by titrating against 0.1N hydrochloric acid. The end point is the disappearance of pink colour (Gaur et al. 1971).

Estimation of Cellulose

To 1 ml of the sample 10ml of anthrone reagent was added and mixed well in a test tube. The tubes were heated in a boiling water bath for 10 min. These were cooled and the color developed was measured at 630 nm. A blank with anthrone reagent and distilled water was also tested (Sadasivam and Manickam 2005).

RESULTS AND DISCUSSION

The physico-chemical characteristics of the selected (dump yard and dumping yard) soils are given in Table 1. The results, i.e. pH in the range of 7.8 to 7.9 – slightly basic, show the favorable conditions for the growth of fungi. Fungal species, which colonize in the dumping yard soils, have been isolated, and their biodegradation potentials were estimated through CO_2 release method, the results of which are presented in Fig. 1. From these results it can be concluded that *Penicillium chrysogenum* and *Aspergillus flavus* are fast cellulose degrading fungi, and as *Aspergillus flavus* is abundant in occurrence. Further work was carried out to compare the biodegradation potential of *Aspergillus flavus* with a standard *Phanerochaete chrysosporium*.

Table 1. Physico-Chemical Parameters of Dumping Yard Soils

Name of sampling Area	pH	Conductivity (milli mhos)	Moisture content (%)	Organic matter (%)
Paper recycling unit, Vellanki (top layer)	7.84	0.25	207.8	0.9
Paper recycling unit, Vellanki (30cm)	7.90	0.23	144.3	1.26
Sea horse junction Dump yard (top layer)	7.8	0.29	69.4	1.8
Sea horse junction Dump yard (30cm)	7.87	0.27	28.9	2.64
Dumping yard at Kapulauppada(top layer)	7.65	0.89	45.6	2.36
Dumping yard at Kapulauppada(30cm)	7.48	0.78	28.7	2.64
Dump yard at Shivaji Park(top Layer)	7.02	0.77	72.8	1.45
Dump yard at Shivaji Park(30cm)	7.23	0.72	65.8	1.68
Gitam College (Top layer)	6.98	0.25	36.9	1.62
Gitam College (30cm)	7.06	0.28	35.2	1.74

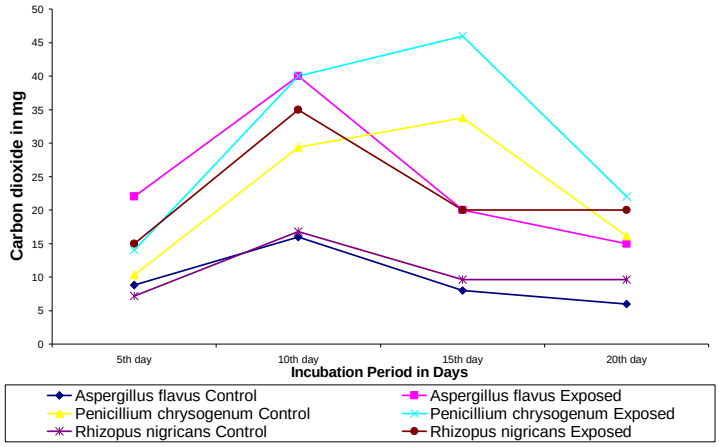

Fig. 1. CO_2 release (mg) during biodegradation of fungi isolated from soil samples

The various levels of biodegradation are due to the activities of fungal enzymes. These fungi are capable of producing enzymes such as endoglucanase, exoglucanase, and β-glucosidase in their system (Fahnrich et al. 1981; Schabel 1981; Kannan et al. 1990). Cellulose enzyme is capable of degrading crystalline forms of cellulose of endo β-1,4glucanase, and exo-β-1,4glucanase (cellobiohydrolase) and β-glucosidase (cellobiase). The net effect of these three enzymes is to rapidly decrease the polymer length with a slow increase in reducing group (Conghlan 1989).

Figure 2 summarizes the CO_2 released during biodegradation in a comparative account for *P.chrysosporium* and *A.flavus*. On the fifth day of degradation the CO_2 release by *P.chrysosporium* was observed to be 11.29 mg, whereas it was 6.46 mg in *A.flavus*. On the tenth day of degradation CO_2 evolved by *P.chrysosporium* was at a maximum of 18.26 mg, and that of *A.flavus* was 16.06 mg. On the fifteenth day of degradation the CO_2 release by *P.chrysosporium* and *A.flavus* was 10.88 and 10.12, respectively. After the twentieth day of degradation the CO_2 release was observed to be 7.04mg by *P.chrysosporium* and 6.82mg by *A.flavus*.

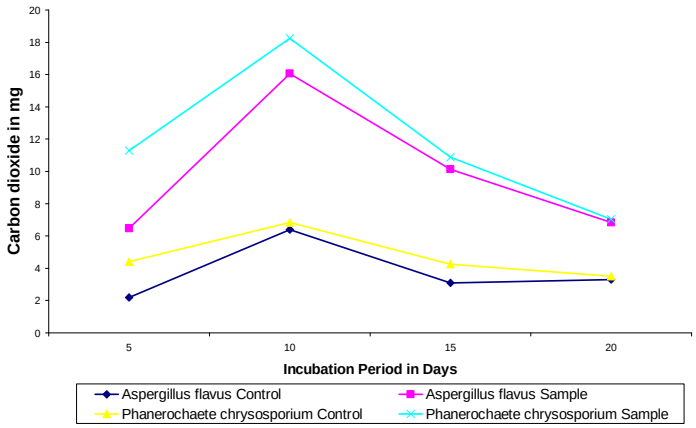

Fig. 2. CO_2 release during degradation by *Phanerocheate sp and Aspergillus sp.*

Figure 3 reveals the extent of cellulose (paper) in µg degraded by the two species *P.chrysosporium* and *A.flavus*, from which it can be observed that cellulose degradation was effectively achieved by *Phanerochaete* sp. and *Aspergillus* sp., wherein *Phanerochaete* sp. showed the greater effect. From the results shown in Figs. 2 and 3, the experiment was further carried out for application of these fungal species in the natural environment by introducing them in the soil, and the results are described below.

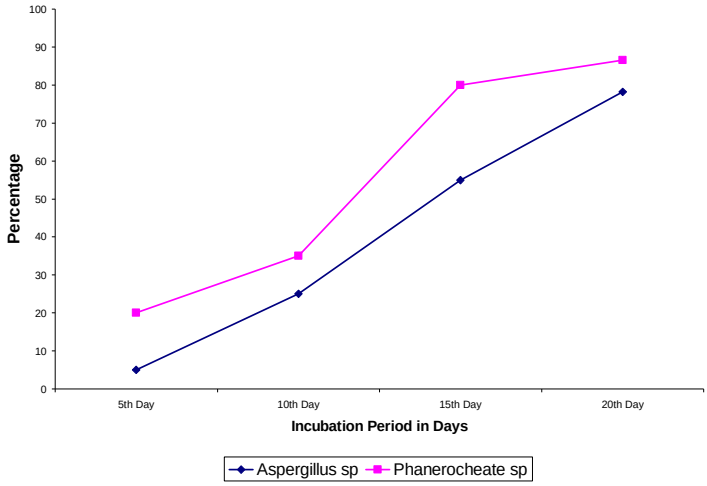

Fig. 3. Percent degradation of cellulose (paper) by fungi in potato dextrose agar

Figure 4 presents the CO_2 release during the biodegradation of baggase in soil. The results observed can be summarized as follows: The CO_2 release during the 5th, 10th, 15th, and 20th days was in the order of sterilized *P.chrysosporium*, showing the greatest effect, followed by sterilized *A.flavus*, the next being unsterilized *P.chrysosporium*, followed by unsterilized *A.flavus*, and the least effective being the unsterilised soil with the native species.

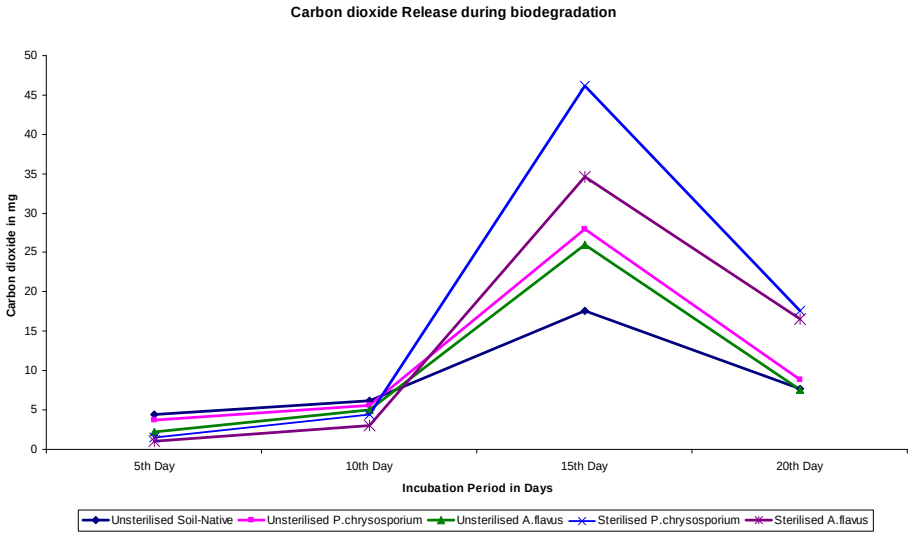

Fig. 4. CO_2 release (mg) during biodegradation of cellulose by selected fungi in soil samples (Temperature $28^o \pm 1^oC$)

Figure 5 elucidates the biodegradation of cellulose by fungi, and it was observed that the degradation achieved a maximum with sterlized soils having *P.chrysosporium* and *A.flavus*, and then by unsterilized soils, in which the maximum was shown by *P.chrysosporium* , followed by *A.flavus*, and then by unsterilized soil.

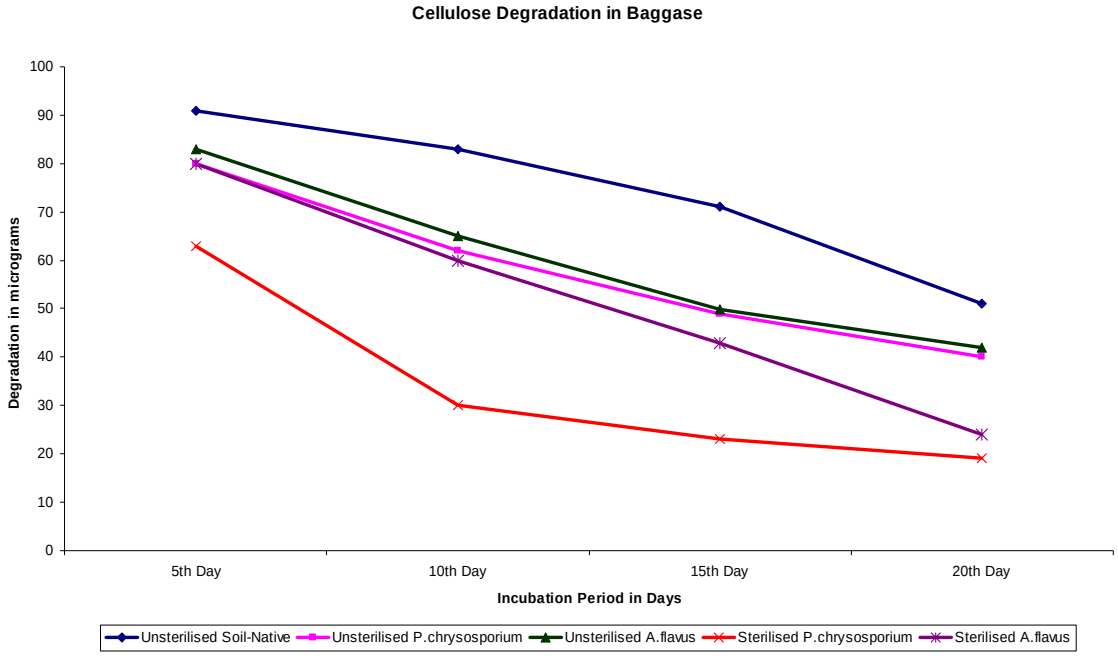

Fig. 5. Decrease in Cellulose content due degradation (µg) in soil samples

The fact that there were different levels of biodegradation in sterilized vs. unsterilized soils is attributed to the competition with indigenous species and the nutrition requirements available. The unsterilized soils have microflora indigenous to them, and the types of microbes present depend on upon many factors such as the type of feed, storage conditions, and weather conditions, etc. On wetting the substrate, the microbes become active and start growing by getting nutrients from the substrate. When the pure cultures of the fungi are inoculated, the inoculum has to compete with the indigenous Microflora. The competition between the two decides the fate of treatment process (Kamra et al. 1998).

Table 2 represents the physico-chemical characteristics of soil of pre- and post-degradation studies, and from these results it can be seen that there was a decrease in the pH of the soils, which is attributed to the degradation by the fungal species. Similar results were observed in the experiments conducted by Kaviyarasan et al. (2003). The degradation in terms of decrease in pH was also observed in the studies conducted by Artuchela et al. The decrease in the carbon content and nitrogen content in the soil characteristics is due to the degradation process that has been taken place, which further gives fertility to the soil by altering the C/N ratio of the soil.

Table 2. Soil Characteristics during Pre- and Post-Exposure to the Biodegradation

Type of soil	Name of the species	pH		Conduc-tivity		Organic Carbon		Nitrogen		C/N RATIO	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Un-steril-ised	*P.chrysosporium*	7.5	5.28	.77	1.33	.66	0.45	.03	0.12	8.25	3.75
	A.flavus		5.46		1.03		0.58		0.1		5.80
Steril-ised Soil	*P.chrysosporium*		5.19		1.70		0.40		0.15		2.66
	A.flavus		5.26		1.67		0.38		0.11		3.45

CONCLUSIONS

The measurement of carbon dioxide release during the biodegradation process may be used as an index of cellulose decomposition (Moore and Landecker 1972). From this study it is to be concluded that fungi such as *Phanerocheate chrysosporium* and *Aspergillus flavus* are capable of producing cellulose enzymes at a faster rate to decompose the substrate cellulose and release more CO_2. Hence, these fungi potentially can be utilized effectively as agents of biodegradation in waste recycling processes. All these observations confirm that the test fungi produced the enzyme *in vitro*. But these fungi, when inoculated to the lignocellulosic substrates, showed enhanced production of enzymes, as reported by Ardon et al. (1997). He also demonstrated that *P. ostreatus* exhibited enhanced enzymatic activity by the addition of cotton stalk extract.

The results of the research conducted so far indicate that there do exist some strains that are able to degrade lignin and cellulose and cause an increase in digestibility of lignocellulose in comparison to *P.chrysosporium*. Therefore, we need microbes that have specific properties as discussed earlier (Kamra 1998). From the present results one can conclude that *P.chrysosporium* and *A.flavus* are fast cellulose biodegrading fungi. This study reveals that fungi such as *P.chrysosporium* and *A.flavus* are capable of producing cellulose-degrading enzymes at a faster rate to decompose cellulose and release more CO_2, and hence these fungi can be utilized effectively as agents of degradation in waste recycling process. The soil fertility can be easily amended from fertility viewpoint with the help of locally available fungi.

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