



Comparative evaluation of different pretreatment methods on biogas production from paddy straw

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Abstract: The present investigations observed the effect of chemical, enzymatic, biological and microwave pretreatment on paddy straw for enhancement of biogas production. Chopped and soaked paddy straw was subjected to chemicals Na₂CO₃ (1%) and NaOH (2%) concentrations, microwave irradiation (720 watt, 30 min), fungal (spawn impregnated, 7 days) and crude silicase (24 hrs) pretreatment. The proximate and chemical analysis showed 16.0% and 12.1% reduction in lignin and silica content in the case of Na₂CO₃ pretreated paddy straw whereas 23.0% and 46.8% reduction was observed in enzymatic pretreatment with 43.7% and 31.1% enhancement in biogas production respectively. This clearly indicates that *Pleurotus ostreatus* MTCC 142 is silicolytic as well as ligninolytic in nature. Enzymatic pretreatment was also compared with, microwave (30 min) and fungal pretreatment which showed 31.2% and 32.8% reduction in silica content enhancing biogas production by 19.7% and 42.6% respectively. NaOH pretreatment showed a maximum increase in biogas production i.e. 49.7% as compared to 1% pretreated sample which showed 28.5% enhancement. The results indicated that the NaOH pretreatment was one of the potential methods to increase biogas production of paddy straw.

Keywords: Biogas production, Ligninolytic, *Pleurotus ostreatus*, Pretreatment, Silicolytic

INTRODUCTION

Rice, one of the most staple food, provides nutrition and calorie intake to nearly half of the world's population (Arvanitoyannis *et al.*, 2008). Every ton of rice harvesting produces 0.8 tons of straw (595.92 million tonnes 2014) which stands in the field as waste biomass but with high energy potential (FAO Rice and Market monitor, 2014). According to the global status energy report approximately 147 GW power from biomass was generated at the end of 2015 and over 8.1 million jobs were estimated to be increased in relation to the renewable energy sector (www.ren21.net). Rice straw possesses various uses such as paper making, resin binders and also as livestock feed but the higher silica and lower phosphorus content makes it a poor feedstock for animals ([http://www.carrb.com/84rpt/Straw Uses. htm](http://www.carrb.com/84rpt/Straw%20Uses.htm)), therefore burning of straw is a common practice mainly in the harvest season which emits about 144719 mg of total particulate matter from burning of rice straw in the field (Gadde *et al.*, 2009). A total of 3 kg particulate matter, 60 kg CO, 1460 kg CO₂, 199 kg ash and 2 kg SO₂ is produced via open burning of one ton rice straw, which not only reduces soil productivity but substantially contributes to air

pollution (Streets *et al.*, 2003 and Akhter *et al.*, 2015). Rice straw is one of the most abundant lignocellulosic biomass in the world (Saini and Saini, 2015). It is mainly composed of (32–47%) cellulose and (19–27%) hemicelluloses that can be readily hydrolysed into fermentable sugars (Saha, 2003 and Raeisi *et al.*, 2016) via of anaerobic digestion, a series of biological processes which involves the conversion of organic material into biogas and digestate in the presence of anaerobic microorganisms and in the absence of oxygen (Kangle *et al.*, 2012). The rate and extent of degradation are the two important parameters that affect the anaerobic digestion process. High biodegradation rate reduces the reactor size and makes it economically attractive (Contreras *et al.*, 2012). High amount of organic acids such as lactic acid, butyric acids and various amino acids have also been produced using rice straw as a substrate as described by Younas *et al.*, (2016) who produced 58.1% lactic acid from 1M NaOH pretreated rice straw while 2.6 g/(L.d) butyric acid was produced from paddy straw via consolidate bioprocessing (Ai *et al.*, 2016).

The major challenge is to digest the multilayer structure of rice straw shielded by the presence of lignin (Jafri *et al.*, 2011 and Werle *et al.*, 2013). Therefore

effective methods are required to degrade the straw at higher rate for maximum biogas production. Over the past decades a large number of chemical (acid, alkali, oxidizing agents, and organic solvents), physico-chemical (steam explosion, ammonia fiber explosion), physical (comminution, milling and grinding), biological, or a combination of these pretreatment methods had been investigated on variety of feedstock (Kumar *et al.*, 2009) which have enhanced biogas production at a higher rate. As the chemicals used are not economically cheap and also provokes a hazardous threat towards the environment, therefore biological methods have gained researcher's attention in the current era. A wide range of bacteria and fungi are known to solubilize insoluble silicates by producing minerals, organic acids and a variety of chelating agents (Henderson and Duff, 1965). Fungi such as *Pleurotus ostreatus*, *Pleurotus florida*, *Phanerochaete chrysosporium*, *Cyathosstercoreus*, *Ceriporiopsis subvermispora*, were reported to elevate the digestibility of paddy straw (Blanchette *et al.*, 1992, Chen *et al.*, 1996, Gammal *et al.*, 1998 and Taniguchi *et al.*, 2005), however *Phanerochaete* and *Pleurotus* showed beneficial effects, degrading lignin by 40% and 60% in cotton straw (Platt *et al.*, 1984). The present study was aimed to find out maximum paddy straw degradation and biogas yield by various pretreatment methods.

MATERIALS AND METHODS

Substrate and chemicals: The paddy straw was procured from the research field of Punjab Agricultural University, Ludhiana after harvesting of crop and was chopped (3-5 cm) with a chopping machine for its easy handling, which was later stored in polythene bags at room temperature. Cattle dung was procured from dairy farm of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) Ludhiana. Digested cattle dung slurry was obtained from a nearby working biogas plant in the School of Renewable Energy and Engineering, PAU Ludhiana.

All the chemicals used for proximate and chemical analysis, media and solutions preparation were of analytical grade and were purchased from Hi-Media, SRL, Sigma and S D fine chemicals Pvt. Ltd.

Silicase production and extraction: Silica peptone broth was inoculated with *P.ostreatus* MTCC 142 culture and incubated at $28^{\circ}\text{C}\pm 2^{\circ}\text{C}$ at 200 rpm. After 0, 2, 4, 6 and 8 days of incubation the broth was filtered and the filtrate was then centrifuged at 15000 rpm for a period of 15 min in a cooling centrifuge (Remi Instruments Ltd.). The clear supernatant was used as crude enzyme extract. The silicase activity in the crude enzyme extract was determined by the method described by Toender and Borchert, (2014).

Pretreatment of paddy straw

Chemical pretreatment of paddy straw: Two chemicals sodium carbonate (Na_2CO_3) and sodium hydrox-

ide (NaOH) of concentrations 1% and 2% each were used. Two hundred and fifty gram of chopped and soaked paddy straw was suspended in these chemical solutions for 24 hrs at room temperature. After the desired period of soaking, the solution was decanted off and the paddy straw was washed under tap water till the washings were clean, colourless and neutral to pH paper. A small amount as a sample was taken and dried overnight in a laboratory oven at 70°C - 80°C . After drying the sample was ground in order to get uniform and reproducible results. Otherwise, the paddy straw formed clumps on pretreatment and was not fully accessible to different chemicals/solutions used in proximate analysis. The samples were stored in polythene bags for proximate and chemical analysis and the rest straw was fed to the digesters for biogas production.

Microwave pretreatment of paddy straw: A domestic microwave oven MC-2681DS produced by LG Electronics India Pvt. Ltd was used in this study for microwave pretreatment. The microwave frequency was 2550 MHz and the power was set at 720 W. Two hundred and fifty gram of chopped and soaked paddy straw was suspended in 500 ml of water in an autoclavable bag. The bags were pierced with pins to avoid excess pressure inside the bag and placed at the centre of microwave oven on the circular plate for its pretreatment for 30 min. The bags were allowed to cool and required sample was washed and dried for proximate and chemical analysis whereas rest straw was fed to the reactors for biogas production.

Biological pretreatment of paddy straw

Preparation of inoculum: The inoculum needed for pretreatment of paddy straw was prepared on wheat grains. The grains were washed under tap and boiled for 25-35 min until tender. The surplus amount of water was decanted off. The grains were then mixed with 4% calcium carbonate (CaCO_3) and 2% gypsum (CaSO_4) which were later dispensed into empty glucose bottles (250g/bottle) and were autoclaved for 90 minutes. After cooling the bottles were inoculated by placing two 5 mm bits of the freshly prepared culture of *P.ostreatus* MTCC 142 each on opposite side of the bottle. The bottles were incubated at $28^{\circ}\text{C}\pm 2^{\circ}\text{C}$, till mycelium completely impregnates the wheat grains (7-10 days).

Mixing of inoculum with paddy straw: The chopped paddy straw (250 g) was soaked in water overnight. The excess water was drained off and the paddy straw was then incorporated with the inoculum (fungus impregnated wheat grains) at the rate of 10% w/w ratio (25g in 250 g paddy straw). The bags were allowed to incubate at room temperature for 1 week. After completion of the incubation period, the samples of pretreated paddy straw were taken and dried at 70°C in an oven. The paddy straw was grounded to powder and stored in polythene bags which were later used for

determining the change in proximate and chemical composition of paddy straw.

Enzymatic hydrolysis

Silicase pretreatment: Silicase was produced from *P.ostreatus* MTCC 142 under optimal conditions the crude enzyme extract in different concentrations i.e. 15U/10g, 30 U/10g and 45 U/10g was used to pretreat the paddy straw. Two hundred and fifty gram of chopped (3-5 cm size) and soaked paddy straw was mixed with crude enzyme extract with different concentrations separately and was allowed to react for a period of 24 hrs at room temperature. The samples were dried in an oven at 80°C overnight, grinded to powder and were stored in polythene bags for determining the variation in proximate and chemical analysis. The samples for proximate and chemical analysis were studied and the remaining straw were fed to the digesters for biogas production.

Solid state anaerobic digestion setup for biogas production (SSAD): Chopped and soaked paddy straw pretreated with different methods chemical, microwave, biological and enzymatic respectively was subjected to biogas production. The untreated (control) and pretreated paddy straw were digested in batch anaerobic digesters. The batch system composed of a 2L glass reactor, 2L glass bottle for gas collection which was filled with dilute hydrochloric acid and solutions (pH <3 to avoid CO₂ dissolution) and 1L liquid collection beaker. Biogas production was measured by water displacement method i.e. by measuring the amount of water displaced by the gas produced in a millilitre. Eight groups (each of control, chemical, microwave, biological and enzymatic pretreated paddy straw) each

containing triplicate anaerobic digesters were set up at 35°C ±2°C. The digesters were mixed with 50 g cattle dung (acting as inducer) and 25 ml cattle dung slurry (acting as inoculums). Reactors were then carefully examined for any leaks to inspect close tight rubber stopper and screw caps. The samples were taken both at the initial stage and after a period of 45 days for proximate and chemical analysis. Biogas production for each reactor was measured every day and level of water was maintained in gas collecting chamber.

Analytical methods: Standard methods of AOAC (2000) were followed for the determination of Total solids, Volatile solids, Total organic carbon, ash, cellulose, hemi-cellulose, lignin and silica content.

Statistical analysis: The experimental data which was generated from the experiments was analysed using the SAS system. The data was analysed by Tukey’s HSD method for multiple comparisons. Standard deviation was also calculated for the data.

RESULTS AND DISCUSSION

Effect of different pretreatment methods on paddy straw digestibility and biogas production

Na₂CO₃ pretreatment: Pretreatment of rice straw by Na₂CO₃ was applied at room temperature to enhance the degradation of rice straw. Table 1 presents the hydrolytic behaviour of Na₂CO₃ pretreatment on paddy straw digestibility with maximum total solids, volatile solids and total organic carbon reduction by 2.2%, 8.9% and 8.9% in 2% pretreated sample respectively. On the contrary ash content was increased by 34.7% which can be due to the decrease in volatile solid content. Maximum reduction in cellulose by 10.5% and

Table 1. Change in proximate and chemical composition of Na₂CO₃ and NaOH pretreated paddy straw.

Composition of PS %	Control		Na ₂ CO ₃ concentration (%)				NaOH concentration (%)			
	Initial	Final	1		2		1		2	
			Initial	Final	Initial	Final	Initial	Final	Initial	Final
Total solids	96.2±0.4 ^a	95.4±0.7 ^b (0.8 ↓)	96.0±0.8 ^a	94.8±0.4 ^d (1.25 ↓)	94.2±0.9 ^e	92.1±1.0 ^d (2.2 ↓)	96.1±0.6 ^a	95.0±0.2 ^b (1.1 ↓)	95.7±0.4 ^b	94.0±0.6 ^c (1.7 ↓)
Volatile solids	86.8±0.5 ^a	85.5±0.3 ^b (1.4 ↓)	84.4±0.2 ^c	78.5±0.7 ^e (6.9 ↓)	82.6±0.7 ^d	75.2±0.8 ^f (8.9 ↓)	82.6±0.2 ^d	75.5±0.15 ^f (8.5 ↓)	83.8±0.6 ^c	71.2±0.1 ^g (15.0 ↓)
Total organic carbon	48.2±0.4 ^a	47.5±0.7 ^a (1.4 ↓)	46.8±1.1 ^b	43.6±0.5 ^d (6.8 ↓)	45.8±1.2 ^c	41.7±0.5 ^e (8.8 ↓)	45.8±0.8 ^c	41.9±0.6 ^e (8.5 ↓)	46.5±0.2 ^b	39.5±0.2 ^f (15.1 ↓)
Ash	13.2±0.6 ^b	14.5±0.6 ^h (9.8 ↑)	15.6±0.6 ^g	22.5±0.2 ^c (44.2 ↑)	18.4±0.4 ^c	24.8±0.6 ^b (34.7 ↑)	17.4±0.7 ^f	24.5±0.3 ^b (40.8 ↑)	19.8±0.6 ^d	28.8±0.4 ^a (45.4 ↑)
Cellulose	46.2±0.5 ^b	45.8±0.2 ^b (0.8 ↓)	44.0±0.8 ^c	40.1±0.6 ^e (8.8 ↓)	42.6±0.9 ^d	38.1±0.8 ^g (10.5 ↓)	45.0±0.6 ^a	39.4±0.2 ^f (12.4 ↓)	44.8±0.9 ^a	36.9±0.3 ^h (17.6 ↓)
Hemicellulose	25.0±0.3 ^a	24.6±0.1 ^b (1.6 ↓)	24.2±0.5 ^b	22.6±0.4 ^d (6.6 ↓)	24.4±0.9 ^b	21.5±0.2 ^e (11.8 ↓)	26.4±0.7 ^a	23.6±0.4 ^e (10.6 ↓)	26.2±0.4 ^a	20.1±0.2 ^f (23.2 ↓)
Lignin	6.5±0.2 ^a	6.2±0.2 ^a (4.6 ↓)	5.8±0.09 ^b	5.5±0.1 ^b (5.1 ↓)	5.6±0.04 ^b	4.7±0.1 ^c (16.0 ↓)	6.2±0.4 ^a	5.7±0.3 ^b (8.0 ↓)	5.5±0.6 ^b	4.3±0.1 ^c (21.8 ↓)
Silica	7.9±0.1 ^a	7.7±0.1 ^a (2.5 ↓)	7.6±0.07 ^a	7.0±0.2 ^b (7.8 ↓)	7.4±0.09 ^a	6.5±0.09 ^c (12.1 ↓)	6.2±0.1 ^c	7.0±0.7 ^b (12.9 ↑)	5.0±0.09 ^d	6.0±0.2 ^c (20.0 ↑)

*Control: Untreated paddy straw; PS: Paddy straw; values are means ± standard deviation (n=3); Initial indicate sample taken after pretreatment; Final indicate sample taken after 45 days of pretreatment; value in parenthesis indicates % increase (↑); % decrease (↓) w.r.t initial value; Within the same row, different small letters indicate a significant difference between parameters under the different pretreatments based on Tukey’s HSD test (p<0.05)

Table 2. Silicase production in submerged fermentation.

Incubation period (days)	Silicase activity (U/ml)	Carbonic anhydrase activity (U/ml)
0	0.046±0.09	0.05±0.01
2	0.45±0.06	0.52±0.02
4	0.67±0.04	0.70±0.02
6	0.85±0.03	0.87±0.03
8	1.05±0.06	1.10±0.02
10	0.73±0.04	0.76±0.01

*Experimental conditions; Medium used silica peptone broth; Incubation temperature: 30°C±2°C; pH 6.5; mycelial bit size 5 mm; values are means ± standard deviation (n=3)

Table 3. Change in proximate and chemical composition of paddy straw by pretreatment with crude silicase enzyme

Parameters (%)	Control	15 U/10 g PS	30 U/10 g PS	45 U/10 g PS
Total solids	96.2±0.4 ^c	96.7±0.5 ^c (0.5 ↑)	97.3±0.7 ^b (1.1 ↑)	98.0±0.4 ^a (1.8 ↑)
Volatile solids	86.8±0.5 ^a	84.5±0.5 ^b (2.6 ↓)	82.6±0.1 ^c (4.8 ↓)	81.0±0.6 ^d (6.6 ↓)
Total organic carbon	48.2±0.4 ^a	46.9±1.0 ^b (2.7 ↓)	45.8±0.8 ^c (4.9 ↓)	45.0±0.3 ^d (6.6 ↓)
Ash	13.2±0.6 ^d	15.5±0.7 ^c (17.4 ↑)	17.4±0.6 ^b (31.8 ↑)	19.0±0.5 ^a (43.9 ↑)
Cellulose	46.2±0.5 ^a	44.2±0.6 ^b (4.3 ↓)	41.1±0.6 ^c (11.0 ↓)	38.4±0.8 ^d (16.8 ↓)
Hemicellulose	25.0±0.3 ^d	27.6±0.5 ^c (10.4 ↑)	29.6±0.8 ^b (18.4 ↑)	31.9±0.6 ^a (27.6 ↑)
Lignin	6.5±0.2 ^a	6.2±0.5 ^a (4.6 ↑)	5.8±0.8 ^b (10.7 ↓)	5.0±0.4 ^c (23.0 ↓)
Silica	7.9±0.1 ^a	7.0±1.1 ^b (11.3 ↓)	6.5±0.4 ^c (17.7 ↓)	4.2±0.2 ^d (46.8 ↓)

*Experimental conditions; soaking period 24 hrs; values are means ± standard deviation (n=3); value in parenthesis indicates % increase (↑); % decrease (↓) w.r.t initial value; Within the same row, different small letters indicate a significant difference between parameters under the different pretreatments based on Tukey's HSD test (p< 0.05)

Table 4. Change in proximate and chemical composition of silicase pretreated paddy straw after biogas production

Parameters	Control		Pretreated straw (45 units/10g PS)	
	Initial	Final	Initial	Final
Total solids	96.2±0.4 ^c	95.4±0.7 ^d (0.8 ↓)	98.0±0.4 ^b	98.6±0.4 ^a (0.6 ↑)
Volatile solids	86.8±0.5 ^a	85.5±0.3 ^b (1.4 ↓)	81.0±0.6 ^c	77.2±0.4 ^d (4.6 ↓)
Total organic carbon	48.2±0.4 ^a	47.5±0.7 ^b (1.4 ↓)	45.0±0.3 ^c	42.8±0.2 ^d (4.8 ↓)
Ash	13.2±0.6 ^d	14.5±0.6 ^c (9.8 ↑)	19.0±0.5 ^b	22.8±0.1 ^a (20.0 ↑)
Cellulose	46.2±0.5 ^a	45.8±0.2 ^b (0.8 ↓)	38.4±0.8 ^c	34.5±0.3 ^d (10.1 ↓)
Hemi-cellulose	25.0±0.3 ^b	24.6±0.1 ^b (1.6 ↓)	31.9±0.6 ^a	28.0±0.1 ^c (12.2 ↓)
Lignin	6.5±0.2 ^a	6.2±0.2 ^a (4.6 ↓)	5.0±0.4 ^b	4.5±0.2 ^b (10.0 ↓)
Silica	7.9±0.1 ^a	7.7±0.1 ^a (2.5 ↓)	4.2±0.2 ^b	3.9±0.2 ^b (7.1 ↓)

*Experimental conditions; Control: Untreated paddy straw; values are means ± standard deviation (n=3); Initial indicate sample taken after pretreatment; Final indicate sample taken after 45 days of pretreatment; value in parenthesis indicates % increase (↑); % decrease (↓) w.r.t initial value; Within the same row, different small letters indicate a significant difference between parameters under the different pretreatments based on Tukey's HSD test (p< 0.05)

Table 5. Change in proximate and chemical composition of microwave and biological pretreated paddy straw

Composition of PS (%)	Control		Microwave (30 min)		Biological pretreatment with <i>Pleurotus ostreatus</i> MTCC 142	
	Initial	Final	Initial	Final	Initial	Final
Total solids	96.2±0.4 ^a	95.4±0.7 ^b (0.8 ↓)	94.2±0.3 ^c	91.4±0.12 ^d (2.9 ↓)	93.8±0.2 ^c	90.2±0.1 ^c (3.8 ↓)
Volatile solids	86.8±0.5 ^a	85.5±0.3 ^b (1.4 ↓)	79.4±0.1 ^d	73.2±0.2 ^e (7.8 ↓)	80.5±0.3 ^c	71.5±0.1 ^f (11.1 ↓)
Total organic carbon	48.2±0.4 ^a	47.5±0.7 ^b (1.4 ↓)	44.1±0.2 ^d	40.6±0.1 ^e (7.9 ↓)	44.7±0.4 ^c	39.7±0.4 ^f (11.1 ↓)
Ash	13.2±0.6 ^c	14.5±0.6 ^d (9.8 ↑)	20.6±0.1 ^c	24.8±0.5 ^b (20.3 ↑)	20.5±0.2 ^c	28.5±0.2 ^a (39.0 ↑)
cellulose	46.2±0.5 ^a	45.8±0.2 ^a (0.8 ↓)	42.8±0.4 ^b	38.6±0.4 ^d (9.8 ↓)	39.1±0.2 ^c	35.8±0.3 ^c (8.4 ↓)
Hemicellulose	25.0±0.3 ^a	24.6±0.1 ^a (1.6 ↓)	22.2±0.3 ^b	20.3±0.3 ^c (8.5 ↓)	20.2±0.4 ^c	17.3±0.1 ^d (14.3 ↓)
Lignin	6.5±0.2 ^a	6.2±0.2 ^a (4.6 ↓)	6.2±0.18 ^a	4.9±0.4 ^b (20.9 ↓)	6.0±0.4 ^a	5.2±0.4 ^b (13.3 ↓)
silica	7.9±0.1 ^a	7.7±0.1 ^a (2.5 ↓)	6.4±0.2 ^c	4.4±0.4 ^c (31.2 ↓)	7.3±0.1 ^b	4.9±0.2 ^d (32.8 ↓)

*Experimental conditions: ± values indicate standard error of the triplicate data; Control: Untreated paddy straw; PS: Paddy straw; Initial indicate sample taken after pretreatment; Final indicates sample taken after 45 days of pretreatment; value in parenthesis indicates % increase (↑); % decrease (↓) w.r.t initial value; Within the same row, different small letters indicate a significant difference between parameters under the different pretreatments based on Tukey's HSD test (p< 0.05)

Table 6. Biogas production from different pretreatments of paddy straw

Biogas Production	Different Pretreatments							
	Control	Na ₂ CO ₃		NaOH		Microwave Pretreatment (30 min)	Biological Pretreatment (7 days)	Enzymatic Pretreatment (24 hrs)
		1%	2%	1%	2%			
Biogas (ml/250g PS)	14.0±1.0 ^h	16.30±1.1 ^g	20.12±1.4 ^b	18.0±1.1 ^e	21.12±1.8 ^a	16.8±1.3 ^f	20.0±0.9 ^c	18.4±2.2 ^d
Biogas (L/Kg PS)	56.1±4.0 ^h	65.2±4.4 ^g (16.4 ↑)	80.5±5.6 ^b (43.7 ↑)	72.0±4.4 ^e (28.5 ↑)	84.4±7.2 ^a (49.7 ↑)	67.2 ±5.2 ^f (19.7↑)	80.0±3.6 ^c (42.6↑)	73.6±8.8 ^d (31.1↑)

*Experimental conditions: PS: paddy straw; control: untreated paddy straw; ± values indicate standard error of the triplicate data; value in parenthesis indicates % increase (↑); % decrease (↓) w.r.t control; Within the same row, different small letters indicate a significant difference between parameters under the different pretreatments based on Tukey's HSD test ($p < 0.05$)

hemicellulose by 11.8% was observed in 2% pretreated sample whereas lignin and silica were decreased by 16.0% and 12.1% respectively. The decrease in cellulose and hemicellulose is a clear cut indication of use of these components for biogas production by anaerobic microorganisms. Wang *et al.* (2001) studied proximate and chemical analysis of *P. ostreatus* impregnated spent grain samples at 20°C for 7 days and reported that spent grains consists of 14% cellulose, 9.1% lignin and 3.3% ash. Paddy straw rich in dry organic matter and cannot be consumed in one cycle. The proximate and chemical composition of residual paddy straw after biogas production indicate that there is still considerable amount of cellulose, hemicellulose and other nutrients which can be used for other applications like composting, vermi-composting etc.

Yang *et al.* (2013) observed 40.3% delignification when straw was pretreated with 2% sodium carbonate whereas 9.6% delignification was reported by Khaleghian *et al.* (2015) when rice straw was pretreated with 1M sodium carbonate for 7h. However Kaur and Phutela, (2016) reported 32% *desilicification* when paddy straw was pretreated with 2% Na₂CO₃. Park and Kim, (2014) also showed 19% delignification with sodium carbonate pretreatment of eucalyptus residues. However a higher proportion of lignin removal ranging from 39.5% to 72.5% was observed in Na₂CO₃ pretreated rice straw with temperature rising from 120°C to 150°C respectively (Yang *et al.*, 2012).

Biogas production from Na₂CO₃ pretreated paddy straw: Table 6 presents the volumetric contents of methane in the reactor using rice straw as a substrate. The 2% sodium carbonate pretreatment of paddy straw increased the biogas production by 43.7 % from control. This may be due to lignin and silica reduction, however 1% Na₂CO₃ pretreated sample produced only 65.2 litre biogas/Kg paddy straw with 16.4% increase. Kaur and phutela, (2016) reported 20.7% increase in biogas production when paddy straw was pretreated with 4% Na₂CO₃. Dehghani *et al.* (2015) pretreated rice straw with 0.5 M sodium carbonate at 110°C for 2 h which led to the improvement in biogas production due to significant reduction in the crystallinity of

cellulose and lignin.

NaOH pretreatment: Maximum total solids were decreased in 2% pretreatment of paddy straw i.e. 1.7% and the decrease was significant ($p < 0.05$) as shown in table 1. Volatile solids also decreased significantly i.e. by 15.1% whereas 15.0 % reduction was observed in total organic carbon content. The ash content was increased maximum by 45.4 % in 2% pretreated sample. Cellulose and hemicellulose were consumed by 17.6 % and 23.2% in 2% pretreated sample respectively. Maximum amount of lignin reduced was 21.8% and silica was found to be increased by 20.0%. The increase in silica may be due to the result of breakdown of silica in its free form. Van Soest, (2006) also reported that NH₃ and urea cracks the silicified cuticular layer of rice straw but did not digest silica in contrast to the action of NaOH. Similar results were obtained by Zhang and Cai, (2008) who found that NaOH pretreatment (2%) for 1 hour at 85°C could reduce lignin and hemicellulose by 36.24 % and 61.07% respectively. Wang *et al.*, (2012) pretreated coastal Bermuda grass with of 0.5% to 3% NaOH solution for a time period of 15 to 90 min which resulted in 86% lignin removal, however treatment of wheat straw with 1% NaOH for 1.5 hrs decrease the hemicelluloses and lignin content by 44.15% and 42.52% respectively. However Harun and Goek, (2016) reported 79.0% *desilicification* of sodium hydroxide (12% w/v) pretreated paddy straw. He *et al.* (2008) showed that rice straw pretreated with 6% NaOH solution reduced cellulose, hemicellulose, and total lignocellulose by 88.0%, 75.0%, and 72.3% respectively. A close observation was made by Jaisamut *et al.* (2013) who reported 59.7% lignin removal from 0.05g of NaOH pretreatment of wheat straw at 140°C for 85 min. Cheng *et al.* (2010) also showed delignification rate from 8.6% to 23.1% respectively. Similar results were observed when Omidvar *et al.* (2016) pretreated rice straw with 2.6 M NaOH at 80°C for 150 min resulting in 31.3% delignification.

Decrease in total solids might be due to the loss of some of the components of paddy straw as a result of their solubilisation. The decrease in cellulose was the

clear-cut indication of the breakdown of lignin-hemicellulose complex thereby resulting in a greater loss in the hemicellulose and lignin content while silica reduction might be due to the fact that NaOH solubilized silica, converting silica to its monomeric forms thus releasing free silicic acids.

Biogas production from NaOH pretreated paddy straw: The hydrolysis of NaOH pretreated rice straw showed a 49.7% hike in biogas production with increase in NaOH concentration from 1% to 2% (table 6). A maximum of 84.4 litre biogas/kg paddy straw was obtained in case of 2% NaOH concentration, whereas in untreated paddy straw only 56.1 litre biogas/kg paddy straw was produced. Similar results were obtained by Dai *et al.* (2014) in which they found that rice straw pretreated with 4%, 6% and 8% NaOH solutions gave a total biogas production of 12.1, 18.7 and 15.0 litre respectively. A total of 64.5% increase in biogas production was observed by He *et al.* (2009) when rice straw was pretreated with 6% NaOH solution. Similarly Qiu *et al.* (2011) pretreated peanut shells with 4% and 8% sodium hydroxide solutions which led to 28.0 litre and 17.6 litre biogas production.

Silicase production in submerged fermentation: Silicase production from *P.ostreatus* MTCC 142 was studied in submerged fermentation for a period of 10 days in silica peptone broth and results are depicted in table 2. With the increase of incubation period, the enzyme activity increased. The maximum silicase activity was found to be 1.05 U/ml on 8th day of incubation and a reduction in enzyme activity was observed after 8th day, this might be due to the fact that peptone which acts as nitrogen source is consumed by 8th day for carrying out metabolic activities for the growth of the microorganism. Report on silicase production by *Pleurotus ostreatus* is not available however, Negi and Kumari, (2014) observed maximum laccase activity of 210 U/g produced from *P.ostreatus* MTCC 1802 at 25°C on 7th day of incubation by using wheat bran and neem bark as substrate. Similar trend was also observed for carbonic anhydrase (silica solubilization) activity (Schroder *et al.*, 2003) which was marginally higher as compared to silicase.

Silica is especially observed in outer epidermal walls and possesses a complex chemical structure which makes it resistant to enzymatic attacks. Armstrong *et al.* (1966) demonstrated that silicase also exhibits carbonic anhydrase activity and can be measured by the colorimetric assay.

Wang *et al.* (2010) reported that silicase is a member of the family of carbonic anhydrases. Comparison of sponge silicase sequence with human carbonic anhydrase (II) shows that most of amino acid characteristics to CA's are also present in sponge silicase. As carbonic anhydrase catalyses very fast reversible reaction, so further studies were conducted by determining the silicase activity by the method of Toender and Borchert, (2014).

Enzymatic hydrolysis

Pretreatment with crude silicase: Chopped and soaked paddy straw was pretreated with crude enzyme extract with different enzyme concentrations i.e. 15, 30 and 45 units/10g of paddy straw for 24 hrs. A significant increase in total solids was noticed. The volatile solids and total organic carbon were reduced by 6.6% and 6.6% respectively (table 3). The ash content drastically increased in all cases but maximum increase was found to be 43.9% with 45 U silicase/10 g paddy straw in the treated sample, which may be due to the decrease in volatile solids. Cellulose decreased maximally by 16.8% but hemicellulose was found to increase by 27.6% with 45 U silicase/10 g paddy straw. However lignin and silica showed maximum reduction i.e. 23.0% and 46.8% respectively. This large change in silica demonstrates that *P.ostreatus* MTCC 142 is silica degrading fungi and can improve biogas production. Our results are in corroboration with that of Kaur, (2013) with 45% silica reduction in paddy straw in *P.ostreatus* MTCC 142 impregnated rice straw at 28° ±2°C for a period of 8 days.

Biogas production from paddy straw pretreated with crude silicase: Pretreatment of paddy straw with crude silicase (45 U/g PS) resulted in the increase in biogas production by 31.1% as compared to control which produced only 56.1 litre biogas/Kg paddy straw (table 6). Kaur, (2009) also showed 15% increase in biogas production from paddy straw pretreated with *P. florida* on 15th day of incubation.

Proximate and chemical composition of silicase pretreated spent paddy straw: A significant change ($p < 0.05$) was observed in the total solid content, whereas volatile solids and total organic carbon reduced by 4.6% and 4.8% respectively as depicted in table 4. This may be due to the fact that organic fraction of paddy straw is utilized for biogas production. Ash content increased by 20.0% whereas cellulose and hemicellulose content were reduced by 10.1% and 12.2% respectively. The lignin and silica reduced by 10.0% and 7.1% respectively. Similar results with a 19.1% lignin reduction were observed in *Coriolus versicolor* MTCC 138 pretreated paddy straw after 25 days of incubation (Phutela and Sahni, 2013).

Microwave and biological pretreatment of paddy straw: In order to investigate the different pretreatment methods the straw was irradiated with microwaves and biological pretreatment. The physical, structural as well as the chemical properties were changed during the microwave pretreatment, as discussed in table 5. There was a significant ($p < 0.05$) loss in total solids volatile solids and total organic carbon content with 2.9%, 7.8% and 7.9% after pretreatment, but the ash content was enhanced by 20.3% which may be due to the reduction in volatile solids. The respective decomposition rates of cellulose and hemicellulose were 9.8% and 8.5% respectively. Higher amount of lignin and silica was dissolved i.e. 20.9% and 31.2% which may be due to the reaction caused by radiation, but the entire mechanism is still unclear. Zhu *et al.* (2005) reported cellulose reduction by 69.2%, lignin 4.9% and hem-

icellulose 10.2% after 30-min microwave/alkali pretreatment. Similarly Akhtar *et al.* (2014) showed 39% lignin reduction with assisted microwave-acid pretreatment of leaf litter.

All the above mentioned methods like chemical and microwave pretreatments are harsh and cost intensive, therefore biological degradation of biomass is preferred now days which is not only cost prone but also enlist eco-friendly behaviour. The method mainly involves the use of actinomycetes, bacteria, white and soft rot-fungi which corrupts the lignin structure, a highly complex polymer present in biomass. The total solid and volatile solid content were reduced by 3.8% and 11.1% in biological pretreatment while the ash content was increased by 39.0%. There was a profound loss in cellulose and hemicellulose content with 8.4 % and 14.3% respectively. The high loss in hemicellulose might be due to the fact that wood-decaying fungi are able to metabolize and decompose all plant cell constituents (lignin, hemicellulose and cellulose) by their enzymes. Lignin and silica also found to be minimized by 13.3% and 32.8% respectively. Some white-rot fungi are able to decompose free phenolic monomers and break the bonds with which lignin is cross-linked to the polysaccharides in rice straw (Chen *et al.*, 1996) which makes it more susceptible to microbial attack. The results were in corroboration with Phutela and Sahni, (2013) who found that paddy straw pretreated with *Coriolus versicolor* MTCC 138 decreased cellulose by 19.3% whereas lignin and silica by 19.1% and 32.5%, respectively. Thakur *et al.* (2013) also showed that pretreatment of wheat straw and banana straw with *P.ostreatus* HP-1 which led to the removal of 49.2% lignin, 12.4% cellulose and 21.8% hemicelluloses. Many other researchers also focused on paddy straw pretreatment using fungal mechanisms. Mustafa *et al.* (2016) pretreated rice straw with *P.ostreatus* (DSM 11191) which led to 33.4% lignin removal. Similar results were reported by Balasubramaniam and Rajarathinam, (2013) who showed 27.8% and 24.4% lignin and cellulose reduction respectively by *P.ostreatus* pretreatment of paddy straw.

Biogas production from Microwave and biological pretreated paddy straw: A summary of results from the biogas potential approach of the microwave pretreated as well as the fungal pretreated straw with respect to the controls is shown in table 6. A total of 67.2 litre biogas/Kg of paddy straw was produced in the microwave irradiated sample corresponding to the biological pretreatment method which produced 80.0 liters biogas/Kg paddy straw. Both pretreatments showed 19.7% and 42.6% increase in biogas production with respect to control. There is possibility of conversion of lignin into other soluble components which may further be utilized by the methanogens as a substrate after the consumption of simpler/preferred substrates i.e. cellulose/hemicellulose. The result justified

that biological pretreatment could enhance the methane yield by removing more cellulose, hemicellulose and silica content from paddy straw as compared to microwave treated and untreated paddy straw.

Conclusion

Lignin and silica reduction significantly affected the biogas production in chemically pretreated straw with 43.7% increase in Na₂CO₃ and 49.7% in NaOH with respect to control. However crude enzymatic pretreatment enhanced biogas yield by 31.1% with 23.0% and 46.8% decrease in lignin and silica content. For *P.ostreatus* pretreatment lignin and silica removal was found to be 13.3% and 32.8% at 7 days of incubation time resulting in 42.6 % increase in methane level. Both chemical and fungal pretreatments showed a linear interrelationship with similar values for biogas production but weakly related to silica degradation during chemical pretreatment. It was concluded that cellulose and hemicellulose breakdown in higher proportion leads to less degradation of lignin and silica content in NaOH pretreated paddy straw. Although chemical pretreatment can efficiently increase biogas production but can be detrimental to the environment, therefore fungal pretreatment is preferred for digestibility of rice straw and biogas yield.

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REFERENCES

- Ai, B., Chi, X., Meng, J., Sheng, Z., Zheng, L., Zheng, X. and Li, J. (2016). Consolidated Bioprocessing for Butyric Acid Production from Rice Straw with Undefined Mixed Culture. *Front Microbiol.* 7 :1-10.
- Akhtar, N., Jain, A. K., Goyal, D. and Goyal, A. (2014). Surfactant assisted microwave-acid pretreatment of leaf litter biomass for enhanced enzymatic release of sugars. *Proc 7th International Conference on Environmental Science and Technology* Department of Biotechnology, Thapar University, Patiala, 147004, Punjab, India
- Akhter, S., Kotru, R., Dar, N. A., Rasool, R. and Din, R. M. U. (2015). Resource conservation technology in rice wheat cropping system. *Best: JHAMS.* 1 (2) : 25-28
- AOAC (2000) Association of Official Analytical Chemists, Official methods of Analysis, *JAOAC Int.* 83 (4): 1020-25, 17th Edition, Maryland, USA
- Armstrong, J. M., Myers, D. V., Verpoorte, J. A. and Edsall, J. T. (1966). Purification and properties of human erythrocyte carbonic anhydrase. *J. Biol Chem.* 241 : 5137-4519
- Arvanitoyannis, I. S. and Tserkezou, P. (2008). Corn and rice waste: A comparative and critical presentation of methods and current and potential uses of treated waste. *Int J Food Sci. Tech.*, 43 : 958–988

- Balasubramaniam, M. K. and Rajarathinam, R. (2013). Implementation of White rot fungal Pretreated Rice straw for Sustainable Bioethanol Production by *Saccharomyces cerevisiae*. *IJERT*, 2 (11) : 4047-4053
- Blanchette, R. A., Burnes, T. A., Eerdmans, M. M. and Akhtar, M. (1992). Evaluating isolates of *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora* for use in biological pulping purposes. *Holzforschung*. 42 : 109-115
- Chen, J., Fales, S. L., Varga, G. A. and Royse, D. J. (1996). Biodegradability of free monomeric cell-wall-bound phenolic acids in maize stover by two strains of white-rot fungi. *J. Sci. Food Agr.*, 71 : 145-150
- Cheng, S. Y., Zheng, Y., Yu, C. W., Zheng, Y., Yu, C. W., Dooley, T. M., Jenkins, B. M., Jean, S. and Gheynst, V. (2010). Evaluation of High Solids Alkaline Pretreatment of Rice Straw. *Appl. Biochem Biotechnol.*, 162 : 1768–1784
- Contreras, L. M., Schelle, H., Sebrango, C. R. and Pereda, I. (2012). Methane potential and biodegradability of rice straw, rice husk and rice residues from the drying process. *Wat Sci Tech.*, 65 (6) : 1142-1149
- Dai, B., Zhu, A., Mu, F., Xu, N. and Wu, Z. (2014). Experiments on anaerobic digestion of rice straw for biogas production under NaOH pretreatment. *Adv Mat Res.*, 953-954 : 220-223
- Dehghani, M., Karimi, K. and Sadeghi, M. (2015). Pretreatment of rice straw for the improvement of biogas production. *Energ Fuels*. 29 : 3770-3775
- Economic uses of rice straw, Cultural rice research board <http://www.carrb.com/84rpt/StrawUses.htm>, accessed online: 18/11/16
- FAO and Rice Market Monitor (2014). Trade and Markets Division Food and Agriculture Organization of the United Nations. 17: 4
- Gadde, B., Bonnet, S., Menke, C. and Garivait, S. (2009). Air pollutant emissions from rice straw open field burning in India, Thailand and the Philippines. *Environ Pollut.*, 157 : 1554–1558
- Gammal, A., Kamel, Z., Adeeab, Z. and Helmy, S. M. (1998). Biodegradation of lignocellulosic substances and production of sugars and lignin degradation intermediates by four selected microbial strains. *Polym.Degrad Stab.*, 61 : 535-542
- Harun, S. and Goek, S. K. (2016). Effect of Sodium Hydroxide Pretreatment on Rice Straw Composition. *Indian J Sci Technol.*, 9 (21): 1-9
- He, Y., Pang, Y., Liu, Y., Li, X. and Wang, K. (2009). Physico chemical characterization of rice straw pretreated with sodium hydroxide in the solid state for enhancing biogas production. *Energ. Fuels*, 22 : 2775–2781
- Henderson, M. E. K. and Duff, R. B. (1965). The release of metallic and silicate ions from mineral rocks and soils by fungal activity. *J. Soil Sci.*, 14 : 236-246
- Jafri, U. A., Javed, M. T. and Chughtai, I. R. (2011). Process investigation for conversion of municipal solid waste into liquid fuel. *JPICHE*. 39 : 23–27
- Jaisamut K., Paulová L., Patáková P., Rychtera M. and Melzoch, M. (2013). Optimization of alkali pretreatment of wheat straw to be used as substrate for biofuels production. *Plant Soil Environ.*, 59 (12) : 537-542
- Kangle, K. M., Kore, S. V., Kore, V. S. and Kulkarni, G. S. (2012). Recent trends in anaerobic co-digestion: A Review. *UJERT*. 2 (4) : 210-219
- Kaur, K. (2009). Effect of chemicals and biological treatment of paddy straw on biogas production. M.Sc Thesis, Punjab Agricultural Univeristy, Ludhiana
- Kaur, K. (2013). Development of lignin and silica solubilizing microbial consortium for paddy straw pretreatment to enhance biogas production Ph.D. dissertation, Punjab Agricultural Univeristy, Ludhiana
- Kaur, K. and Phutela, U. G. (2016). Sodium carbonate pretreatment: an approach towards *desilicification* of paddy straw and enhancement in biogas production. *Paddy Water Environ.*, 14 (1) : 133-221
- Khaleghian, H., Karimi, K. and Behzad, T. (2015). Ethanol production from rice straw by sodium carbonate pretreatment and *Mucorhiemalis* fermentation. *Ind Crop Prod.*, 76 : 1079-1085
- Kumar, P., Barrett, D. M., Delwiche, M. J. and Stroeve, P. (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind Eng Chem Res.*, 48 : 3713–3729
- Mustafa, A. M., Poulsen, T. G., and Sheng, K. (2016). Fungal pretreatment of rice straw with *Pleurotostreatus* and *Trichoderma reesei* to enhance methane production under solid-state anaerobic digestion. *Appl. Energy*, 180 : 661–671
- Negi, S. and Kumari, J. (2014). Development of bioprocess for the production of laccase by *Pleurotus ostreatus* MTCC 1802 using evolutionary optimization technique. *Indian J Exp Biol.*, 52 (11) : 1106-1111
- Omidvar, M., Karimi, K. and Mohammadi, M. (2016). Enhanced ethanol and glucosamine production from rice husk by NaOH pretreatment and fermentation by fungus *Mucorhiemalis*. *Biofuel Res J*. 11 : 475-481
- Park, C. Y. and Kim, S. J. (2014). Comparison of various alkaline pretreatment methods of lignocellulosic biomass. *Energy*, 47 (1) :31-35
- Phutela, U. G. and Sahni, N. (2013). Microscopic structural changes in paddy straw pretreated with *Trichoderma reesei* MTCC 164 and *Coriolus versicolor* MTCC 138. *Indian J. Microbiol.*, 53 (2) : 227–231
- Platt, M. W., Hadar, Y. and Chet, I. (1984). Fungal activities involved in lignocellulose degradation by *Pleurotus spp.* *Appl. Microbiol. Biotechnol.* 20 (2): 150-154
- Qiu, Y. S., Ling, Q., Yong, S., Tao, L. and Fang, Y. D. (2011). Experiments on anaerobic digestion of NaOH-pretreated peanut shell for biogas production. *J. Agro-Environ Sci.*, 30 (3) : 573-578
- Raeisi, S. M., Tabatabaei, M., Ayati, B., Ghafari, A. and Mood, S. H. (2016). A novel combined pretreatment method for rice straw using optimized EMIM [Ac] and Mild NaOH. *Waste Biomass Valor.*, 7 : 97-107
- Renewables Global Status Report (2016). Renewable Energy Policy Network for the 21st Century (REN 21), www.ren21.net. ISBN 978-3-98181-0-7
- Saha, B. C. (2003). Hemicellulose bioconversion. *J Ind Microbiol Biotechnol.*, 30 : 279–291
- Saini, J. K. and Saini, R. (2015). Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. *Biotechnol.*, 5 : 337-353
- Schroder, H. C., Krasko, A., Pennec, G. L. and Muller, W. E. G. (2003). Silicase, an enzyme which degrades biogenous amorphous silica: Contribution to the metabolism

- of silica deposition in the demosponge *Suberites domuncula*. *Prog Mol Subcell Biol.*, 33 : 249-268
- Soest, V. (2006). Rice straw, the role of silica and treatments to improve quality. *Anim Feed Sci Technol.*, 130 (3-4) : 137-171
- Streets, D. G., Yarber, K. F., Woo, J. H. and Carmichael, G. R. (2003). Biomass burning in Asia: Annual and seasonal estimates and atmospheric emissions. *Glob. Biogeochem Cycles* 17 : 1099–1119
- Taniguchi, M., Suzuki, H., Watanabe, D., Sakai, K., Hoshino, K. and Tanaka, T. (2005). Evaluation of pretreatment with *Pleurotus ostreatus* for enzymatic hydrolysis of rice straw. *J Biosci Bioeng.*, 100 : 637-643
- Thakur, S., Shrivastava, B., Ingale, S., Kuhad, R. C. and Gupte, A. (2013). Degradation and selective ligninolysis of wheat straw and banana stem for an efficient bioethanol production using fungal and chemical pretreatment. *Biotechnol.*, 3 (5) : 365-372
- Toender, E. J. and Borchert, T. M. (2014). Use of enzymes having silicase activity US20140335577A1
- Wang, D., Sakoda, A. and Suzuki, M. (2001). Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain. *Bioresource Technol.*, 78:293-300
- Wang, X., Hu, S., Gan, L., Wiens, M. and Muller, W. E. G. (2010). Sponges (Porifera) as living metazoan witnesses from the Neoproterozoic: biomineralization and the concept of their evolutionary success. *Terra Nova.*, 22 (1) : 1–11
- Wang, Y., Peng, Z., Liao, Q. and Chen, R. (2012). Effect of magnesium ions on biogas production characteristics of lettuce and potato peelings in anaerobic digester. *T Chi Soc of Agric Eng.*, 28 (6) : 217-221
- Wang, Z., Keshwani, D. R., Redding, A. P. and Cheng, J. J. (2010). Sodium hydroxide pretreatment and enzymatic hydrolysis of coastal Bermuda grass. *Bioresource Technol.*, 101 (10) : 3583-3585
- Werle, L. B., Garcia, J. C., Kuhn, R. C., Schwaab, M., Folletto, E. L., Cancelier, A., Sergio, L. J. and Mazutti, M. A. (2013). Ultrasound-assisted acid hydrolysis of palm leaves (*Roystonea oleracea*) for production of fermentable sugars. *Ind. Crops Prod.* 45 : 128–132
- Yang, L., Cao, J., Jin, Y., Chang, H. M., Jameel, H., Phillips, R. and Li, Z. (2012). Effects of sodium carbonate pretreatment on the chemical compositions and enzymatic saccharification of rice straw. *Bioresource Technol.* 124 : 284-291
- Yang, L., Cao, J., Mao, J. and Jin, Y. (2013). Sodium carbonate–sodium sulphite pretreatment for improving the enzymatic hydrolysis of rice straw. *Ind Crop Product.*, 43 : 711-717
- Younas, R., Zhang, S., Zhang, L., Luo, G., Chen, K., Cao, L., Liu, Y. and Hao, S. (2016). Lactic acid production from rice straw in alkaline hydrothermal conditions in presence of NiO nanoplates. *Catal. Today.*, 274: 40–48
- Zhang, Q. and Cai, W. (2008). Enzymatic hydrolysis of alkali pretreated rice straw by *Trichoderma reesei* ZM4-F3. *Biomass Bioeng.*, 32 : 1130-1135
- Zhu, S., Wu, Y., Yu, Z., Liao, J. and Zhang, Y. (2005). Pretreatment by microwave/alkali of rice straw and its enzymatic hydrolysis. *Process Biochem.*, 40: 3082-3088