Research Article

Evaluation of Chemo-Micobial Methods for Cellulose Hydrolysis in Sugarcane Bagasse and Saw Dust

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Abstract | Saw dust and sugarcane bagasse were treated with acids/bases, microbial cultures and their enzyme extracts to check their effect on cellulose hydrolysis. Microbes were isolated from soil by using serial dilution method and were selected on efficiency basis (screening of microbes on the basis of their activities as cellulose degrading agents). The cellulose and related constituent of saw dust and sugarcane bagasse showed that cellulose (49 %), hemicelluloses (23.5 %) and ash (1.8 %) were higher in sugarcane bagasse, while lignin (7.9 %) was higher in saw dust. The data regarding microbe isolation showed highest colonies of *Aspergillus* sp. (8 CFU × $10^4 g^{-1}$) followed by *Penicillium* sp. and *Trichoderma* sp. (7 CFU × $10^4 g^{-1}$ and 5 CFU × $10^4 g^{-1}$), while the lowest colonies were noted for *Candida ablican* and *Saccharomyces cerevisiae*. The screening test of these microbes for cellulose degradation showed higher degradation for *Trichoderma* sp. (6 % and 8 %) followed by *Penicillium* sp. (4 % and 4.5 %) in both of the samples (saw dust and sugarcane bagasse) respectively. Similarly the combined effect of acids, bases and microbes showed higher result of NaOH, *Trichoderma* sp. (29.5 % and 41 %) in both samples (saw dust and sugarcane bagasse). The optimization of incubation time and temperature showed 72 hrs of incubation time and tempreture of 50 ˚C was best for cellulose degradation. From the data, it was concluded that *Trichoderma* sp. among microbes, HCl in acid and NaOH in bases were best for cellulose hydrolysis in samples while, incubation of 72 hrs at 50 ˚C showed higher cellulose degradation rate.

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Introduction

Plants cell wall has cellulose as its major component which serves to maintain their structure. Cellulose is also present in bacteria, fungi, algae, and even in animals (Mayer and Staples, 2002). Cellulose content was worked out by French chemist named Anselm Payne during 1838. He extracted the cellulose from green plants. Cellulose due to its digestibility in to fermentable sugars, capture the attention of energy scientist in the world. These sugars can easily be converted in to biofuels (Bansal *et al.*, 2012). Cellulose is also important for its uses in pharmaceutical industry. The derivatives of cellulose are used as a film coating agent on tablets, which reduces its vulnerability to environmental attack and help in transportation (Florencio *et al.*, 2007). The cellulose is also present in agricultural residues which can be used

in biofuel production, the world scientists tend to use agricultural residues in different valuable biomass energy products such as oil, generating electricity and other biodegrading products at the same time (Wiselogel *et al*., 1996). The fermentation of cellulose is carried out in two steps that are pretreatment and hydrolysis. In nature many microorganisms for example bacteria, fungi and actinomycetes produces enzymes (hydrolytic and oxidative) that degrade the lignocellulosic material. These enzymes are important in agricultural and industrial use (Perez *et al*., 2002). The objective of this study is the digestion of cellulose which is complicated and still need to be standardized for economical use in various industries, especially in biofuel technology.

Materials and Methods

The present study is related to cellulose digestion of two samples viz. saw dust and sugar cane bagasse. First microbes were isolated from soil samples and were screened for their cellulose digestion efficiency in the form of whole cells and aqueous enzymatic extracts. Isolated species were tested for cellulolytic enzyme production on basal medium. The efficiency of microbial enzymes was checked in the form of reducing sugar in the samples which was determined by dinitrosalicylic acid (DNS) method using glucose as standard.

Secondly the samples of saw dust and sugar cane baggasse were treated with 1 normal solution of various acids (HCl, H_2SO_4, H_3PO_4) and bases (NaOH, $Ca(OH)_2$, NH_4OH) and they then were subjected to enzymatic digestion with crude extract of microbes selected for their high cellulose digestion efficiency screening. microbial isolation was carried out by using PDA media. For preparation of PDA media 200gm of potatoes were washed and peeled properly and then boiled in 300 ml distilled water until it become soft. Then it was filtered through muslin cloth. After this 20 gm of agar and dextrose were added and volume was made to 1 liter by adding distilled water. Then media was sterilized in an autoclave at 121°C for 15 min. Saw dust was collected from saw machines and was brought in polythene bags to the laboratory. Dust was passed through 1mm sieve and used for further analysis. Sugarcane bagasse was collected from cane juice extractors, selling cane juice in local markets. The sample was squeezed to remove the sweet liquid and soften the fiber for further treatment. Then the pulp sample after squeezing was soaked overnight and washed thoroughly in distilled water. After washing, sample was dried in the lab oven first at 70°C and then at temperature of 100°C for complete dryness. After drying the pulp samples was milled with lab grinder and was passed through a sieve of about 1 mm pore size. The samples were stored in polythene bags. The fine powder was used for subsequent analysis.

Cellulose and hemicelluloses in samples were determined as acid detergent fiber (ADF). Neutral detergent fiber (NDF) were used as a source of lignin and ash. Method by Goering and Van Soest (1970) with some modification was used for the analysis of Acid detergent fiber (ligno-cellulose) and Neutral detergent fiber in the representative samples of sugar cane bagasse and saw dust.

Chemical treatment

The samples were subjected to chemical treatment of acid and bases in order to make accessible to microbial activity by the procedure of Detroy *et al*. (1981) and Lynd *et al*. (2002) with some modifications.

Acid treatment

Sawdust and sugarcane baggasse of 20 g was taken in conical flasks. Each sample was dissolved in 200 ml of 1N HCl, H_2SO_4, H_3PO_4 and were kept at 85 °C for 1 hour. After acid treatment samples was washed with distilled water and placed in an oven at 70 °C for complete dryness.

Base treatment

Sawdust and sugarcane baggasse of 20 g was taken. Dissolved each sample in 200 ml of 1N NaOH, $Ca(OH)_2$, NH_4OH and was kept at 85 °C for 1 hour. After base treatment samples was washed with distilled water and placed in an oven at 70 °C for complete dryness.

After acid and base treatment, all treated and non-treated samples were placed in an autoclave 121 °C for 15 min. These treated samples were further used for enzyme hydrolysis.

Statistical analysis

Data were subjected to the Completely Randomized Design (CRD) by Gomez and Gomez (1984) with LSD Test at 5% probability level (Steel and Torrie, 1997).

Results and Discussion

Cellulose is useful constituent in agricultural raw material specially those which go into waste. For this purpose, two samples of cellulosic raw materials, saw dust and sugar cane baggase were subjected to microbial degradation alone where microbial species isolated from soil were screened for their hydrolyzability on the samples mentioned above. The effect was observed in form of cellulose degradation and reducing sugar.

Results in Table 1 showed that saw dust contained 1.3 % ash, 41 % cellulose, 19 % hemicellulose and 24 % lignin. Sugar cane bagasse contained 1.8 % ash, 49 % cellulose, 23.5 % hemicellulose and 7.9 % lignin. The study of Chinedu *et al.* (2008) also supported the present data who found ash content in sawdust and sugarcane pulp that were 1.1 % and 1.8 % respectively. These results reflect the fact that saw dust is a good source of cellulose.

Table 1: *Cellulose content in sample of sawdust and sugar cane bagasses.*

Samples	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)
Sawdust	41.0	19.0	24.0	1.3.0
Sugarcane bagasses	49.0	23.5	7.9.0	1.8.0

The data presented in Table 2 showed that maximum colonies were observed for *Aspergillus* sp. (8.0 CFU × 10^3g) followed by *Penicillium* sp. (7.0 CFU × 10^4g), while the colonies of *Candida ablican* and *Saccharomyce cerevisiae* were same in amount and the lowest one. The study of Sharma *et al.* (2004) could be related to the present study who isolated fungal species from soil and examined maximum species that of *Aspergillus* group.

Table 2: *Isolated microbes (CFU × $10^4 g^{-1}$) from soil.*

S/No	Species	CFU × 104g-1
1	*Alternaria*	2.0
2	*Aspergillus*	8.0
3	*Penicillium*	7.0
4	*Saccharomyces cerevisia*	1.0
5	*Candida ablican*	1.0
6	*Fusarium*	4.0
7	*Absidia*	1.5
8	*Trichoderma*	5.0

Screening of microbial species for cellulose hydrolysis in saw dust and sugar cane bagasse

Table 3 showed that maximum degradation in saw dust was observed in sample treated with *Trichoderma* sp. (up to 35 %), while degradation in saw dust samples treated with *Saccharomyces cerevisiae, Absidia, Candida ablican* and *alternaria* was negligible. Similarly, the highest degradation in sugarcane bagasse was observed in sample treated with *Trichoderma* sp. (up to 41 %) this might be due to the fact that *Trichoderma* sp. is enzyme common use for hydrolysis of lignocellulosic biomass, while sugarcane bagasse samples treated with *Saccharomyces cerevisiae, Absidia, Candida ablican* and *alternaria* showed negligible cellulose degradation. The present work is closely related with the findings of Lennox *et al.* (2010) who worked on degradation of cellulose content of saw dust and observed maximum degradation by *Trichoderma* sp., *Penicillium* sp., *Aspergillus* sp., while lowest degradation by *Absidia*.

Table 3: *Cellulose content (%) in saw dust and sugarcane baggase incubated with fungal species.*

Microbial isolates	% Saw dust*	% Sugarcane bagasse**
Alternaria	40.9	48.8
Aspergillus sp.	37.5	44.0
Penicillium sp.	37.0	44.5
Saccharomyces cerevisiae	40.5	48.6
Candida ablican	40.9	48.9
Fusarium sp.	40.0	47.5
Absidia sp.	40.9	48.9
Trichoderma sp.	35.0	41.0

** Cellulose content of saw dust (41 %); ** Cellulose content of sugarcane baggase (49 %).*

Acid hydrolysis

For acid hydrolysis saw dust and sugar cane bagasse was treated with 1 N HCl, 1 N H_2SO_4 and 1 N H_3PO_4. These acid treated samples were further digested with microbial species. Data presented in Table 4 showed the highest amount of reducing sugars (32.90 %) for sugar cane bagasse treated with 1 N HCl followed by 1 N H_2SO_4 (26 %) by *Trichoderma* sp., while the lowest hydrolysis (1.1 % and 1.5 %) for sugar cane bagasse was observed in non treated sample and treated sample with H_3PO_4 by *Fusarium* sp. That might be due to saw dust contain high level of lignin content which could reduce the efficiency of enzymatic hydrolysis. Similarly, the highest amount of reducing sugars (29 %) for saw dust was found in sample treated with 1 N HCl by *Trichoderma* sp. while the lowest (0.98 % and

1.2 %) amount of reducing sugars for saw dust was observed in non treated sample followed by treated sample with H_3PO_4 by *Fusarium* sp. Laopaiboon *et al*. (2010) was in line with previous study who tested different acids for hydrolysis of sugarcane bagasse and found best results with HCl. generally, acid is useful in hydrolysis of saw dust and sugarcane biomass at an optimum condition of pH and temperature.

Table 4: *Percent reducing sugar of saw dust and sugarcane baggase samples hydrolyzed with acids and microbial species.*

Sample	Treatment	Species				Mean
		Penicillium	***Fusarium***	***Trichoderma***	***Aspergillus***	
Saw dust	Control	1.10op	0.98p	5.20k	03.50mn	2.70h
	H_2SO_4	12.80g	3.50mn	29.00b	12.10g	7.58e
	HCl	11.20h	3.90lm	1.90o	12.20g	14.08c
	H_3PO_4	3.30mn	1.20op	10.10i	5.20k	4.95f
Sugarcane baggase	Control	2.90n	1.10op	7.30j	4.10lm	3.85g
	H_2SO_4	14.50f	4.70kl	26.00c	24.20d	17.35b
	HCl	17.90e	4.50kl	32.90a	23.90d	19.80a
	H_3PO_4	7.10j	1.50op	14.30f	14.30f	9.30d
Sample x Species						
Saw dust		7.10f	2.40h	11.55c	8.25e	7.32b
Sugarcane baggase		10.60d	2.95g	20.125a	16.625b	12.58a
Treatment x Species						
	Control	2.00j	1.04k	6.25g	3.80i	3.27d
	H_2SO_4	13.65d	4.10i	13.95cd	18.15b	12.64b
	HCl	14.55c	4.20i	30.95a	18.05b	16.99a
	H_3PO_4	5.20h	1.35k	12.20e	9.75f	7.13c
Mean		8.85c	2.67d	15.84a	12.44b	

*Values within columns sharing a common letter are not significantly different at P<0.01. *Incubation time=72 hrs; *Incubation temperature=50°C*

Table 5: *Percent reducing sugar of saw dust and sugarcane baggase samples hydrolyzed with bases and microbial species.*

Sample	Treatment	Species				Mean
		Penicillium	***Fusarium***	***Trichoderma***	***Aspergillus***	
Saw dust	Control	1.10rs	0.98s	5.20l	3.50no	2.70h
	NaOH	15.00f	4.85lm	29.50b	18.90e	17.06 b
	Ca(OH)2	9.00j	2.50pq	18.10e	10.10i	9.93d
	NH_40H	5.08l	1.30rs	9.20j	4.76lm	5.09f
Sugarcane baggase	Control	2.90op	1.10rs	7.30k	4.10lm	3.85g
	NaOH	15.00f	5.10l	41.00a	27.90c	23.75a
	$Ca(OH)_2$	11.40h	3.76n	21.30d	15.20f	12.92c
	NH_40H	5.38l	1.90qr	13.50g	7.45k	7.06e
Sample x Species						
Saw dust		7.55f	2.41h	15.50b	9.32e	8.69b
Sugarcane baggase		10.17d	2.97g	20.78a	13.67c	11.89a
Treatment x Species						
	Control	2.00l	1.04m	6.25h	3.80j	3.27d
	NaOH	18.00d	4.98i	35.25a	23.40b	20.41a
	$Ca(OH)_2$	10.20g	3.13k	19.70c	12.65e	11.42b
	NH_40H	5.23i	1.60lm	11.35f	06.11h	6.07c
Mean		8.86c	2.69d	18.14a	11.49b	

*Values within columns sharing a common letter are not significantly different at P< 0.01. *Incubation time=72 hrs; *Incubation temperature=50°C*

Alkali hydrolysis

Maximum amount of reducing sugars (41 %) for sugarcane bagasse was showed by sample treated with 1N NaOH by *Trichoderma* sp. (Table 5). while the lowest amount of reducing sugar (1.1 % and 1.9 %) for sugar cane bagasse was observed in non treated sample followed by treated sample with 1 N NH_4OH by *fusarium* sp. Similarly, for saw dust reducing sugar (29.5 %) was higher in sample treated with 1 N NaOH by *Trichoderma* sp. while the lowest amount of reducing sugar (0.98 % and 1.3%) for saw dust was observed in non treated sample followed by treated sample with 1N NH_4OH by *fusarium* sp. The work of Sharma *et al*. (2004) also supported the present study who treated the sample of sunflower hulls with 0.5 % (w/v) sodium hydroxide autoclaved at 121 ˚C and showed maximum saccharification of 59.8 % by hydrolysis enzyme of *Trichoderma reesei*.

The effect of different temperature (control, 30˚C, 40˚C, 50˚C and 60˚C) for an incubation period of 72 hrs is shown in Table 6. The data presented in table 6 showed the highest amount of reducing sugar (45%) for sugarcane bagasse was produced by crude enzyme extract of *Trichoderma* sp. at 50 ˚C while the lowest amount of reducing sugar (1.5 % and 3.5 %) for sugarcane bagasse was observed in control sample followed by incubated sample at 30˚C by *Fusarium* sp. Similarly, the highest amount of reducing sugar (29.9%) for sawdust was observed in sample incubated at 50˚C by *Trichoderma* sp. while the lowest amount of reducing sugar (0.99 % and 1.9 %) for saw dust was observed in control sample followed by incubated sample at 30 ˚C by *Fusarium* sp. The present work is in agreement with Rajesh *et al*. (2012) who determined the effect of temperature on enzymatic activity by using *Trichoderma reesei* in solid state fermentation process.

The data presented in Table 7 showed the highest amount of reducing sugar (46.2 %) for sugarcane bagasse was produced in sample incubated for 72 hrs at 50˚C by crude enzyme extract of *Trichoderma* sp., while the lowest amount of reducing sugar (0.98 % and 1.30 %) for sugarcane bagasse was observed in control sample followed by sample, incubated

Table 6: *Percent reducing sugar of saw dust and sugarcane baggase samples hydrolyzed with NaOH and microbial species at different incubation temperature.*

Sample	Treatment	Species				Mean
		Penicillium	***Fusarium***	***Trichoderma***	***Aspergillus***	
Saw dust	control	2.90rs	0.99t	3.900opqr	3.50pqr	02.84i
	30 °C	6.90m	1.90st	9.20l	5.30n	05.83g
	40 °C	19.30h	4.20nopqr	18.10e	19.10h	17.23d
	50 °C	16.10j	4.47nopq	29.90e	21.23g	17.93c
	60 °C	7.20m	3.10rs	18.10hi	11.30k	09.93e
Sugarcane baggase	control	5.15no	1.50t	4.20nopqr	3.90opqr	03.69h
	30 °C	6.70m	3.50qr	12.00k	07.00m	07.30f
	40 °C	17.10ij	4.90nop	38.40b	26.50f	21.73b
	50 °C	21.50g	5.30n	45.00a	31.50d	25.83a
	60 °C	11.16k	3.30qr	33.80c	18.90h	16.79d
Sample x Species						
Saw dust		10.48d	2.93f	17.48b	12.10c	10.75b
Sugarcane baggase		12.32c	3.70e	26.68a	17.56b	15.07a
Treatment x Species						
	Control	4.02jkl	01.25n	04.05jkl	03.73kl	03.26e
	30 °C	6.80i	02.70m	10.60g	06.15i	06.56d
	40 °C	18.20e	04.55jk	32.35b	22.80d	19.48b
	50 °C	18.80e	04.88j	37.45a	26.37c	21.87a
	60 °C	9.18h	03.20lm	25.95c	15.10f	13.36c
Mean		11.40c	3.32d	22.08a	14.83b	

*Values within columns sharing a common letter are not significantly different at P < 0.01. *Incubation time=72 hrs.*

Table 7: *Percent reducing sugar of saw dust and sugarcane baggase samples hydrolyzed with base (NaOH) and microbial species at different incubation time.*

Sample	Treatment	Species				Mean
		Penicillium	*Fusarium*	*Trichoderma*	*Aspergillus*	
Saw dust	Control	1.10q	0.55q	1.50q	1.35q	1.12g
	24 hrs	4.10p	1.10q	9.40mn	04.12p	4.68f
	48 hrs	9.80lmn	1.10q	24.90ef	18.10j	13.47d
	72 hrs	21.10h	4.30p	31.00d	23.20fg	19.90b
	96 hrs	9.10n	1.10q	23.10g	20.30hi	13.40d
Sugarcane Baggase	Control	3.71p	0.98q	1.90qr	1.30q	1.97g
	24 hrs	4.80op	1.10q	11.00lm	06.30o	5.85e
	48 hrs	16.20k	1.90q	33.40c	19.20ij	17.67c
	72 hrs	25.10e	5.10op	46.20a	30.50d	26.72a
	96 hrs	11.47l	1.80q	35.80b	19.80hij	17.21c
Sample x Species						
Saw Dust		9.04f	1.63g	17.98b	13.41d	10.52 b
Sugarcane Baggase		12.25e	2.21g	25.66a	15.42c	13.89 a
Treatment x Species						
	Control	2.40j	0.76k	1.70jk	1.32jk	1.549d
	24 hrs	4.45i	1.20k	10.20h	5.21i	5.265c
	48 hrs	13.00g	1.50jk	29.15b	18.65f	15.575b
	72 hrs	23.10d	4.70i	38.60a	26.85c	23.313a
	96 hrs	10.28h	1.45jk	29.45b	20.05e	15.31b
Mean		10.649c	1.923d	21.82a	14.42b	

*Values within columns sharing a common letter are not significantly different at P < 0.01. *Incubation temperature=50 °C*

for 24 hrs at 50°C by *Fusarium* sp. Similarly the highest amount of reducing sugar (31.0%) for sawdust was observed in sample incubated for 72 hrs at 50°C by *Trichoderma* sp., while the lowest amount of reducing sugar (0.55% and 1.10%) for saw dust was observed in control sample followed by sample, incubated for 24 hrs at 50°C by *Fusarium* sp. Iqbal *et al.* (2010) studied that beyond the optimum incubation time (72 hrs) resulted decrease in reaction activity that might be due to depletion of nutrients or accumulation of some inhibitory compound in media. Results showed that *Aspergillus* sp. and *Penicillium* sp. also showed maximum hydrolysis for saw dust and sugarcane bagasse after 72 hrs of incubation period, while lowest amount of reducing sugar was obtained at control and 24 hrs of incubation time by *Fusarium* sp. followed by *Penicillium* sp. for saw dust and sugarcane bagasse. The present study agreed to Juliet *et al.* (2013) who observed that *Trichoderma* sp. was found with highest enzyme activity as compared to other isolated species and reported maximum hydrolysis of 35 % after 72 hrs of incubation time at 6.5 pH, respectively.

Conclusions and Recommendations

We concluded that cellulosic content in sugar cane bagasse was hydrolyzed more efficiently than saw dust during various treatments. Among different acids hydrochloric acid was more efficient in hydrolysis of cellulose content, while among bases sodium hydroxide was efficient. Combine effect of chemical and fungi (pretreated sample) was more efficient than individual effect of fungi (non-pretreated sample). In fermentation process, incubation time of 72 hrs and temperature at 50 °C were best for cellulose hydrolysis.

Novelty Statement

Digestion of cellulose using economically low-cost procedure for biofuel industries been specially given consideration during this research article.

Author's Contribution

Samra Aftab: Conducted experiment, did data analysis and compilation.

Saleem Ullah: Supervised all the activities.
Farida Anjum: Original draft writing and data analysis.

Conflict of interest
The authors have declared no conflict of interest.

References

Bansal, N., R. Tewari, R. Soni and S.K. Soni. 2012. Production of cellulases from Aspergillus niger NS-2 in solid state fermentation on agricultural and kitchen waste residues. J. Waste Manage., 32: 1341–1346. https://doi.org/10.1016/j.wasman.2012.03.006

Chinedu, S.N., S.C. Yah, O.C. Nwinyi, V.I. Okochi, U.A. Okafor and B.M. Onyegeme- Okerenta. 2008. Plant waste hydrolysis by extracellular enzymes of aspergillus niger and Penicillium chrysogenum: Effect of ammonia pretreatment. Nigerian J. Biochem. Mol. Biol., 23(1): 1-7.

Detroy, R.W., L.A. Lindenfelser, S. Sommer and W.L. Orton. 1981. Bioconversion of wheat straw to ethanol: Chemical modification, enzymatic hydrolysis, and fermentation. J. Biotech. Bioeng., 23: 1527-1335. https://doi.org/10.1002/bit.260230712

Florencio, A.P.S., J.H.L. Melo, C.R.F.C. Mota, M.R. Melo-Junior and R.V.S. Araujo. 2007. Progress in bioethanol processing. J. Rev. Eletron. Farmacia, 1: 61-65.

Goering, H.K. and P.J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agriculture Handbook 379 (pp. 1–20). U.S. Department of Agriculture. Washington DC.

Gomez, K.A. and A.A. Gomaz. 1984. Statistical procedure for agriculture research. 2nd Edn. pp. 8-22.

Iqbal, M.N.T., A.M. Ishtiaq and H. Shahbaz. 2010. Media optimization for hyper-production of carboxymethylcellulase using proximally analysed agro-industrial residues with *Trichoderma harzianum* under solid state fermentation. J. Agro Vet. and Med. Sci. 4(2): 47-55.

Juliet, B.A., A.O. Fabunmi and O.O. Olaniyi. 2013. Effect of variations in growth parameters on cellulase activity of Trichoderma viride NSPR006 cultured on different wood-dusts. J. Mic., 9(3): 193-200.

Laopaiboon, P., A. Thani, V. Leelavatcharamas and L. Laopaiboon. 2010. Acid hydrolysis of sugarcane bagasse for lactic acid production. Biores. Tech., 101: 1036–1043. https://doi.org/10.1016/j.biortech.2009.08.091

Lennox, J.A., C. Abriba, B.N. Alabi and F.C. Akubuenyi. 2010. Comparative degradation of sawdust by microorganisms isolated from it. J. Micro. Res., 4(13): 1352-1355.

Lynd, L.R., P.J. Weimer, W.H. VanZyl and I.S. Pretorius. 2002. Microbial cellulose utilization: Fundamentals and biotechnology. Micro. Mol. Biol. Rev., 66: 506–577. https://doi.org/10.1128/MMBR.66.3.506-577.2002

Mayer, A.M., and R.C. Staples. 2002. Laccase: New functions for an old enzyme Phytochemistry. J. Sci., 60: 551–565. https://doi.org/10.1016/S0031-9422(02)00171-1

Perez, J., J. Munoz-Dorado, T. Rubia and J. Martinez. 2002. Biodegradation and biological treatments of cellulose, hemicellulose, and lignin: an overview. J. Microbiol., 5: 53-63. https://doi.org/10.1007/s10123-002-0062-3

Rajesh, M.J., L. Rajesh and L.W. Abachire. 2012. Optimization of solid state fermentation conditions for the production of cellulase by using trichoderma reesei. Euro. J. Appl. Eng. Sci. Res., 1(4): 196-200.

Sharma, S.K., K.L. Kalra and G.S. Kocher. 2004. Fermentation of enzymatic hydrolysate of sunflower hulls for ethanol production and its scale-up. J. Biomass Bioener., 27: 392-402. https://doi.org/10.1016/j.biombioe.2004.03.005

Steel, R.G.D. and J.H. Torrie. 1997. Principles and procedures of statistics. M.C. Graw Hill Pub. Comp. Inc. New York.

Wiselogel, A., S. Tysonand and D. Johnson. 1996. Biomass feedstock resources and composition. Handbook of bioethanol: Production and utilization. Taylor and Francis, Washington, DC, USA, Chapter 6: 1-56032-553-4.