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Full Length Research Paper

Biodegradation of some agricultural residues by fungi in agitated submerged cultures

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Digestibility of agricultural residues in animal feeding is deeply dependent on the amounts and types of their fibers. Biological treatment of agricultural residues is a new method for improvement of digestibility. Therefore, the capacity of a few fungi in biodegradation of some agricultural residues was studied. Losses of crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) of wheat, barley, rice, wood, and pea straw were investigated in agitated submerged culture during biodegradation by fungi. Biodegradation of the plant residues is dependent on the plant and fungus species. The biodegradation order of plant residues was pea>barley>wheat>rice>wood. *A. terreus* and *T. reesei* were more able to degrade the easy degradable plant residues. Rice and wood were degraded more by *Armillaria* sp., *Polyporus* sp. and *P. chrysosporium*. Crude fiber, NDF and ADF of agricultural residues were reduced more by *P. chrysosporium*. Generally, the reduction of agricultural residues NDF by fungi was more than their ADF. However, *Polyporus* sp. decreased ADF of wheat straw more. Thus, for improvement of digestibility of agricultural residues, the treatment by white-rot fungi may be recommended.

Key words: Agricultural residues, basidiomycetes, deuteromycetes, fibers.

INTRODUCTION

Lignocellulosic materials are the most abundant agricultural residues in the world. They are constantly being replenished by photosynthesis. Thus, microorganisms able to degrade these compounds, including fungi as well as actinomycetes and other bacteria, have an important role in increasing their digestibility. Biological treatment of agricultural residues is a new method for the improvement of their digestibility (Jalk et al., 1998).

Although cellulolytic fungi occur in all major fungal taxa (Coughlan, 1985), there are relatively few groups of microorganisms that can produce the ligninolytic enzymes. The most efficient lignin degrading microorganisms are the white-rot fungi (Falcon et al., 1995; Orth and Tien, 1995). Hence much of the research on the lignin-degrading enzyme systems has centered upon the enzymes of the white-rot fungi specially *Phanerochaete Chrysosporium*. However, there is considerable documentation on the bioremediation and biodegradation of the lignin-related compounds by the imperfect fungi (Betts and Dart, 1988; Iyayi and Dart, 1982). The objectives of this study were to characterize and directly compare the biodegradation capacities of a number of saprophytic fungi on some agricultural residues.

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Abbreviations: CF, crude fiber, NDF, neutral detergent fiber, and ADF, acid detergent fiber.

MATERIALS AND METHODS

Microorganisms

Fungi including the deuteromycetes, *Aspergillus terreus*, *Trichoderma reesei*; the basidiomycetes, *Armillaria* sp., *Polyporus* sp., and *Phanerochaete chrysosporium* were obtained from the Microbiology Laboratory of the Science Faculty of Isfahan University in Iran.

Media and culture conditions

Stock cultures of fungi were maintained at 4°C on commercially prepared Potato Dextrose Agar (PDA, Merck). The vegetative mycelia of fungi were inoculated in 250-ml Erlenmeyer flasks containing 1.0 g of milled agricultural residues (<2 mm) in 100 ml distilled water. Agricultural residues were wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), rice (Oryza sativa), pea (*Pisum sativum*) straws and wood shavings. All inoculated cultures were incubated at 28°C with (120 rev min-1) agitation.

Biodegradation Measurements

After 3 weeks with filtration of culture media, agricultural residues and fungal biomasses remaining on the filter paper were dried in an oven at 75°C. Weight, crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) losses of each sample were calculated with respect to their blanks. Crude fiber, NDF, and ADF of these samples were measured according to the gravimetric methods.

Gravimetric methods for the analysis of dietary fiber have been divided into two groups. The first (detergent acid and neutral methods, ADF and NDF) consists in the gravimetric determination of residues previously treated with acid and neutral detergent solutions. These methods determine the insoluble fraction and their individual components (Van Soest´s methods). The second group uses amylolytic and/or proteolytic enzymes determination of insoluble and soluble fraction. Although, the first methods sometimes leads to overestimation due to the incomplete removal of starch, proteins and fats (Robertson and Horvath, 1992), in this study samples were analyzed for acid and neutral detergent fibers by the method of Van Soest (Van Soest and Wine, 1967, 1968) and crude fiber by the gravimetric method #7071 described in AOAC (1984, 1995). After the treatments, all samples were ashed 2 h at 550°C, cooled and weighed. Crude Fiber, ADF, and NDF were assayed without residual ash.

Statistical analyses

The experimental design was completely randomized with 3 replications. Data was statistically analyzed for standard deviation. Means were calculated and Duncan's new multiple range test was used to compare the biodegradation of the agricultural residues by fungi.

RESULTS AND DISCUSSION

Mean of the culture pHs during 3 weeks biodegradation of agricultural residues is shown in Table 1. Culture pH was as an index of fungal activity, since wherever the pH was low, fungal activity was high, and vice versa. Easily degradable (pea, wheat, and barley) culture media had a good growth condition for fungi. With the exception of pea culture medium, they had significantly lower pHs compared to wood and rice cultures. In addition, the imperfect fungi compared to the basidiomycetes, decreased pH of these cultures more effectively. On the other hand, the basidiomycetes especially *Polyporus* sp. decreased pH of wood and rice cultures effectively. Overall the order of pH of cultures was: pea>wood>rice>barley>wheat.

Biodegradation of plant residues by fungi is dependent on the plant and fungus species. Weight loss of residues varied markedly with plant species (Table 2). The order of residues reduction was pea>barley>wheat>rice>wood. This is in agreement with the other report in which legumes i.e. pea, degrades faster than the other crops (Blackshaw and Lindwall, 1996). The N content and C/N ratio of wheat, barley, and pea were about 0.28, 0.47, 0.73% and 156, 88, 58, respectively. It has been shown that lignin, C/N ratio and N contents of crop residues would affect their decomposition rate (Muller et al., 1988; Summerell and Burgess, 1989). However, Christensen (1985) has reported that barley and wheat had similar rates of disappearance. Residues like rice straw and wood shavings, with high lignin and low readily available C and N contents generally have slow decomposition rates (Janzen and Kucey, 1988; Parr and Papendick, 1978). Our results indicate that the decomposition rates of rice straw and wood shavings by the *Polyporus* sp. and *P.chrysosporium* were higher than the other fungi (Table 2). Wheat, barley, and pea were decomposed easily by all fungi, though *Armillaria* sp. had a lower capacity. Wheat and barley decomposition rate by the imperfects were considerably higher (about 30% in 3 weeks).

Crude fiber loss of wheat, barley and rice straws are shown in Table 3. Crude fiber reduction by *P. chrysosporium* was significantly high among the fungi. In addition, wheat and rice NDF and ADF decreased by this fungus effectively (Table 4). Wheat and rice straws NDF decreased more than their ADF by all fungi. However, wheat straw ADF decreased more by *Polyporus* sp., surprisingly.

Biodegradation of crop residues by fungi is dependent on the plant and fungus species. Residues reduction varied markedly with plant species. However, there was a relatively good relation between residues biodegradation and fungi lignocellulolytic enzyme activities. The deuteromycetes grew fast and produced more hemicellulolytic and cellulolytic enzymes in easily degradable plant residues like wheat, barley, and pea (result not shown). Therefore, they reduced more of these crop residues. The basidiomycetes *Armillaria* sp., *Polyporus* sp. and *Phanerochaete chrysosporium* could produce equal or higher amounts of the lignocellulolytic enzyme on the plant residues with high lignin and C/N ratio (result not shown) compared to the other fungi. Consequently, the basidiomycetes degraded these plant residues more.

Table 1. Extract pH of agitated submerged cultures of agricultural residues treated with different fungi.

Fungi	Wheat	Barley	Rice	Wood	Pea
A.terreus	5.1 b	6.4 ab	6.53 ab	7.27 a	7.2 a
T.reesei	5.4 b	6.47 ab	6.5 ab	7.17 a	7.13 a
Armillaria sp.	5.8 b	6.97 ab	7.37 a	7.2 a	7.17 a
Polyporus sp.	5.75 b	5.77 b	6.1 b	6.3 b	7.7 a
P.chrysosporium	5.7 b	5.2 b	5.57 b	7.13 a	6.97 a

Values within a row followed by different letters are significantly different at the 0.05 probability level.

Table 2. Weight loss (%) of agricultural residues in agitated submerged cultures treated with different fungi.

Fungi	Wheat	Barley	Rice	Wood	Pea
A.terreus	29.9 ab	33.1 ab	21.1 b	1 c	40.4 a
T.reesei	33.2 ab	32.2 ab	22 b	1.3 c	42.4 a
Armillaria sp.	24.1 ab	22.5 ab	13.5 b	2.6 c	30.4 a
Polyporus sp.	28 a	28 a	25.5 a	8.77 b	20.5 a
P.chrysosporium	30 a	36.9 a	30.9 a	5.84 b	40.1 a

Values within a row followed by different letters are significantly different at the 0.05 probability level.

Table 3. Crude fiber loss (%) of agricultural residues in agitated submerged cultures treated with different fungi.

Fungi	Wheat	Barley	Rice
A.terreus	15.38 a	13.38 a	4.17 b
T.reesei	19.89 a	16.89 a	1.67 b
Armillaria sp.	5 a	5 a	4 a
Polyporus sp.	14.92 a	13.92 a	6.46 b
P.chrysosporium	45 a	50 a	10.21 b

Values within a row followed by different letters are significantly different at the 0.05 probability level.

Table 4. Losses (%) of neutral detergent and acid detergent fibers (NDF and ADF) of agricultural residues in agitated submerged cultures treated with different fungi.

Fungi	Wheat		Rice	
	NDF loss (%)	ADF loss (%)	NDF loss (%)	ADF loss (%)
A.terreus	2.84 a	1.67 ab	0.82 ab	0.37 b
Polyporus sp.	1.61 a	3.23 a	1.88 a	1.42 a
P.chrysosporium	11.1 a	10.9 a	8.01 b	6.86 b

Values within a row followed by different letters are significantly different at the 0.05 probability level.

Plant residues CF, NDF, and ADF decreased by *P. chrysosporium* more than the other fungi. Thus, this fungus is the most effective fibrilolytic microorganism. Generally, wheat and rice straws NDF (cell wall + hemicellulose) decreased more than their ADF (cell wall) during biodegradation by the microorganisms. However, *Polyporus* sp. by producing lower amounts of xylanase and higher carboxymethyl cellulase and ligninase reduced wheat straw ADF more than its NDF. This finding shows that how these fungi can increase the digestibility of plant residues for nutrition of domestic animals.

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