

Full Length Research Paper

Role of lignocellulolytic thermophilic fungus *Thermoascus aurantiacus* MTCC 375 in paddy straw digestibility and its implication in biogas production

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Paddy straw was pretreated with *Thermoascus aurantiacus* MTCC 375, a ligno-cellulolytic thermophilic fungus, to enhance its biodegradability. The potential of microbial pretreatment under aerobic condition on paddy straw digestibility was investigated at regular intervals of 1, 2, 3, 4 and 5 days by determining the change in proximate (total solids (%)) and volatile solids (%) and chemical composition (cellulose, hemicellulose, lignin and silica content). The pretreatment of 5 days significantly ($P \leq 0.05$) reduced the concentrations of cellulose, hemicelluloses, lignin and silica content in the paddy straw by 34.25, 39.19, 34.12 and 10.59%, respectively. A maximum of 30% increase in biogas production was observed from one day pretreated paddy straw as compared to untreated paddy straw. However, biogas production from paddy straw supplemented with enzyme containing digested biogas slurry without giving aerobic treatment was found to be more than the samples given aerobic treatment. In both cases, biogas production was greater than the control by 63.2 and 30.7%, respectively.

Key words: Paddy straw, pretreatment, lignin, *Thermoascus aurantiacus*, biodegradability, enzyme containing digested biogas slurry, biogas production.

INTRODUCTION

Rising energy consumption, depletion of fossil fuels and increased environmental concerns has shifted the focus of energy generation towards biofuel use. Due to increasing fuel prices and environmental concerns, it has become necessary to develop alternative energy sources

like biogas. Among potential alternative bioenergy resources, agricultural wastes such as paddy straw, sugarcane bagasse, blends of cassava, potato peels, etc. have been identified as the prime source of biofuels and other value added products.

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Rice being a major cereal crop in India, leads to the production of much larger quantity of paddy straw. About 150 million tonnes of paddy straw were produced in India during 2011-12 (Anonymous, 2012). From such a large quantity of paddy straw, only a minor portion is used as animal feed and household fuel while the remaining paddy straw is disposed off by burning. One tonne paddy straw burning releases 3 kg particulate matter, 60 kg CO, 1460 kg CO₂, 199 kg ash and 2 kg SO₂ (Jenkins and Bhatnagar, 2003), which causes many respiratory diseases.

For effective utilization of paddy straw, intensive research and development studies are being carried out throughout the world. High concentration of organic matter in paddy straw makes it a suitable substrate for biogas production. However, high lignin (6-14%) content in the cell wall of paddy straw hinders the accessibility of cellulase to cellulose and hemicellulases to pentoses, thereby reducing the hydrolysis efficiency significantly. In addition to lignin, high concentration of silica (8-12%) in its epidermal surface acts as a physical barrier preventing microbial attachment (Wiidyastuti et al., 1987) for hydrolytic process.

Therefore, the paddy straw needs to be pretreated, to enable cellulose to be more accessible to the microbial / enzymatic attack. Many physical (mechanical and non mechanical), chemical (alkaline hydrolysis, acid hydrolysis, oxidative delignification and solvent extraction), physicochemical (ammonia fibre explosion, CO₂ and steam explosion) and biological pretreatments (lignocellulolytic microorganisms and the enzymes) have been proposed (Saratale et al., 2008; Hendricks and Zeeman, 2009). However, the physical and chemical pretreatments require high energy and corrosion resistant, high pressure reactors, which increase the cost of pretreatment. Furthermore, the chemical pretreatments can be detrimental to the methanogens apart from generating acidic or alkaline water, which needs pre-disposal treatment to ensure environment safety (Keller et al., 2003).

Some of these problems can be solved by biological pretreatment of paddy straw which involves the use of either whole micro-organisms or enzymes produced by microbes to enhance its digestibility. Both fungi and bacteria are being used for biotreatment of paddy straw. Fungal pretreatment of paddy straw has been well employed for improving its digestibility (Sahni, 2010; Sinigani et al., 2005). Advantages of biological pretreatment include inexpensive, low energy requirement and mild environmental conditions (Saratale et al., 2008). Fungi degrade lignin by secreting enzymes collectively termed as lignolytic enzymes (laccase, lignin peroxidase and manganese peroxidase). Most of the research concerning biodegradation of lignin has been focused on *Phanerochaete chrysosporium*, *Streptomyces viridosporus*, *Pleurotus eryngii*, *Trametes trogii*, *Fusarium proliferatum*, etc. (Regalado et al., 1997).

No doubt, reports are available on biological pretreatment of paddy straw; however, the effect of pretreatment on biogas production is less explored. Therefore, the present study was undertaken to optimize the conditions for biological pretreatment of paddy straw by thermophilic *Thermoascus aurantiacus* MTCC 375, a lignocellulolytic fungus and to study the implications of enhanced paddy straw digestibility on biogas production.

MATERIALS AND METHODS

Procurement of the materials

Paddy straw was procured from the research field of Punjab Agricultural University, Ludhiana after harvesting the crop. The paddy straw was chopped to 3-4 cm with a chopping machine and was stored in polythene bags at room temperature. Microbial culture of *T. aurantiacus* MTCC 375 was procured from Institute of Microbial Technology, Chandigarh India and was maintained on potato dextrose agar slants at 45±2°C by monthly transfers. Digested biogas slurry was procured from a working biogas plant of School of Energy Studies for Agriculture, PAU, Ludhiana, India.

Pretreatment of paddy straw

For pretreatment of paddy straw, lignolytic enzymes were produced from *T. aurantiacus* MTCC 375 using digested biogas slurry as medium and paddy straw as a substrate. Sterilized digested biogas slurry medium (250 g) was inoculated with 10⁷ spores/ml and incubated at 45±2°C for 4 days as per the method described by Dar and Phutela (2013). The lignin degrading enzymes like laccase, manganese peroxidase and lignin peroxidase were measured by the method of Shandilya and Munjal (1983), Paszczynski et al. (1988) and Tien and Kirk (1988) respectively. Two hundred and fifty grams of paddy straw was soaked and mixed in polybags with enzyme containing digested biogas slurry (250 g). The mixture was dispensed in polybags and incubated at 45°C for aerobic pretreatment studies. Five sets of such bags in triplicate were prepared. A control was also used where uninoculated digested biogas slurry was mixed with paddy straw. Samples from one set of bag was taken from incubator at an interval of 24 h, dried at 100°C overnight and was used for proximate and chemical analysis of pretreated straw.

Biogas production from pretreated paddy straw

Biogas production experiments were carried out in two litre capacity digesters following monophasic method and biogas produced was measured by water displacement method. Pretreated paddy straw (250 g) containing digested biogas slurry was mixed with 100 ml cattle dung and was fed to biogas digester. The digested biogas slurry acts as inoculum for biogas production whereas cattle dung acts as inducer for enhancing biogas production from paddy straw. The digester was properly sealed with rubber cork and araldite. The biogas production data was taken for a period of 35 days.

Biogas production from paddy straw supplemented with enzyme containing digested biogas slurry

Lignolytic enzyme containing digested biogas slurry (250 ml) was mixed with soaked paddy straw (250 g) and 100 g of cattle dung. The mixture was put in the digester without any pretreatment and

Table 1. Change in chemical and proximate composition of paddy straw by enzyme containing media.

Treatment (Day)	Composition of paddy straw (%)							
	TS	VS	TOC	Ash	Cellulose	Hemicellulose	Lignin	Silica
Control [#]	98.5±0.38	86.39±0.35	47.98±0.43	16.15±0.58	36.2±0.41	27.3±0.69	8.5±0.29	11.8±0.32
1	95.55±0.57	82.82±0.55	46.01±0.56	17.38±0.49	34.6±0.78	24.2±0.51	7.4±0.38	11.35±0.43
2	93.56±0.96	81.07±0.90	45.04±0.37	17.85±0.37	34±0.35	23.8±0.51	7.1±0.27	11.2±0.33
3	92.13±0.88	80.05±0.63	44.47±0.61	18.05±0.50	31.8±0.61	23.2±0.41	6.8±0.32	10.8±0.55
4	91.04±0.89	78.15±0.36	43.42±0.42	18.93±0.64	28.6±0.74	20.6±0.43	6.2±0.54	10.65±0.43
5	90.12±0.69	77.5±0.28	43.06±0.33	19.95±0.39	23.8±0.43	16.6±0.68	5.6±0.32	10.55±0.39
Percentage change from control	8.5 (↓)	10.30 (↓)	10.25 (↓)	23.53 (↑)	34.25(↓)	39.19 (↓)	34.12 (↓)	10.59 (↓)
CD (5%)	2.33	1.72	1.61	1.55	1.74	1.69	1.13	NS

Control: control contains soaked paddy straw, uninoculated digested biogas slurry and cow dung; TS: total solids; VS: volatile solids; TOC: total organic carbon; CD: critical difference at 5% level; ± values indicate % standard error for triplicate data; (↓): decrease ; (↑): increase.

biogas produced was measured for a period of 35 days.

Chemical analysis

Standard methods of AOAC (2000) were followed for the determination of proximate and chemical composition of paddy straw, that is, total solids, volatile solids, cellulose, hemicellulose, lignin and silica.

Statistical analysis

The standard error (SE at 5% level) and critical difference (5% level) were calculated for triplicate data.

RESULTS AND DISCUSSION

Effect of enzymatic pretreatment conditions on paddy straw digestibility

Chopped and soaked paddy straw was pretreated with enzyme containing digested biogas slurry (DBS) and its effect on paddy straw digestibility was determined. The change in chemical (TS, VS, Ash and TOC contents) and proximate (cellulose, hemicellulose, lignin and silica contents) composition of paddy straw with enhanced biogas production was taken as criteria for paddy straw digestibility. The results are presented in Table 1.

Results from Table 1 indicate that there was a smooth decrease in total solids and volatile solids with increase in the incubation period. The total solids decreased from 95.25 (in control) to 90.12% in 5 days treatment. Volatile solids also decreased from 82.62 (in control) to 77.5%. However, ash content was found to be increasing with a maximum increase of 10.30% in 5 days treated sample. The cellulose content decreased from 36.2 (control) to 23.8% and hemicellulose decreased from 27.3 (control) to 16.6% in 5 days. The decrease in cellulose and hemicellulose

content might be the result of breakdown or hydrolysis of cellulose and hemicellulose into fermentable sugars (Jalc et al., 1998). This observation clearly indicates that the fungus has active cellulases and hemicellulases. Lignin content also decreased from control (8.5%), showing maximum reduction of 34.12% in 5 days treated sample (5.6%). There was decrease in silica content but the decrease is not significant as critical difference came out to be non significant. This demonstrates that the *T. aurantiacus* MTCC 375 is lignocellulolytic in nature.

Similar results were found by Huang et al. (2007) who used two lignolytic micro-organisms viz. *P. chrysosporium* (white-rot fungi) and *Streptomyces badius* (actinomycetes) for bio-delignification of rice straw and found that lignin was degraded by 41 and 31% by *P. chrysosporium* and *S. badius*, respectively. Zafar et al. (1980) also observed that cellulose content of rice straw treated with *Pleurotus sajor caju* decreased from 45.0 to 17.8%. Shi et al. (2009) pretreated cotton stalks with *P. chrysosporium* and found a significant decrease in lignin, that is, 19.38 and 35.33% for submerged and solid state cultivation, respectively.

Biogas production from enzymatically pretreated paddy straw under aerobic condition

Results from Table 2 show that the biogas production was enhanced in pretreated straw as compared to untreated straw. However, with the increase of pretreatment period, there was decrease in biogas production. Highest biogas production (168.6 l/Kg PS) was found in 1 day pretreated paddy straw which showed an increase of 30.7% over control. This increase in biogas production might be due to the increase in paddy straw digestibility by enzyme pretreatment. The presence of anaerobic fungi and methanogens in cow dung and digested biogas slurry (Davies et al., 1993) might be other contributing

Table 2. Biogas production from enzymatically pretreated paddy straw.

Parameter	Control [#]	Pretreatment period (Day)					CD (5%)
		1	2	3	4	5	
Biogas (l/250 g PS)	32.3±3.85	42.2±2.40	40.0±1.10	39.3±2.35	35.2±2.65	34.4±2.60	2.13
Biogas (l/kg PS)	129.3±2.35	168.6±2.10	160.4±2.30	157.0±2.00	140.8±2.40	137.7±2.65	7.99
Biogas (l/Kg TS)	339.1±3.95	451.1±2.45	427.5±1.90	407.0±2.50	365.3±2.60	349.2±2.20	9.28
Biogas (l/Kg VS)	390.9±3.55	514.9±2.55	499.7±1.96	478.1±2.02	428.5±3.25	424.3±3.60	10.05
%age change from control	0.0	30.7(↑)	23.8(↑)	21.7(↑)	9.0(↑)	6.5(↑)	2.63

Control: soaked paddy straw, uninoculated digested biogas slurry and cow dung; T S: total solids; VS: volatile solids; PS: paddy straw; CD: critical difference at 5% level; ± values indicate % standard error for triplicate data; (↓): decrease ; (↑): increase.

Table 3. Biogas production and chemical composition of paddy straw supplemented with enzyme containing digested biogas slurry.

Parameter	Control	Paddy straw + enzyme containing digested biogas slurry
TS (%)	98.5±0.38	96.35±0.55
VS (%)	86.39±0.35	83.44±0.61
Ash (%)	16.15±0.58	17.2±0.40
Cellulose (%)	36.2±0.41	35±0.44
Hemicellulose (%)	27.3±0.69	26.6±0.61
Lignin (%)	8.5±0.29	8±0.35
Silica (%)	11.8±0.32	11.6±0.46
Biogas (l/250g PS)	32.3±3.85	52.7±2.54
Biogas (l/kg PS)	129.3±2.35	210.8±2.72
Biogas (l/kg TS)	339.1±3.95	563.1±3.31
Biogas (l/kg VS)	390.9±3.55	643.1±2.44
%age change from control	0.0	63.2(↑)

TS: Total solids; VS: volatile solids; PS: paddy straw; ± values indicate % standard error for triplicate data; (↓): decrease ; (↑): increase.

factors for increase in biogas production.

In the present study, there may have been more transfer of H₂ between methanogenic bacteria and anaerobic fungi in one day pretreated paddy straw, thus resulting in more carbon flow than others through hydrogenosomes which ultimately shift the end product formation towards acetate, H₂ and formate (though the latter two are utilized by the methanogens as precursors of methane production). While in the case of the samples given the 2, 3, 4 and 5 days aerobic pretreatment, with the increase in pretreatment period, more death of methanogens and anaerobic fungi may have occurred, thus resulting in less

flow of carbon through the hydrogenosomes and ultimately less production of acetate, H₂ and formate and ultimately less biogas production.

Biogas production from paddy straw supplemented with enzyme containing digested biogas slurry

Results from Table 3 showed that there was 63.2% enhancement in biogas production from the paddy straw which was directly put in the digester after mixing with enzyme containing digested biogas slurry (DBS) than that of the control. It produced 210.8 l biogas/kg of paddy

straw which was quite higher than the control (129.3 l biogas/kg of paddy straw) and other samples pretreated aerobically by enzyme activated digested biogas slurry (Table 2). High biogas production might be due to utilization of volatile fatty acids by the microorganisms (anaerobic fungi and methanogens), thus not inhibiting the methanogenesis as was found in the case of aerobically treated samples (Davies et al., 1993).

Conclusion

The thermophilic fungus *T. aurantiacus* MTCC 375 is both cellulolytic as well as lignolytic in nature as it degrades both cellulose and lignin. There was decrease in silica content but the decrease was not significant as critical difference was not significant. Biogas production from paddy straw supplemented with enzyme containing digested biogas slurry without giving aerobic treatment was found to be more than the samples given aerobic treatment, although biogas production was enhanced as compared to the control in both cases.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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