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Full Length Research Paper

Effects of *Aspergillus niger* (K8) on nutritive value of rice straw

M. F. Jahromi[1], J. B. Liang[1]*, M. Rosfarizan[2], Y. M. Goh[3], P. Shokryazdan[1] and Y. W. Ho[1]

[1]Laboratory of Industrial Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.
[2]Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.
[3]Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.

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The objective of this study was to evaluate the use of solid state fermentation for the improvement of the quality of rice straw as animal feed. Rice straw was fermented using Aspergillus *niger (K8)* with and without additional nitrogen source (urea). Cellulose, hemicelluloses, organic matter (OM), dry matter (DM), acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) contents of rice straw were determined before and after 10 days of fermentation. Fermentation has significant (P < 0.01) effect on NDF, but not ADF and ADL contents. Addition of urea as nitrogen source significantly reduced (P < 0.01) the NDF and hemicellulose contents of fermented rice straw. Cellulose content of the rice straw was not affected (P > 0.05), but crude protein (CP) increased significantly (P < 0.01) after fermentation. *In vitro* gas production technique was used to evaluate the effect of the biological treatment on activity of rumen microorganisms. Fermentation of rice straw using *A. niger* significantly reduced total gas production (P < 0.01), DM disappearance (P < 0.01) and acetate, propionate and total volatile fatty acids (VFA) production (P < 0.05). Results of the present study showed that solid state fermentation of rice straw using *A. niger* reduced lignocellulose content, but has negative effect on microbial activity in the rumen ecosystem, presumably due to antagonistic activity of *A. niger*, or other intermediate products from the fermentation, on the rumen microorganisms.

Key words: *Aspergillus niger*, biomass, solid state fermentation, biological treatment, *in vitro* gas production.

INTRODUCTION

Lignocellulosic biomass is a potential raw material for microbial production of feed, food, fuel and chemicals (Desgranges and Durand, 1990; Purkarthofer et al., 1993; Ruiz et al., 2006). One of the key problems for the effective utilization of this resource as raw material for chemical reactions and/or animal feeds is the low ability of its lignocelluloses to hydrolysis, which is attributable to the crystalline structure of cellulose fibrils surrounded by the hemicellulose and the presence of the lignin which prevents the degrading enzymes to act on. Biological conversion of major polymers in biomass to simpler constituents is preferred over chemical conversion and investigations have shown that nutritive values of lignocellulosic materials could be improved by fermentation (Panaloza et al., 1985). Cellulases are hydrolytic enzymes capable of degrading cellulose into smaller units. Various bacteria and fungi produce extra-cellular celluloses when they grow on cellulosic substrates (Moldoveanu and Kluepfel, 1983; Harchand and Singh, 1994). Madamwar et al. (1989) reported that solid state fermentation (SSF) using *Aspergillus niger* exhibited 30 to 80% higher enzymatic activities than conventional submerged

*Corresponding author. E-mail: jbliang@ibs.upm.edu.my. Tel: +60 3-8947 2132. Fax: +60 3 8947 2213.

Abbreviatins: OM, Organic matter, **DM,** dry matter, **ADF,** acid detergent fibre, **NDF,** neutral detergent fibre; **ADL,** acid detergent lignin; **CP,** crude protein; **SSF,** solid state fermentation; **VFA,** volatile fatty acids; **FID,** flame ionization detector; **GLM,** general linear model.

fermentation. The objective of the present study was to evaluate the potential of using the cellulolytic strain of *A. niger* (K8) for the degradation of cellulose and hemicellulose in SSF to improve quality of rice straw as animal feed. In order to determine the actual usefulness of the fermented rice straw as ruminant feed, *in vitro* gas production technique was used to evaluate the effect of this biological treatment on the fermentation activities of rumen micro organisms.

MATERIALS AND METHODS

Substrate and micro organism

Rice straw was collected from the local rice fields from the State of Selangor, Malaysia. The material was dried and ground to uniform size (mesh size 6) and stored in plastic bags at 4°C for use as the substrate in this study. *A. niger* (K8), a local strain isolated by the Laboratory of Industrial Biotechnology, Institute of Bioscience, Universiti Putra Malaysia was used for this study.

Preparation of spore suspension

A. niger was cultured in potato dextrose agar. Spore suspension of the fungal strain was prepared by washing 5-day old culture slants with sterilized saline solution (0.9% NaCl) with shaking vigorously for 1 min. Spores were counted by a haemocytometer to adjust the count to approximately 10^7 spores/ml.

SSF

SSF of rice straw was carried out in 500 ml Erlenmeyer flasks. 30 g of rice straw was put in individual flasks with 60 ml distilled water added to give moisture content of about 66%. No additional mineral was added to the culture. For the study on the effect of additional nitrogen source on the activity of *A. niger*, urea (1% of DM) was added to the rice straw as one of the treatments. The flasks were plugged with cotton and autoclaved at 121°C for 15 min. Each flask was inoculated with 10% (v/w) inoculums containing 10^7 spores per ml for the fermentation. Each of the 3 treatments; namely unfermented rice straw (control), fermented rice straw and fermented rice straw with urea were replicated 3 times. The flasks were incubated at 32°C for 10 days.

Chemical analysis

Dry matter (DM) content was determined by drying to constant weight at 103°C for 12 h followed by equilibration in a desiccator. Ash was determined after incineration for 4 h at 550°C (AOAC, 1990) while crude protein (CP) was determined by micro-Kjeldahl technique (AOAC, 1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the detergent system (Van Soest et al., 1991) and acid detergent lignin (ADL) was determined by method described by AOAC (1990). Hemicellulose content was estimated as the difference between NDF and ADF while cellulose content was estimated as the difference between ADF and ADL.

In vitro gas production

Gas production was determined by the procedure described by Menke and Steingass (1988). 500 mg of each treatment sample (triplicate) were weighed into 100 ml calibrated plastic syringes. Buffered mineral solution (Menke and Steingass, 1988) was prepared and placed in a water-bath at 39°C under continuous flushing with CO_2. Rumen fluid was collected after the morning feeding from two ruminally fistulated male cows that were fed an equal weight mixture of 40% concentrate and 60% hay twice daily at 08:00 and 18:00 h. Rumen fluid was collected from the rumen with a manually operated vacuum pump and transferred into two pre-warmed bottle. pH was determined immediately and after which the rumen fluid was transported to the laboratory, filtered through eight layers of cheesecloth and flushed with CO_2. Rumen fluid was added to the buffered mineral solution with constant stirring, while maintained in a water bath at 39°C. About 30 ml of buffered rumen fluid was dispensed into syringes containing 500 mg of treatments. The above procedures were conducted under continuous flushing with CO_2. After closing the clips on the silicon tube at the syringe tip, syringes were gently shaken and the clips were opened to remove the gas by pushing the piston upwards to achieve complete gas removal. The clip was closed, the initial volume recorded and the syringe was placed in an incubator at 39°C. Incubation was completed in triplicate within each run. Gas production was recorded at 2 h intervals, at which time, each syringe was gently shaken and at the end of the incubation, the liquid layer of each syringe was sampled for subsequent volatile fatty acids (VFA) analysis and determination of pH.

VFA detection

After incubation, samples were centrifuged at 1340 *g* for 10 min and 3 ml of the supernatant fluid were collected into 15 ml centrifuge tube and 600 µl of 24% methaphosphoric acid was added for acidification of the samples allowing the volatile fatty acids to be vaporized in the gas chromatography (GC) injection port and the samples were kept for 24 h at room temperature. The samples were then centrifuged in 1340 *g* for 20 min and 0.5 ml of supernatant and 0.5 ml on internal standard were transferred into 2 ml glass tube and were kept at 4°C pending analyses. 4-methyl-valeric acid was used as internal standard. The concentrations of VFA were determined by gas chromatography (Model GC6890, Agilent Technologies, USA) with a flame ionization detector (FID) and fused silica capillary column (Erwin et al., 1961).

Statistical analysis

Data were analysed using the general linear model (GLM) procedure of SAS 6.12 (1988). All multiple comparisons among means were performed using Duncan's new multiple range test.

RESULTS AND DISCUSSION

Nutrient components

The effects of biological treatment on the various nutrient components of rice straw are shown in Table 1. There were differences among fermented and non fermented rice straw in NDF ($P < 0.01$), hemicellulose ($P < 0.01$), CP ($P < 0.01$), ash ($P < 0.05$) and organic matter ($P < 0.05$). *A. niger* reduced the NDF content of rice straw from 82.98 to 78.18 and 75.97% in the culture medium with or without urea treatments, respectively. This was due to the reduction of hemicellulose and cellulose

Table 1. Effect of biological treatment on composition of rice straw (% DM)

Composition	Non fermented	Fermented	Fermented plus urea	Significant level
Natural detergent fibre	82.98 ± 0.06[a]	78.18 ± 0.23[b]	75.97 ± 0.70[c]	**
Acid detergent fibre	52.81± 0.42	53.58 ± 0.80	54.09 ± 0.69	NS
Acid detergent lignin	6.23 ± 0.30[b]	7.01 ± 0.32[ab]	7.69 ± 0.61[a]	NS
Cellulose	46.58 ± 0.73	46.57 ± 1.12	46.4 ± 0.57	NS
Hemicellulose	30.17 ± 0.48[a]	24.60 ± 0.57[b]	21.88 ± 1.07[c]	**
Crude protein	5.01 ± 0.16[c]	7.54 ± 0.11[b]	9.01 ± 0.44[a]	**
Ash	7.78 ± 0.18[b]	9.780.28[a]	9.760.71[a]	*
Organic mater	92.22 ± 0.18[a]	90.22 ± 0.28[b]	90.24 ± 1.71[b]	*

NS: Not significantly different (P > 0.05), *significantly different at 5% level (P < 0.05), **significantly different at 1% level (P < 0.01).
[a, b and c:] Means with different letter within a row differed significantly.

Table 2. Total gas production (ml/g DM), pH and dry mater disappearance with incubation of fermented and non fermented rice straw.

Incubation time (h)	Non fermented	Fermented	Fermented and urea	Significant level
8	15.67 ± 1.11	16.33 ± 1.78	16.00 ± 1.33	NS
12	24.67 ± 1.11	25.33 ± 1.78	19.67 ± 1.11	NS
24	69.33 ± 2.44[a]	48.00 ± 2.67[b]	36.67 ± 3.56[c]	**
32	86.67 ± 2.89[a]	60.00 ± 0.67[b]	48.67 ± 4.89[c]	**
48	102.00 ± 2.00[a]	78.67 ± 0.89[b]	62.67 ± 6.22[c]	**
Gas production rate (ml/g DM/h)	2.13 ± 0.04[a]	1.64 ± 0.02[b]	1.31 ± 0.13[c]	**
pH	6.54 ± 0.06	6.75 ± 0.05	6.79 ± 0.04	NS
DM disappearance (%)	29.17 ± 3.11[a]	23.21 ± 1.53[b]	22.17 ± 1.45[b]	**

NS: Not significantly different (P > 0.05), *significantly different at 5% level (P < 0.05), **significantly different at 1% level (P < 0.01).
[a, b and c:] Means with different letter within a row differed significantly.

contents of rice straw after fermentation. Reduction of lignocelluloses content in the rice straw is assumed to be the activities of cellulase, beta glucosidase and xylanase enzymes of the *A. niger* as have been reported in the different studies (Pothiraj et al., 2006; Madamwar et al., 1989; Bailey and Poutanen, 1989). The best result for the reduction of hemicellulose was obtained in rice straw with urea supplementation. The present result also showed that addition of non protein nitrogen source (urea) has beneficial effect on reduction of NDF content of rice straw.

Nitrogen is essential for biological activity of micro-organisms and the supplementation of the appropriate nitrogen source improves enzyme production by microbes. Although rice straw contains about 5% CP, this nitrogen sources are protected by the cellulose, hemicellulose and lignin, therefore, they are not easily available to the microbes. Additional nitrogen source improved the microbial ability to degrade the lignocellulose content of biomass during biological treatment. In addition, biological treatment significantly (P < 0.01) increased the CP content of the fermented rice straw from 5.01 to 7.54 and 9.01%, respectively, for treatments of non-urea and with additional urea.

In vitro gas production

The effect of biological treatment of rice straw on total gas production, pH and DM disappearance are shown in Table 2. There were differences among treatments in total gas production at 24, 32 and 48 h incubation (P < 0.01). Non fermented rice straw produced higher volume of gas during the different incubation times than fermented rice straw. Fermented rice straw with and without urea produced 23 and 33% lower total gas than non fermented rice straw, respectively. The rate of gas production indicated that there were significant differences (P < 0.01) between treatments (2.13, 1.64 and 1.31ml/g DM/h for non fermented, fermented and fermented rice straw with urea, respectively). Biological treatment of rice straw has no significant effect on gas production in 8 and 12 h of incubation and pH (P > 0.05). There was a significant (P < 0.01) difference between treatments for DM dis-appearance, with non-fermented rice straw showing higher DM disappearance in compression to fermented samples.

Volatile fatty acid production (mM/l) by rice straw and ratio of VFA as percentage of total VFA in the different treatments are shown in Table 3. Acetate, propionate and

Table 3. Production of volatile fatty acid (mM/l) in ruminal fluid during incubation of fermented and non-fermented rice straw.

VFA	Non fermented	Fermented	Fermented and urea	Significant level
Acetate	37.81 ± 1.17[a]	30.74 ± 2.3[b]	31.97 ± 0.75[b]	*
Propionate	15.68 ± 0.52[a]	12.80 ± 1.04[b]	12.75 ± 0.69[b]	*
Iso-butyrate	0.78 ± 0.01[ab]	0.72 ± 0.04[b]	0.79 ± 0.01[a]	NS
Butyrate	7.26 ± 0.12	6.09 ± 0.38	5.34 ± 1.31	NS
Iso-valerate	1.73 ± 0[b]	1.64 ± 0.08[b]	1.84 ± 0.05[a]	*
Valerate	0.76 ± 0.01[a]	0.68 ± 0.04[b]	0.73 ± 0.01[ab]	NS
Caproate	0.03 ± 0.01	0.07 ± 0.05	0.090.06	NS
A/P	2.41 ± 0.01	2.40 ± 0.02	2.51 ± 0.07	NS
Total	64.06 ± 1.84[a]	52.73 ± 3.89[b]	54.50 ± 1.33[b]	*
VFA (as % of total VFA)				
Acetate	59.05 ± 0.13[a]	58.27 ± 0.23[b]	58.65 ± 0.16[ab]	*
Propionate	24.48 ± 0.11	24.26 ± 0.23	23.38 ± 0.62	NS
Iso-butyrate	1.22 ± 0.03[b]	1.36 ± 0.03[a]	1.45 ± 0.05[a]	**
Butyrate	11.35 ± 0.13	11.56 ± 0.21	9.86 ± 2.6	NS
Iso-valerate	2.70 ± 0.08[b]	3.12 ± 0.10[a]	3.38 ± 0.18[a]	**
Valerate	1.19 ± 0.01[b]	1.28 ± 0.02[ab]	1.34 ± 0.06[a]	*
Caproate	0.05 ± 0.01	0.15 ± 0.10	0.16 ± 0.11	NS

NS: Not significantly different ($P > 0.05$), *significantly different at 5% level ($P < 0.05$), **significantly different at 1% level ($P < 0.01$).
[a, b and c:] Means with different letter within a row differed significantly.

total VFA production from non fermented rice straw were significantly higher than fermented rice straw ($P < 0.05$). There were no significant differences between fermented rice straw, with or without urea, for VFA production ($P > 0.05$). Isovalerate, an indicator for protein degradation, increased in fermented rice straw with urea ($P < 0.05$), while percentage of isovalerate was significantly increased for the fermented rice straw ($P < 0.01$). The acetate to propionate ratio, an index of efficiency of feed utilization, did not differ among treatments.

Although biological treatment significantly reduced ligno-cellulose content of rice straw, it reduced gas production, which has positive correlation to DM digestibility. Results of the present study showed that it is not sufficient to use lignocellulose content alone as indicator to evaluate the improvement of the nutritive value of biomass as animal feed after biological treatment. The reduction of the *in vitro* gas and VFA productions from the fermented rice straw suggested some forms of suppression on the fermentative activities of the rumen microbial population. The above suppression on microbial activity could be due to antagonistic effect of *A. niger* and/or secondary products of this microorganism on rumen cellulolytic bacteria and other microorganisms in the rumen ecosystem. Several recent studies reported the antagonistic activity of *A. niger* on different plant pathogens (Kandhari et al., 2000; Rai and Upadhyay, 2002). Rai and Upadhyay (2002) have shown that colonization of pigeon-pea substrate by *Fusarium udum* (as plant pathogen) was highly suppressed by antagonism from *A. niger* when this was used in inoculums mixtures with *Fusarium udum*. Gamble et al. (1994) in their study on the treatment of Bermuda grass using 2 white rot fungi reported that treatment using *Ceriporiopsis subvermispora* increased while treatment using *Phanaerochaete chrysosporium* reduced total *in vitro* gas production in comparison with the untreated samples.

It can be concluded that, although there are many reports of the high ability of *A. niger* to produce lignocellulolytic enzymes, biological treatment of low quality biomass, such as rice straw for animal feed using *A. niger* may not be effective. The reduction in lignocellulosic content for improvement in the biodegradability of the material needs to be evaluated together by rumen microorganisms using *in vitro* and/or *in vivo* studies.

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