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Bacterial pretreatment of paddy straw for enhancing biogas production

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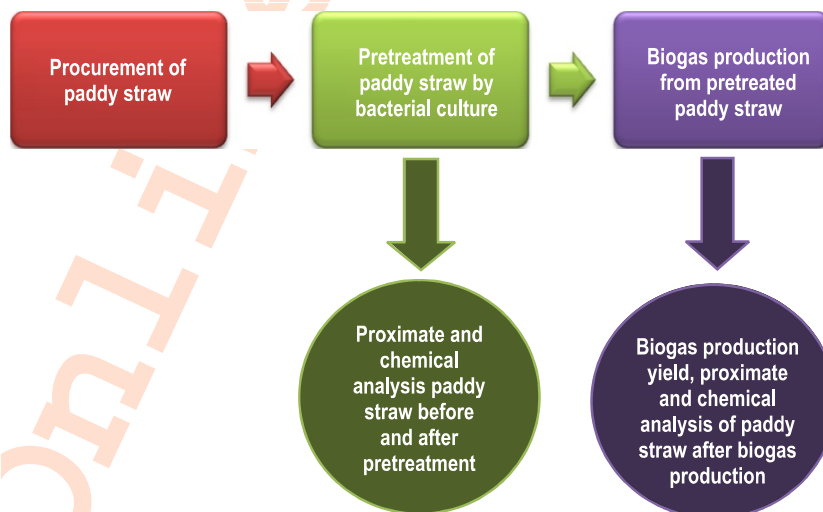
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Abstract

Aim : Increasing the digestibility of paddy straw and biogas production by pretreating it with bacterial culture, *Delftia* sp. PP4_S3.

Methodology : The chopped (3-5 cm) and soaked paddy straw in different sets (each with 250 g PS) were pretreated with bacterial culture i.e., *Delftia* sp. PP4_S3 suspension for different durations and was further utilized for biogas production.

Results : Biogas yield was highest (180 l kg⁻¹ PS) in paddy straw treated with *Delftia* sp. PP4_S3 for 3 days showing an increase of 66.1% from untreated paddy straw. Chemical analysis approximately showed that maximum reduction of lignin (45.7%) and silica (17.7%) occurred in 5 days of pretreatment.



Interpretation : Treatment of paddy straw with *Delftia* sp. PP4_S3 enhance the digestibility of paddy straw by lowering the lignin and silica content. These observations showed that *Delftia* sp. PP4_S3 is a good lignocellulosic degrader and can be efficiently used for enhancing biogas production.

Key words: Biogas production, *Delftia* sp. PP4_S3, Lignin, Paddy straw, Silica

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Introduction

Use of agricultural by-products instead of high input dedicated crops is currently the area of interest in sustainable biogas production. These by-products represent unused form of lignocellulosic biomass which helps to increase the biogas production. The resultant mixture of gases from anaerobic digestion consists of CH₄ (50-75%), CO₂ (20-45%), H₂ (3%), CO (0.3%), H₂S (0.1-0.6%), N₂ (1-3%) and water vapours (in traces) (Rassi *et al.*, 2007). Biogas production involves a group of micro-organisms *i.e.*, hydrolytic, acidogenic and methanogenic bacteria. Hydrolytic bacteria break the carbohydrates, proteins and fats into simple forms *i.e.*, sugar, amino acids and fatty acids. Acidogenic bacteria further convert these forms to acetic acid, hydrogen and carbon dioxide. These act as substrates for methanogenic bacteria and converts them in to biogas. As the calorific value of biogas is about 6kWh m⁻³ (this corresponds to half a litre of diesel oil), the process would save enormous amount of fuel per year (Kashyap *et al.*, 2003). Due to high concentration of methane in biogas, it is considered as one of the cleanest and efficient alternative sources.

Paddy straw, corn stover, wood and sugarcane are rich sources of lignocellulosic biomass. In India, paddy (*Oryza sativa*) is cultivated in about 43.95 million ha producing about 106.54 million tonnes of rice and approximately 160 million tonnes of straw (Chandra *et al.*, 2017). Paddy straw mainly contain cellulose (30-40%), hemicellulose (20-25%), lignin (6-23%), silica (6-12%) and other trace compounds which form complex network due to cross-bonding between them (Nigam *et al.*, 2009, Saha *et al.*, 2003). Cellulose, a homopolymer, is most abundant material of lignocellulosic biomass and also most abundant renewable resource whereas hemicellulose the second abundant component is a heteropolymer. The packed microfibrils of cellulose are stabilized by hydrogen bonding. Cellulose is attacked by microbes when it is present in non-crystalline form. Cellulose has high molecular weight as compared to hemicellulose and is unbranched in nature. Paddy straw is low in lignin content as compared to other cereal straws but have high silica content which protects the plant cell wall carbohydrates (Van Soest, 2006). Lignin is associated with cellulose and hemicellulose by forming ether linkages with them. Lignin is degraded by delignification (the process by which lignin breakdown) through pre-treatment methods like chemical, physical and biological.

Farmers burn paddy straw after harvesting which causes serious problems. The stubble burning not only affects the soil fertility but also results in loss of essential nutrients and causes respiratory problems in humans (Wang and Christopher, 2003). Due to low calorific value and high silica content in paddy straw as compared to other crop-residues, it is difficult to utilize different applications such as energy conversion, animal fodder *etc.* (Calvo-Flores and Dobado, 2010; Menon and Rao, 2012; Anwar *et al.*, 2014). Pre-treatment of biomass is an important tool to alter the structure of paddy straw and make cellulose more available to the enzymes. Different methods used for pretreatment are

physical methods which include mechanical or non-mechanical methods (UV-radiations, microwave, gamma rays, irradiations, electron beam), chemical methods (acid or alkali treatment like H₂SO₄ or NaOH) and biological methods (using biological materials). However, these physio-chemical methods require pressurized and anti-corrosive reactors along with high energy consumption which increase the cost of equipment. Furthermore, in chemical pretreatment method toxic compounds are released into the environment which causes many health hazards. The biological methods are more convenient than other pretreatment methods as they are cost-effective and require less energy (Saratale *et al.*, 2008). In biological pretreatment processes, mainly white rot, brown rot fungi and moulds are used, however, researchers have isolated lignolytic and cellulolytic bacteria that are capable of degrading lignocellulosic biomass. Due to bacterial diversity, fast reproduction rate and short life cycle of cellulolytic bacteria can be preferred for biological treatment of lignocellulosic substrates as compared to other micro-organisms like fungi, microalgae, moulds *etc.* Moreover, cellulolytic bacteria in the marine environment are capable of degrading cellulose from wood. There are wide range of bacterias like *Pseudomonas aeruginosa*, *Serratia marcescens*, *Nocardia*, *Arthrobacter*, *Micrococcus* *etc.* that have been identified as competent degraders.

Delftia is a gram-negative, rod-shaped, motile, non-sporulating, oxidase and catalase positive, non-pigmented bacterium belongs to Comamonadaceae family which may occur singly or in pairs. The genus *Delftia* has been isolated from different environments like fresh and marine water, clinical samples, infected plants and activated sludge (Wen *et al.*, 1999). Han *et al.* (2005) and Morel *et al.* (2011) reported the capability of *Delftia* sp. to fix N₂, produced phytohormones and are used as biofertilizers. *Delftia* sp. also considered as good agent for cleaning of contaminated environments (Ubalde *et al.*, 2012). They have a marked ability to convert or degrade many organic pollutants including aniline, chloroniline (Benndaof *et al.*, 2002), linurin and diuron (Bazot *et al.*, 2007).

Bai *et al.* (2017) used *Bacillus megaterium* MYB3 for the decomposition of lignocellulosic biomass of corn stover and rice straw. Similarly, Zhao *et al.* (2014) identified BMC-9 strain of bacteria which shows potential to rapidly degrade the lignocellulosic residue of rice straw under relatively inexpensive conditions as *Clostridium* sp., *Bacillus* sp. and *Geobacillus* sp. There are numerous reports available on paddy straw treatment with fungi but least on bacteria for enhancing biogas production. In view of the above, the present study focused on the potential of *Delftia* sp. PP4_S3 to enhance paddy straw digestibility and biogas production.

Materials and Methods

Fresh paddy straw was procured from the research field of Punjab Agricultural University, Ludhiana, after harvest. Paddy straw was chopped into small sized pieces (3-5 cm) using Toka

machine and was stored in polythene bags at room temperature. The chemicals used in the experiments were purchased from SD fine, Molychem Chemicals and Hi-Media Chemicals Pvt. Ltd.

Bacterial culture of *Delftia* sp. PP4_S3 (GenBank Accession number JF274923.1) was procured from the Department of Microbiology, Punjab Agricultural University, Ludhiana and was grown in Nutrient Broth for 24 hr at $37 \pm 2^\circ\text{C}$ in BOD incubator. The cell density of bacterial suspension was determined by measuring OD at 600 nm using UV-Visible spectrophotometer (Hitachi model 2800) and taking cell count on haemocytometer. Chopped paddy straw (250 g) was soaked overnight and extra water was drained off. It was then inoculated with 40% inoculum concentration of bacterial culture, i.e., 100 ml culture broth in 250 g paddy straw in different treatments and each treatment was kept for different time period i.e. 1d, 2d, 3d, 4d and 5d. Treated paddy straw was washed and dried, after completion of incubation time. After drying, paddy straw was stored in polythene bags was further used for deducing the change in composition. Untreated paddy straw was kept as a control.

The morphological changes occurred after pre-treatment was discerned by Scanning Electron Microscope (SEM) Imaging according to Bazzola and Russel (1999) at Nano and Electron Microscope Laboratory (NEML), Punjab Agricultural University, Ludhiana. The samples were processed by dehydrating, followed by mounting on carbon tape. Thereafter, sputter coating was carried out with the gold particles and then viewed under Scanning Electron Microscope (Hitachi S-3400N, Germany) at accelerating voltage of 15000 V. Monophasic method was used for determining the biogas production and experiments were carried out in 2 l capacity digesters, while biogas produced was measured by water displacement method. Two fifty gram of pre-

treated paddy straw was mixed with 25 ml of slurry and 25 g of cattle dung. Bio-digested slurry functioned as inoculum whereas cattle dung functioned as inducer to enhance the biogas production. A pH of 7.2 was maintained and was checked with a pH meter. The digester was properly sealed with rubber cork and araldite. This was further connected to the water displacement system for measuring biogas production. Simultaneous experiment using untreated paddy straw and cattle dung was also carried out. The biogas digesters were then incubated at room temperature and biogas was measured daily for a period of 45 days. The proximate and chemical analysis experiments were conducted in triplicate. One set of untreated paddy straw that served as control was also conducted simultaneously for biogas production. Standard methods of AOAC (2000) were followed for determining proximate and chemical composition of paddy straw. The critical difference (5%) with CRD Design was performed using CPCS1 software (developed by Department of Statistics, PAU, Ludhiana) and standard error was calculated manually for all the experiments.

Results and Discussion

Degradation of straw was enhanced with inoculation of *Delftia* sp. PP4_S3 from the beginning of fermentation as shown in Table 1. The decrease in the weight of paddy straw indicates its degradation by treatment which was also observed in SEM images from untreated paddy straw. The results showed that both total solids and volatile solids decreased with increase in the incubation period. The total solids ranged from 99.1% to 91.7% in control and 5 day treatment, with a significant decrease of 2.54% in 5 day treatment as compared control. Volatile solids also decreased from 84.6% (control) to 79.8% during 5 day treatment. However, ash content was found to increase with a maximum

Table 1: Proximate and chemical composition of paddy straw pretreated with bacterial culture for different incubation period

Treatment (Days)	Composition of paddy straw (%)							
	Total Solids	Volatile Solids	TOC	Ash	Cellulose	Hemicellulose	Lignin	Silica
Control#	99.1 \pm 0.50	84.6 \pm 0.21	47.0 \pm 0.08	15.5 \pm 0.20	36.4 \pm 1.21	24.8 \pm 0.17	12.9 \pm 0.40	11.8 \pm 0.08
1	97.9 \pm 0.46 (1.21 ↓)	82.7 \pm 0.14 (2.24 ↓)	45.9 \pm 0.05 (2.34 ↓)	17.4 \pm 0.14 (12.2 ↑)	31.2 \pm 0.23 (14.2 ↓)	22.4 \pm 0.25 (9.67 ↓)	8.9 \pm 0.54 (31.0 ↓)	11.5 \pm 0.17 (2.54 ↓)
2	95.7 \pm 0.29 (3.43 ↓)	82.5 \pm 0.40 (2.54 ↓)	45.9 \pm 0.23 (2.34 ↓)	17.5 \pm 0.41 (12.9 ↑)	27.7 \pm 0.23 (23.9 ↓)	22.5 \pm 0.89 (9.27 ↓)	8.9 \pm 0.17 (31.0 ↓)	10.8 \pm 0.20 (8.47 ↓)
3	94.8 \pm 0.34 (4.33 ↓)	82.2 \pm 0.43 (2.83 ↓)	45.7 \pm 0.20 (2.76 ↓)	17.9 \pm 0.43 (15.4 ↑)	24.3 \pm 0.31 (33.2 ↓)	21.3 \pm 0.02 (14.1 ↓)	8.5 \pm 0.17 (34.1 ↓)	10.1 \pm 0.44 (14.4 ↓)
4	92.8 \pm 0.23 (6.35 ↓)	81.5 \pm 0.37 (3.66 ↓)	45.3 \pm 0.20 (3.61 ↓)	18.6 \pm 0.37 (20.0 ↑)	23.0 \pm 0.31 (36.8 ↓)	21.0 \pm 1.35 (15.3 ↓)	8.0 \pm 0.14 (37.9 ↓)	10.0 \pm 0.08 (15.2 ↓)
5	91.7 \pm 0.28 (7.46 ↓)	79.8 \pm 0.83 (5.67 ↓)	44.4 \pm 0.50 (5.53 ↓)	20.3 \pm 0.83 (30.9 ↑)	21.6 \pm 0.17 (40.6 ↓)	19.2 \pm 1.21 (22.5 ↓)	7.0 \pm 0.34 (45.7 ↓)	9.7 \pm 0.02 (17.7 ↓)
CD (5%)	2.17	0.82	1.32	1.11	3.29	2.12	1.99	1.28

Control: uninoculated soaked paddy straw; 2% urea in 40% inoculated culture in soaked paddy straw. Cultural conditions: pretreated straw (250 g soaked paddy straw+ 2% urea), Inoculum concentration 40% equivalent to total cell count of 8×10^7 cells per ml.; values are mean of three replicates \pm SD; (↓): decrease; (↑): increase; Critical difference (CD) at 5% level

Table 2: Biogas production profile (45 days) of pretreated paddy straw with different treatments

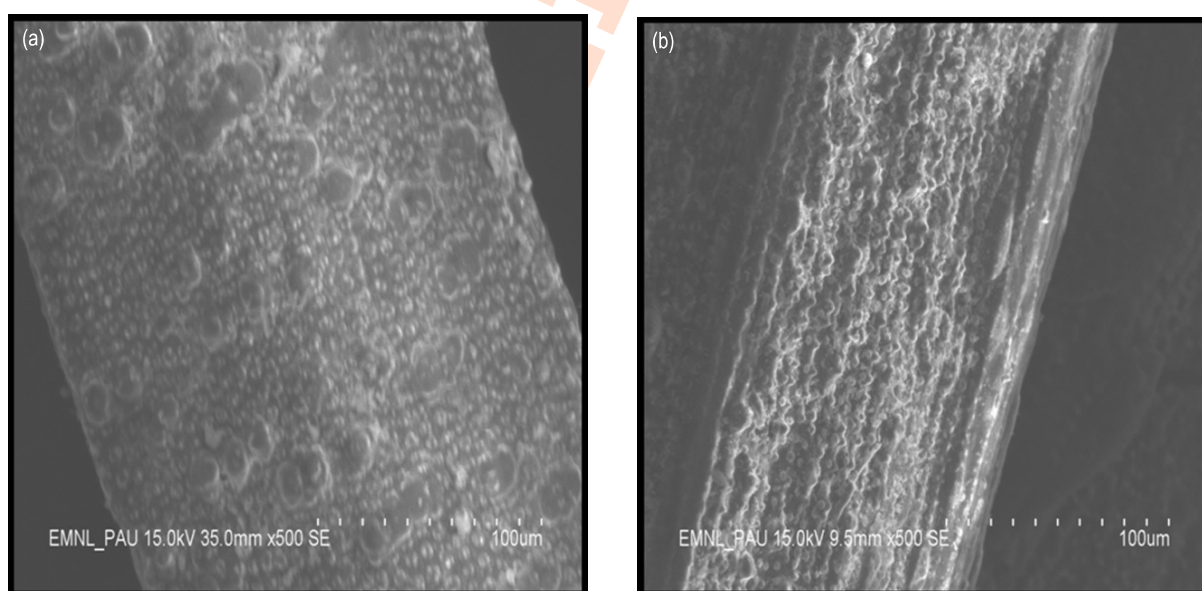
Parameters	Control#	Pretreatment period (days)					CD (5%)
		1	2	3	4	5	
Biogas (l 250 g ⁻¹ PS)	27.2±0.08	29.3±0.05	29.5±0.08	45.2±0.57	40.9±0.17	39.2±2.21	0.87
Biogas (l kg ⁻¹ PS)	108.6±0.34	117.2±0.23	117.8±0.34	180±2.3	163.6±0.69	157.0±8.89	5.64
Biogas (l kg ⁻¹ TS)	268.6±0.25	357.3±2.60	340.4±7.52	560.5±5.66	558.1±7.54	553.2±2.88	22.5
Biogas (l kg ⁻¹ VS)	314.6±1.78	426.4±3.67	403.5±12.0	673.2±8.66	679.2±4.64	681.2±5.62	28.2
% change from control	0.0	7.91(↑)	8.47(↑)	66.1(↑)	47.7(↑)	44.5(↑)	3.2

#Control: Untreated paddy straw; PS: Paddy straw; TS: Total solids; VS: Volatile solids. Composition of paddy straw mixture: 250 g paddy straw +25 g biogas digester slurry +25 g cattle dung; Biogas digester: 2 litre capacity; incubation temperature: 28±37°C; incubation period: 45 days. Values are mean ± SD of three replicates; (↓) decrease; (↑): increase; Critical difference (CD) at 5% level

increase of 30.9% in 5 day treated sample. As the incubation period increased, there was a decrease in cellulose content, accompanied by maximum reduction of 40.6% after 5 day of treatment. As compared to control, hemicelluloses also decreased to 19.2% after 5 days.

A decline in hemicellulose and cellulose content may be attributed to degradation of hemicelluloses and celluloses into fermentable sugars (Jalc *et al.*, 1998). Hence, it clearly indicates

that bacteria have more active cellulases than hemicellulases. Similarly, as compared to control, Lignin content also decreased from control (12.9%) showing maximum reduction of 45.7% in 5 day treated sample (7%). The ratio between cellulose and lignin degradation mainly represent the lignin degrading capability of micro-organism (Mustafa *et al.*, 2016). Silica content also decreased significantly with a maximum reduction of 17.7% from control after 5 day treatment. These observations indicate that *Delftia* sp. PP4_S3 is a lignocellulolytic and silicolytic bacterium.

**Fig.1:** Scanning Electron Micrographs of paddy straw (a) Control and (b) Pretreated with bacterial culture.

However, the extreme reduction in lignin and cellulose content indicates that *Delftia* sp. PP4_S3 may be a potential organism for degradation of cellulose and lignin. Removal of hemicellulose might result in improving surface area accessible to cellulose. Kaur and Phutela (2016) observed maximum reduction of lignin content (65%) and silica (54.7%) by treating paddy straw with NaOH in a microwave. Pretreating paddy straw with *Pleurotus ostreatus* and *Trichoderma reesei* showed maximum reduction of lignin (33.4% and 23.6%) in 20 days. Prasad et al. (2014) reported maximum cellulose degradation by *Mesorhizobium* sp. and *Bacillus pumilus* with other substrates within 5 weeks and lignin degradation in 3 weeks of inoculation. Chengbin et al. (2009) reported degradation of aniline by *Delftia* sp. XYJ6 which convert aniline into catechol 1,2-dioxygenase within 22 hr of inoculation. Xiong et al. (2014) observed the rice straw degradation capacity in endophytic bacterial strain *Pantoea* sp. SD-1, this specie. reduce the rice straw weight by 54.5% after 6 days and 69.1% lignin content after 4 days of pretreatment.

The SEM images of paddy straw before and after its pretreatment with *Delftia* sp. PP4_S3 is show in Fig. 1. The distinct changes in surface structure of paddy straw are visible in the basic tissue whereas untreated paddy straw showed a rigid and highly compact structure. In bacterial pretreated sample, sluffing of material was observed whereas in fungal pretreated sample more pores along with the reduction in size of silica nodules were observed. Zhang and Cai (2008) also reported similar changes in structure of paddy straw after 2% NaOH pretreatment. Sahni and Phutela (2012) reported that pretreatment of rice straw with *Humicola fuscoatra* MTCC 1409 resulted in opening of holo-cellulose fibrils due to pores of various sizes. Xu et al. (2007) reported that the structure and surface area of pretreated straw favours enzymatic hydrolysis.

Table 1 shows that biogas production from pre-treated paddy straw increased till 3d day. Biogas production on 1st and 2nd day of treatment were almost same i.e., 117.2 l kg⁻¹ PS (426.4 l kg⁻¹ VS) and 117.8 l kg⁻¹ PS (403.6 l kg⁻¹ VS). The maximum biogas production was found on 3rd day of treatment with 66.1% increase from control. However, after increasing the pre-treatment period i.e., above optimum period the biogas production started decreasing. The maximum biogas yield was observed on 4th and 5th day of pretreated paddy straw as compared to 1st and 2nd day of pretreatment. This may be attributed to enhanced paddy straw digestibility due to lower silica and lignin content from breakage of bonds between cellulose, hemicellulose and lignin with increase in treatment period (Fox and Noike, 2003). However, the decrease in biogas production after 3rd day treatment can be attributed to decrease cellulose content with increasing incubation period. Digestibility is mainly restricted by lignin, hence cellulose is the preferred substrate for producing biogas. When straw was treated, a part of lignin was removed, and the cellulose structure was significantly modified. This could be the reason for higher overall biogas yield. In lignocellulosic biomass, methane production depends on two important factors viz. rate at which degradation occur and extent of degradation. The fungal

pretreatment improved the biogas yield by 120% (*P. ostreatus*) and 78% *T. Reesi* as compared to untreated paddy straw (Mustafa A.M. et al., 2016) probably due to the more intense lignolytic activity of this fungus (Taniguchi et al., 2005). While wheat straw pretreated with *C. subvermispura* for 10 weeks showed increase in biogas production both in mono and in co-digestion with pig slurry (18% and 17%) (Vasmara et al., 2015).

Incubation time plays an important role in bacterial pretreatment of paddy straw by *Delftia* sp. in order to remove cellulose and lignin content during pretreatment and enhancing the biogas production during anaerobic digestion. Bacterial pretreatment caused a significant decrease in cellulose (40.6%) and lignin (45.7%) content as compared to hemicellulose (22.5%) and silica (17.7%) content. Three day pretreated paddy straw resulted in 66.1% increase in biogas production as compared to untreated paddy straw. Thus, bacterial pretreatment of paddy straw can significantly increase the biogas production. So, hence it is important to degrade the cellulose and lignin content in paddy straw to increase biogas production.

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