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Farm Scaling up Biological Treatment by Solid State Fermentation to Invest Rice Straw as a Livestock Feed in Egypt

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ABSTRACT

Feeding only with a highly lignified material such as rice straw does not provide enough nutrients to ruminants due to the low-produced nutritive value. To improve the feeding value of rice straw for ruminants, to overcome their inherent barriers to rumen microbial fermentation, farm scaling up for fifty tons of rice straw was subjected to biological treatment by *Trichodermaharzianium* F-418 for 8 days under solid state fermentation system. Chemical analysis revealed that, acid detergent fiber, natural detergent fiber, cellulose and hemicellulose were decreased during treatment by 13.80, 20.06, 16.81, and 32.79% respectively. The enzymes: Fpase, CMCase, cellobiase and xylanase included during fermentation were estimated at periodic intervals, and reached 237.40, 144.72, 11.22, and 3490.35 U/g DM, respectively, after 8 days. Soluble sugars increased by fermentation time from 6.47 to 17.63 mg/g DM. The dry matter loss was 12.27 %. The end product contained 9.92% crude protein. Using ligninolytic fungi may be one potential alternative to provide more practical and environmental-friendly approach for enhancing the nutritive value of rice straw.

KEY WORDS: Rice straw, feeding,*Trichodermaharizianum*F-418, biological treatment, solid state fermentation.

1.INTRODUCTION

Huge quantities of agro-industrial biomass are produced worldwide annually, that is including about 900 million tons of rice straw (RS). These materials are potential feed resources for ruminant livestock. However, their use is limited because of the high indigestible fiber components. The burning of these materials in the field is, environmentally, of pollution concerns. Biodegradation of cellulose and hemicellulose content of agro-biomass and convert it into microbial mass and bioactive materials is an alternative method for enhancement the quality of these materials for used as animal feed. Solid state fermentation (SSF) is the growth of microorganisms on moist solid materials, in the absence or near absence of free water (1), has been used for enhancement of nutritive value of agricultural biomass as animal feed(2). Rice straw can be used as a potential substrate for biomaterial production in SSF. Cellulose and hemicelluloses are the main components in this agricultural biomass and during the solid state fermentation process, these materials break down into compounds of smaller molecules and high quality components (such as glucose and microbial protein) and their digestibility were improved for use as animal feed.

Microorganisms that are selected for solid state fermentation should have the capability to produce sufficient quantity of the appropriate enzymes to degrade the specific cellulose and hemicellulose in the substrate. These enzymes are the most important catalyzers in feed industry, and consumption of these enzymes can increase the degradability and digestibility of agro-biomass for ruminants. The objective of this study was to apply Farm scaling up biological treatment by solid state fermentation for reduction of lignocellulose content in rice straw at short incubation time to assess the potential use of this method to improve the nutritive value of RS for its investment as animal feed, to overcome nutritional Gabe in Egypt.

2.MATERIALS AND METHODS

2.1Substrate

Rice straw, collected from the local rice field, was obtained from the collection zone of Arab Authority of Manufacturing at El-Khattara-Fakous -El Sharkia. Governorate, Egypt. Sugar beet pulp (SBP), a by–product of beet sugar industry, was obtained from Abokorkas Sugar Factory, El-Minia, Egypt. Molasses was obtained from Egyptian Sugar and integrated company El-Hawamdia, Giza, Egypt. Urea tri-supper phosphate and magnesium sulfate, fertilizer Grade, were purchased from local market. Crusher/Chopper, Loader and Backhus machines were from Arab Authority for Manufacturing, Kader Factory, Egypt.

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Fig.1. Rice straw crusher /chopper

Fig. 2.crusher /chopping rice straw

Fig. 3.Loader

Fig. 4.Backhus

2.2 Microorganism and preparation of spore suspension

T. harziamum F-418, used in this study, was obtained from Microbial Chemistry, Department. National Research Center, Egypt. It was maintained on potato dextrose agar (PDA) slants, stored at 4^{o}C, and sub-cultured at 30^{o}C for 3 days every two weeks. For the preparation of spore suspension, 10 ml of sterilized water with 0.1% Tween-80 solution was added to a 5-day old slant of the fungus and the surface of the culture was scratched with sterilized loop and agitated thoroughly using a shaker to suspend the spores. The number of spores was adjusted to a 10^{6} spores/ml and used as inoculum throughout the study.

2.3 Fungal strain preparation

Sugar beet pulp (SBP)was employed as a complete medium for cultivating *T. harziamum* F-418 under solid state fermentation system as follows: 50 g of SBP was introduced in each 500 ml capacity conical flask, after which 100 ml water was added to moisten the SBP to be nearly 65% (v/w). The flasks were sterilized by autoclaving at 121^{o}C for 20 min. Cooled sterilized flasks were inoculated with a 5 ml of fungal spore suspension (10^{6} spores/ml) and the flasks were incubated statically at 30^{o}C for 96 hrs.

2.4 Propagation of fungal inoculum

Each flask from the above was transferred to 10 l capacity conical flasks contained 1 kg sterilized moistened SBP and incubated statically at 32^{o}C for 96 hrs.

2.5 Rice straw preparation

Rice straw was crouched and chopped to 15-20 cm pieces using the crusher/chopping machine (Fig. 1and 2). An amount of 50 tons were bedded on cement plotted ground at high about 90 cm. Two Rows were prepared, using a loader (Fig. 3), at a length of 75 meters.

2.6 Mixing and moistening the prepared rice straw

A machine called Backhus (Fig. 4), prepared and provided with water source, was used for mixing, homogenizing and moistening the two Rows of chopped straw (the water quantity was calculated to give solid: liquid ratio 1: 2. At the end of moistening, liquid medium containing fungal culture, molasses, urea, tri-calcium phosphate, magnesium sulfate (patent) was sprayed on the moistening rice straw and then mechanically mixed. The mixing and homogenizing were done at intervals every 12 hrs to get rid of the inlet heat formed during fermentation.

After 8 days samples were taken for enzymes assay from the fermented straw. At end of fermentation the fermented straw was mixing at intervals every 4 hrs for solar drying. The end product was subject to chemical analysis.

2.7Chemical analysis

Dry matter (DM) loss was determined by the difference between dry weight before and after fermentation and described as percentage of initial weight. Acid detergent fiber (ADF)and neutral detergent fiber (NDF) were determined by the detergent system (3), and acid detergent lignin (ADL) by the method described by A.O.A.C (4). Hemicellulose contents were estimated as the difference between NDF and ADF, while cellulose content was the difference between ADF and ADL. Crude protein was measured by micro-Kejldahel method described in A.O.A.C (5). Ash was carried out on dried sample at 105C by ignition 3 samples each 50 g in muffle furnace at 800 C for 5 hours , and the residual ash was calculated as percentage from the dried initial weigh according to the method described in A.O.A. C (6).

2.8Enzyme extraction

At periodic intervals, 20 samples each of about one kg were taken from different location and mixed well. Five samples each of 50 g were taken, and 500 ml of citrate buffer (50 mM, pH 5.0) were added to each 50 g fermented substrate and shaken at 200 rpm for 1h. The mixture of all samples was filtered through Whatman No.1 filter paper. The filtrate was stored at 4^{o}C for assays of enzymes activity.

2.9Enzymes assay

Activity of cellulase (using filter paper; FPase)was determined as described previously (7). Activity of FPase was assayed by incubating 1 ml of extracted enzyme, 50mg of Whatman filter paper No.1 (1×6 cm) as a substrate, and 1 ml citrate buffer (50 mM, pH 5) incubated at 50^{o}C for 30 min in a shaking water bath. Activity of carboxymethyl-cellulase (CMCase) was determined by estimation of the reducing sugars liberated by the action of the extracted enzyme on carboxymethylcellulose (CMC) as a substrate. A reaction mixture containing one ml of 1% CMC,1 ml citrate buffer (50 mM, pH 5), and1 ml of the extracted enzyme was incubated at 37^{o}C for 30 min in a shaking water bath. The reaction was stopped by adding 1 ml of potassium sodium tartrate (Rochelle salt). Cellobiase activity was estimated using glucose oxidase kit (9). Xylanase activity was estimated by a reaction mixture containing 1 ml of 1% xylan(oat, Sigma) as a substrate, 1 ml of the extracted enzyme, and 1 ml citrate buffer (50 mM, pH 5), and incubated at 37^{o}C for 30 min (10). At the end of the incubation time the concentration of xylose was determined by DNS method with xylose as the standard. One unit of enzyme activity was described as the amount of enzyme that produce 1miro-mol of reducing sugars in 1 min under standard assay conditions. Concentration of glucose units was estimated using the DNS method (8).Amino acids were analyzed by Amino Acid Analyzer (11) after 0.5 g of protein was hydrolyzed with 10 ml of 6 N HC1 under vacuum at 110 C for 24 h. Cystine plus cysteine was determined after pretreatment with performic acid.

3.RESULTS AND DISCUSSION

3.1Enzymatic activities

Enzyme activities of *T.harzinium*F-418 in solid state fermentation of rice straw are shown in Table 1. Cellulase activity reached 237.4 U/g DM after 8 days of fermentation. Activity of CMCase reached 144.72 U/g DM after 8 days, whereas cellobiase activity was11.22 U /g DM. The highest activity obtained, in this study, was for xylanase, which reached 3490 U/gDMafter 8 days of fermentation. Xylanasecatalyzes xylan to xylose(15,16).Many reports have shown the high ability of *Tricoderma sp*. for production of xylanase (17, 18, 19,20). On other hand, higher activities of cellulases and β-glucosidase were reported by *Aspergillusniger* grown under solid state fermentation conditions. Kang *et al*. (2004)have reported that, 129 units of CMCase activity using *Aspergillusniger KK2* and RS as substrate in solid state fermentation (12).Kalogeris*et al*. (2003) have reported that, 170 units of CMCase activity was obtained using *Thermoascusaurantiacus* and wheat straw as substrate(13). Different high levels of xylanase activity were produced by *A.carneus*M34 when cultivated under SSF technique (14). Other studies have reported the ability of *T.harzinium*F-418for production of FPase, CMCase, β-glucosidase and xylanase (17,19,21) These enzymes are important in the process of cell wall degradation of biomass. The data clearly indicated high variation for production of enzyme using solid state fermentation. Carbon source and microorganism are the most important factors for production of these enzymes in SSF. In the present study, results show that, rice straw is a more suitable carbon source for production of lignocellulolytic enzymes by *T. harzinium*F-418in solid state fermentation.

Table 1: Enzymes activity of *T.harzinium*F-418 grown on rice straw after 8 days under solid state fermentation

Incubation time (day)	Enzyme activity (U/gDM)			
	FPase	CMCase	cellobiase	**Xylanase**
2	89.25	64.85	2.95	1362.55
4	142.60	96.84	6.55	1967.73
6	186.35	119.64	8.82	3425.20
8	237.40	144.72	11.22	3490.35

3.2Lignocelluloses content

The effects of *T. harizianium*F-418 on lignocellulose contents of rice straw are shown in Table 2. Fermentation has aneffect on acid detergent fiber (ADF), neutral detergent fiber (NDF), cellulose and hemicellulose contents of RS but not on acid detergent lignin (ADL). The results also suggested that, 8 days fermentation by *T. harizianium* F-418 is appropriate to achieve reduction of lignocelluloses with low loss in DM. Duration of fermentation is of practical importance in the biological treatment of biomass, as the growth rate of microorganism used for this purpose should be high, and thus, achieved the target of improving quality of the biomass in a short time. White rot fungi are widely studied in biological treatment, however, their growth rates are slow and need a long incubation period, often more than 21 days and even up to 2 to 3 months to be effective (22). Long incubation period is of disadvantage under farm-scale conditions because space and storage is limited in the farm and it is not economically acceptable forfarmers to keep the feed for a long time. On the other hand, long time incubation will increase the dry mater loss of fermented samples,which may reach more than 50%(23), and may also permitother undesirable microorganisms to grow.Results of this study show that, *T. harizianium*F-418 has faster growth rate than white rot fungi in biological treatment for reduction of lignocellulose contents in biomass.

Reduction rates of different components of lignocelluloses are reflected by the activities of the specific enzymes. Based on the obtained data of fermentation, 32.86% of the hemicelluloses were degraded, while only 16.32% of the cellulose was degraded within the same duration (Table 2). The higher degradation rate of the hemicelluloses than the cellulose is reflected by the significantly higher activity of xylanase, purportedly to hydrolyze xylan in hemicelluloses to xylose, when compared with the total activity of the cellulases.

Table 2: Effect of fermentation period on lignocellulose composition of rice straw (% of dry matter) by *T. harizianium*F-418

Incubation time (days)	Neutral detergent fiber	Acid detergent fiber	Acid detergent lignin	Hemicellulose	Cellulose
0	79.26	53.64	5.47	25.62	48.17
2	73.92	52.47	5.54	21.45	46.93
4	67.68	47.69	5.99	19.99	41.70
6	64.95	46.93	5.53	18.02	41.40
8	63.44	46.24	5.93	17.20	40.31

3.3Dry matter loss and reducing sugars

The effects of *T. hariziamum* F-418 on DM loss, and reducing sugar are shown in Table 3, as discussed previously, based on the enzyme activity and lignocellulose degradability after 8 days fermentation for the biological treatment of RS. At this incubation period, the DM loss was 12.27%, which is lower than that reported using white rot fungi (24).

Table 3: Effect of fermentation period by *T. harizianium*F-418on dry matter loss and sugar content of rice straw

Incubation time (day)	Reducing sugar (mg/g DM)	Dry matter loss (%)
0	6.47	-
2	8.52	3.74
4	15.16	7.77
6	16.o4	10.33
8	17.63	12.27

One of the problems of biological treatment using white rot fungi is the high DM loss as a result of long incubation period. The effect of five white rot fungi (*basidiomycetes*) for the improvement of the quality of oat straw was studied. Although 30 days of fermentation using *Phanerochaetechrysosporium* had shown enhancement in *in vitro* DM digestibility, but 42.3% of DM was lost due to the long incubation time required. Loss of 43% DM after biological treatment of wheat straw using *Daedaleaguercina* (white rot fungi) was reported (23,24).

Solid state fermentation using *T. harizianium*F-418 had good effect on increasing reducing sugar content of rice straw (Table 3). Enhancement of reducing sugar over incubation times was correlated with enzyme activity and reduction of lignocelluloses described earlier, cellulose and hemicelluloses are converted to soluble (partly sugars) materials during the incubation time. Reducing sugars, include glucose, xylose and mannose, have higher digestibility for the animals than their original macromolecules (cellulose and hemicelluloses), therefore, enhancing the concentration of these sugars is an additional advantage besides the reduction of lignocelluloses in improvement of the quality of biomass through biological treatment (25). Extensive attachment of mycelium spores of *T. harizianium*F-418 on the surface of treated rice straw, when compared with the untreated sample, suggesting the efficacy of *T. harizianium*F-418for production of lignocellulolytic enzymes and degradation of lignocellulose in rice straw.

One of the applications of solid state fermentation is the use of this technique for enhancement of biomass quality as animal feed. The microorganisms to be selected for SSF should be able to produce the appropriate lignocellulolytic enzymes at sufficient quantity to effectively degrade the fibers contents. The enzymes that had been

determined in this study, i.e.,cellulases and xylanase are among the widely used enzymes in animal feed additive for the degradation of lignocelluloses in feed. Huge quantities of agricultural biomass are produced worldwide annually and are potential sources of pollution if not appropriately managed. Although, these biomass can be used as animal feed, their high cell wall content is the inhibiting factor for their practical use; because cellulose, hemicellulose and lignin contents of these materials have negative correlation with their degradability and digestibility.

3.4Changes in chemical analysis of rice straw after biological treatment

Biological treatment under farm scale in the present study shows positive changes in the constituents of rice straw related to its feed value.Reduction of the cell wall, i.e. ADF, NDF, hemicellulose, and cellulose components, as shown in Table 4, is a useful indicator for improving the quality of lignocellulotic materials using biological treatment under SSF technique. In addition, the cellulolytic enzymes present in the treated biomass have an additional potential benefit when actively continued, in the rumen ecosystem, to further degrade the lignocellulosic contents of feed in the rumen. Enhancement in crude protein to be 9.98 (411.80%) is very important indicator to replace RS than concentrates in animal rations, whereas the percentage of reducing sugars after fermentation reached 270.77 %. Moreover, this enrichment will enable rumen microorganisms to colonize asrumen flora need nutrients for growth and metabolism (26). The present study emphasized that, biological treatment of rice straw effectively increased its nutritive value. Treated rice straw contained fermentable sugars (occurred from the action of enzymes), amino acids from the formed protein, and minerals from the additives of molasses as well as tri-super phosphate and magnesium sulfate due to an efficient microbial growth.

Table 4: Changes in chemical analysis of rice straw after biological treatment

Component	Before fermentation	After fermentation	Difference	% Changes
Dry matter	100	87.73	- 12.27	12.27
Organic matter	88.42	85.40	-3.02	3.42
Crude protein	1.95	9.98	+8.03	411.80
ADF	53.64	46.24	-7.40	13.80
NDF	79.92	63.44	-15.90	20.06
ADL	5.47	5.93	+0.46	7.07
Cellulose	48.17	40.31	-8.10	16.81
Hemicelullose	25.62	17.20	-8.40	32.79
Reducing sugars (mg /gDM)	0.65	1.76	+1.11	270.77

3.5The amino acid composition of microbial compared to alfalfa protein.

A comparison for the amino acid composition of microbial and alfalfa protein is represented in Table 5. The amino acid profiles of *T. harizianium*F-418 culture grown on rice straw have advantage in lycinie, arginine, glutamiic acid, proline, alanine and methionine and less in cysteine.

Table 5. Amino acid composition of microbial protein (g/100 g of protein) compared to Alfalfa protein

Amino acid	*T .harezianum* F-418	Alfalfa	Amino acid	*T .harezianum* F-418	Alfalfa
Lysine	8.00	6.70	Alanine	8.12	6.33
Histidine	1.96	2.53	Valine	6.79	6.70
Arginine	6.18	5.54	Methionine	1.99	1.36
Aspartic acid	8.30	12.54	Isoleucine	4.12	5.54
Threonine	4.70	5.12	Leucine	8.66	8.43
Serine	4.11	5.25	Tyrosine	2.41	3.72
Glutamic acid	18.49	11.33	Phenylalanine	3.69	5.75
Proline	7.51	5.10	Cystine/cysteine	0.41	1.40
Glycine	4.17	5.73			

CONCLUSIONS

Although several treatments have been used to improve th**e** degradability and voluntary intake of rice straw, such as physical or chemical treatments, the practical use of these treatments is still restricted in terms of safety concerns, costs and potentially negative environmental consequences. Using ligninolytic fungi may be one potential alternative to provide more practical and environmental-friendly approach for enhancing the nutritive value of rice straw. Moreover, the application of ligninolytic fungi to rice straw may be an alternative way to shorten the period of the incubation time, and decreasing the amount of chemicals, affecting some synergy. Many of treatments are carried out on Lab-scale and not practical for use on small- farms, or on industrial scale. This makes these treatments in many cases economically unprofitable for farmers as the benefits may be too low. However, small machines to grind or chop rice straw may be feasible. In our study, biological treatment of rice straw appears to be the most practical for use on farm- or on industrial- scale as no expensive machinery is required, the chemicals are relatively cheap, and the procedures to use them are relatively simple. In addition, the chemicals used in this treatment are not harmless and no safety precautions are needed for their use.

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