Department of Renewable Energy Engineering
College of Agricultural Engineering and Technology
P A U, Ludhiana

Summary of The Major Research Project entitled, "Development of thermophilic and stable fungal consortium for enhancing biogas production from paddy straw" 41-1158/2012(SR), 26 July 2012

1.	NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR	Dr. Urmila Gupta, Principal Scientist (REE) **Department of Renewable Energy Engineering** (School of Energy Studies for Agriculture) College of Agricultural Engineering and Technology, PAU Ludhiana
2.	NAME AND ADDRESS OF THE INSTITUTION	PAU, Ludhiana
3.	UGC APPROVAL NO. AND DATE	41-1158/2012(SR), 26 July 2012
4.	DATE OF IMPLEMENTATION	21 September 2012
5.	TENURE OF THE PROJECT	3 years and 3months
6.	TOTAL GRANT ALLOCATED	9,76,800/-
7.	TOTAL GRANT RECEIVED	1st Installment :5,32,800/- 2nd Installment : 2,64,726/- **Total: 797,526/-**
8.	FINAL EXPENDITURE	**7,97,269/-**
9.	TITLE OF THE PROJECT	**Development of thermophilic and stable fungal consortium for enhancing biogas production from paddy straw.**

10.	OBJECTIVES OF THE PROJECT	• To develop a thermophilic, stable, fungal consortium having efficient lignin-silica degrading capabilities • To develop an efficient, cost effective, robust and user friendly technology for biogas generation from paddy straw through application of lignin–silica degrading/ solubilizing fungal consortium • Fabrication and evaluation of paddy straw based biogas plant
11.	WHETHER OBJECTIVES WERE ACHIEVED(GIVE DETAILS)	**Yes** • A thermophilic, stable, fungal consortium having efficient lignin-silica degrading capabilities has been developed. • An efficient, cost effective, robust and user friendly technology for biogas generation from paddy straw through application of lignin–silica degrading/ solubilizing fungal consortium has been developed. • Paddy straw based biogas digester has been fabricated and evaluated using thermophilic consortium
12.	ACHIEVEMENTS FROM THE PROJECT	Thermophilic, stable, fungal consortium having efficient lignin-silica degrading capabilities has been developed during the

		project, which can be further upgraded to field scale for enhancing paddy straw digestibility and biogas production.
13.	SUMMARY OF THE FINDINGS(IN 500 WORDS)	**See Annexure 1**
14.	CONTRIBUTION TO THE SOCIETY(GIVE DETAILS)	
	As rice is a major cereal crop in India, it leads to the production of much larger quantity of rice straw. 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 ton of 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). Lung and respiratory diseases caused by burning adversely affects public health. Repeated burning of paddy straw also results in soil erosion. Paddy straw, being a lignocellulose, predominantly contains cellulose (35-40%), hemicellulose (20-24%), lignin (8-12%), ash (14-16%) and extractives (10-12%) which are associated with each other. Although, paddy straw has high holo-cellulose content but the lignin complex and silica incrustation shields the holo-cellulose components, thereby hindering its utilization. The present project clearly shows that silica can be removed along with lignin by using a fungal consortium which not only increases paddy straw digestibility but also its utilization for energy generation. This will be helpful in managing environmental pollution also.	
15.	WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT	Two M.Sc. and one Ph.D

		One (Development of thermophilic fungal consortium for enhancing biogas production from paddy straw) **M.Sc: Two** 1. Production and partial purification of cellulase from H*umicola fuscoatra* mtcc 1409 for enhancing paddy straw digestibility 2. Isolation and screening of silica solubilizing thermophilic fungi for enhancing paddy straw digestibility and biogas production.
16.	Details of the Publications resulting from the project work (please attach re-prints) letter of Acceptance of paper/communicated.	▪ Nidhi Sahni and Urmila Gupta Phutela (2013) Isolation and Preliminary screening of paddy straw degrading thermophilic fungi. *Indian Journal of Applied Research* 3 (10):1-3 ▪ Nidhi Sahni and Urmila Gupta Phutela (2013) Effect of thermophilic fungus *Humicola fuscoatra* MTCC 1409 on Paddy Straw digestibility and biogas production. *International Journal of Scientific Research* 2(9): 25-27. ▪ Nidhi Sahni and Urmila Gupta Phutela (2013) Comparative profile of paddy straw pretreated with standard and isolated lignocellulolytic fungal cultures. *Journal of Yeast and Fungal Research* 4(7): 92-97. ▪ Baldeep Kaur, Surekha Bhatia and **Urmila Gupta Phutela** (2015)Production of cellulases from *Humicola fuscoatra* MTCC

		1409: Role of enzymes in paddy straw digestion *African Journal of Microbiology Research*, .**9(9):** 631-638

SUMMARY OF THE FINDINGS

The present study was aimed at developing and enriching thermophilic lignolytic and silica degrading fungal consortium for efficient utilization of paddy straw. The effect of developed consortium on paddy straw digestibility and biogas production was studied.

A total of 80 thermophilic fungal cultures were isolated, purified and screened qualitatively for ligno-cellulolytic and silicolytic potential on agar plates. Isolate T22 showed maximum cellulase activity whereas nineteen cultures showed positive results for lignin degradation. The twenty isolates were further screened quantitatively for cellulolytic and lignolytic enzyme production. The growth and zone formation on Silica-Peptone agar medium was also observed for these cultures. . For the screening of silica solubilising thermophilic fungi, liquid broth method, zone method, well method were used. The isolated fungal cultures were screened qualitatively for their silica solubilizing potential by adding methyl red in the Silica Peptone agar (SPA) whereby change in yellow/orange color to pink/transparent color was considered as a positive indication for silica solubilizing potential. Eleven consortia were developed by growing 5 isolates namely T5, T10, T14, T17 and T22 in close association on agar plates in different combinations. Paddy straw was pretreated with individual cultures and developed consortia along with standard culture namely *Humicola fuscoatra* MTCC 1409. *H fuscoatra* pretreated paddy straw showed 27.7% increase in biogas production while T14, T17 and T22 pretreated paddy straw showed increase in biogas production by 37%, 41.6% and 25.2%, respectively. The lignolytic enzyme production and change in chemical composition of paddy straw pretreated with consortium (C10) made of isolates T14 (*Aspergillus* sp), T17 (*Paecilomyces* sp) and T22 (*Penicillium* sp) was studied. Cellulose, hemicellulose, lignin and silica contents decreased significantly with 57.01% increase in biogas production in 6 d pretreated paddy straw. Various parameters like incubation temperature, pH, incubation time, metal ion (Cu^{+2}) and nitrogen source (yeast extract) were optimized for lignolytic enzyme

production by Box Benhken model using Response Surface Methodology (RSM) from consortium (C10). Maximum laccase (315.1 U/mg), lignin peroxidase (355.8 U/mg) and manganese peroxidae (238.7 U/mg) was produced from consortium (C10) when media was supplemented with 0.3% Cu^{+2} and 0.1% yeast extract at pH 6.0, incubated at 50°C for 6 days. The lignolytic enzymes were partially purified using ammonium sulphate precipitation, dialysis and ion exchange chromatography using DEAE cellulose. There was 2.23 fold purification for laccase enzyme with specific activity of 289.98 U/mg proteins. The partially purified enzyme remained stable up to 96 h at 4°C. Hence the selected thermophilic fungal consortium (C10) was found to be suitable for increasing paddy straw digestibility and biogas production.

Period - 2012-13 to
2015-16

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

Utilization certificate

Certified that the grant of Rs. Rs. **7,97,526/- (Rupees Seven lakh ninety seven thousand five hundred and twenty Six only)** received from the University Grants Commission under the scheme of support for Major Research Project entitled, **"Development of thermophilic and stable fungal consortium for enhancing biogas production from paddy straw"** vide UGC letter No. F. **41-1158/2012(SR) dated 26 July, 2012 and Dated June 2015.** Out of which **Rs 7, 97,269/-** has been utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission. (Audited Report attached)

21.4.16

PRINCIPAL INVESTIGATOR

Senior Scientist (Biogas)
School of Energy Studies for Agriculture
PAU, Ludhiana.

22/4/16

Director of Research
Punjab Agricultural University,
Ludhiana

DIRECTOR OF RESEARCH

27/5/16

Comptroller
Punjab Agricultural University
LUDHIANA.

PRINCIPAL STATUTORY AUDITOR/COMPTROLLER