Research Article **ISSN: 2393 – 9532**

International Journal of Arts and Science Research

Journal home page: www.ijasrjournal.com

DEGRADRATION OF AGRICULTURAL SUBSTRATES BY CELLULOLYTIC BACTERIA ISOLATED FROM TERMITE GUT

N. Hemashenpagam*[1], M. Pratheeba[1], R. Karuppusamy[1]

[1*]Post Graduate and Research Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore-641028, India.

ABSTRACT

Lignocelluloses biomass like wood and agricultural crops residue, e.g., straw and sugar beet pulp are potential raw materials for producing several high value products like fuel ethanol and biodiesel. Up to 80% of the lignocelluloses are polysaccharides (Kaparaju *et al*, 2009)[1]. Consequently lignocellulose genes from various organisms have been explored. Termites possess varied sets of efficient micro-scale lignocellulose degrading systems. In this study, bacteria that degraded cellulose were isolated from termite gastrointestinal tract. The bacterial enzyme cellulase showed the maximum activity at 40^{o}C and pH 6.0. The agricultural substrates were hydrolyzed by cellulase and more sugar was released from corn stover than paddy straw and sugarcane baggase. After direct hydrolysis and fermentation of agricultural substrates end products were analyzed using HPLC. Thus, termite gut bacteria can efficiently hydrolyze hemicellulose and cellulose and these bacteria also have the potential to convert the fermentable sugars.

KEYWORDS

Lignocellulose, Cellulase, Termite and Cellulose.

Author for Correspondence:

Hemashenpagam N,
Department of Microbiology,
Hindusthan College of Arts and Science,
Coimbatore-641028, India.

Email: drhemashenpagam@gmail.com

INTRODUCTION

Lignocelluloses biomass like wood and agricultural crop residues, e.g., straw and sugar beet pulp are potential raw materials for producing several high value products like fuel ethanol and biodiesel. Upto 80% of the lignocelluloses are polysaccharides. These renewable raw materials look promising for replacing environmentally unfriendly fossil hydrocarbon raw materials and hence creating "green" products.

There is a need for low-cost materials, effective enzymes, and pretreatment methods to decrease the expenditure for Bioethanol production (Sanchez and Cardona *et al*, 2008)[2]. Cellulosic biomass is a low-cost, renewable and abundantly available material throughout the world. These materials include wood chips, residues of crops, grasses etc. (Binod *et al*, 2010)[3]. In terms of quantity, sugarcane baggase, paddy straw, corn stover, wheat straw are the most accessible agricultural wastes (Kim and Dale *et al*, 2004)[4].

During hydrolysis, monomeric sugars are generated via depolymerization of hemicelluloses and cellulose (Sarkar *et al*, 2012)[5]. Complete hydrolysis of cellulose to glucose requires the synergistic action of three enzymes. Cellulase system consists of three enzymes. Cellulase system consists of three classes of soluble extracellular enzymes, i.e. 1, 4-β-endoglucanases, 1, 4-β exoglucanases and β-glucosidases. Cellulolytic is a biological process which controlled and processed with cellulase system. This enzyme cocktail is needed to establish a cost-effective technology in addition to the lower price of biomass (Arantes and Saddler *et al*, 2011)[6].

Insects have evolved effective strategies to utilize lignocellulosic substrates as sources of energy which makes them optimal resources of prospecting for novel cellulolytic enzymes. Extensive efforts have characterized lignocellulose degradation in termites. Molecular phylogenetic analysis has revealed that termites harbor more than 200 species of symbiotic microorganisms, which produce enzymes that degrade cellulose and hemicelluloses (Brune *et al*, 2007)[7]. The gut of wood eating termite is a bioreactor where a number of microbes utilize cellulose and hemicellulose content of lignified plant materials and convert them to fermentable products. Without these microorganisms termites are unable to hydrolyze cellulose, which is their main food.

In this study organism was isolated from termite gut. The isolate was screened for cellulolytic activity. The crude enzyme activity was checked at different temperature, pH and by different carbon, nitrogen sources. The agricultural substrates were hydrolyzed with the enzyme produced by the isolate. The substrates were directly hydrolyzed and fermented with the isolate to find the end products.

MATERIAL AND METHODS

Insects and Agricultural Substrates

Termites were collected from infested logs around the surroundings. The agricultural substrates were collected and ground to produce small size particles.

Isolation and Screening of Bacteria

Termites were sterilized with 70% ethanol and then washed in sterile distilled water. The gut was seperated crushed and mixed with 10mL 0.85% NaCl. The suspension was mixed with nutrient broth containing 1% CMC and incubated at 30^0C. Then the culture was plated on Nutrient agar+ 1% CMC agar plates using well diffusion method and incubated for 24hrs (Pourrameza *et al*, 2012)[8].

To visualize the hydrolysis zone the plates were flooded with an aqueous solution of 0.5% Congo red for 15mins and washed with 1M NaCl (Apun, Jong *et al*, 2000)[9].

Enzyme Production and Activity Assay

The enzyme production media containing nutrient broth with 1% CMC, pH 6.8 (Dheeran *et al*, 2012[10], Bashir *et al*, 2013)[11] was prepared. The media were inoculated with termite gut bacteria and incubated at mild rotation for 48hrs at 30^{o}C.

The enzyme activities of CM Case of the bacteria were studied by using CMC as substrate. The effect of the various ranges of temperatures 30, 40, 50 and 60^{o}C, and also a pH at 5.0, 6.0, 7.0, 8.0 and 9.0 was assessed using crude enzymes. The effects of various carbon sources like glucose, sucrose, lactose, maltose and fructose and nitrogen sources like yeast, peptone, ammonium sulphate and urea were also assessed. Cellulase activity was measured with a reaction mixture composed of 0.2mL of crude enzyme solution plus 1.8mL of 0.5% carboxymethyl cellulose (CMC) in 50mM sodium phosphate buffer (pH 7) and 3ml of DNS reagent, boiled in water bath for 10mins. The reaction was stopped by adding Rochelle salt and OD of sample was measured at 575nm against a blank containing all the reagents minus the crude enzyme. One unit (U) of enzyme activity was defined as the amount of enzyme that

released 1 µmol of reducing sugars per min during the reaction.

Saccharification of Corn Stover, Sugarcane Baggase, Paddy Straw

First the contents of cellulose, hemicelluloses (Agblevor *et al*, 2003)[12], and lignin (Anwar *et al*, 2012)[13] were determined for corn stover, sugarcane baggase and paddy straw.

Then corn stover, sugarcane baggase and paddy straw were taken 5% by dry weight, which means 5gm in 100ml of distilled water (w/v). The ratio of crude enzyme of organism (CM Case) to substrates was 1:1, means 100ml of crude enzymes were added. With mild rotation, the reaction mixture was placed at 50ºC for 24hrs. The combined effect of enzyme was also studied. Agricultural substrates treated with distilled water were used as controls.

End Product Analysis

The agricultural substrates were directly treated with bacterial isolate for saccharification and fermentation. Corn stover, sugarcane baggase and paddy straw were used at 5% dry weight (w/v) and supplemented (with, in g/L H_2O: KH_2PO_4 1.5, $MgSO_4$ 0.3, NaCl 0.01, $CaCl_2$ 0.1, $FeSO_4$ 7, H_2O 0.005, NH_4Cl 0.3, and yeast extract 0.05) (Rastogi *et al*, 2009)[14]. The agricultural substrates were inoculated with 1% of cultured isolates (1ml of culture upto 100ml of 5% substrates). The reaction mixture was incubated at 30ºC for 5 days at mild rotation and microaerophilic conditions.

The fermentative medium from corn stover and sugarcane baggase was centrifuged at 14,000rpm for 2min and 4ºC to remove the remaining substrates and bacterial cells. The supernatant filtered through 0.22µm membranes, and the filterate was stored at -20ºC for high-performance in liquid chromatography analysis. The isolates were tested for their efficiency to produce secondary metabolites.

RESULTS AND DISCUSSION

Isolation and Screening of Bacteria

In this study, the termite gut was explored to identify bacteria producing enzymes that degrade cellulose and hemicelluloses and to determine the role that these bacteria play in this small ecological niche. Termites harbor microbes that produce cellulases and hemicellulases, which hydrolyze lignocellulosic material (Scharf and Tartar *et al*, 2008[15], Zhang *et al*, 2009)[16].

Enzyme Activity Assay

The optimization for enzyme activity by pH and temperature is shown in Figure No.3 and Figure No.4.

The isolate showed maximum endoglucanase activity at 40ºC and pH 6. (Immanuel *et al*, 2006)[17] observed that *Micrococcus, Bacillus,* and *Cellulomonas* species obtain maximum cellulase activity at neutral pH. Generally, cellulases isolated from microbes from mesophilic environments have an optimum pH of 4.0 to 8.0 (Dutta *et al*, 2008)[18]. These results are close those of (Bakare *et al*, 2005)[19] who found that the cellulase enzyme produced by *Pseudomonas fluorescence* was activated at 30 to 35°C showing the optimum temperature at 35°C. (Ray *et al*, 2007)[20] reported that minimum cellulase yield was observed when fermentation was carried out at 45°C, while maximum yield was obtained at 40°C by *Bacillus subtilis* and *Bacillus circulans*.

The cellulase activity was optimized with different carbon and nitrogen sources. This is illustrated in Figure No.5 and Figure No.6.

Among the optimization with different sugars with different concentration, glucose with 5% as carbon source shows the maximum production than other carbon sources (Figure No.5).

Among the various nitrogen sources tested, ammonium sulphate was found to be the best nitrogen source for cellulase production and is shown in Figure No.6. According to (Mandels *et al*, 1975)[21]. Nitrogen is one of the major cell proteins and stimulation of cellulase activity by ammonium sulphate salt might be due to their direct entry in protein synthesis.

Saccharification of Corn Stover, Sugarcane Baggase, Paddy Straw

The agricultural substrates corn stover, sugarcane baggase, paddy straw was hydrolyzed by the enzyme with diverse efficiency. Corn stover was a potential substrate for this enzyme. (Saha and Cotta, 2006)[22] reported that the cellulose and hemicelluloses contents in corn stover were 42.6 and 21.3%,

respectively. The contents of cellulose and hemicelluloses are high in corn stover as compared to sugarcane baggase and paddy straw. Therefore the sugar content produced by corn stover is higher than other substrates. This was identified using DNS method of agricultural substrates supplemented with fermentation media. The reducing sugar value is 3.684µmol. The least amount of sugar content was released and is 0.164 µmol from paddy straw. The sugar content released from sugarcane baggase is 2.489µmol. This is shown in Figure No.7.

End Product Analysis

Compared to standard the Sample No.1 (cornst over with inoculum) was found to be degraded which is illustrated in peak of HPLC analysis.

Compared to standard the Sample No.2 (sugarcane baggase with inoculum) was found to be degraded which is illustrated in peak of HPLC analysis.

The production of sugars and end products can be increased by optimizing the different conditions to achieve the maximum potential of the bacterial isolate.

Figure No.1: Isolation of cellulolytic bacteria

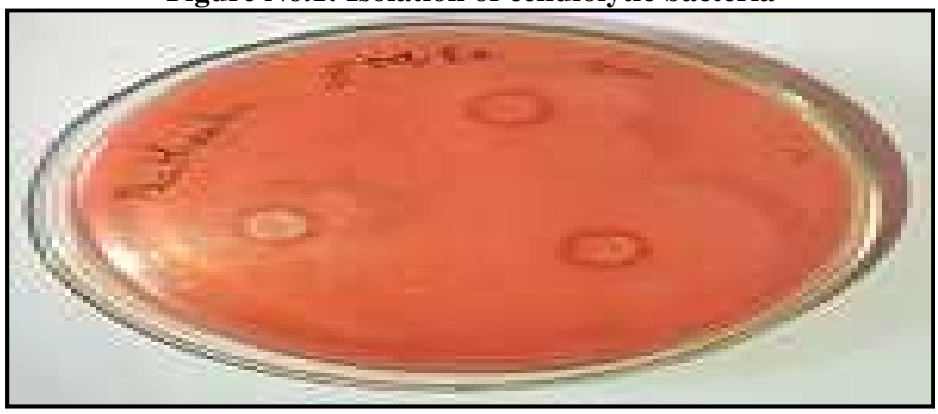


Figure No.2: Congo red screening method

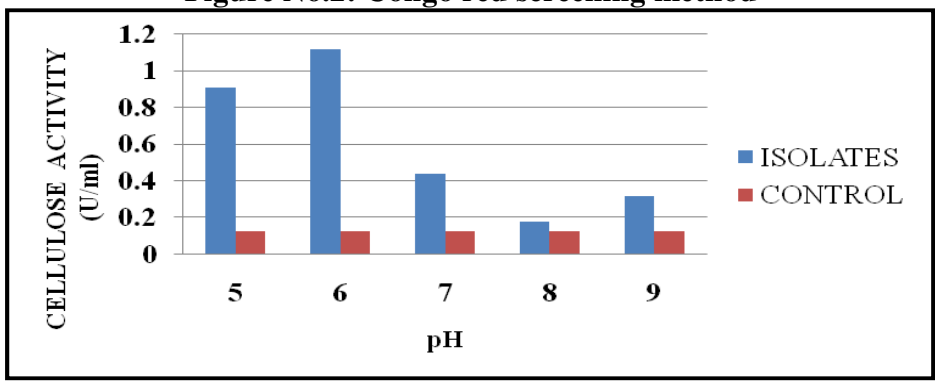


Figure No.3: Optimization of pH for CMCase (cellulase) activity U/mL

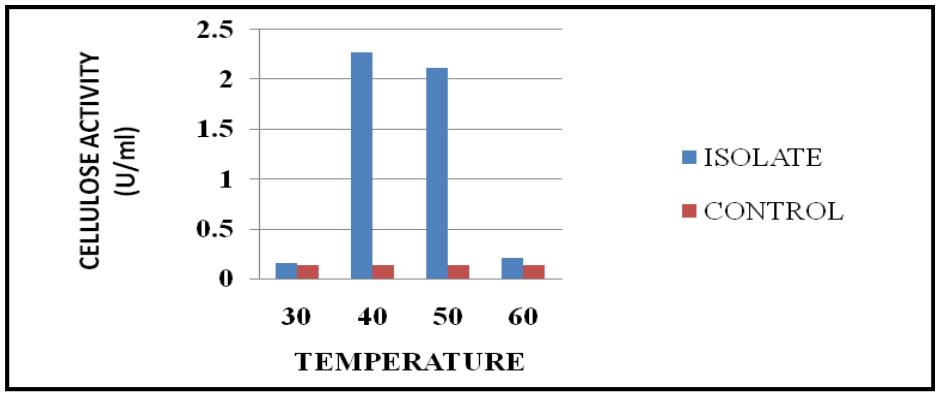


Figure No.4: Optimization of pH for CMCase (cellulase) activity U/mL

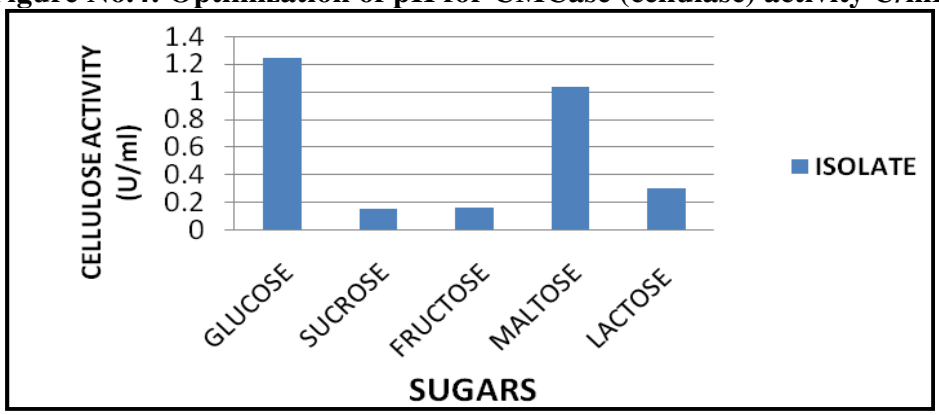


Figure No.5: Optimization of carbon sources for CMCase (cellulase) activity U/mL

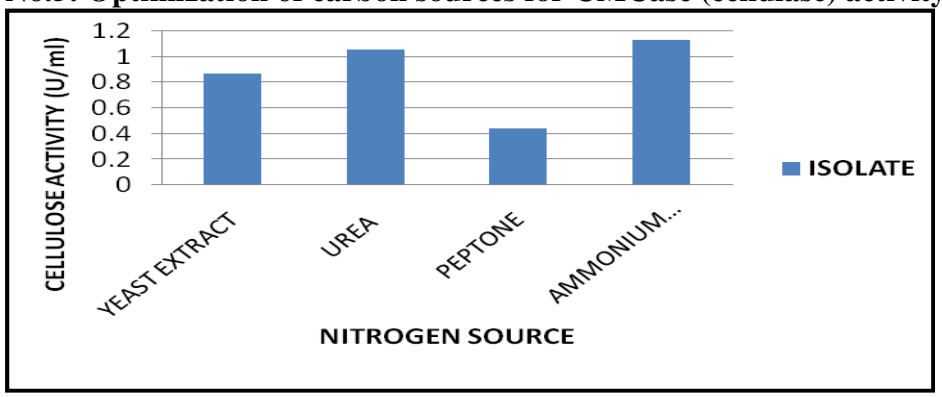


Figure No.6: Optimization of Nitogen sources for CMCase (cellulase) activity U/mL

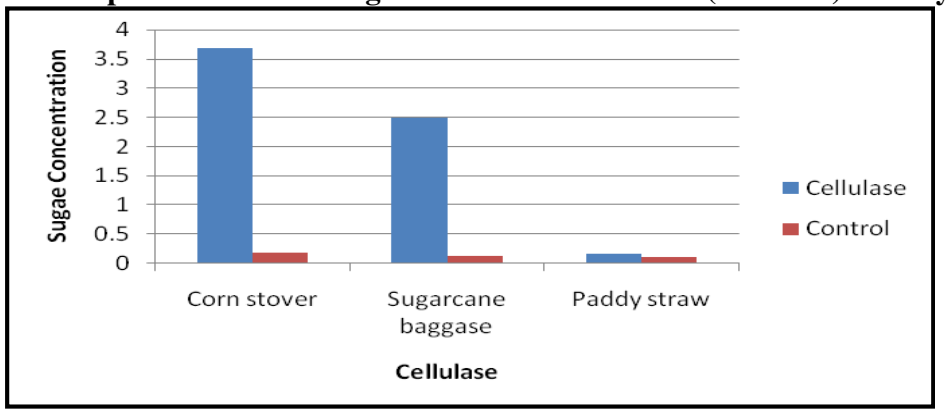
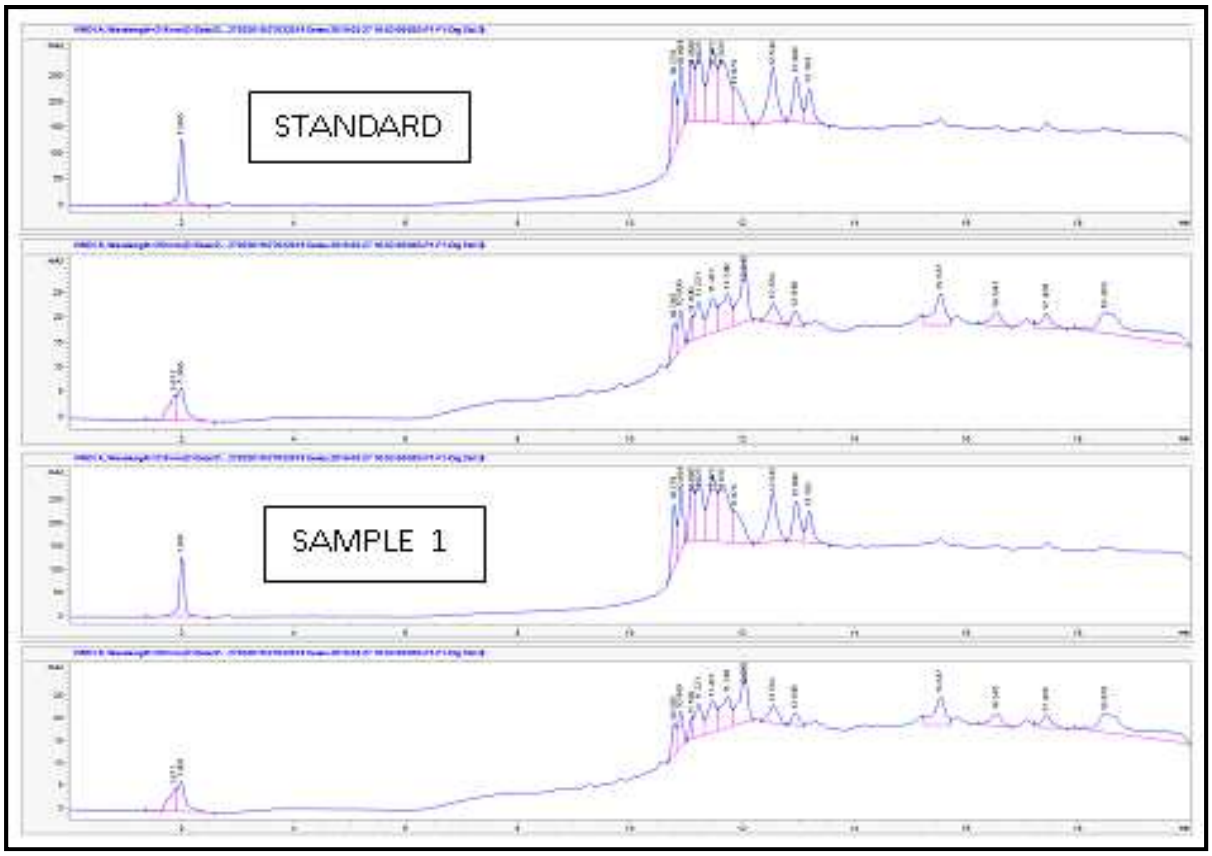
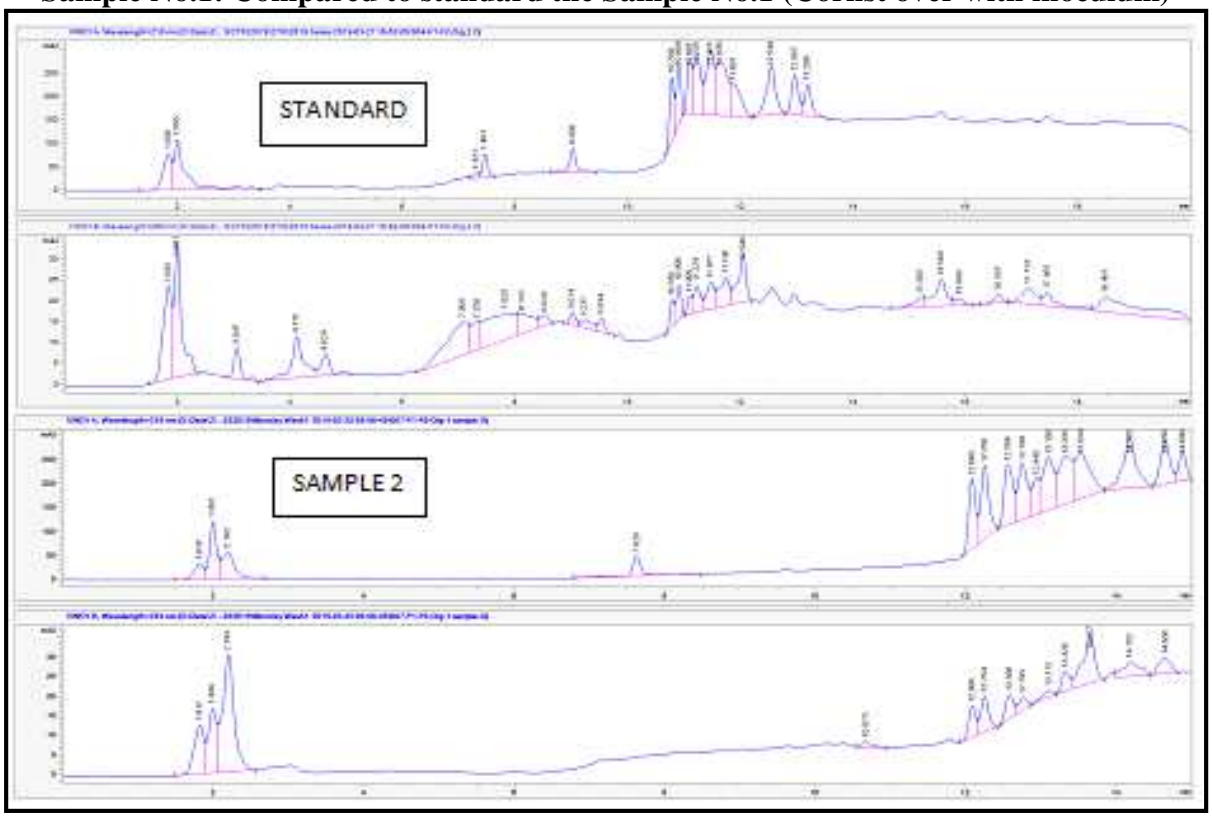


Figure No.7: Sugar concentration of cellulase to hydrolyze agricultural biomass



Sample No.1: Compared to standard the Sample No.1 (Cornst over with inoculum)



Sample No.2: Compared to standard the Sample No.2 (Sugarcane baggase with inoculum)

CONCLUSION

The cellulose producing bacteria was isolated from the termite gut with the screening method using congo red. It was then allowed to produce cellulase enzyme and the bacterial isolate was used to hydrolyze the corn stover, sugarcane baggase and paddy straw and end product was analysed. This enzyme has the potential to hydrolyze pure substrates and degrade agricultural substrates without any chemical pretreatment.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Post Graduate and Research Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore-641028, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Kaparaju P, Serrano M, Thomsen A B, Kongjan P, Angelidaki I. Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept, *Bioresour Technol,* 100(9), 2009, 2562-2568.
2. Sanchez O J and Cardona C A. Trends in biotechnological production of fuel ethanol from different feedstocks, *Bioresource Technology,* 99(13), 2008, 5270-5295. Sanderson K. Lignocelluloses. A chewy problem, *Nature,* 474(7352), 2011, 12-14.
3. Binod P, Sindhu R R, Singhania R, Vikram S, Devi L, Nagalakshmi S. Bioethanol production from rice straw: An overview, *Bioresource Technology,* 101(13), 2010, 4767-4774.
4. Kim S and Dale B E. Global potential bioethanol production from wasted crops and crop residues, *Biomass and Bioenergy,* 26(4), 2004, 361-375.
5. Sarkar N, Sumanta K G, Satarupa B, Kaustav A. Bioethanol production from agricultural wastes: An overview, *Renewable Energy,* 37(1), 2012, 19-27.
6. Arantes V and Saddler J N. Cellulose accessibility limits the effectiveness of minimum cellulase loading on the efficient hydrolysis of pretreated lignocellulosic substrates, *Biotechnology for Biofuels,* 4(1), 2011, 79.
7. Brune A. Woodworker's digest, *Nature,* 450(7169), 2007, 487-488.
8. Pourramezan Z, Ghezelbash G R, Romanic B, Ziaeid S, Hedayatkhah A. Screening and identification of newly isolated cellulose degrading bacteria from the gut of xylophagous termite *Microcerotermesdiversus* (Silvestri), *Microbiology,* 81(6), 2012, 796-802.
9. Apun K, Jong B C, Salleh M A. Screening and isolation of a cellulolytic and amylolytic Bacillusfrom sago pith waste, *Journal of General and Applied Microbiology,* 46(5), 2000, 263-267.
10. Dheeran P, Nandhagopal N, Kumar S, Jaiswal Y K, Adhikari D K. A novel thermostable xylanase of *Paenibacillusmacerans* IIPSP3 isolated from the termite gut, *Journal of Industrial Microbiology and Biotechnology,* 39(6), 2012, 851-860.
11. Bashir Z, Kondapalli V K, Adlakha N, Sharma A, Bhatnagar R K, Chandel G, Yazdani, S S. Diversity and functional significance of cellulolytic microbes living in termite, pill-bug and stem-borer guts, *Scientific Reports,* 3, Article No. 2558, 2013, 1-11.
12. Agblevor F A, Batz S, Trumbo J. Composition and ethanol production potential of cotton gin residues, *Applied biochemistry and biotechnology,* 105(1-3), 2003, 219-230.
13. Anwar Z, Gulfraz M, Imran M, Asad M J, Shafi A I, Anwar P, Qureshi R. Optimization of dilute acid pretreatment using response surface methodology for bioethanol production from cellulosic biomass of rice polish, *Pakistan Journal of Botany,* 44(1), 2012, 169-176.

14. Rastogi G, Muppidi G L, Gurram R N, Adhikari A, Bischo K M, Hughes S R, Apel W A, Bang S S, Dixon D J, Sani R K. Isolation and characterization of cellulose-degrading bacteria from the deep subsurface of the Homestake gold mine, Lead, South Dakota, USA, *Journal of Industrial Microbiology and Biotechnology,* 36(4), 2009, 585-598.
15. Scharf M E and Tartar A. Termite digestomes as sources for novel lignocelluloses, *Biofuels, Bioproducts and Biorefining,* 2(6), 2008, 540-552.
16. Zhang D, Lax A R, Raina A K and Bland J M. Differential cellulolytic activity of native-form and C-terminal tagged form cellulase derived from *Coptotermesformosanus* and expressed in *E. coli*, *Insect Biochemistry and Molecular Biology,* 39(8), 2009, 516-522.
17. Immanuel G, Dhanusha R, Prema P, Palavesam A. Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment, *International Journal of Environmental Science and Technology,* 3(1), 2006, 25-34.
18. Dutta T, Sahoo R, Sengupta R, Ray S S, Bhatta C A, Ghosh S. Novel cellulases from an extremophilic filamentous fungi *Penicillium citrinum*: Production and characterization, *Journal of Industrial Microbiology and Biotechnology,* 35(4), 2008, 275-282.
19. Bakare M K, Adewale I O, Ajayi A, Shonukan O O. Purification and characterization of cellulase from the wild-type and two improved mutants of Pseudomonas fluorescens, *African Journal of Biotechnology,* 4(9), 2005, 898-904.
20. Ray A K, Bairagi A, Sarkar Ghosh K, Sen S K. Optimization of fermentation conditions for cellulase production by Bacillus subtilis CY5 and Bacillus circulans TP3 isolated from fish gut, *Acta Ichthyologica Et Piscatoria,* 37(1), 2007, 47-53.
21. Mandels M. Microbial source of cellulose, *Biotechnology and Bioengineering,* 5, 1975, 81-105.
22. Saha B C, and Cotta M A. Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw, *Biotechnology Progress,* 22(2), 2006, 449-453.
23. Karmakar M and Ray R R. Current trends in research and application of microbial cellulases, *Res J Microbiol,* 6(1), 2011, 41-53.

Please cite this article in press as: Hemashenpagam N *et al.* Degradration of agricultural substrates by cellulolytic bacteria isolated from termite gut, *International Journal of Arts and Science Research,* 6(2), 2019, 24-31.