Enzymatic Depolymerisation of Hemicellulose into Arabinose and Xylose by Aerobic Bacteria

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Abstract— *The cellulotic and hemicellutotic materials found in plant can be utilized for generating bio-fuel by breaking them into simple sugar form. The depolymerisation of hemicellulose has been investigated, since it is second highest sugar component after cellulose. Hemicellulolytic microorganisms were obtained by enrichment of samples taken from aerated composting piles essentially composed of lignocellulosic plant wastes. The isolated Microorganism demonstrated the appreciable hemicellulolytic activity in depolymerising hemicellulose into simple pento sugars under the favorable conditions. The extent of endoxylanase (107.76 IU/ml) and sugar (2088.81 µg/ml) were found to be produced in the present investigation.*

Keywords— Beech wood, Hemicellulose, Enzymatic Hydrolysis, Pentose, Endoxylanse, Endoglucanase.

I. INTRODUCTION

Increasing global energy necessities and greater environmental consciousness have resulted in increasing emphasis on alternatives to fossil fuels as energy sources. Lignocellulosic biomass such as agricultural residues, forestry waste and municipal solid waste are sustainable and renewable resources for the production of liquid biofuels such as bioethanol [1]. As most often being a by-product from food and feed production, lignocellulosic biomass does not compete with the production of edible crops [2, 3] and has the potential to be the feedstock for the production of a considerable proportion of transport fuels if cost effective conversion processes are available [4]. The major components in lignocellulosic biomass are cellulose, hemicellulose and lignin. Hemicellulose sugars are the second most lavish carbohydrates in nature and its transformation to ethanol could provide a substitute liquid fuel source for the future [5]. The carbohydrates and lignin make up a major portion of biomass sample, carbohydrates can be structural or non structural. During the hydrolysis the polymeric carbohydrates are hydrolysed into the monomeric forms, which are soluble hydrolysis liquid. They are then measured by HPLC. A measure of acetyl content is necessary for biomas containing hemicellulose with a xylan backbone, but also biomass containing a mannan backbone. Acetate is measured by HPLC [6]. The degradation of cellulose and hemicellulose is carried out by microorganisms that can be found either free in nature or as part of the digestive tract of higher animals. The variable structure and organization of hemicellulose require the intensive action of many enzymes for its complete degradation. In many niches, this process is very slow because of the insoluble rigid structure of the plant cell wall and the limited availability of efficient cellulolytic and hemicellulolytic microorganisms.

Many studies on cellulose have acknowledged cellulolytic bacteria as discussed above, but not many on hemicellulose which has been the historical dogma/backlash. In this study attempt is made to depolymerize the hemicellulose by using aerobic bacteria isolated from aerated compost.

II. MATERIALS AND METHODS

The hemicellulose, as xylan, from beech wood ≥ 90% HPLC, cell wall polysaccharides of 250 gram was procured from Sigma Aldrich. The material has the Product No. X4252, appearance (color) Faint yellow to brown, HPLC w/pulsed Amperometry is ≥ 90% xylose after hydrolysis.

LCMS XBRIDGE BEH AMIDE COLUMEN of 130 A^o, 100 mm X 2.1 mm X 3.5 µm was procured from HYM BROTHER ANLYTICAL SOLUTIONS P. LTD. All the required standard chemicals and sugar standards are procured from Himedia. Enzymatically hydrolysed xylan were analysed for pento sugar at Shilpa Medicare Ltd (R&D Centre), Raichur, India using LCMS separation.

III. ISOLATION AND EXTRACTION OF BACTERIA

Hemicellulolytic microorganisms were obtained by enrichment of samples taken from aerated composting piles mainly composed of lignocellulosic plant wastes. Samples of 5 g (fresh weight) were added to 250 ml flasks containing 95 ml of mineral basal medium (MBM) supplemented with xylan (0.5%, w/v). Enrichment cultures were incubated at 120 rpm and 30^0C for 7 days. A 5 ml aliquot was taken from the culture and transferred into fresh medium. This process was repeated three times and isolated on MBM agar and xylan. A halo around colonies indicated hemicellulolytic activity. The bacteria were identified by standard microbiological techniques [7].

IV. ESTIMATION OF ENZYMES

Hemicellulases production assays performed in 250 ml Erlenmeyer flasks with 25 ml MBM, 5 ml of Hemicellulolytic microorganism and supplemented with 8 g (w/v) Xylan as carbon source. The culture was incubated at 30^oC for 48 hours. After the incubation, it was centrifuged at 8000 x g for 10 min. The supernatants of the centrifuge were subjected to enzyme assays [8].

The enzymes activity was determined by measuring release of reducing sugar from Xylan substrate. For endoxylanase activity, the reaction mixture was prepared by 250 µl of supernatant with enzyme activity and 250 µl of 8g XYL on 25 mM citrate phosphate buffer (pH 6.5). The estimation is based on international Xylanase unit (IU). corresponds to 1 µmol/ml of reducing sugar as xylose produced equivalents per min at standard assay conditions. Endoglucanase (CMCase) activity was measured by adding 8 g of CMC in 25 mM sodium acetate buffer (pH 5). One unit of CMCase activity was defined as the amount of enzyme that released 1 mol/ml of glucose equivalents per min at 37^0C and pH 5. All the reactions were stopped by boiling for 5 min. The amount of reducing sugar levels in the supernatant obtained after centrifugation at 10000 x g for 15 min at 4^0C were determined by the dinitrosalicyclic acid method [9]. Enzyme and substrate controls were routinely included. All assays performed in triplicate.

V. ESTIMATION OF SUGAR BY LCMS

The pentose sugar, which depicts the concentrations of Arabinose, Xylose, Mannose and Rhamnose were analysed using the LCMS. Analyses were conducted using a QTRAP 3200 mass spectrometer (AB/Sciex) equipped with detector AB Sciex 3200 QTRAP LC/MS/MS with Shimadzu UFLC [(LC-20AD Pump, Prominence auto sampler (SIL-20ACHT), and column oven (CTO-20AC)] [10].

VI. RESULTS

Hemicellulolytic microorganisms were successfully isolated from the source. Twenty five different obligatory aerobic and facultative aerobes were isolated. Majority of them were Gram negative bacilli. Out of the 25 bacteria isolated, 16 exhibited significant hemicellulolytic activity on solid medium. The isolates were screened for endoxylanase and endoglucanase production. Out of 19 isolates, 10 showed significant amounts of endoxylanase activity and least endoglucanase activity. The enzymatic activities of these isolates on the substrate are shown in the table 1. Isolate BMS P02 and P15 showed highest endoxylanase activity 107.76 and 94.70 IU/ml respectively and not much activity was observed for endoglucanase.

6.1 LCMS, Separation of Pento sugars.

The standard solutions of Rhamnose, Mannose, Xylose and Arabinose were prepared at 1.0, 5.0, 10.0, 15.0 and 25.0µg/mL in 50/50 Acetonitrile/Water. Linearity Standards were analyzed in at the beginning of batch of samples. Samples enzymatically hydrolysed were analyzed under the linearity curve.

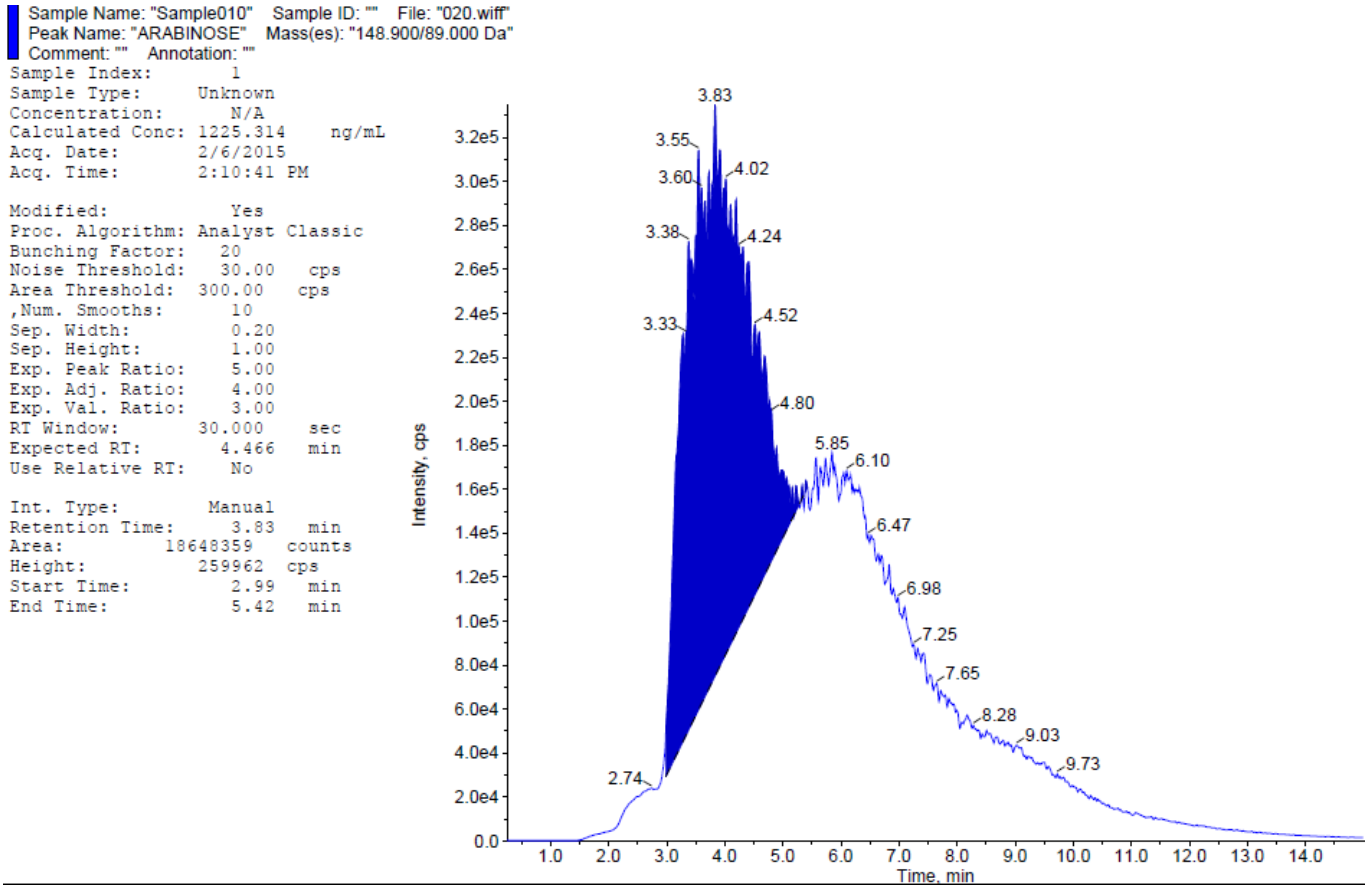
Calibration curves were generated by integrating MRM chromatogram peaks for each target analyte, and the area was graphed as a function of analyte concentration. Linear regressions were performed using 1/X*X weighting, and by forcing the calibration line through the origin

The different pento sugars degraded by the hemicellulytic bacteria are depicted in the table 2. The highest concentration of degradation of hemicellulose was observed in the bacterial Isolate in as BMS 01 (isolate no. 10) in the present study. The concentration of sugars obtained for Arabinose (Fig. 1a), Xylose (Fig.1b) and Rhamnose (Fig.1c) were 1225.31, 855.90 and 7.60 µg/ml respectively with a retention time of 4.50, 4.72 and 5.95 mints respectively with a total pento sugar concentration of 2088.81 µg/ml (table 2).

TABLE-1

ENDOXYLANSE AND ENDOGLUCANASE ACTIVITIES (IU/ML) OF SELECTED HEMICELLULOTIC MICROORGANISM FROM XYLAN FROM BEECH WOOD.

Bacterial Isolates	Endoxylanase, IU/ml	Endoglucanase, IU/ml
BMS P01	2.28	0.01
BMS P02	107.76	0-07
BMS P03	54.43	0.03
BMS P04	24.29	0.01
BMS P05	4.96	0-01
BMS P06	74.29	0.03
BMS P07	18.67	0.02
BMS P08	50.35	0-06
BMS P09	8.49	0.03
BMS P10	3.66	0.02
BMS P11	6.27	0-01
BMS P12	0.89	0.00
BMS P13	1.87	0.00
BMS P14	44.18	0-04
BMS P15	94.70	0.06
BMS P16	58.89	0.04



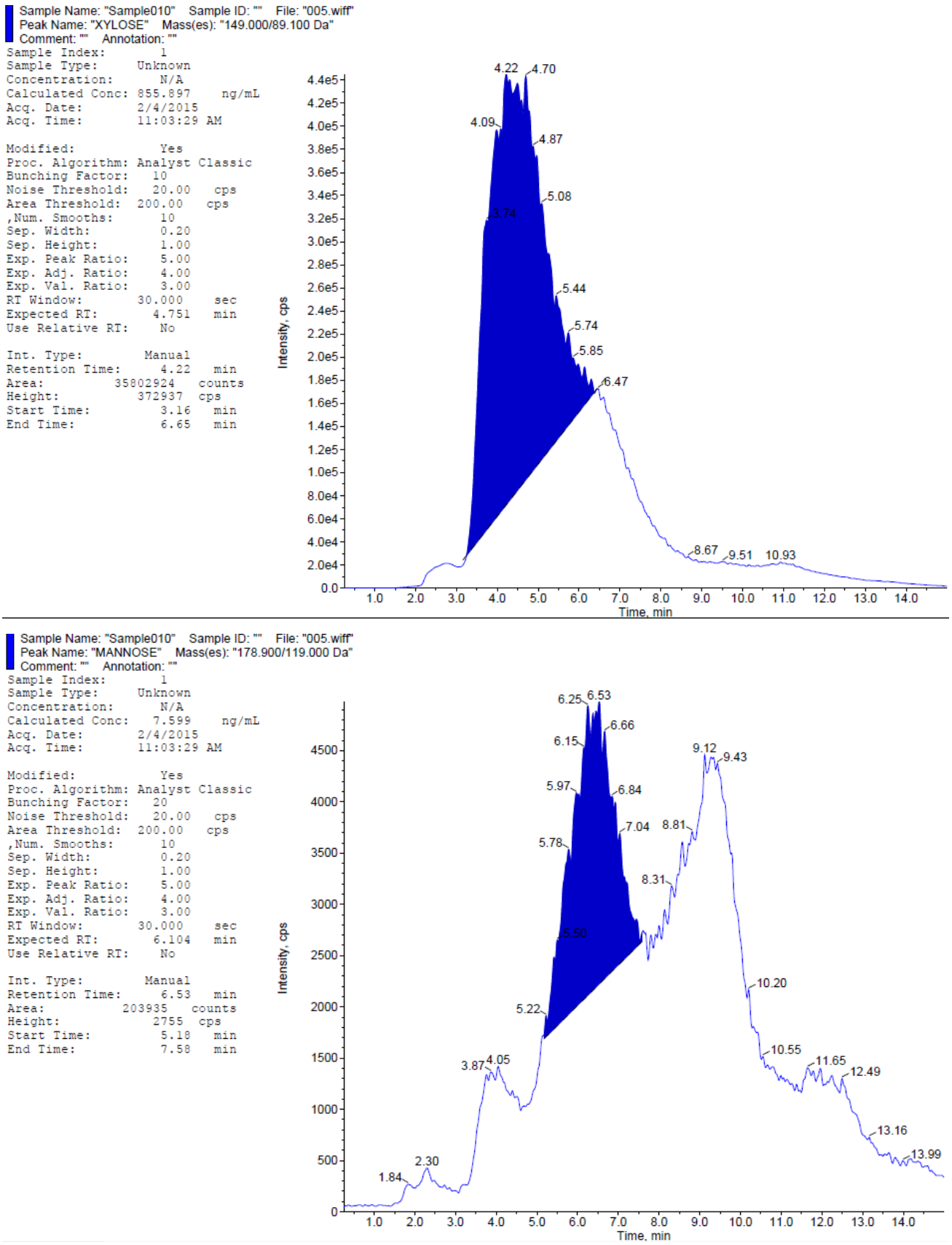
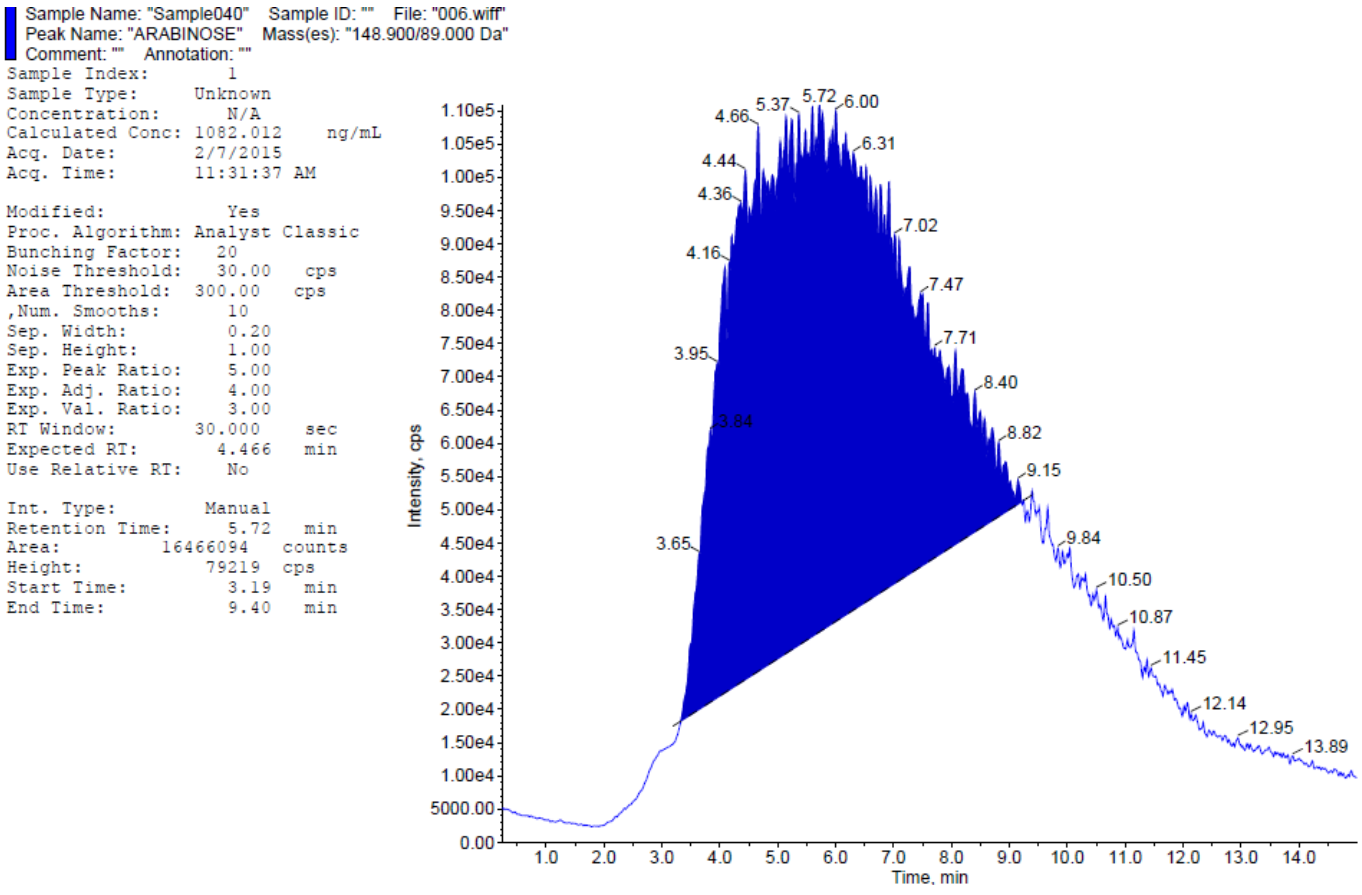


FIG. 1. LCMS CHROMATOGRAMS CORRESPONDING TO SUGAR CONCENTRATIONS OF A) ARABINOSE, B) XYLOSE AND C) MANNOSE FOR BACTERIAL ISOLATES BMS P02

Similarly for the bacterial isolates BMS 02. The concentration of sugars obtained for Arabinose (Fig.2a) and Xylose (Fig.2b) are 1082.012 and 691.73 µg/ml respectively with a retention time of 4.50 and 4.72 mints respectively with a total pento sugar concentration of 1773.74.81 µg/ml (table 2).

TABLE -2
SUGAR CONCENTRATIONS IN (µG/ML)

Bacterial Isolates	Mannose (µg/ml)	Rhamnose (µg/ml)	Xylose (µg/ml)	Arabinose (µg/ml)	Total Concentration (µg/ml)
BMS P01	0.55	0.73	15.27	26.10	42.65
BMS P02	7.60	0.00	855.90	1225.31	2088.81
BMS P03	20.29	0.00	498.74	612.51	1131.54
BMS P04	0.05	0.00	394.25	260.16	654.45
BMS P05	0.00	0.60	19.99	57.93	78.52
BMS P06	0.00	0.00	441.49	856.89	1298.38
BMS P07	6.54	0.00	84.09	217.11	307.74
BMS P08	8.46	0.82	366.29	574.79	950.35
BMS P09	0.00	0.00	176.32	87.85	264.17
BMS P10	4.04	0.00	546.38	0.00	550.41
BMS P11	1.81	0.00	500.31	35.13	537.25
BMS P12	0.98	0.00	3.90	10.31	15.19
BMS P13	1.67	0.00	23.98	20.46	46.11
BMS P14	0.23	0.00	396.81	498.91	895.94
BMS P15	0.00	0.00	691.73	1082.01	1773.74
BMS P16	0.00	5.84	315.11	681.74	1002.68



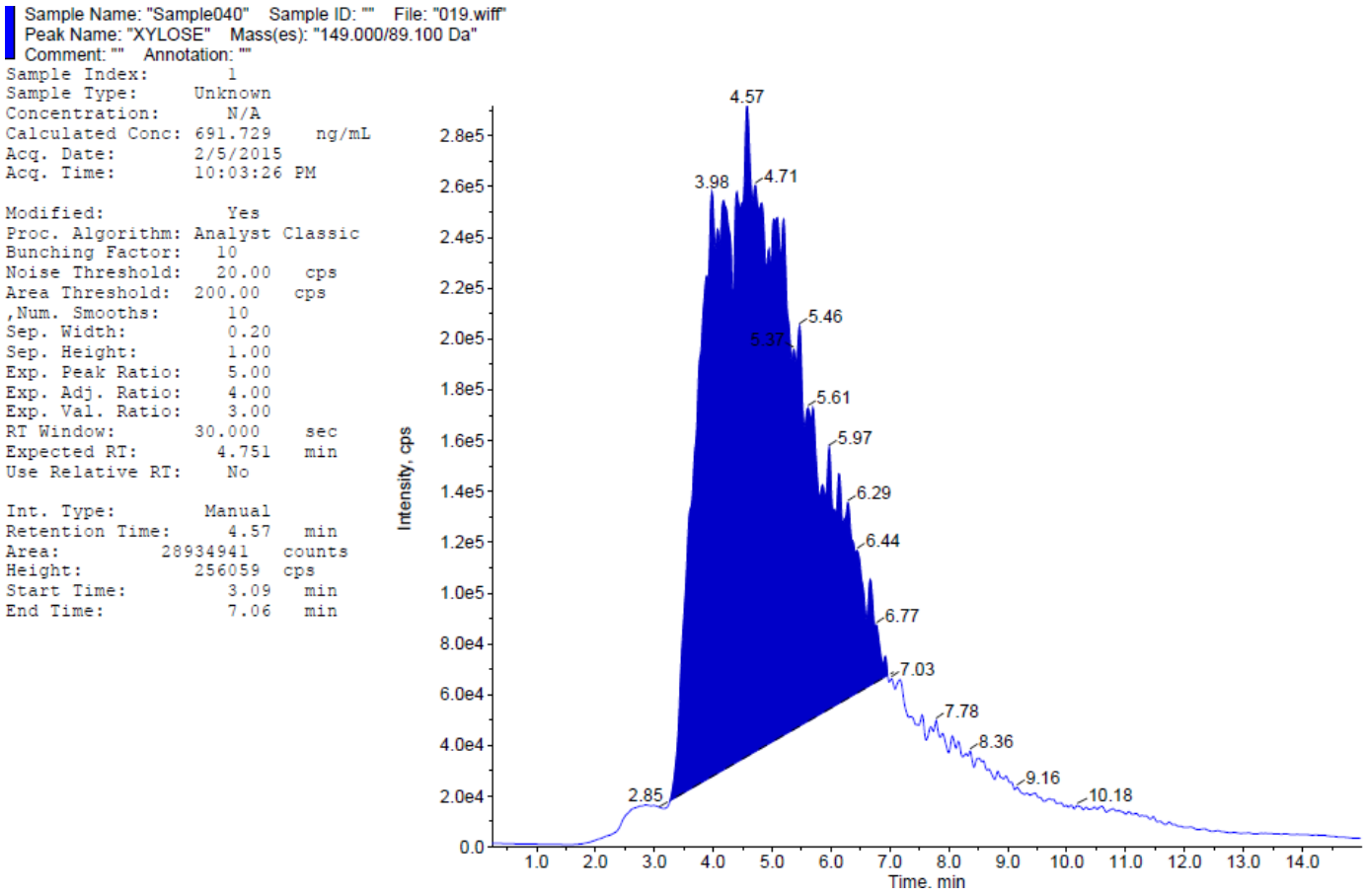


FIG. 2. LCMS CHROMATOGRAMS CORRESPONDING TO SUGAR CONCENTRATIONS OF A) ARABINOSE, B) XYLOSE FOR BACTERIAL ISOLATES BMS P15

VII. DISCUSSION

In the present study, it has been demonstrated that microbially mediated solublisation of hemicellulose aerobically. The bacteria isolated were aerobic in nature and can grow efficiently at the temperature 35^{o} C and can degrade Xylan effectively. BMS P01 and BMS P15 could efficiently solubilise the xylan compared to other bacteria isolated from the same source. The ability of few strains to utilize the hemicellose as an energy source and inability of other strains appears to be either physiologically related or due to the lack of energy required for further metabolism of these products. Highest enzymatic activity was seen in the fungi isolated from the soil (3.80 IU/ml). Study conducted by Guisado, Almeria, Spain isolated 13 cellulolytic fungi and 2 cellulolytic bacteria and highest hemicellulytic activity was seen in the fungi (2.49 IU/ml) [11]. There are not many studies to compare the bacterial enzmymatic activities, but study done by Abdul Jalil Kader at Uniersiti Kebaangsaan, Malaysia demonstrated enzymatic activities of four fungi [12].

In our study the bacteria isolated showed highest enzymatic property. The total enzyme production was seen in BMS PO2 and P15.

VIII. CONCLUSION

The enzymatic conversion of carbon source, such as Xylan, extracted from beech wood into C5 sugars such as Arabinose and Xylose. The simultaneous conversion of high concentrations of xylanolytic enzymes isolated from aerated composting piles mainly composed of lignocellulosic plant wastes. The number of microorganism with hemicellulosic activity showed that it can grow and produce xylanolytic enzymes on simple compost waste sources such as lignocellulosic biomass.

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