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Original Research Article

Production of Ethanol from Groundnut Shell using Saccharomyces cerevisiae

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Abstract

Bioethanol is produced through distillation of ethanol waste emanating from fermentation of biomass derived sugars. Ethanol is the most widely used liquid bio-fuel. This research thesis designed to produce ethanol from groundnut shell agro-waste. To carry out the experiment, groundnut shell was obtained from Eastern Hararge zone, babile district. The shell was washed and dried in an oven and ground to powder form by grinding machine. The powders of shell with different concentrations (25gm, 50gm, 75gm, and 100gm) were treated by dilute sulphric acid in case of treated part. Inoculum (0.5% and 1% yeast) with 100ml nutrient solution was added in each substrate concentration separately and the pH of solution was adjusted to 4.5 and allowed to ferment for 16days for ethanol production. The reducing sugar contents decreased gradually as the fermentation period increased. With increased time of fermentation the bio-ethanol yield and cell density also increased up to the 8th day of the fermentation. In this study, the effect of pretreatment on bio-ethanol production was also investigated. The result showed that the pretreated samples yielded more ethanol than in untreated samples.

Keywords: Bio ethanol, groundnut shell, fermentation, distillation, Saccharomyces cerevisiae

INTRODUCTION

A transition from the utilization of petroleum-derived fuels in the transportation sector to renewable fuels, such as bio-ethanol, can be beneficial to society in many ways. . Combustion of renewable materials will not contribute to a net increase of the greenhouse gas CO2 in the atmosphere. Carbon dioxide is believed to contribute to global warming[12]. Bio-ethanol can also contribute to the national energy security by reducing the dependency of petroleum imports. However, the strongest argument for switching to alternative fuels is that the global supply of petroleum is running low[3]. Ethanol can be produced from renewable raw materials and will function in the automobiles and buses of today without any major modifications of the engine. Groundnut shells are abundant and widely available as a lignocellulosic material. Maloney[13] stated that lignocellulosic materials are the most abundant polymers mainly constituted from three natural polymers namely: cellulose, lignin and hemicelluloses in varying proportions depending upon the specific plant in which they occur[4]. The use of lignocellulosic materials is a base for the production of chemical substance. It has been less expensive to derive carbon based chemicals from fossil fuels, especially natural gas and petroleum.

Groundnut is one the five widely cultivated oilseed crops in Ethiopia[18], Eastern Hararghe zone of Oromia region hold primary position in producing and supplying both.

Domestic and export markets as compared to other parts of the nation (unpublished Ethiopia Export Promotion report, EEP 2004).Widely cultivation of groundnut results in high amount of waste as a groundnut shell. Since these wastes are available as a lignocellulosic material, it is the basis for the production of chemical substance. With this point in mind this study was designed to produce bio-ethanol from an agro waste, groundnut shell, with the following objectives.

General Objective

- To produce ethanol from groundnut shell cultivated around Eastern Hararge zone

Specific objectives

- To evaluate the effect of substrate concentration (groundnut shell) on bio-ethanol production.
- To test the effect of acid pretreatment on ethanol production from groundnut shell.

MATERIALS AND METHODS

Description of Study Area

The experiment was conducted in Microbiology laboratory, Department of Biology at Haramaya University, which is located at latitude of 9°26' N, longitude of 42°03'E and an altitude of 1980 m.a.s.l [9]. The mean annual temperature is 17°C with mean minimum and maximum temperatures of 3.8 and 25°C, respectively.

Sample Preparation

The samples (groundnut shells) were collected from Babile town, Eastern Hararge zone. After the collection process, the sample was grounded into small pieces and then allowed to dry in an oven at 650C for 48hr. Four kilogram of the sample was used for sample preparation. Among the 4kg of substrate, 1kg was pulverized with a blender, packed in plastic container and stored in the freezer for subsequent analysis[7]. Dried baker's yeast (S. cerevisiae) was obtained from local market.

Pretreatment of Substrates

The purpose of the pretreatment was to remove lignin, reduce cellulose crystallinity and increase the porosity of the materials. Pretreatment must meet the following requirements: improve the formation of sugar, avoid the degradation or loss of carbohydrate, avoid the formation of by-product inhibitors and must be cost effective.

Substrate pretreatment was carried out in 500ml conical flasks by using dilute sulphuric acid hydrolysis to enhance the rate of conversion of glucose into bio-ethanol by the action of baker's yeast (*S. cerevisiae*). During this hydrolysis, dilute H_2SO_4 (1.25%) was added with each gram of substrate and the mixture was shaken & heated up to $100c^o$ for 15 minutes. Sodium hydroxide (NaOH) was added for removing lignin and to neutralize until reaching the pH of 4.5 for fermentation[5]. The effect of pretreatment on fermentation was compared with fermentation on untreated substrates.

Preparation of Nutrient Solution

Nutrient supplements that enhance the growth of yeast was prepared by adding 0.1 g KH_2PO_4, 0.5 g $CaCl_2$, 0.05 g $MgSO_4$, 0.1 g Na_2SO_4 and 0.1 g $(NH_4)2SO_4$ per litter.

Sterilization

The reactor and all the equipment that were used for fermentation purposes were sterilized (autoclaved). The sterilization was carried out at a temperature of 121^oC for 15 minutes.

Experimental Design

Experiment was conducted in a factorial design in three replications for each treatment and control. Fermentation (experiment) was carried out using the substrates (groundnut shell) and inoculums (baker's yeasts) in a proportion shown in Table 1 below. For each substrate amount, un-pretreated substrate mixed with each of inoculums was used as control. The pH of the mixture in each case was adjusted to 4.5 and then pasteurized. The fermentation process was allowed to proceed for 16 days at 30^oC. The production of ethanol was estimated at the interval of 4 days starting from the beginning of fermentation.

Table-1: Treatments showing the different substrate and yeast concentration combinations for bioethanol production from Groundnut shell.

Batch No	Substrate Concentration	Volume of Nutrient	Volume of Inoculums
Groundnut shell-1	25 gram	100 ml	0.5% Yeast
Groundnut shell -2	25 gram	100ml	1% Yeast
Groundnut shell – 3	50 gram	100ml	0.5% Yeast
Groundnut shell -4	50 gram	100ml	0.1% Yeast
Groundnut shell -5	75 gram	100ml	0.5% Yeast
Groundnut shell-6	75 gram	100ml	1% Yeast
Groundnut shell -7	100 gram	100ml	0.5% Yeast
Groundnut shell -8	100 gram	100ml	1% Yeast

Analytical Procedure

The sample was collected from each flask (Thirty cubic centimeters ($30cm^3$)) at the interval of 4days and centrifuged at 400 rpm for 30 minutes to remove the cells suspension. The supernatant fluid was filtered through Whatman No 1 filter paper and the filtrate was used for determining ethanol and reducing sugar concentrations. The remaining was used to determine the cell density[1].

Determination of Cell Density (Biomass)

Cell density was measured at 4^{th}, 8^{th}, 12^{th} and 16^{th} days of fermentation using spectrophotometer (Humas Think HS 3300, Korea) at 600 nm absorbance [17].

Quantitative Analysis of Reducing Sugar

Reducing sugar like glucose concentration was determined by using 3, 5-dinitrosalicylic acid (DNS) reagent as described by[14]. A sample (0.05ml) was taken from filtrate, and citrate buffer (0.35ml) (pH=6.5) and 0.6ml of DNS were added and the sample was boiled for 5 minutes immediately to stop the reaction. The absorbance was measured for reducing sugar at 540nm using spectrophotometer[2].

Quantitative Estimation of Bio-ethanol Production

The amount of bio-ethanol produced from substrates (groundnut shell) was analyzed from filtrate and compared with the bio-ethanol produced from dextrose as a source of glucose. The quantitative analysis was carried out by determining the densities of the distillates as follows[1].

Data Analysis

Data obtained from laboratory experiment were analyzed using one way analysis of variance (ANOVA) utilizing SAS (SAS version 9.1).LSD (least significant difference) test were used to identify significant differences among treatment means. P values < 0.05 were considered significant in all cases.

RESULTS AND DISCUTION

Effects of acid pretreatment and inoculums concentration on ethanol production from different concentrations of substrates

In both acid pretreated and untreated substrates ethanol production was observed on the fourth day of fermentation (Table 2). Previous works of some researchers (e.g., Ruchi et al., 2011; Nyachaka et al., 2013) show that ethanol production commences on 4th day of fermentation of plant material under similar conditions set for this experiment. The amount of ethanol produced, however, is significantly higher from the substrates treated with acid than untreated substrates at all concentrations and fermentation duration (Table 2). Pretreatment processes of lignocellulose are necessary to break down lignin and increase accessibility of enzymes and microbes to carbohydrates [6, 11]. Esteghlalian et al.[8] also reported that dilute sulfuric pretreatment can result in high reaction rates and significantly improve cellulose hydrolysis. The amount of ethanol produced peaked on the 8th day of fermentation in both acid pretreated and untreated substrates, but found to decline thereafter (Table 2). This could be due to depletion of substrates by yeast to be converted to ethanol. In both acid treated and untreated substrates, ethanol production found to increase with increasing concentration of inoculums (Table 2), suggesting more enzyme from yeast facilitating conversion of more substrates into ethanol. In both acid pretreated and untreated substrates, the highest amount of ethanol production was observed when 50g of substrate was mixed with 1% inoculums (Table 2), suggesting this mix ration is found to be optimum compared to the rest of substrate-inoculum concentrations used in this experiment. Previously, Reddy (2006) reported that ethanol production increases with substrate concentration up to certain level and decreases thereafter. This finding is in agreement with those of Hoyer et al., (2009) and Grubb and Mawson[10]. According to these people, the decrease in ethanol production at high substrate concentration beyond the optimum might be attributed to various factors including increments of viscosity in fermentation medium, osmotic pressure, and production of other compounds such as glycerol or acetic acid that have inhibitory effect on yeast growth.

Table-2: Ethanol production from groundnut shell using yeast (mean ± SD, n=3)

Substrate yeast	treatment	Ethanol produced from groundnut shell (%)			
		4^{th} day	**8^{th} day**	**12^{th} day**	**16^{th} day**
A	Treated	2.05 ± 0.08^{Aa}	7.01 ± 0.08^{Ab}	3.85 ± 0.08^{Ac}	2.88 ± 0.1^{Ad}
	Untreated	1.93 ± 0.23^{Aa}	5.7 ± 0.25^{Bb}	3.03 ± 0.12^{Aa}	2.17 ± 0.25^{Ad}
B	treated	2.84 ± 0.08^{Aa}	7.09 ± 0.09^{Bb}	4.71 ± 0.16^{Bc}	3.34 ± 0.14^{Bd}
	untreated	2.37 ± 0.17^{Aa}	6.41 ± 0.02^{Cb}	3.73 ± 0.17^{Ac}	2.62 ± 0.04^{Ad}
C	treated	9.31 ± 0.10^{Ca}	17.04 ± 0.15^{Db}	15.94 ± 0.17^{Cc}	14.57 ± 0.25^{Cd}

	untreated	7.34 ± 0.27^{Da}	15.28 ± 0.13^{Eb}	12.38 ± 0.5^{Dc}	11.70 ± 0.3^{Dd}
D	treated	11.43 ± 0.05^{Ea}	19.50 ± 0.47^{Fb}	17.68 ± 0.36^{Ec}	16.70 ± 0.06^{Ed}
	untreated	8.53 ± 0.25^{Fa}	18.32 ± 0.31^{Gb}	15.14 ± 0.49^{Cc}	14.45 ± 0.36^{Cd}
E	treated	5.91 ± 0.11^{Ga}	14.93 ± 0.08^{Eb}	14.51 ± 0.13^{Cc}	12.65 ± 0.10^{Dd}
	untreated	4.83 ± 0.11^{Ga}	14.77 ± 0.11^{Eb}	12.73 ± 0.16^{Dc}	10.32 ± 0.30^{Dd}
F	Treated	6.82 ± 0.15^{Ga}	17.30 ± 0.28^{Gb}	16.07 ± 0.15^{Cc}	14.63 ± 0.23^{Cd}
	Untreated	5.82 ± 0.13^{Ga}	15.58 ± 0.29^{Eb}	14.11 ± 0.36^{CFc}	12.24 ± 0.22^{Dd}
G	Treated	3.76 ± 0.11^{Aa}	10.99 ± 0.06^{Hb}	9.77 ± 0.15^{Hc}	6.25 ± 0.18^{Hd}
	Untreated	2.95 ± 0.07^{Aa}	10.08 ± 0.21^{Hb}	7.45 ± 0.36^{Ic}	4.66 ± 0.1^{Id}
H	Treated	4.52 ± 0.27^{Ha}	12.29 ± 0.28^{Ib}	10.44 ± 0.69^{Hc}	7.38 ± 0.36^{Hd}
	Untreated	3.5 ± 0.08^{Aa}	11.54 ± 0.21^{Hb}	7.91 ± 0.66^{Ic}	5.6 ± 0.21^{Id}

A= 25g + 0.5% yeast, B=25g + 1% yeast, C= 50g + 0.5% yeast, D= 50g + 1% yeast, E= 75g + 0.5% yeast, F= 75g + 1% yeast, G= 100g + 0.5% yeast and H= 100g + 1% yeast

Effects of acid pretreatment and inoculums concentration on cell density different concentrations of substrates

Cell density was found significantly higher in acid pretreated substrates than untreated substrates at all concentrations and fermentation duration (Table 3). Pretreatment processes of lignocellulose are necessary to break down lignin and increase the availability of sugar for microbes to grow on convert it to ethanol[6,11]. This result goes in line with the observed ethanol production. Like that of ethanol production, cell density peaked on the 8th day of fermentation in both acid pretreated and untreated substrates, but found to decline thereafter (table 3). This may be due to high alcohol content and decrement in fermentable sugar. In both acid pretreated and untreated substrates, the highest amount of cell density was observed when 50g of substrate was used.

Table-1: Cell density (mg/ml) measured at 600nm after fermentation of groundnut shell. Values are mean±SD, n =3

Substrate+yeast	treatment	cell density observed from fermented groundnut shell at 600nm			
		4th day	8th day	12th day	16th day
A	treated	0.76 ± 0.01^{Aa}	1.69 ± 0.25^{Ab}	1.17 ± 0.17^{Ac}	0.52 ± 0.03^{Ad}
	untreated	0.64 ± 0.01^{Aa}	1.41 ± 0.23^{Bb}	1.09 ± 0.15^{Ac}	0.42 ± 0.03^{Ad}
B	treated	1.46 ± 0.1^{Ba}	2.28 ± 0.13^{Cb}	1.67 ± 0.11^{Bc}	1.36 ± 0.11^{Ba}
	untreated	1.16 ± 0.15^{Ca}	2.03 ± 0.06^{Db}	1.42 ± 0.14^{Cc}	1.19 ± 0.1^{Ca}
C	treated	0.78 ± 0.01^{Aa}	2.32 ± 0.2^{Cb}	1.85 ± 0.09^{Bc}	1.39 ± 0.16^{Bd}
	untreated	0.75 ± 0.01^{Aa}	1.95 ± 0.07^{Eb}	1.64 ± 0.11^{Dc}	1.17 ± 0.17^{Cd}
D	treated	1.78 ± 0.01^{Da}	2.65 ± 0.08^{Fb}	2.33 ± 0.17^{Ec}	1.60 ± 0.04^{Da}
	untreated	1.56 ± 0.02^{Ba}	2.37 ± 0.09^{Cb}	2.13 ± 0.11^{Ec}	1.4 ± 0.05^{Ba}
E	treated	0.77 ± 0.01^{Aa}	1.96 ± 0.06^{Eb}	1.72 ± 0.04^{BDc}	1.2 ± 0.16^{BCd}
	untreated	0.73 ± 0.02^{Aa}	1.79 ± 0.15^{Eb}	1.39 ± 0.11^{Cc}	1.03 ± 0.08^{Cd}
F	treated	1.6 ± 0.06^{Ba}	$2.49\pm0.04C^{Fb}$	2.15 ± 0.15^{Ec}	1.46 ± 0.07^{BDa}
	untreated	1.39 ± 0.04^{Ba}	2.15 ± 0.1^{Cb}	1.97 ± 0.01^{Bc}	1.31 ± 0.04^{Ba}
G	treated	0.76 ± 0.01^{Aa}	1.85 ± 0.1^{AEb}	1.68 ± 0.01^{BDb}	1.11 ± 0.1^{BCc}
	untreated	071 ± 0.01^{Aa}	1.63 ± 0.05^{Eb}	1.29 ± 0.12^{Cc}	1 ± 0.05^{BCd}
H	treated	1.56 ± 0.09^{Ba}	2.36 ± 0.11^{Cb}	2.07 ± 0.07^{Ec}	1.37 ± 0.1^{BCDd}
	untreated	1.27 ± 0.08^{Ba}	2.1 ± 0.05^{Cb}	1.84 ± 0.04^{BCDc}	1.22 ± 0.13^{BCd}

n = number of experimental replicates; SD = standard deviation; means with the same letter (lower case) in the same row are not significantly different; means with the same letter (upper case) in the same column are not significantly different. A= 25g + 0.5% yeast, B=25g + 1% yeast, C= 50g + 0.5% yeast, D= 50g + 1% yeast, E= 75g + 0.5% yeast, F= 75g + 1% yeast, G= 100g + 0.5% yeast and H= 100g + 1% yeast

Effects of acid pretreatment and inoculums concentration on reducing sugar concentration of the different substrate concentrations

Concentration of reducing sugar measured on the 4th day of fermentation was found to be significantly higher in both acid pretreated and untreated substrates, but found to decline with increasing days of fermentation (Table 4). The reduction in sugar concentration indicated that some sugar contained in groundnut shell has been consumed by yeast as a substrate to grow and converted into ethanol. Comparison of reducing sugar concentration between acid pretreated and untreated substrates showed that the amount of reducing sugar was significantly higher when substrates are pretreated with acid (Table 4), suggesting the facilitation of hydrolysis of lignocellulosic material by the acid for conversion into

sugar. Authors such as Demirbas[5] and Hendriks and Zeeman,[11] reported previously that acid pretreatment of lignocellulose is important to break down lignin and increase the availability of sugar for microbes to grow on convert it to ethanol. Rate of decrement of reducing sugar agrees with the amount of ethanol production, suggesting that the more is the reduction in reducing sugar means the more it is converted to ethanol by yeast. Increasing yeast concentration from 0.5 to 1% resulted in significant increase in ethanol concentration and decrease in sugar concentration at all-time.

Table-2: Reducing sugar concentration (mg/ml) measured at 540nm from fermented groundnut shell. Values are mean SD, n=3.

Substrate yeast	treatment	Reduced sugar concentration (g/ml))			
		4th	8th	12th	16th
A	Treated	12.64 ± 0.11^{Aa}	6.76 ± 0.18^{Ab}	3.78 ± 0.06^{Ac}	2.32 ± 0.22^{Ad}
	untreated	10.70 ± 0.30^{Ba}	3.02 ± 0.15^{Bb}	2.08 ± 0.97^{Bc}	1.81 ± 0.16^{Bd}
B	Treated	11.47 ± 0.45^{Ca}	5.60 ± 0.22^{Cb}	2.04 ± 0.06^{Bc}	1.04 ± 0.04^{Cd}
	untreated	9.61 ± 0.31^{Da}	2.77 ± 0.21^{Dd}	1.18 ± 0.25^{Cc}	0.94 ± 0.08^{Cd}
C	Treated	20.79 ± 0.38^{Ea}	11.41 ± 0.16^{Eb}	5.59 ± 0.18^{Dc}	4.72 ± 0.04^{Dd}
	untreated	19.07 ± 0.02^{Fa}	9.09 ± 0.1^{Fb}	5.15 ± 0.11^{Ec}	3.44 ± 0.22^{Ed}
D	Treated	19.46 ± 0.42^{Ga}	8.67 ± 0.07^{Gb}	3.56 ± 0.2^{Fc}	1.66 ± 0.11^{Fd}
	untreated	17.4 ± 0.11^{Ha}	6.8 ± 0.1^{Ab}	2.97 ± 0.21^{Gc}	1.27 ± 0.05^{Cd}
E	Treated	15.13 ± 0.11^{Ia}	9.14 ± 0.04^{Hb}	4.26 ± 0.1^{Hc}	3.41 ± 0.21^{Gd}
	untreated	12.12 ± 0.05^{Ja}	4.4 ± 0.2^{Ib}	3.3 ± 0.25^{Fc}	2.6 ± 0.11^{Ad}
F	Treated	13.23 ± 0.27^{Ka}	7.48 ± 0.1^{Jb}	2.83 ± 0.24^{Gc}	1.18 ± 0.06^{Cd}
	untreated	10.59 ± 0.29^{Ba}	4.08 ± 0.37^{KB}	2.22 ± 0.22^{Bc}	1.07 ± 0.09^{Cd}
G	Treated	13.02 ± 0.01^{Ka}	8.45 ± 0.02^{GB}	$4\pm0.02A^{Hc}$	2.95 ± 0.08^{Hd}
	untreated	11.83 ± 0.35^{Ja}	3.83 ± 0.09^{Ib}	2.6 ± 0.29^{Gc}	2.1 ± 0.19^{Ad}
H	Treated	12.19 ± 0.11^{Ja}	6.72 ± 0.13^{Ab}	2.37 ± 0.08^{BGc}	1.05 ± 0.03^{Cd}
	untreated	10.36 ± 0.14^{Ba}	3.15 ± 0.08^{Ba}	1.79 ± 0.11^{Ic}	1.01 ± 0.01^{Cd}

n = number of experimental replicates; SD = standard deviation; means with the same letter (lower case) in the same row are not significantly different; means with the same letter (upper case) in the same column are not significantly different.
A= 25g + 0.5% yeast, B=25g + 1% yeast, C= 50g + 0.5% yeast, D= 50g + 1% yeast, E= 75g + 0.5% yeast, F= 75g + 1% yeast, G= 100g + 0.5% yeast and H= 100g + 1% yeast

SUMMARY, CONCLUSION AND RECOMMENDATIONS

Summary

Biomass is seen as an alternative energy source for several reasons. The main reason is that bio-energy can contribute to sustainable development. Resources are often locally available and conversion into secondary energy carriers is feasible without high capital investments. The Current study was focused on bioethanol production from groundnut shell using effective fermenting microorganism, *Saccharomyces cerevisiae*. Groundnut shell was found to be an effective lignocellulosic material for bio ethanol production by *Saccharomyces cerevisiae*. Bio-ethanol tech-nology is a modern and eco-friendly technology based on the fermentation of organic materials in anaerobic condition at suitable temperature and pH by anaerobic yeast. Conversion of lingo-cellulosic biomass into fermentable sugars is the key step in lingo cellulosic - ethanol production. Pretreatment of substrates is very important in order to open up the structure of the biomass to release fermentable sugars on which yeasts act to produce bio-ethanol. Although recently ethanol has been produced mainly from crops, there is great interest in utilizing cheaper lignocellulosic materials as a feedstock for bio-ethanol production. Lignocellulosic material is known as an abundant, renewable and widely available resource. Agricultural wastes are considered as environmental polluters over the world. However, this study identified that some agro wastes are applicable for production of renewable fuel.

CONCLUSION

The finding of present study revealed that groundnut shell that collected from Babile district exhibited significant result for the production of bioethanol. Bio-ethanol production from these substrates was statistically significant at $p<0.05$. Amount of substrate that used for the production of ethanol were different. Among the different substrate concentration, 50 gram of treated substrate showed the highest % of ethanol production with high yeast concentration.

Therefore, substrate concentration and inoculums concentration are directly proportional until it reached the optimum level for ethanol production. Comparatively the reducing sugar utilization was more in pretreated substrates than untreated ones. Bio-ethanol production increased slightly when initial substrate concentration increased. But, it was decreased after 8th day of fermentation. This may be due to substrate limitation and decrease in cell biomass (yeast).

Recommendation

Based on the findings of the experiments conducted in the present study, the following points are recommended.

- The study revealed that it is possible to produce bio-ethanol from groundnut shell waste. Further studies should be conducted on pretreatment of other agro waste materials to release high fermented sugars to increase ethanol yield from agro wastes.
- Further work is again necessary to look at the effect of inhibitor on bio-ethanol production as a result of pretreatment
- Efforts should also be made to check the bio-ethanol quality of groundnut shell waste by Gas chromatography too.
- Further study is very important to describe how absolute bio-ethanol can be produced from groundnut wastes by using rotary evaporator, because it is difficult to make pure ethanol since there are other chemicals that can evaporate below the boiling point of ethanol (78^{o}C).
- Most of the solid wastes including groundnut shell waste in our country have no or very low conversion to different usable products and as such among the major problems of health. Hence, it recommended that government or other investor's to recover this very valuable product as well as to contribute to the country in reducing the highly rising quantity of wastes.

REFERENCES

1. Amadi, B. A., Agumuo, E. N., & Ibegbulem, C. O. (2004). Research Methods in Biochemistry Supreme Publisher. Owerri, Nigeria, 130-133.
2. Bennett, C. (1971). Spectrophotometric acid dichromate method for the determination of ethyl alcohol. The American journal of medical technology, 37(6), 217-220.
3. Bentley, R. W. (2002). Global oil & gas depletion: an overview. Energy policy, 30(3), 189-205.
4. Casey, G. (1980). Composition of Lignocellulosic materials: Cellulose, lignin and hemicelluloses in varying proportions depending up on the specific plant in which they occur.
5. Cheng, C., Hani, H. H., & Ismail, K. S. K. (2007). Production of bioethanol from oil palm empty fruit bunch. ICoSM2007, 69-72.
6. DEMİRBAŞ, A. (2005). Bioethanol from cellulosic materials: a renewable motor fuel from biomass. Energy sources, 27(4), 327-337.
7. Joshi, S. S., Dhopeshwarkar, R., Jadhav, U., Jadhav, R., D'souza, L., & Dixit, J. (2001). Continuous ethanol production by fermentation of waste banana peels using flocculating yeast.
8. Esteghlalian, A., Hashimoto, A. G., Fenske, J. J., & Penner, M. H. (1997). Modeling and optimization of the dilute-sulfuric-acid pretreatment of corn stover, poplar and switchgrass. Bioresource technology, 59(2-3), 129-136.
9. FAO (Food and Agricultural Organization of the Unites Nations) .2003. FAO state statistics data base, Agriculture, Rome, Italy.
10. Grubb, C. F., & Mawson, A. J. (1993). Effects of elevated solute concentrations on the fermentation of lactose by Kluyveromyces marxianus Y-113. Biotechnology Letters, 15(6), 621-626.
11. Hendriks, A. T. W. M., & Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource technology, 100(1), 10-18.
12. IPCC-WG, I. (2001). Climate Change 2000, Third Assessment Report.
13. Maloney, M. (1978). The lignocellulosic materials are the most abundant polymers on earth which are obtainable as the original biomass.
14. Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chemistry, 31(3), 426-428.
15. Gupta, N. (2009). Hydrolysis of lignocellulosic materials for ethanol production (Doctoral dissertation).
16. Onuki, S. (2006). Bioethanol: Industrial production process and recent studies. J. Chem. Technol. Biotechnol, 5, 1À12.
17. Summer, J.B. and Somers, G.F. 2004. Laboratory Experiments in Biological Chemistry. Academic Press, Inc., New York.
18. Wijnands, J. H. M., Biersteker, J., & Hiel, R. (2007). Oilseeds business opportunities in Ethiopia. Ministry of Agriculture, Nature and food quality.