Original Research Article

Feasibilitiy study of bioethanol production: A case of spent mushroom and waste paper as potential substrate.

ABSTRACT

Aims: The aim of the present study is to produce ethanol from waste paper and spent mushroom using *Saccharomyces cerevisiae* as enzymes for the fermentation process.

Study design: Waste paper and spent mushroom samples were subjected to fermentation and hydrolysis by *Aspergilus niger* and *Saccharomyces cerevisiae* to produce bioethanol.

Place and Duration of Study: This study was carried out in the Environmental Microbiology
Laboratory, University of Port Harcourt, Nigeria, between May and October 2017.

Methodology: Waste paper and spent mushrooms samples were hydrolyzed by *Aspergillus niger*, and the hydrolysate from each set up subjected to fermentation by *Saccharomyces cerevisiae.* Ethanol was extracted by fractional distillation, and qualitatively determined by Gas Chromatography with Mass Spectrometry.

Results: After 8 days of fermentation, there was decrease in glucose content in waste paper hydrolysate ranging from (0.51-0.1 mg/l), and spent mushroom substrate (0.3-0.07mg/l). Upon extraction of the bioethanol, the highest yield was recorded for waste paper hydrolysate which after characterization with GC-MS ethanol concentration was 28.01 mg/l, followed by spent mushroom hydrolysate 26.8mg/l.

Conclusion: This study revealed that bioethanol can be obtained from fermentation of waste paper using *Saccharomyces cerevisiae* and ethanol can be obtained after the paper has been used in growing edible mushroom; if adopted, this could be a way to achieving environmental sustainability.

Keywords: [Fermentation; bioethanol; hydrolysis; spent mushroom}

1. INTRODUCTION

Waste management has been a major environmental challenge everywhere since the industrial revolution. About 35% of municipal solid waste by weight is paper and paper products [1]. It is very imperative though challenging task in handling these solid wastes as the option employed would be incineration; this burning of waste papers can result in environmental and health challenges to living things [1].

Most people dump their garbage and other domestic solid wastes on the street and roadsides and around their houses[1]. This practice constitute not only a nuisance but also a hazard to public health as they increase air and water pollution. This has led to increase in rodent and insect infestation which are often vectors of causative agents of many diseases which adversely affect community life and development[1]. The wastes dumped into open drains cause blockage of drainage systems leading to stagnant pools which harbors the

breeding of vectors like mosquitoes. During decomposition, toxic gases like sulphur (IV) oxide are released to the atmosphere which can give rise to health and environment hazards with health implications as bronchitis, tuberculosis, food poisoning and yellow fever [2]. On the other hand, most people resort in burning their garbage, which also leads to pollution of the atmospheric environment, and release of green house gases.

Waste paper can be used for the cultivation of mushroom (*Pleurotus ostreatus*)[3]. After mushroom cultivation, the left substrate is regarded as spent mushroom. In nature, *Pleurotus* species live on parts of plants which are generally poor in nutrients and vitamins. *Pleurotus* species have extensive enzyme systems capable of utilizing complex organic compounds which occur as agricultural wastes and industrial by-products. For this reason, it is not necessary to process substrates for cultivation of *Pleurotus* species [4,5].

In this work, bioethanol was produced from spent mushroom and waste paper substrate by fermenting hydrolysates from the two substrates using *Saccharomyces cerevisiae.*

2. MATERIALS AND METHODS

2.1 Source of samples

Waste paper samples were collected from littered lecture halls and printing press within the University of Port Harcourt, and soil sample from a dumpsite along Elimgbu-Eneka road was aseptically collected into sterile container and transported to the laboratory for isolation of *Aspergillus niger*. Palm wine sample for *Saccharomyces cerevisiae* isolation was purchased from local market in Alimini Community in Emouha L.G.A of Rivers State.

2.1.1 Isolation/identification of *Aspergillus niger* from soil

One Gram of the soil sample was serially diluted and plated on PDA media in duplicates incorporated with Streptomycin to inhibit bacterial growth. Inoculated media were incubated at room temperature at 25^0 C for 5 days in the dark [5].
Macroscopic and microscopic identification of fungal growth of *Aspergillus niger* was done with reference from manual of Larones (1995) and this was subcultured to get pure culture.

2.1.2 Isolation/identification of *Saccharomyces cerevisiae* from palm wine

A 10-fold serial dilution of 1ml sample of the palm wine was made, and 1ml plated out on PDA media in duplicates. This was incubated at 30^0C for 24 hrs and sub cultured to get the pure culture of the isolate[5].
Macroscopic and microscopic identification were done and recorded according to Larone's manual (1995).

2.2Collection and tissue culture of *Pleurotus ostreatus*

The mushroom *Pleurotus ostreatus* was procured from mushroom unit of Agricultural demonstration farm at Choba Campus of University of Port Harcourt, and Tissue of the mushroom was cultured on Potato Dextrose Agar media at 27 degree C for 10 days.

2.2.1Spawning of *Pleurotus ostreatus*

Spawning of the mushroom was done in spawn bottles [4]. Mushroom spawn was inoculated into the guinea corn grain and incubated.

The guinea corn was washed in clean water, drained and put into spawn bottles and mixed with 5g of calcium carbonate (CaCO3) after which they were autoclaved at 121^{0}c (at a pressure of 15 p.s.i) for 2 hours each day, for 3 consecutive days. The guinea corn in the bottles were then inoculated with three 9mm mycelial discs per bottle (in triplicate) under aseptic condition [4] and incubated at 27^{0}C.

2.2.2Mushroom cultivation

The method for mushroom cultivation was adopted from Baysal *et al*., (2003) [6]. The waste paper was cut into small pieces and pretreated using steam explosion method[7]. The pretreated paper was then allowed to cool. 40g of the spawn was inoculated into 1kg of the pretreated paper and incubated in the dark for three weeks at 25^{o}c, after which it was monitored for full mycelia colonization. The setup was then opened to light, and watered daily for two weeks till the pinhead was observed. It was further allowed for 4days till it grew to maturity and harvested. The setup was incubated for another 4weeks for the second fructification.

2.3Hydrolysis of waste paper and spent mushroom

The proximate analysis of the waste paper was carried out before and after cultivation of the mushroom on the waste paper to monitor the changes in proximate composition of the waste paper. The resulting spent mushroom substrate and waste paper were further set up for hydrolysis. Hydrolysis of substrate was carried out following the method used by Byadgi and Kalburgib (2016) [8]. Pure culture of the isolated *Aspergillus niger* were inoculated into the pretreated substrate in set up 1, consisting of sterilized waste paper and water; set up 2, consisting of sterilized spent mushroom and water. The setup was incubated at room temperature for 72hs and glucose monitored using dinitrosalicylic acid (DNS) method.

2.4Fermentation of waste paper hydrolysate, and spent mushroom hydrolysate

Submerged fermentation of hydrolysed broth was carried out in a shake flask following the method of Byadgi and Kalburgib (2016) [8]. The fermentation procedure consisted of two setups; set up 1 (waste paper hydrolysate), and set up 2 (spent mushroom hydrolysate). The setups were inoculated with *Saccharomyces cerevisiae*. The fermentation was carried out until maximum sugars were converted into bioethanol. The set up was agitated regularly to aerate the medium [9,10].

2.5Extraction of Ethanol

Fractional distillation method was used for separation of ethanol from the fermentation setups and ethanol production was determined using Gas Chromatography with Mass Spectrometry according to the methods of ASTDM 3921.

2.6Statistical Analysis

Two way ANOVA was used to check for significant difference in the values of the various fermentation setup to check if there is significant difference in the alcohol produced.

3. RESULTS AND DISCUSSION

The proximate composition of the waste paper and spent mushroom substrate before hydrolysis are shown in table 1.

Table 1. Proximate composition of Waste paper and Spent Mushroom substrate

Parameter(%)	Spent Mushroom	Waste Paper
Ash	13.08± 0.30	19.50±0.20
Moisture	15.92±0.30	16.10±0.22
Lipid	3.66±0.25	1.90±0.20
Protein	26.94±0.10	5.80±0.18
Fiber	6.16±0.30	12.05±0.37
Carbohydrate	34.26±0.29	45.29±0.35

The changes in the proximate composition of the spent mushroom before and after hydrolysis are represented in Fig 1. The results clearly demonstrated an increase in ash content from 13.1 to 49.5%, increase in moisture from 15.9 to 29.9%, a reduction in lipid from 3.66 to 0.54%, protein 26.9 to 12.2%, fiber 6.16 to 2.4%, and carbohydrate 34.3 to 5.32%. These results suggest that the lipid, protein, fiber and carbohydrate were reduced by the action *Aspergillus niger* during the hydrolysis.

Waste paper substrate was hydrolyzed biologically through the action of *Aspergillus niger.* The following changes were observed in the proximate composition of the waste paper before and after the 72hr hydrolysis period. There was an increase in ash content from 19.5 to 56.9%, and 16.1 to 30.7% for moisture, while a decline from 1.9 to 0.55% was recorded for lipid, 5.8 to 0.2% for protein, 12.1 to 1.82% for fiber, and carbohydrate from 45.3 to 10.2. The continuous decline in these parameters further explains that the organism was able to hydrolyze the waste paper sample. This result is represented in fig.2.

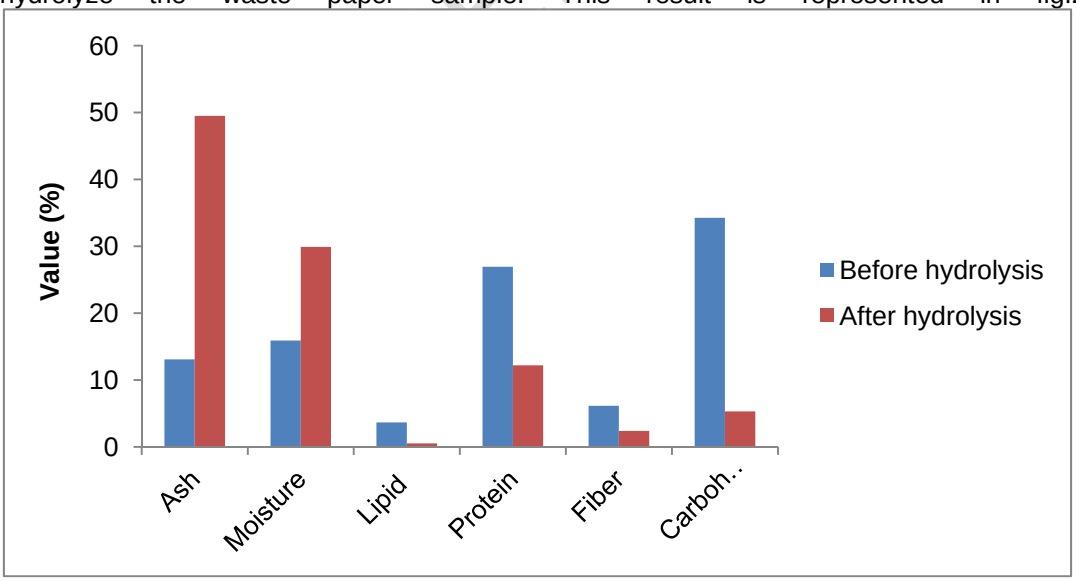

Fig 1: Proximate composition of Spent Mushroom before and after hydrolysis

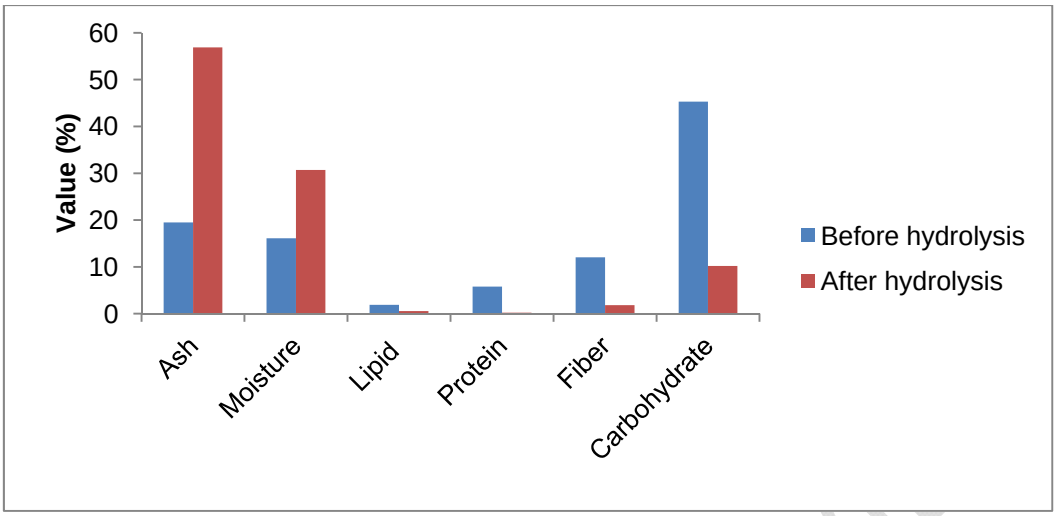

Fig 2. Proximate composition of Waste Paper before and after hydrolysis

During the period of hydrolysis of the waste paper and spent mushroom substrate, the glucose concentration in each medium was monitored. The results of the changes in glucose concentration during hydrolysis are presented in fig. 3. This serves as a biomarker indicating that the organism was able to break down cellulose present in the medium, thereby releasing glucose. The glucose concentration in both setups of waste paper and spent mushroom substrate increased with increasing time. The waste paper set up ranged from 0.26 to 0.51mg/l, while the spent mushroom setup from 0.20 to 0.30mg/l for 72hrs respectively.

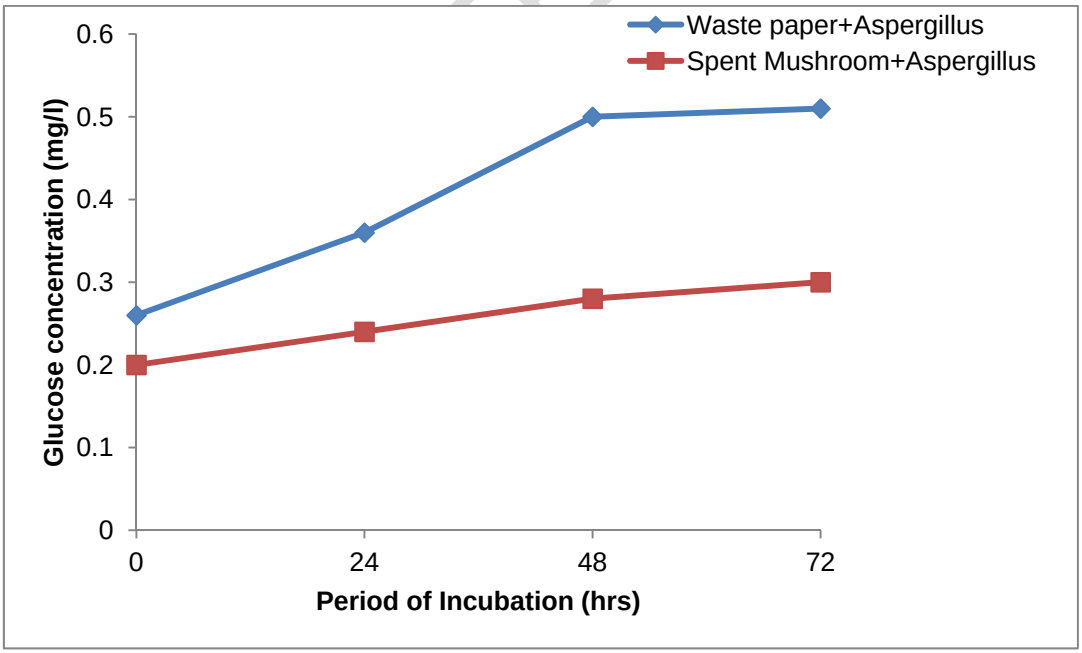

Fig 3: Glucose concentration during Hydrolysis of waste paper and spent mushroom substrate

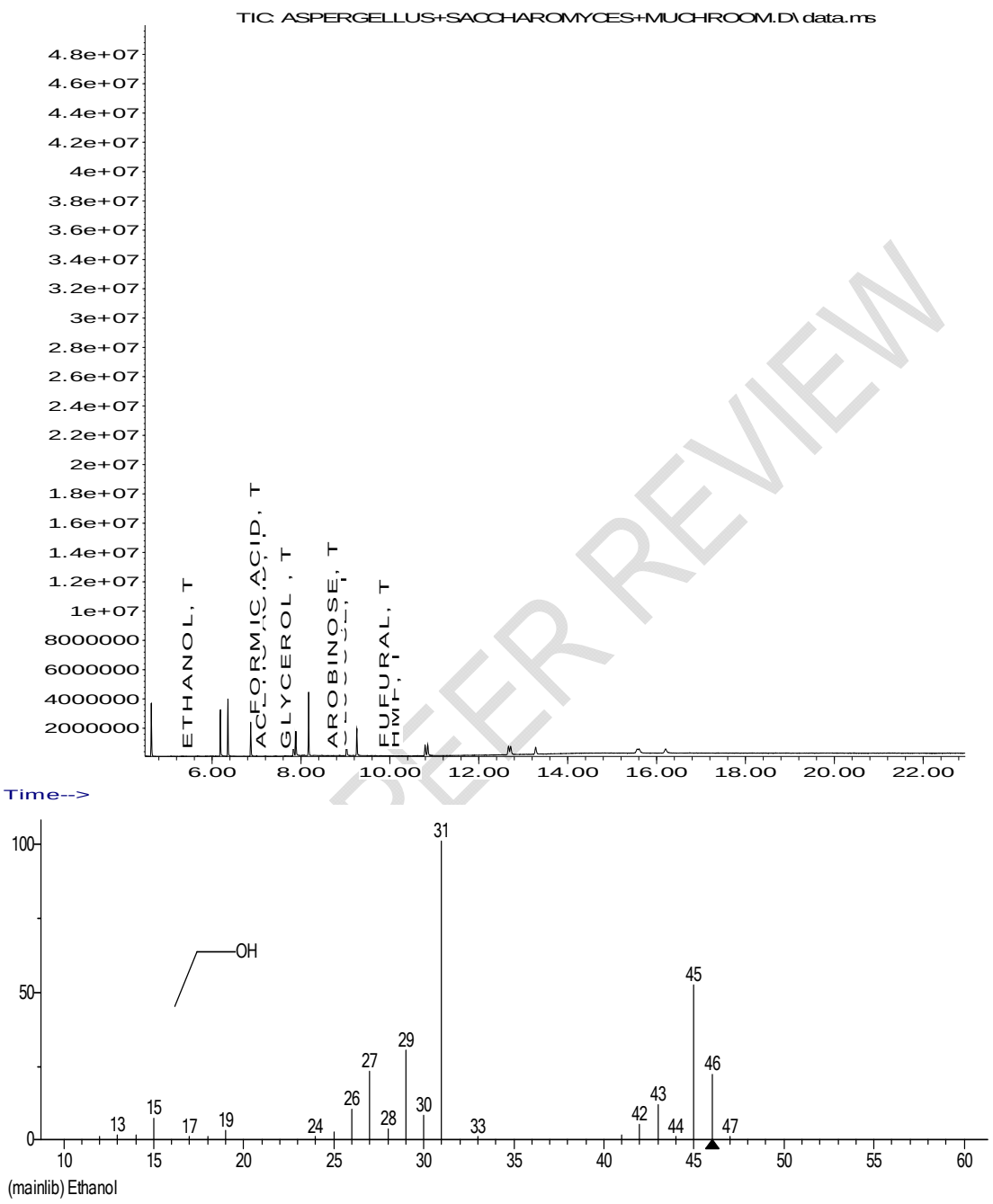

Fig 4:GC-MS of ethanol concentration from spent paper hydrolysate + *Saccharomyces cerevisiae* fermentation

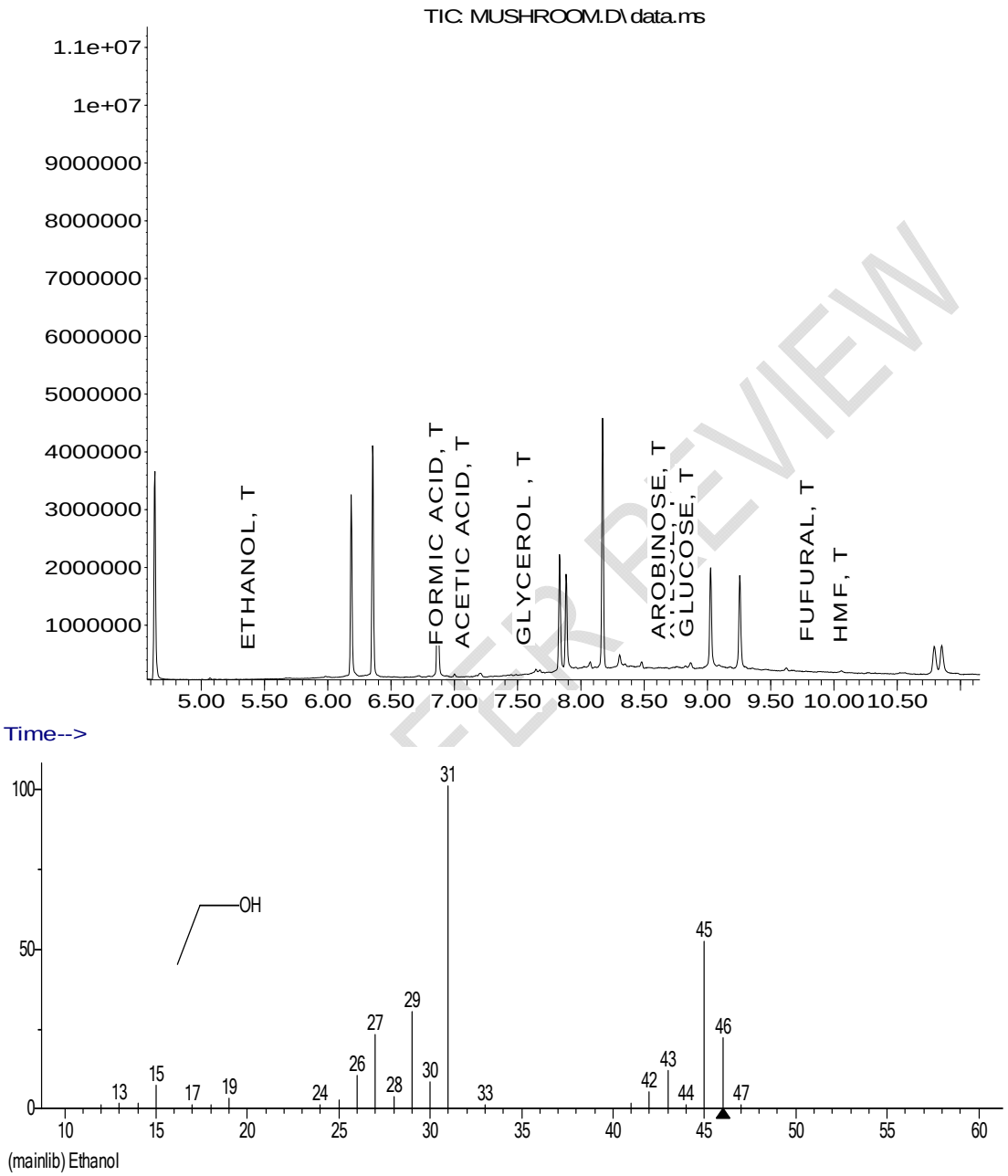

Fig 5: GC-MS for ethanol concentration from Spent mushroom hydrolysate + *Saccharomyces cerevisiae* fermentation

The chromatogram representing the ethanol produced from waste paper hydrolysate and its quality is represented in fig. 4. The amount of ethanol produced was recorded to be 28.01mg/l with retention time of 5.323. Other compounds produced were formic acid (0.01mg/l), Acetic acid (5.64mg/l), Glycerol (5.18mg/l), Arobimose (1.74mg/l), Glucose (10.41mg/l), Fufural (8.38mg/l) and HMF (13.06mg/l). While the chromatogram representing the ethanol produced from spent mushroom and its quality is represented in fig. 5. The

amount of ethanol produced was recorded to be 26.8mg/l with retention time of 5.356. Other compounds produced were Acetic acid (6.03mg/l), Glycerol (4.37mg/l), Arobimose (2.08mg/l), xylose (0.02), Glucose (9.86mg/l), Fufural (9.12mg/l) and HMF (10.32mg/l).

4. CONCLUSION

In conclusion, waste paper serves as a readily available feedstock for cultivation of *Pleurotus ostreatus.* Spent mushroom and waste paper serves as good feedstock for the production of bioethanol, as the hydrolysate from these substrates yielded bioethanol when subjected to fermentation by *Saccharomyces cerevisiae* .

REFERENCES

1. Ndukwe C, Joseph O, Nwuzor CI. Urban Solid Waste Management And Environmental Sustainability In Abakaliki Urban, Nigeria. European Scientific Journal. 2016;12:(23)155-158
2. Adeyemo I.A, Sani A. Hydrolysis of tuber Peels and Sorghum Chaff by Cellulolytic culture filtrates of *Aspergillus niger* AC4 Isolated from Agricultural Waste Dumpsites. Journal of Biology, Agriculture and Healthcare. 2013;3:66 – 71.
3. Ha TI, Chun-Li W, Chong-Ho W. The Effects of Different Substrates on the Growth, Yield, and Nutritional Composition of Two Oyster Mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). Mycobiology 2015;43:423-434
4. Fasidi IO, Kadiri M. Use of grains and agricultural waste for the cultivation of lentinus subnudus in Nigeria. Rivista Biol trop. 1993;41:411 – 415.
5. Larone D.H. *Medically Important Fungi, a Guide to Identification*, 3rd ed. American Society for Microbiology Press, Washington, DC. 1995
6. Baysal E, Peker H, Yalinkilic M.K, Temiz A. Cultivation of oyster mushroom on waste paper with some added supplementary materials. Bioresource Technology.2003;89: 95–97.
7. Sánchez C. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. Appl Microbiol Biotechnol. 2010;85:1321–1337.
8. Byadgi SA, Kalburgi PB. Production of Bioethanol from waste newspaper. *Procedia Environmental Sciences*. 2016;35:555-562
9. Stanley HO, Ezeife CO, Onwukwe CD. Bioethanol Production from Elephant Grass. Nigerian Journal of Bioethanol. 2017; 32: 1-6.
10. Yalinkilic M.K, Altun L, Baysal E, Demirci Z. Development of mushroom cultivation techniques in Eastern Black Sea Region of Turkey. Project of the Scientific and Technical Research Council of Turkey (TUBITAK), No TOAG-875, 287.1994;55-70.