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Bioethanol Production from Neem Tree Leaves *(Azadirachtaindica)* Using Saccharomyces cerevisiae as Fermenting Agent

Yusuf Muhammad[2], Bashar Hadi A.[1], Ahmad M. Bello[1] and B.B S.Dogarai[1]
[1]*Department of Chemistry, ShehuShagariCollege of Education, Sokoto Nigeria*
[2]*National Food and Drugs Administration and Control Agency. Nigeria*

Abstract: *The following study was aimed to investigate the potential of Neems tree leaves (Azadirachtaindica) in bioethanol production using Baker's yeast (Saccharomyces cerevisiae) as fermenting agent. Dried powdered leaves of neem tree (Azadirachtaindica) were hydrolyzed using Conc. 2% 5%, and 10% H_2S0_4. And the hydrolyzed samples were it was fermented using Baker's yeast (Saccharomyces cerevisiae). After fermentation the broths formed were distillated to obtained ethanol. Acidified$K_2Cr_2O_7$was used to determine the bioethanol produced. The absorbance of the ethanol produce using UV-Visible Spectrophotometer was extrapolated with the series of standard prepared glucose solution. Alsothe FTIR Spectroscopy analysis of bioethanol produced confirms the presence of alcohol content in the sample. The results of the bioethanol content from the leaves using 10%, 5% and 2% H_2S0_4 were 3.30%, 6.25%, and 5.34% yield respectively. The results showed that the neem tree leaves that are lignocelluloses can be used to generate appreciable percentage of bioethanol. Among the samples 5% H_2S0_4 shown the highest yield of 6.25%.*

Keywords: *Acid Hydrolysis, Azadirachtaindica, Bio-ethanol, Saccharomyces cerevisiae.*

I. Introduction

Recently, as to address the uncertain fuel supply, in an efforts to reduce carbon dioxide emissions and to provide feasible alternative to fossil transport fuel all over the world bioethanol production has attracted the attention of the world [1]. Bioethanol can be produced from various sources, like starch crops, sugar crops, household waste, agricultural waste, fruit juices, fruit wastes etc. Among these sources, non-edible source seems to be the best options [2]. Bioethanol is seen as a good alternative to fossils fuel and its termed as renewable energy source since its source crops can be grown renewably within almost all climates around the world with considerable lower emissions of poisonous gasses to our environment [3],[4] Sufficient use of bioethanol as an energy source for transportation or in industries can considerably reduce the nauseating greenhouse gas emissions from transport and industries emission [5]. More job opportunities can be created through bioethanol production as well as economic income and energy security [6].

To prevent fuel food crisis lignocelluloses biomass, particularly agricultural residues is converted to useful products such as bioethanol [7], [8] [9]. In this research, on edible plants source that is neem tree leaves is used in bioethanol production using beakers yeast as fermenting agent prior to acid hydrolyzed substrate. Lignocelluloses biomass consists mainly of lignin, cellulose and hemicelluloses that are present in a different percentage according to the plant type and its parts. It was reported that,the plant leaves contain 15-20% cellulose, 80-85% of hemicelluloses and 0% of lignin[10]. Presently, more researches are focused on non-edible biomass due to their availability and low cost in procurement [11]. Bioethanol is a volatile and flammable liquid produced through microbial fermentation process, which has a molecular formula of C_2H_5OH [6].

Neem tree *(Azadirachtaindica)*, is a tree in the mahoganyfamilyMeliaceaethat is shown in Figure 1and it is one of two species in the genus *Azadirachta*. It is a native to India, Pakistan and Bangladesh which is widely growing in tropical and semi-tropical regions. Neem tree is the official tree of the Sindh Province and is very common in all its cities. Neem tree also grow in islands of the southern part of Iran (USDA, 2014).It grows widely in northern Nigerian region, its fruits and seeds are the source of Neem oil. The neem tree leaves were reported to have contained 60% of H_2O, 23% carbohydrates, 7% proteins and more than 3% minerals and 1% fat [12].

Figure 1: Neem tree leaves

The major process for conversion of lignocelluloses to bioethanol requires the following process; delignification to liberate the cellulose and hemicelluloses, depolimerisation of carbohydrate polymers to produce free sugars (which is known as hydrolysis of cellulose to simple sugar) and finally, the fermentation of mixed simple sugar to ethanol [13]. The Pretreatment of lignocelluloses biomass in bioethanol production is done not only to break the β-1,4-glycosidic linkage of the polysaccharide but alsocontribute in generating high yield of bioethanol. However, the picture of the process is shown in Figure 2also the equation 1.1 shown the pretreatment process[14].

$C_{12}H_{22}O_{11} + H_2O \longrightarrow C_6H_{12}O_6 + C_6H_{12}O_6$ ……..……1.1
Sucrose Water Fructose Glucose

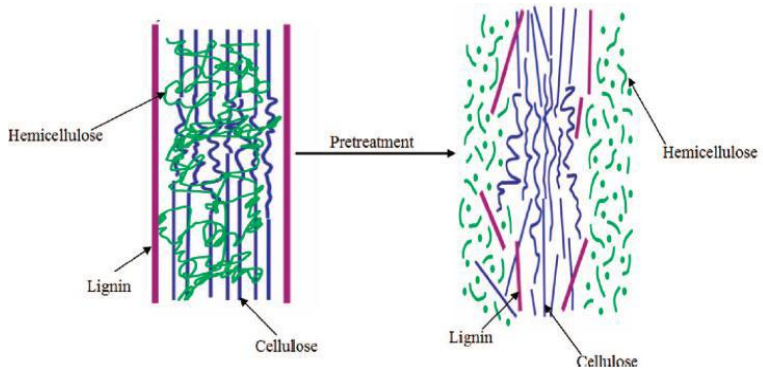
II. Material And Methods

Sample Collection and Sample Preparation

Three different types ofNeem tree leaves were collected in polythene bags and taken to the laboratory for analysis atUsmanuDanfodiyo University permanent site.The *Saccharomyces cerevisiae*used (dry baker's yeast).Neemleaves were air dried and grounded to powder followed by sieved.

Pretreatment of the sample with Acid

Thirty (30g) of powdered leaves of neem sample were taken into three conical flasks and 300cm^3 of 10% H_2SO_4 was added. The flasks were plugged with cotton wool and wrapped in aluminum foil and sterilized at 121^{o}C for 15 minutes then allowed to cool. The same procedure was repeated using 5% and 2% H_2SO_4. The content of each flask was filtered and their pH values were then adjusted to 4.5 beforethe fermentation[15].



Source: Manta *et al*., 2013

Figure 2: pretreatment of lignocelluloses Biomass

Reducing sugar determination

For reducing sugar determination series of glucose as standard were prepared. Then, the Hydrolysatesfiltrate samples were treated with 3cm^3 dinitrosalicylic acid (DNS) reagent and the mixture was boiled in water bath for 10 minutes to develop red brown colour and 1cm^3 of 40% Potassium sodium titrate solution was added to stabilize the colour. The absorbance of the portion of the mixture from each test tube was measured using UV-visible spectrophotometer at 540nm. The corresponding concentrations of the samples were extrapolated with the standard glucose curve [4], [16].

Fermentation process

In this analysis conical flaskcontaining the hydrolyzed filtrate sampleswere covered with cotton wool wrapped in aluminum foil and sterilized at 121^{o}C. After coolingof the flasks at room temperature the pH of each

flask was adjusted to 4.5 then3gof the *Saccharomyces cerevisiae*(dry baker's yeast) was taken into them and incubated aerobically at 37^{o}C for five days. After that, the broth obtained wasdistillated by taken the fermented brothinto a round-bottom flask fixed to a distillationcolumn enclosed in with a running tap water and a heating mantle with the temperature adjusted to 78.3°C. To collect the distillate, a conical flask was fixed to theother end of the distillation column [17], [18] the process is shown using equation 2.1.

$C_6H_{12}O_6 \xrightarrow{Yeast} 2C_2H_5OH$ + $2CO_2$ ……2.1
Glucose Ethanol Carbon dioxide

Qualitative and quantitative test for Ethanol

Two (2) drops of acidified 0.1M $K_2Cr_2O_7$ was added to the 2cm^3 of distillate produced and heated for 30 minutes on a water bath. The content of the test tube changed to green colour indicating the presence of ethanol. Equation of the reaction is represented usingequation 2.2 [19].

$2K_2Cr_2O_7 + 8H_2SO_4 + 2C_2H_5OH \rightarrow 2K_2SO_4 + 2Cr_2(SO_4)_3 + 3CH_3COOH + H_2O$……..2.2

However, the quantity of ethanol produced was determine using UV-visible quantitative analysis of alcohols using potassium dichromate VI an Oxidizing reagent in whereby the ethanol will be oxidized to ethanoic acid.This was carried out using UV-VIS quantitative analysis. 1cm^3 of absolute ethanol (98% v/v) was to prepare series of standard solution. The content of each test tube was then heated in water bath for five (5) minutes, for the colourto development. The absorbance of each concentration was measured at 585nm using UV-VIS Spectrophotometer and the reading was used to develop standard ethanol curve.Consequently, Five (5cm^3) of each of the sample were taken in the test tubes, and then 2cm^3 of Dichromate reagent was added to each. The content of each test tube was then heated in water bath for five (5) minutes, for the colourdevelopment. The absorbance of each concentration was measured at 585nm, using U-V Visible spectrophotometer [20].

FTIR Spectroscopy

The samples of bioethanol produced wereanalyzed using MB 3000 FTIR Spectroscopy machine to determine the vibrational frequencies of the bioethanolproduced.

III. Result and Discussion.

Table 1: Reducing Sugar concentrations using acid hydrolysis, bio-ethanol produceand percentage yield.

Samples	Reducing Sugar (g/kg)	Bioethanol Produced (g/kg)	% Yield
2% H_2SO_4	1.80± 0.52^{bc}	53.41 ± 19.26^{ab}	5.34
5% H_2SO_4	3.20± 0.24^{a}	65.24 ± 0.86^{a}	6.52
10% H_2SO_4	1.23± 0.04^{c}	32.96 ± 14.26^{c}	3.30

The results in Table1 indicated that 5% acid hydrolysis gives the highest reducing sugar (3.20g/kg), followed by 2% (1.80g/kg) and 10% (1.23g/kg) which gives the lowest concentration. The significant variation was observed ($P< 0.05$), hence using 5% acid hydrolysis is the optimum amount required for the break down the complex sugar contained in the neem sample. However, no significant ($P>0.05$) variation was observed using either 2% or 10%, however there is numerical differences.

The highest yield bioethanol obtained that is using 5% acid hydrolysisweresubjected to FTIR Spectroscopy analysis, the samples shown strong broad peak at 3450 - 2850cm^{-1}, therefore indicating -CH_2- and -CH_3 stretching vibrations and well resolved Peak around 3416cm^{-1} can be assigned to alcoholic -OH vibrations. These values are in agreement with the values obtained According to Spectra (2014) free O-H stretching normally occurs at 3550 – 3200 cm^{-1}, while C-H stretch occurs at 3000 – 2840 cm^{-1}. Therefore, the production of ethanol was successful. The results obtained from the present study can be concluded that 5% H_2SO_4 acid hydrolysis gave highest concentration of ethanol production. This indicates that, Baker's yeast (*Saccharomyces cerevisiae*) is a suitable fermenting agent in the production of bioethanol using neem leaves sample. Production of ethanol from ligno-cellulosic materials has received extensive interest due to their availability, abundance and relatively low-cost. Neem leaves (*Azadirachtaindica*) is therefore an abundant and sustainable biomass and non-food material that could be exploited for bio-ethanol production especially in the northern part of the Nigeria. Neem leaves (*Azadirachtaindica*) however could serve this purpose since from the study it is indicated that with proper pretreatment and appropriate method bio-ethanol could be obtained.

IV. Conclusion

The population of human being is increasing on the average worldwide, hence the demand for energy source increases. It is apparent that current fuel bioethanol production from grain-based feedstock is notfavorable as it may lead to food shortage to the teaming world populace. In order to avoid these foreseen worrisome, lignocelluloses biomass should be utilized in the production of bio-ethanol and biofuels in general. The study showed that 5% H_2SO_4 acid hydrolyzed sample has the highest percentage yield of bioethanol production.

References

[1]. Brochure produced by the European Biomass industry Association (2007) which was supported by the European commission under 6th program (FP6). Retrieved on March, 23 from: http://www.europea.eu/rapid/presreleasedaction.

[2]. A.A. Brooks, Ethanol Production Potential of Local Yeast Strains IsolatedFrom Ripe Banana Peels. *African Journal of Biotechnology*. *7 (20)2008*: 3749-3752

[3]. Ahring, B. K., Licht, D., Smith, A.S. Sommer, F. and Thomsen, A.B.Production of ethanol from wet oxidized wheat straw byThermonanaerobactermathranii. BioresourcesTechnology. *68 1999*: 3-9

[4]. Akpinar, O., Erdogan, K. and Bostanci, S. (2009). Production of xylooligosaccharides by controlled acid hydrolysis of lignocellulosic materials. *Carbohydrate Research***344:**660-666

[5]. Balat, M, Balat H. and Oz, C. (2008) Progress in bio-ethanol processing.*Progress in Energy and Combustion Science*, **34**:pp551-573.

[6]. Chandel, A. K., Felipe A. F. A., Priscila V. A., Milessi, T. S. S., da Silvio S. S. and Maria G. (2012) Dilute Acid Hydrolysis of Agro-Residues for the Depolymerization of Hemicellulose: State-of-the-Art. *Springer-Verlag Berlin Heidelberg* DOI: 10.1007/978-3-642-31887-02, pp39-61

[7]. Demirbas, A. (2007) Bioethanol from Cellulosic materials: A Renewable Motor Fuel from Biomass. *Energy Sources***27**: pp327 – 337.

[8]. Graeme, M. W. (2010), Bioethanol: Science and technology of fuel alcohol, VentusPublishers and ApS USA pp1-17

[9]. Heinrich, M.,Prance, G. and M. Nesbitt. (2005). "Plants as Medicines." The Cultural History of Plants. London: Routledge. 228.

[10]. Humphrey, C.N & Caritas, A.O (2007), Optimization of Ethanol Production from Garcinia kola (bitter kola) pulp agro waste. *Africa Journal of Biotechnology*,**6(17)**: pp2033-2037

[11]. Junchen, L., Muhammad, I. & Lin, F. (2012) Bioconversion of agricultural waste to ethanol: A potential source of energy. *Achives Des Sciences***65 (12)** pp626-642

[12]. Mamta, A., Kauri, J. &Ramas, S. (2013), Bioethanol Production through water Hycinth (EichorniaCrassipes) via optimization of the pretreatment conditions. *International Journal of Emerging Technology and Advance Engineering***3 (3)** pp42-46

[13]. Ohgren, K.,Vehmaanpera, J., Siika-Alio, M. Galbe M. Viikari, L. &Zacchi G.(2006) High temperature enzymatic pre-hydrolysis prior to simultaneous saccharification and fermentation of steam pretreated corn Stover for ethanol production. *Enzyme and Microbial Technology***40(4)**:pp607 – 613

[14]. Oyeleke S.B and Jibrin N.M (2009), Production of Bio-ethanol from Guinea Corn Husk and Millet husk. *Africa Journal of Microbiology Research***3(4)**:pp147-152.

[15]. Pramanik, K (2005) Parametric Studies on Grape Waste Alcohol Fermentation Using Sacchromyces Yeast extract from Toddy.*Journal of Chinese Institute of Chemical Engineers.***4(34)**: pp1-23

[16]. Rabah, A. B., Oyeleke, S. B., Manga, S. B. And Hassan, L. G. (2011)Utilization of Millet and Guinea corn husk for bioethanol production. African Journal of l Microbiology Research**5 (31)** pp5721-5724.

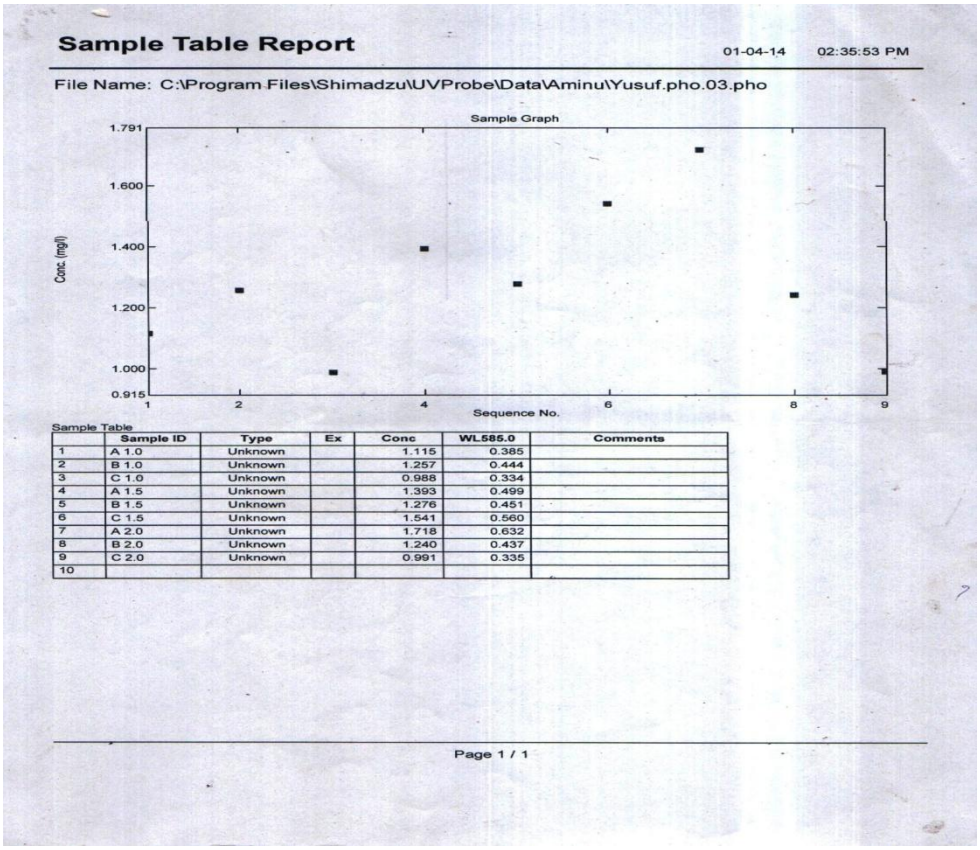
[17]. Shanmugam, P. Sivakumar, V. Sridhar, R and Venkatesa, S (2009) Production of Bioethanol from sugar molasses using Saccharomyces cerevisiae. *Modern Applied Science.***3 (8)**: 32-35

[18]. Uduak, G. A, Adamu, A. A and Udeme, J. J (2008) Production of Ethanol from Organic and Food Wastes. *Journal Practices and Technologies.* **13**:pp1-12

[19]. US Department of Agriculture (2014) Agricultural Research Service, Germplasm Resources Information Network. Retrieved on April 14, 2014. From: http://www.ars.grin-gov/cgi-bin/npgs/taxol.p1?616.

[20]. Miller, G.L. (1959). Use of Dinitrosalicylic Acid reagent for Determination ofReducing. *Analytical Chemistry*, **31**: pp426-471.

[21]. Zhao X, Zhang L & Liu D. (2012) Biomass recalcitrance. Part II: Fundamentals of different pre-treatments to increase the enzymatic digestibility of lignocellulose. Biofuels, Bioprod. Bioref DOI:10.1002/bbb.



Sample Table Report

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Sample Graph

Sample Table

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1	A 1.0	Unknown		1.115	0.385	
2	B 1.0	Unknown		1.257	0.444	
3	C 1.0	Unknown		0.988	0.334	
4	A 1.5	Unknown		1.393	0.499	
5	B 1.5	Unknown		1.276	0.451	
6	C 1.5	Unknown		1.541	0.560	
7	A 2.0	Unknown		1.718	0.632	
8	B 2.0	Unknown		1.240	0.437	
9	C 2.0	Unknown		0.991	0.335	
10						

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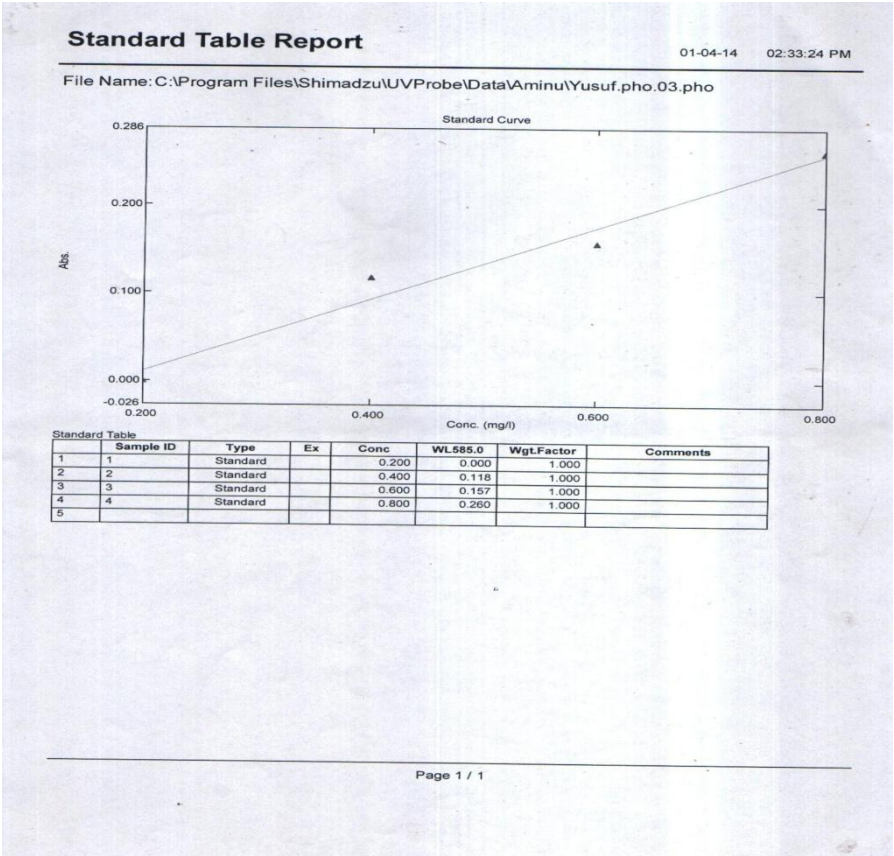

Standard Table Report

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Standard Curve

Standard Table

	Sample ID	Type	Ex	Conc	WL585.0	Wgt.Factor	Comments
1	1	Standard		0.200	0.000	1.000	
2	2	Standard		0.400	0.118	1.000	
3	3	Standard		0.600	0.157	1.000	
4	4	Standard		0.800	0.260	1.000	
5							

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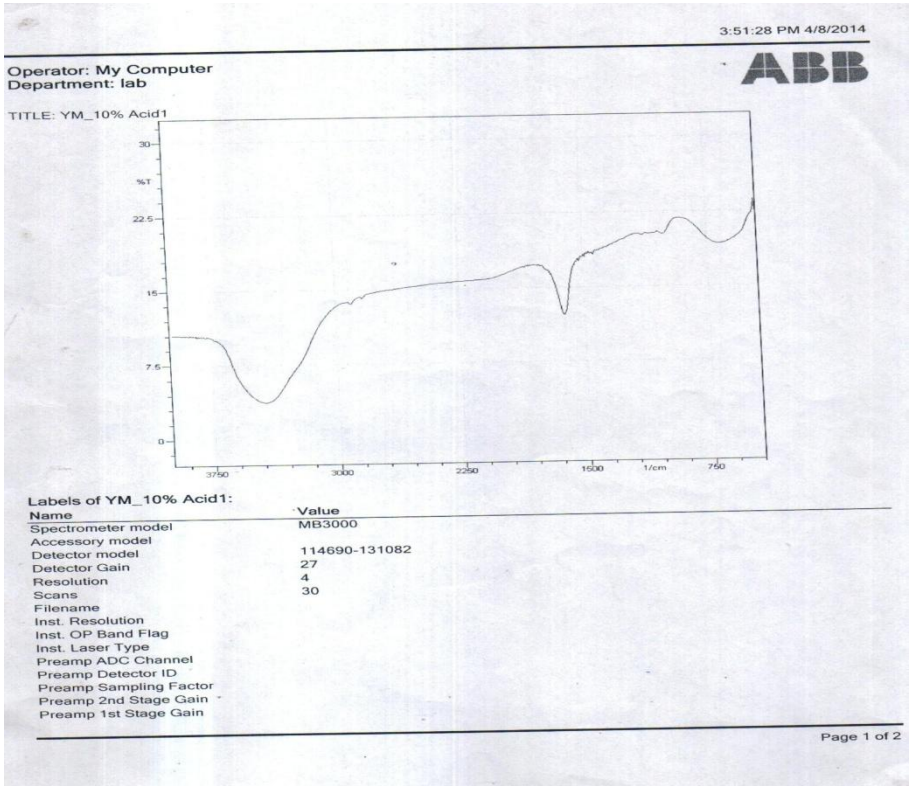

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Accessory model	
Detector model	114690-131082
Detector Gain	27
Resolution	4
Scans	30
Filename	
Inst. Resolution	
Inst. OP Band Flag	
Inst. Laser Type	
Preamp ADC Channel	
Preamp Detector ID	
Preamp Sampling Factor	
Preamp 2nd Stage Gain	
Preamp 1st Stage Gain	

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