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Quantitative Screening of Lignocellulose Degrading Fungi Using Digested Biogas Slurry as a Substrate

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A B S T R A C T

Digested biogas slurry is a rich lignocellulosic substrate, consistent with the idea of sustainable agriculture and development. This work is aimed at utilization of digested biogas slurry as a substrate for the quantitative screening of lignocellulose degrading fungi. In this study, the potential of digested biogas slurry as a substrate to determine the lignocellulolytic enzyme production after 8 days of incubation at 30±2ºC (mesophilic) and 50±2ºC (thermophilic) was investigated. Standard mesophilic culture *Phanaerochaete chrysosporium* MTCC 787 showed maximum enzyme activities (3.9U/ml endoglucanase, 2.09 U/ml exoglucanase, 29.45 U/ml β-glucosidase, 55 U/ml xylanase, 69.5 U/ml mannanase, 48.33U/ml lignin peroxidase, 13.3 U/ml Manganese peroxidase) followed by isolate A5, A4, A12. Among thermophilic cultures, isolate A31 is the best cellulolytic fungal culture. *Thermoascus aurantiacus* (1.97U/ml endoglucanase), isolate A25 (23.08U/ml β-glucosidase, 2.35U/ml exoglucanase, 63U/ml Mannanase) and A31 (48.08 U/ml xylanase, 11.5 U/ml lignin peroxidase, 5.6U/ml laccase) are thus the best lignolytic fungal cultures. The results show that digested biogas slurry, which otherwise is considered as a waste of biogas plants, can be efficiently utilized to screen different fungi for their lignocelluloses degrading potential. Mesophilic cultures *Phanaerochaete chrysosporium,* isolate A4 and A5 and thermophilic cultures *Thermoascus aurantiacus,* isolate A25 and A31 are the best lignocelluloses degraders.

Keywords

Fungi, Digested biogas slurry, Enzymes, Biodegradation, Waste utilization.

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Introduction

Biomass such as animal, forest and agro-industrial wastes are available in abundance and can play a major role in supplementing the energy resources of the country. Easily available cattle dung alone (about 304 million cattle), has an estimated potential of about 18,240 million cubic meter of biogas generation annually that produces gaseous fuel and high quality organic fertilizer (digested biogas slurry)to improve the soil fertility required for sustainable production and improved productivity. This rich organic slurry is free from smell and odour for the field. But due to its semi solid nature, its direct application in fileds is not preferred. Thus, alternate methods of its effective utilization are required.

Microorganisms especially filamentous fungi possess an efficient hydrolytic system capable to convert lignocellulosic material to essential metabolites for growth by producing extracellular enzymes like cellulases (cellobiohydrolases, endoglucanases, β-glucosidases), hemicellulases (xylanases) and lignolytic enzymes (Pothiraj *et al.,* 2006).

These enzymes have numerous industrial applications in chemicals, fuel, food, brewery, animal feed, textile, laundry, paper and pulp industry worth one billion US dollars (Beauchemin *et al.,* 2003). Enzyme sector increase at a 6.6% compound annual growth rate and reached $1.5 billion in 2015 (Binod *et al.,* 2013).

Filamentous fungi like *Aspergillus*, *Penicillium, Trichoderma,* white-rot fungus *Ceriporiopsis subvermispora,* other microbes like *Phanerochaete chrysosporium*, *Streptomyces viridosporous, Pleurotus eryngii, Trametes trogii, Fusarium proliferatum, Aspergillus, Paecilomyces* and *Sporotrichum* are reported to produce lignocellulolytic enzymes (Regalado *et al.,* 1997; Mandhulika *et al.,* 1993). Since, substrate is one of the major cost-determining factors for bulk enzyme production, so relatively low-cost agricultural by-products should be used as substrates for enzyme production (Bajaj and Singh, 2010; Bajaj and Abbass, 2011).

Preliminary work was initiated for production and partial purification of cellulose from digested biogas slurry using *Trichoderma reesei* MTCC 164 (Kaur, 2012).

The aim of this study is to screen some potential fungal isolates which degrade lignocelluloses taken from digested slurry, so that the whole biotechnology industry and enzyme market can be benefitted.

Materials and Methods

Procurement of digested biogas slurry, microbial cultures and chemicals

Digested biogas slurry was procured from a working biogas plant in biogas field laboratory of School of Energy Studies for Agriculture (Punjab Agricultural University), Ludhiana.

Standard fungal cultures *viz. Trichoderma reesei* MTCC 164, *Trichoderma harzianum* MTCC 792, *Coriolus versicolor* MTCC 138, *Pleurotus ostreatus* MTCC 142, *Phanerochaete chrysosporium* MTCC 787, *Thermoascus aurantiacus* MTCC 375 and *Humicola fuscoatra* MTCC were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. *Aspergillus oryzae* NCIM 1212 and *Penicillium roquefortii* NCIM 712 was procured from National Collection of Industrial Microorganisms (NCIM), Pune. These cultures were maintained on potato dextrose agar media slants at 30 ± 2^0C for mesophilic and 45 ± 2^0C for thermophilic cultures and were stored in refrigerator after sub-culturing. Different fungal cultures were isolated at Biogas Laboratory, SESA, PAU.

Chemicals used for solutions preparation for enzymatic analysis were of analytical grade and were purchased from Hi-Media, SRL, Sigma and S.D fine chemicals Pvt. Ltd.

Screening for ligno-cellulolytic activities

Duplicate flasks each containing 100 ml digested biogas slurry were mixed with equal amount of distilled water and were inoculated with 2ml spore suspension of spores of mesophilic and thermophilic isolates as well as with standard cultures @ 10^7spores/ml and incubated at 30±2°C for mesophilic and

45±2°C for thermophilic cultures for the production of enzymes. After an incubation of 8 days, crude enzyme was extracted by centrifugation at 10000 rpm for 15 minutes. Supernatant was analyzed for activities of various lignocellulolytic enzymes *viz.* Endoglucanase, exoglucanase (Mandels *et al.,* 1976) and β-glucosidase (Toyama and Ogawa, 1977)**;** Xylanase (Singh *et al.,* 2000) and Mannanase (Growindhagaer *et al.,* 1999); Laccase, Lignin Peroxidase (LiP by Tien and Kirk, 1988) and Manganese Peroxidase (MnP by Paszczynski *et al.,* 1988). Laccase was determined by measuring oxidation of 2, 2'-Azinobis-3-ethyl benzthiazoline-6-sulphonic acid (ABTS by Mishra *et al.,* 2008).Enzyme activities (U/ml of sample) and protein (mg/ml of sample by method of Lowry *et al.,* 1951) was determined spectrophotometrically using UV-VIS spectrophotometer 2800 model.

Reducing sugars produced by cultures were estimated by Miller's (1959) dinitro-salicylic acid (DNS) method. The enzyme activity was expressed in terms of International units, which is defined as 1 micromole of reducing sugar released per mililitre of enzyme extract, measured as glucose or xylose or mannose. Appropriate dilution factors were used as and when followed during estimation of enzyme activity:

$$\text{Reducing sugars } (\mu \text{ mole/ml/min}) = \frac{\text{mg of reducing produced/ ml}}{0.18 \text{ X incubation period (min)}}$$

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

Results and Discussion

Diluted digested biogas slurry was used as substrate to screen fungi for cellulolytic, hemicellulolytic (xylanases, mannanases) and lignolytic enzyme (laccase, manganese peroxidase, lignin peroxidase) production. The enzymes produced were estimated quantitatively.

Quantitative estimation of cellulolytic enzyme activities of potential fungal isolates

Results from Table 1 shows that among mesophilic cultures, *Phanaerochaete chrysosporium* MTCC 787 showed maximum endoglucanase activity i.e. 3.9U/ml followed by isolate A5 (1.98U/ml) and then A4 (1.12U/ml), respectively. Maximum exoglucanase activity was reported in *Phanaerochaete chrysosporium* MTCC 787 (2.09 U/ml) followed by isolate A5 (1.83 U/ml) and A12 (1.58 U/ml), respectively. β-glucosidase activity of 29.45 U/ml was recorded in *Phanaerochaete chrysosporium* MTCC 787 followed by 28.55 U/ml in isolate A4 and 27.35 U/ml in isolate A2, respectively.

Results from table 2 shows that among thermophilic cultures, isolate A31 is the overall best cellulolytic fungal culture. Maximum endoglucanase activity was recorded in *Thermoascus aurantiacus* (1.97U/ml) followed by 1.35 U/ml in isolate A31. Highest β-glucosidase activity (23.08U/ml) was found in isolate A25 followed by 22.13 U/ml in isolate A31. Exoglucanase activity was low i.e. 2.35U/ml in isolate A25 followed by 1.73U/ml in *Thermoascus aurantiacus.*

Shahriarinour *et al.,* (2011) reported that *Aspergillus* isolate showed highest cellulolytic activity (CMCase 3.05U/ml, Fpase 0.61U and β-glucosidase 1.75U/ml). This isolate was identified as *Aspergillus terrus.* Cellulolytic enzyme activities through solid state fermentation by *Aspergillus terreus* ATCC 74135 were also reported by Jahromi *et al.,* (2011) and were found as maximum

FPase of 410.76 U/g DM, CMCase of 351.96 U/g DM, xylanase of 6166.01 U/g DM, amylo-glucosidase of 425.04 U/g DM on 8th day of incubation from rice straw with 1% urea as nitrogen source and 50% moisture content. 4.68 U/g corn stover CMCase was reported by Zhu *et al.,* (2011) on 21st day of incubation period by *Trametes versicolor* during SSF under optimized conditions.

Quantitative estimation of hemicelluloytic enzyme activities of potential fungal isolates

Table 1 shows that hemicellulolytic enzymes *i.e.* xylanase and mannanase gave maximum enzyme activities in standard fungal culture *Phanaerochaete chrysosporium* MTCC 787 with xylanase activity of 55 U/ml and mannanase activity of 69.5 U/ml, respectively. Among the isolated fungi isolate A3 gave 53U/ml of xylanase activity. A5 isolate gave maximum mannanase activity of 63.5 U/ml.

Table 2 shows that among thermophilic cultures, maximum xylanase activity was recorded in Isolate A31 i.e. 48.08 U/ml followed by 42.72 U/ml in *Thermoascus aurantiacus*. Mannanase was maximum in isolate A25 i.e 63U/ml, followed by 62.5 U/ml in Isolate A31. Overall, isolate A31 is the best hemicelluloses degrading culture.

Palaniswamy *et al* (2008) reported that xylanase and β-D-xylosidase activity ranges from 4.41 to 132.20 U and 48.72 to 1510.32 U, in litter degrading fungi *Aspergillus* sp. and Basidiomycetes sp., respectively. Costa *et al.,* (1994) investigated *A. niger* CCMI 850 for the production of xylanolytic enzymes and a maximum activity of 65 IU/ml of β-xylanase was observed during the experiment. *Aspergillus niger* has been investigated for xylanase synthesis and Gawande and Kamat (1999) reported maximum xylanase activity (26.7 IU/ml) after 48 hours of incubation.

Quantitative estimation of Lignolytic enzyme activities of potential fungal isolates

Tables 1 and 2 indicate that lignin peroxidase activities are higher in comparison to manganese peroxidases and laccase, respectively in all the cultures. Among mesophilic cultures, *Phanaerochaete chrysosporium* showed highest lignin peroxidase activity i.e. 48.33U/ml, followed by 38.33 U/ml in isolate A4. Highest Manganese peroxidase activity of 13.3 U/ml was also showed by standard fungal culture *Phanaerochaete chrysosporium* followed by 11.03 U/ml in isolate A5.

Among thermophilic cultures *Thermoascus aurantiacus*, isolate A25 and A31 are the best lignolytic fungal cultures with 11.5 U/ml of lignin peroxidase activity in isolate A31, followed by 10.83U/ml in *Thermoascus aurantiacus.* Maximum laccase was recorded in isolate A31 (5.6U/ml) followed by 5U/ml in isolate A25. Elisashvili and Kachlishvili (2009) screened basidiomycetes for laccase production using rice straw as a substrate (17.3 U/g). Dhaliwal *et al.,* (1991) reported that production of lignocellulolytic enzymes by *Pleurotuso streatus* with paddy straw. Laccase production in solid state fermentation of selected agro-residues by *Pycnoporus sanguineus* reached a maximum of 480.7 Ug^{-1} (Vikineswary *et al.,* 2006). *Fomessclero dermeus* grown on wheat straw produced 270.5 Ug^{-1} of laccase (Papinutti *et al.,* 2003).

Quantitative estimation of Reducing sugars and protein content

Reducing sugars and protein content from all the cultures were also determined. Figure 1 indicated that *P. chrysosporium* has maximum reducing sugars, thus high potential for lignocellulolytic enzyme production, while isolate A12 has maximum protein, which will lower its specific activity.

Table.1 Quantitative screening of mesophilic cultures grown on digested biogas slurry

MESOPHILIC CULTURES	Enzyme activities (U/ml)							
	Endoglucanase	β-glucosidase	Exoglucanase	Xylanase	Mannanase	Laccase	Lignin Peroxidase	Mangenese Peroxidase
Trichodermareesei MTCC 164	0.76 ± 0.005	16.18 ± 0.005	0.76 ± 0.005	42.0 ± 0.554	65.0 ± 0.033	2.0 ± 0.881	47.5 ± 0.871	3.33 ± 0.587
Trichodermaharzianum MTCC 792	1.11 ± 0.008	18.26 ± 0.580	0.77 ± 0.057	48.0 ± 0.185	68.0 ± 0.120	5.0 ± 0.881	9.17 ± 0.011	10.0 ± 0.577
Pleurotusostreatus MTCC 142	1.22 ± 0.008	18.97 ± 0.571	0.78 ± 0.054	49.0 ± 0.176	68.0 ± 0.057	4.67 ± 0.619	15.0 ± 0.577	1.66 ± 0.293
***Phanaerochaete chrysosporium* MTCC 787**	**3.9 ± 0.288**	**29.45 ± 0.023**	**2.09 ± 0.017**	**55.0 ± 0.317**	**69.5 ± 0.120**	**6.3 ± 0.088**	**48.33 ± 0.909**	**13.33 ± 0.587**
Penicilliumroquefortii NCIM 712	0.98 ± 0.057	19.95 ± 0.005	0.88 ± 0.060	45.0 ± 0.338	61.0 ± 3.756	0.33 ± 0.003	44.17 ± 0.580	1.67 ± 0.727
Coriolusversicolor MTCC 138	0.96 ± 0.351	17.34 ± 0.011	1.17 ± 0.011	47.0 ± 0.057	61.0 ± 1.763	4.0 ± 0.577	4.17 ± 0.580	8.3 ± 0.906
Aspergillusoryzae NCIM 1212	1.12 ± 0.023	26.34 ± 0.008	0.83 ± 0.020	47.0 ± 0.264	68.5 ± 0.550	7.0 ± 0.577	5.83 ± 0.972	3.33 ± 0.587
A1	0.95 ± 0.008	18.63 ± 0.011	1.26 ± 0.005	42.0 ± 0.152	59.0 ± 0.202	0.67 ± 0.088	20.67 ± 1.444	6.67 ± 0.485
A2	0.99 ± 0.040	27.35 ± 0.012	0.66 ± 0.005	44.0 ± 0.173	61.5 ± 0.057	1.66 ± 0.060	19.0 ± 0.057	5.0 ± 0.058
A3	1.1 ± 0.107	26.39 ± 0.011	0.97 ± 0.005	53.0 ± 0.115	59.5 ± 0.461	1.3 ± 0.115	3.33 ± 0.867	7.0 ± 0.881
A4	**1.12 ± 0.006**	**28.55 ± 0.008**	**1.35 ± 0.008**	**47.0 ± 0.057**	**63.0 ± 1.154**	**2.67 ± 0.011**	**38.33 ± 0.909**	**8.33 ± 0.587**
A5	**1.98 ± 0.034**	**26.41 ± 0.008**	**1.83 ± 0.008**	**51.0 ± 0.230**	**63.5 ± 0.793**	**2.66 ± 0.405**	**21.67 ± 1.176**	**11.03 ± 0.909**
A6	1.1 ± 0.067	27.36 ± 0.005	1.14 ± 0.008	41.0 ± 0.145	60.0 ± 1.154	1.0 ± 0.490	2.5 ± 0.600	6.0 ± 0.577
A7	1.03 ± 0.005	23.33 ± 0.008	1.06 ± 0.011	41.0 ± 0.176	59.5 ± 1.128	0.67 ± 0.056	20.0 ± 1.201	5.4 ± 0.881
A8	0.97 ± 0.020	18.97 ± 0.586	1.09 ± 0.026	43.0 ± 0.057	52.5 ± 1.342	0.67 ± 0.010	22.5 ± 1.092	1.67 ± 0.294
A9	1.1 ± 0.070	19.46 ± 0.064	0.76 ± 0.008	47.0 ± 0.145	52.5 ± 0.230	3.0 ± 1.00	17.5 ± 0.866	6.67 ± 0.619
A10	0.58 ± 0.054	16.7 ± 0.260	1.26 ± 0.008	40.0 ± 0.176	59.0 ± 2.603	0.67 ± 0.008	8.33 ± 0.674	3.33 ± 0.399
A11	1.27 ± 0.008	17.99 ± 0.566	1.19 ± 0.028	44.0 ± 0.115	59.0 ± 1.527	1.0 ± 0.881	11.67 ± 1.176	6.67 ± 0.619
A12	0.89 ± 0.014	19.67 ± 0.415	1.58 ± 0.018	38.0 ± 0.120	59.5 ± 1.732	0.67 ± 0.014	19.17 ± 0.580	5.84 ± 0.333
A13	1.03 ± 0.008	23.36 ± 0.008	1.16 ± 0.012	39.0 ± 0.284	61.5 ± 0.057	0.67 ± 0.011	0.83 ± 0.018	5.0 ± 0.331
A14	1.23 ± 0.009	21.14 ± 0.006	1.15 ± 0.008	34.0 ± 0.173	55.0 ± 0.881	2.6 ± 0.493	13.33 ± 0.909	3.33 ± 0.909

*Incubation period, 5 days, Slurry concentration, 50% (with d/w), Incubation temperature,30±2°C; The data represents the mean of three determinations each; ±values indicate standard error

Table.2 Quantitative screening of thermophilic cultures grown on digested biogas slurry

Thermophilic Cultures	**Enzyme activities (U/ml)**							
	Endoglucanase	**β-glucosidase**	**Exoglucanase**	**Xylanase**	**Mannanase**	**Laccase**	**Lignin Peroxidase**	**Manganese Peroxidase**
***Thermoascus aurantiacus* MTCC 375**	**1.97 ± 0.005**	**21.97 ± 0.328**	**1.73 ± 0.289**	**42.72 ± 0.625**	**58.0 ± 0.333**	**2.33 ± 0.078**	**10.83 ± 0.364**	**7.33 ± 0.294**
Humicolafuscoatra MTCC 1409	1.04 ± 0.011	17.98 ± 0.663	1.59 ± 0.290	40.37 ± 0.590	55.0 ± 0.577	2.0 ± 0.577	7.0 ± 0.333	6.67 ± 0.619
A15	1.01 ± 0.012	19.94 ± 0.863	1.43 ± 0.289	37.56 ± 0.606	53.5 ± 0.167	1.0 ± 0.288	3.67 ± 0.294	5.3 ± 0.586
A16	1.03 ± 0.011	16.51 ± 0.288	1.67 ± 0.294	40.93 ± 0.345	51.5 ± 0.927	1.3 ± 0.115	6.83 ± 0.057	5.0 ± 0.577
A17	1.06 ± 0.323	14.13 ± 0.356	0.84 ± 0.053	40.93 ± 0.655	50.0 ± 0.577	1.3 ± 0.115	5.83 ± 0.056	3.33 ± 0.294
A18	1.13 ± 0.313	16.47 ± 0.598	1.01 ± 0.580	33.35 ± 0.479	40.5 ± 0.440	1.6 ± 0.416	4.06 ± 0.333	7.3 ± 0.493
A19	1.11 ± 0.316	14.50 ± 0.288	1.17 ± 0.308	34.93 ± 0.345	45.0 ± 0.333	1.07 ± 0.577	2.13 ± 0.333	1.67 ± 0.399
A20	1.32 ± 0.295	15.00 ± 0.333	1.22 ± 0.303	32.65 ± 0.292	42.5 ± 0.440	1.66 ± 0.402	1.83 ± 0.056	2.67 ± 0.399
A21	1.20 ± 0.305	16.85 ± 0.311	1.59 ± 0.290	38.79 ± 0.304	51.0 ± 0.577	1.0 ± 0.577	4.17 ± 0.276	3.3 ± 0.585
A22	1.08 ± 0.320	12.27 ± 0.584	1.43 ± 0.289	30.01 ± 0.331	54.0 ± 0.333	2.3 ± 0.585	9.17 ± 0.308	6.0 ± 0.577
A23	0.97 ± 0.161	20.25 ± 0.583	1.30 ± 0.296	39.84 ± 0.310	53.5 ± 0.440	3.33 ± 0.110	3.33 ± 0.399	1.67 ± 0.294
A24	1.21 ± 0.304	16.88 ± 0.315	1.01 ± 0.335	36.33 ± 0.294	54.5 ± 0.166	0.67 ± 0.146	1.33 ± 0.294	5.0 ± 0.577
A25	**1.17 ± 0.308**	**23.08 ± 0.577**	**2.35 ± 0.404**	**42.47 ± 0.448**	**63.0 ± 0.333**	**5.0 ± 0.333**	**9.45 ± 0.166**	**8.33 ± 0.294**
A26	1.31 ± 0.295	14.74 ± 0.299	0.38 ± 0.291	33.17 ± 0.640	52.5 ± 0.166	3.33 ± 0.294	5.0 ± 0.577	1.67 ± 0.294
A27	1.03 ± 0.328	16.33 ± 0.587	1.64 ± 0.406	37.74 ± 0.299	51.0 ± 0.333	4.0 ± 0.577	6.67 ± 0.619	4.33 ± 0.399
A29	1.34 ± 0.293	16.13 ± 0.578	1.41 ± 0.464	37.03 ± 0.328	55.0 ± 0.333	0.33 ± 0.078	3.33 ± 0.294	3.33 ± 0.295
A30	1.27 ± 0.298	20.86 ± 0.358	1.17 ± 0.308	39.84 ± 0.310	54.5 ± 0.440	1.67 ± 0.294	5.83 ± 0.640	8.33 ± 0.294
A31	**1.35 ± 0.311**	**22.13 ± 0.578**	**1.68 ± 0.421**	**48.08 ± 0.320**	**62.5 ± 0.600**	**5.6 ± 0.611**	**11.5 ± 0.601**	**8.3 ± 0.392**
A32	0.96 ± 0.160	17.26 ± 0.504	1.45 ± 0.453	37.91 ± 0.319	55.5 ± 0.288	1.0 ± 0.333	5.83 ± 0.640	1.21 ± 0.296
A33	1.07 ± 0.322	20.59 ± 0.418	0.98 ± 0.006	32.65 ± 0.292	58.0 ± 0.577	3.0 ± 0.416	1.67 ± 0.619	1.03 ± 0.577
A34	1.24 ± 0.301	13.53 ± 0.288	0.97 ± 0.162	37.03 ± 0.328	58.0 ± 0.333	6.3 ± 0.585	3.33 ± 0.587	4.33 ± 0.294

*Incubation period, 5 days, Slurry concentration, 50% (with d/w), Incubation temperature,45±2°C; The data represents the mean of three determinations each; ±values indicate standard error.

Table.3 Critical difference at 5% significance

Enzyme Profile	C.D. (5%)
Endoglucanase	0.554
β-glucosidase	1.110
Exoglucanase	0.669
Xylanase	0.952
Mannanase	2.790
Laccase	1.266
Manganese peroxidase	1.537
Lignin peroxidase	1.911
Reducing sugars	1.578
Protein	0.970

Figure.1 Reducing sugar and protein content in mesophilic cultures grown on digested biogas slurry

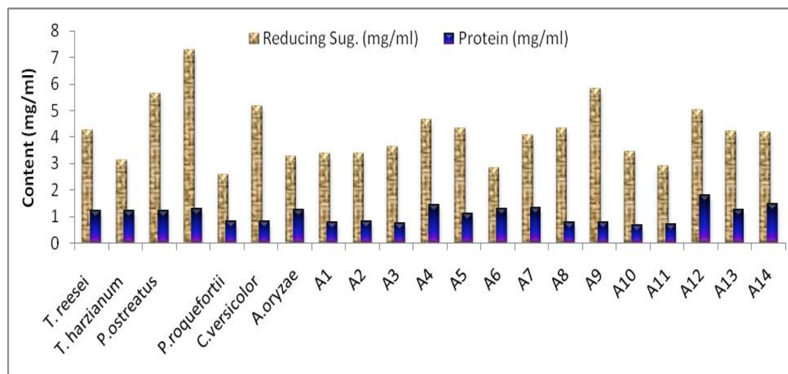
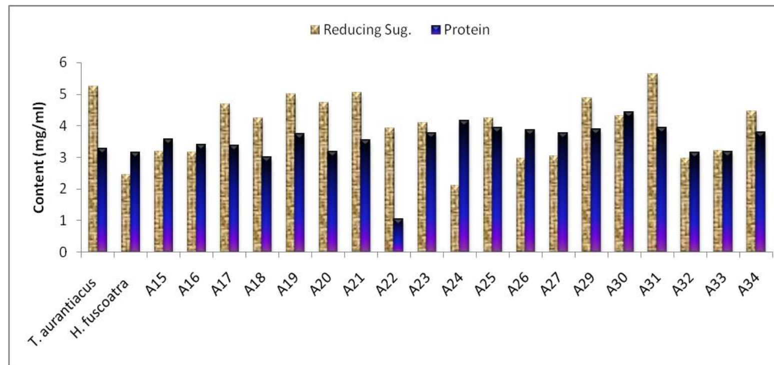


Figure.2 Reducing sugar and protein content in thermophilic cultures grown on digested biogas slurry



For thermophilic cultures, figure 2 showed that *Thermoascus aurantiacus* and isolate A31 have maximum reducing sugars which are validated by the higher values of lignocellulolytic enzymes in both of these cultures. Isolate A 30 has maximum protein, thus lower potential for lignocellulolytic specific activities.

Table 3 indicated that enzyme activity values for all the mesophilic as well as thermohilic cultures are significantly different from the zero as the critical difference at 95% significance is >0.05. Maximum C.D. value was obtained for mannanase activity *i.e.* 2.79, which showed that all the tested cultures vary in production of

mannanase. Minimum C.D. of 0.55 was obtained for endoglucanase activity.

So from the above studies, it may be concluded that different fungal isolates namely A5, A4, A12, A25, A31 and standard fungal cultures *Phanaerochaete chrysosporium* and *Thermoascus aurantiacus* are efficient lignocelluloses degraders.

All the enzyme activities significantly differ from the value of zero. These isolates showed significant ligno-cellulolytic activities by using digested slurry as a substrate without the addition of any nutritional supplements which shows the vast potential of slurry as a cheap and easily available substrate for enzyme production using efficient fungi.

Significance and impact of the study

Digested biogas slurry can be used as manure, but this excellent manure is not always appreciated, due to the threats posed to human health and sanitation facility. Microorganisms like fungi and bacteria can easily inhabit this slurry and can produce different enzymes with different potential.Thus, utilization of this slurry as a substrate for production of lignocellulose degrading enzymes using different fungal cultures presents a novel approach. In this study, lignocellulosic fungi are screened quantitatively using this cost effective substrate.

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