WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 6, 733-753. Research Article ISSN 2277– 7105

MICROBIAL CONVERSION OF CELLULOSE INTO BIOETHANOL

[1]*S. Ashok Kumar, [2]J. Santhosh, [3]C. Venkatesh, [4]Dr. N.G. Ramesh Babu, [5]Dr. N. Saravanan and [6]S. Parvathy

[1,2,3]Biotechnology, Tamil Nadu -635109.

[4]Head of The Department, Adhiyamaan College, Tamil Nadu-635109.

[5]Asst.Professor, Biotechnology Adhiyamaan College, Hosur, Tamil Nadu -635109.

[6]Research Manager, R&D Div, Genewin Biotech, Hosur, Tamil Nadu -635109.

Article Received on
04 March 2019,

Revised on 24 March 2019,
Accepted on 15 April 2019,

DOI: 10.20959/wjpr20196-14773

***Corresponding Author**
S. Ashok Kumar
Biotechnology, Tamil Nadu -635109.

ABSTRACT

Ethanol is a bio degradable and renewable fuel which causes less pollution then other existing fuels. In this study the production of ethanol from Duck weed, Aquatic ferm, grass and Rotten wastes were carried out using three microbes such as *Bacillus thuringensis, Klebsiella pneumonia and Clostridium thermocellum* which are used for the degradation or convertion of cellulose to glucose and the fermentation was carried out using yeast(*Saccharomyces cerevisiae*) and the purification was done by activated carbon and the quantitative and qualitative analysis were done.

KEYWORDS: Ethanol, Duck weed, Aquatic ferm, Grass and Rotten wastes, microbes, Fermentation, purification, degradation, cellulose, glucose.

Aim of the present study

1. To convert the raw mnaterials into cellulose product(cellulose).
2. To convert the cellulose product into reducing sugar (glucose).
3. To convert the glucose into the bioethanol by fermentation.
4. To purify the ethanol using activated carbon.

1. INTRODUCTION

Bio-ethanol is a fuel used as a petrol substitute for road transport vehicles. Bio-ethanol is mainly produced by the sugar fermentation process. The main sources of sugar required to produce ethanol comes from cellulostic biomass. The municipal solid wastes are used to produce ethanol fuel, this is consider as the third generation method of producing bio-ethanol.

Ethanol or ethyl alcohol (C_2H_5OH) is a clear colourless liquid, it is biodegradable, low in toxicity and causes little environmental pollution if spilt. Ethanol burns to produce carbon dioxide and water. lead is replaced by ethanol as an octane enhancer in petrol. By blending ethanol with gasoline we can also oxygenate the fuel mixture so it burns more completely and reduces polluting emissions.

The most common blend is 10% ethanol and 90% petrol. Vehicle engines require no modifications to run on E10 and vehicle warranties are unaffected also. Only flexible fuel vehicles can run on up to 85% ethanol and 15% petrol blends.

1.1.History of ethanol production

Ethanol is an alcohol made from fermentation of sugars which is produced from agricultural food crops and biomass resources. Because of rapid depletion of the world reserves of petroleum, the production of ethanol have grown in recent years .It has emerged as one of the alternative liquid fuel and has generated immense activities of research in the production of ethanol and its environmental impact. Production of alcoholic beverages was used along the human civilization. The production of ethanol assumed to begins in the start of 12-14th century along with improvement of distillation process for purification. During the middle ages, alcohol was used mainly for production of medical drugs but also used for manufacture of painting pigments. The knowledge of using starchy materials for ethanol production was first used in the 12th century in beer production. It was only in the 19th century that the enormous production of ethanol was done due to the economic improvements of the distilling process.[1]

1.2.Microorganisms used as biocatalysts in cellulose biodegradation

Many microbes can produce extracellular enzymes (secondary metabolites) that can be used to break down polysaccharides such as celluloses and convert them in to polymeric compounds like sugars and there are several Gram-positive and Gram-negative bacterias, which have the enzymatic potential to cut down celluloses into other form.[2]

1.3.Effect s of microbial action

Literature evidence[2] says that a range of possible effects of microbial action such as,

- The creation of alteration in their metabolism such as redox reactions.
- The biodegradation of organic matters and
- The production of gases.

1.4. Bio-ethanol feedstock

The raw materials which are used in the production of ethanol from there cellulosic compounds are called as bio-ethanol feedstock. It can be divided into three major groups:

(1) First generation feedstock

First generation bio-ethanol feed stocks come from agricultural crops that are also sources of human and animal food. The bio-ethanol produced by fermentation of sugars such as sugarcane, sugar beet, sorghum, whey and molasses and starchy feedstock such as grains viz. maize, wheat, root crops are commonly known as first generation bio-ethanol.

(2) Second generation feed stocks

Exploitation of first generation feed stocks for bio-fuel production is ultimately unsustainable due to food security and land-use issues. Second-generation bio-ethanol refers to fuel alcohol produced from non-food biomass sources, such as lignocelluloses[3] the most abundant form of carbon on the earth.[4]

- Cellulose is an organic compound with the formula ($C_6H_{10}O_5$).
- A polysaccharide consisting of a linear chain of several hundred to many thousands of β linked D-glucose units.
- Cellulose is an important structural component of the primary cell wall of green plants, many forms of algae and the oomycetes
- Some species of bacteria secrete it to form bio-films. Cellulose is the most abundant organic polymer on Earth.
- The cellulose content of cotton fiber is 90%, that of wood is 40–50%, and that of dried hemp is approximately 57%.[3]

(3) Third generation feed stocks

Third-generation bio-fuels are produce from algal biomass and wastes, which has a very distinctive growth yields as compared with classical lignocelluloses biomass

1.5. What are the benefits of Bioethanol?

Bio-ethanol has many advantages over conventional fuels. It comes from a renewable resource that is from food wastes. Another benefit over fossil fuels is the greenhouse gas emissions is much less. The road transport network accounts for 22% of all greenhouse gas emissions and through the use of bio-ethanol these gases can be reduced.[5] By encouraging bio-ethanol's use, the economy would also receive a boost in its growth. Bio-ethanol is a substance which is biodegradable and less toxic when compared to fossil fuels. By using bio-

ethanol in older engines can help in reducing the amount of carbon monoxide released by the vehicle and thus reduce air pollution.[5] Another advantage of bio-ethanol is it can be used in the existing road transport fuel system. In quantities up to 5%, bio-ethanol can be blended with petroleum fuel without engine modifications. Bio-ethanol is produced using familiar methods, such as fermentation, and it can be distributed using old mediums.[1]

1.6. Ethanol production technologies

Ethanol can be produced by chemical synthesis or by biological method. Fermentation is a reaction induced by enzymes produced by living cells.[2] There are a number of advanced technologies of ethyl alcohol production in the world presently, depending on the raw material subjected to fermentation.[6] The raw materials containing simple sugars and suitable for direct processing through fermentation are white beet and its processing products, sugar cane, domestic and citrus fruits, some tropical plants (punk), juices of certain trees (birches, maple), honey. The group of raw materials containing starch and polysaccharides, such as cellulose and inulin used for the production of ethanol should comprise cereals in form of food grain of rye, barley, corn, oat, wheat, sorghum, besides also vegetable bulbs of potato vegetable roots, seeds of bifoliate plants, fruits, timber, grass, moss, etc. Using current production technology the cheapest bioethanol produced in world comes from sugarcane in Brazil and in Europe from starch crops.[7]

Presently the production of ethanol (for fuel) largely depends on waste materials[8] lignocellulosic biomass such as crop residues, wasted and energy crops (switchgrass), fast-growing trees such as poplar and willow, waste paper[9] and package material, cereals in form of grain unsuitable for consumption, domestic and agricultural waste[10] (maize and wheat stalks).[7-11] However ethanol production from lignocellulosic biomass[6] is not yet at commercial scale, even though many technologies are mooted. The total potential bio-ethanol production from crop residues and wasted crops is about 16 times higher than the current world ethanol production ($31 \cdot 10^9$ dm^3 in 2001).[12]

1.7. Reduction in greenhouse gas emissions

The estimation of greenhouse gas and energy balances of bio-ethanol is complex. For comparison with fossil fuels the full fuel cycle must be considered: production which required inputs and combustion which is considered to be CO_2-neutral. The final accounting is country-specific and is a function of raw material cultivated, the associated agricultural yield and utilisation of by- and co-products.

The Overview of CO_2 emissions (cultivation, production, distribution and vehicle emissions) saving from bio-ethanol compared with reference fossil fuel vehicle was estimated to be low than other fuels.[17][21]

2. MATERIALS AND METHODS

2.1. Collection of substrates

Different rotten fruits and vegetables were collected from the local market.

Garden weeds and duck weeds were collected from Genewin Biotech, R and D division, Hosur.

2.2. Collection of microbes

Bacillus thuringensis

Klebsiella pneumoniae

Clostridium thermocellum were collect from genewin biotech, Hosur

2.3. ENZYME PRODUCTION

The microbes which are transferred to the production media during its stationary phase will produce degradive enzymes after 24hrs form its incubation time. By the centrifuge process the enzyme was extracted.[13]

2.4. PRETREATMENT

2.4.1. Acid pretreatment

The diluted acid (HCL) is used for this process increases the glucose yield.[14]

Experiment methodology

Table 1: Pretreatment for selected substrates.

Substrate	Pretreatment	Time exposure
Vegetables	Acid – HCL	12 hrs
	Alkali – KOH	24 hrs
	Hot water	20 min
Aquatic fern	Acid – HCL	12 hrs
	Alkali – KOH	24 hrs
	Hot water	20 min
Garden grass	Acid – HCL	12 hrs
	Alkali – KOH	24 hrs
	Hot water	20 min
Duck weed	Acid – HCL	12 hrs
	Alkali – KOH	24 hrs
	Hot water	20 min

In 2% concentration the diluted acid was prepared. The diluted acid was sterilized at 121^{o}C for 30mins in autoclave. The sample and the diluted acid were mixed and incubated at 36^{o}C for 24 hours.[14]

2.4.2. Alkali peroxide pretreatment

Alkaline pretreatment is also one of the chemical pretreatment which is used beside acid pretreatment because of its efficiency.[14]

Experiment methodology

Alkaline peroxide pretreatment was performed. The collected substrates were suspended in 7.5% (v/v) H_2O_2 and NaOH was then added to adjust the pH to 11.5. At 35°C the pretreated samples were then incubated, shaken at 250 rpm for 24 hrs. Finally, before enzymatic hydrolysis , the pH of the solution was reduced to 4.8 by adding HCL. The filtration was not needed after the pretreatment. The liquid phase with solubilized hemicellulose and the solid phase with cellulose of the samples were back to back hydrolyzed by the enzymes.

2.4.3. Heat pre-treatment

The thermal-based pre-treatment process was developed primarily for use with the fermentation process and the smaller particle size of biomass materials than the particle size of cellulase enzymes will enhances interaction and hence enzymatic hydrolysis yields.[14]

Table 1: The substrates are pretreated using acid, alkali and hot water method.

2.5. DETERMINATION OF CELLULASE AND XYLANASE

2.5.1. Enzymatic assays

Cellulase activity was, ensured by determining the amount of reducing sugar by benedicts method, 1ml of sample was added to 2ml of benedicts solution and leave it for about 3 to 5mins to observe the color change. Increase in amount of reducing sugar was determined by Somogyi Nelson method.

Xylanase activity was, ensured by determining the amount of reducing sugar released from the spletxylan reaction mixture consisted of 1% xylan in 100mM TrisHCl buffer (pH7), and enzyme to give a final volume of 0.6 ml. Incubation at 50°C is done for 15 min. Increase in amount of reducing sugar was determined by Somogyi Nelson method

2.6. NELSON-SOMOGYI METHOD

Sugars with reducing property which is because of the presence of a aldehyde or keto groups in them. These are called as reducing sugars. Some of the example of reducing sugars are glucose, galactose, lactose and maltose. The Nelson Somogyi method is one of the classical and mostly used method for the quantitative analysis of reducing sugars.

2.6.1. Estimation of Reducing Sugars By Nelson-Somogyi Method

This method tests for the presence of free carbonyl group (C=O), the so called reducing sugars. This involves the oxidation of the aldehyde functional group present in, for example, glucose and the ketone functional group in fructose. Simultaneously, 3,5-dinitrosalicylic acid (DNS) is reduced to 3-amino,5-nitrosalicylic acid under alkaline conditions:

- oxidation

aldehyde group $\xrightarrow{yields}$ carboxyl group

- Reduction

3,5-dinitrosalicylicacid$\xrightarrow{yields}$ 3am**ino,5- nitrosalicylic** acid

Because dissolved oxygen can interfere with glucose oxidation, sulfite, which itself is not necessary for the color reaction, is added in the reagent to absorb the dissolved oxygen.

Experiment methodology

- 3 ml of DNS reagent to 3 ml of glucose samples was added in a capped test tube. (To avoid evaporation)
- The mixture was heated at 90º C for 5-15 minutes until it develops the red brown color.
- 1 ml of a 40% potassium sodium tartrate (Rochelle salt) solution was used to stabilize the color.

Using cold water bath the sample was cooled to room temperature, the absorbance at 575 nm was recorded.

2.7. FERMENTATION

Fermentation of ethanol, is a biological process in which sugars like glucose, fructose, and sucrose are converted into glucose and this can be converted in to ethanol and carbon dioxide as metabolic waste product.[6] Because of yeasts the conversion glucose into ethanol will happen in the absence of oxygen, which is considered as anaerobic process. During

fermentation process, an enzyme from yeast (zymase) changes the simple sugars into ethanol and carbon dioxide.[15]

2.7.1. Procedure

The batch fermentation was used, the EP medium containing 18 Bx was inoculated with respective microbes. The samples were collected from the batch at regular time intervals for analysis and the optimum inoculums was selected for the determination of reducing sugar contents.

2.8. Distillation

Distillation is a separation process for a mixture of liquids or oils. It relies on differences in the boiling points of the component liquids to be separated. The mixture to be separated is added to a Distilling Pot where it is heated to the boiling point. Lower boiling components will preferentially vaporize first. This vapor passes into a Distilling Head and then into a Condensor. Within the Condensor the vapor is cooled and it liquifies. The resulting liquid is then collected in a Receiving Flask. Initially, low boiling components are collected in the Receiving Flask. As the distillation proceeds, these components are depleted from the Distilling Pot and higher boiling components begin to distil over. Switching out the Receiving Flask at the appropriate point allows for the separation of the component liquids of the mixture. Ethyl Alcohol has a boiling point of 78.5^{O}C and that of Water is 100^{O}C. Initially the distillation will be relatively efficient in enriching the Alcohol.

3. RESULT

The cellulose from varies source(weeds) were degraded using microbes and the ethanol is produced by fermentation of glucose which is produced in the cellulose degrading process.[19] The ethanol production of varies source is analysed for its quantity and quality.

Determination the amount of glucose by Benedicts method

Fig 1:The change of color from blue to yellow will confirm the presents of glucose in the solution and it indicates that it has 2% glucose in it.

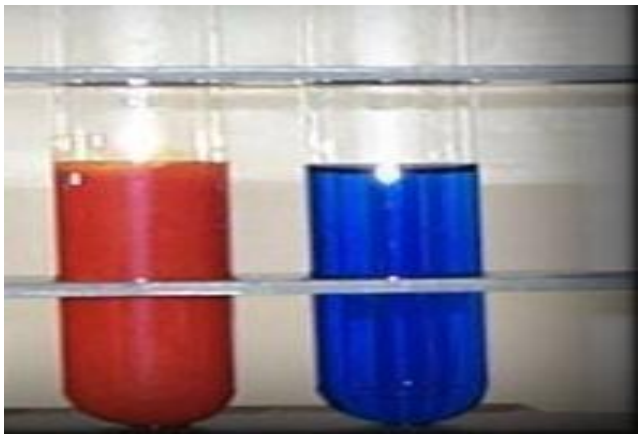

Fig 2: The change of color from blue to brown will confirm the presents of glucose in the solution and it indicate that the solution has more than 4%.

3.1. QUANTIFICATION ANALYSIS

3.1.1. Reducing Sugars (Vegetables As Substrate)

A reducing sugar is any sugar that either has an aldehyde group. The aldehyde functional group allows the sugar to act as a reducing agent. It is measured by the DNS method.

Table 2: Standard curve of Glucose.

Concentration (ppm)	Optical Density
0	0
0.5	0.0206
1.0	0.0511
1.5	0.0520
2.0	0.0948
2.5	1.0141

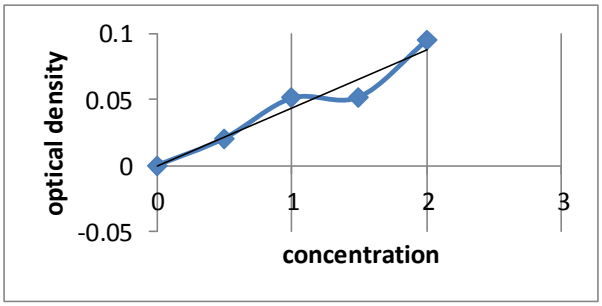

Fig 3: Concentration vs optical density.

Fig 4: The result of Benedict test.

Titration result

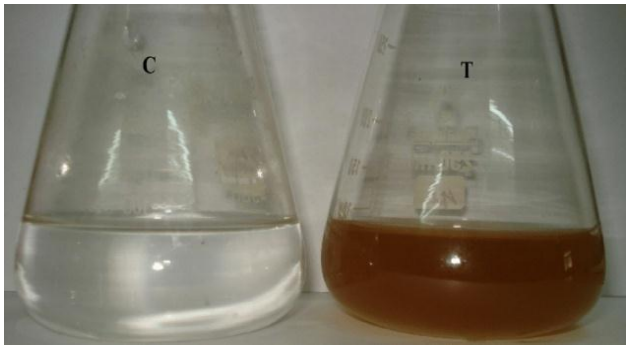

Fig 5:T indicates the iodine solution need to be titrated.

Fig 6: The iodine solution after titration withsodium thiosulfate.

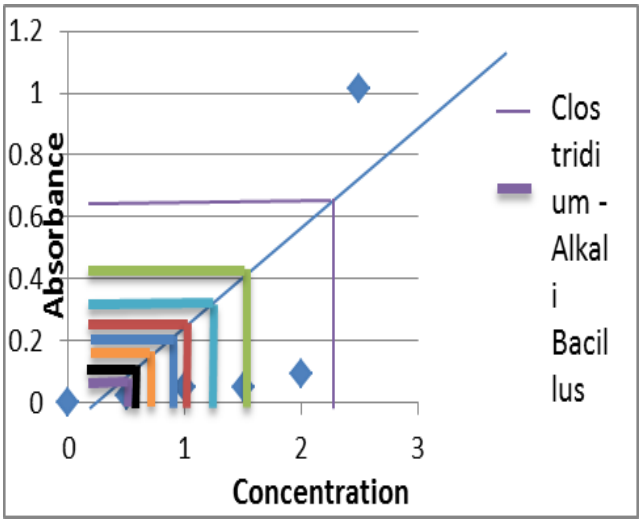

Fig 7: Determination of reducing sugars of rotten wastes.

Table 3: Estimation of reducing sugar.

Microbes	Amount of reducing sugar (mg/ml)
Clostridium – Acid	1.1
Clostridium – Alkali	1.7
Clostridium – hot water	0.61
Bacillus thuringeinsis – Acid	0.8
Bacillus thuringeinsis – Alkali	0.45
Bacillus thuringeinsis – hot water	0.3
Klebsiella – Acid	0.65
Klebsiella – Alkali	0.78
Klebsiella – hot water	0.35

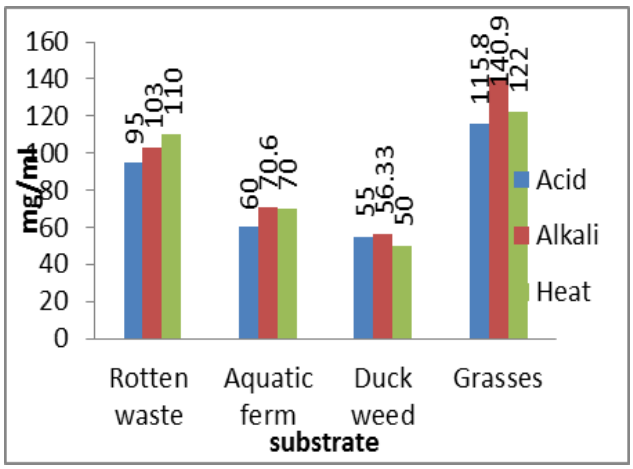

Fig 8: The production of glucose by clostridium from different pre-treatment methods.

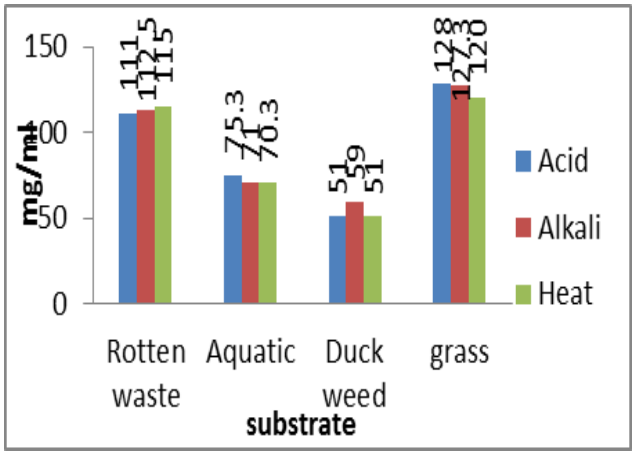

Fig9: The production of glucose by Bacillus thuringensis from different pre-treatment methods .In this the more amount of glucose is produced by grass in acid treatment.

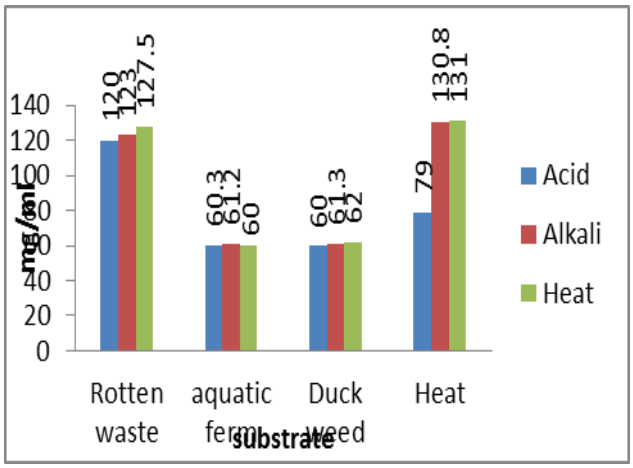

Fig 10: The production of glucose by Klebsiella pneumoniae from different pre-treatment methods In this the more amount of glucose is produced by grass in heat treatment.

3.1.2. AQUATIC FERN

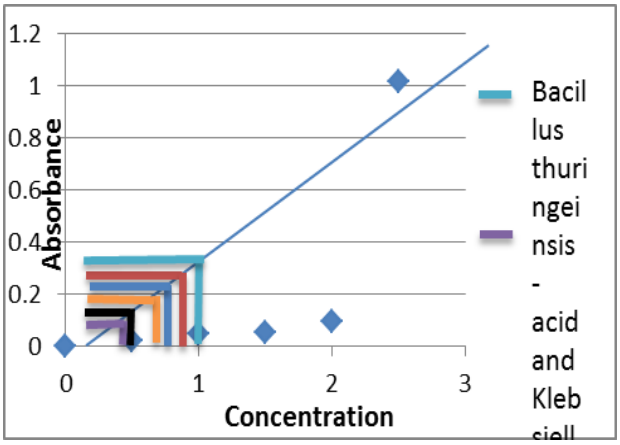

Fig 11: Determination of reducing sugars.

Table 4: Estimation of reducing sugar.

Microbes	Amount of reducing sugar (mg/ml)
Clostridium – Acid	0.5
Clostridium – Alkali	0.7
Clostridium – hot water	0.3
Bacillus thuringeinsis – Acid	0.8
Bacillus thuringeinsis – Alkali	0.5
Bacillus thuringeinsis – hot water	0.3
Klebsiella – Acid	0.6
Klebsiella – Alkali	0.78
Klebsiella – hot water	0.35

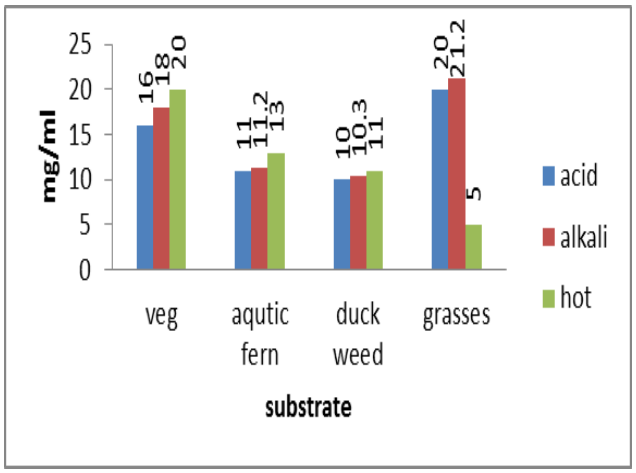

Fig 12: Production of glucose by xylanase enzyme from clostridium In this the more amount of glucose is produced by grass in alkali treatment.

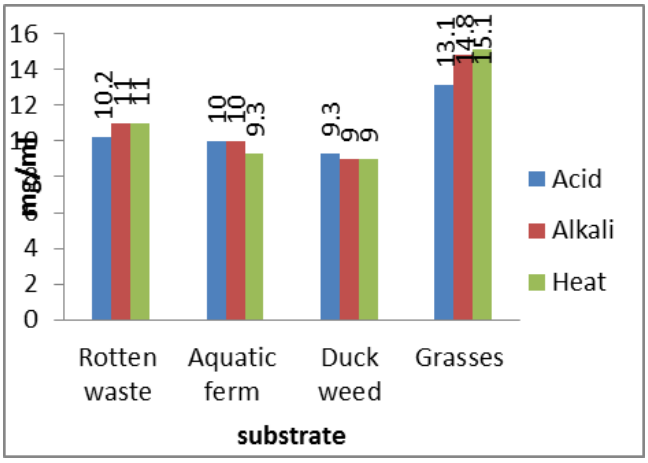

Fig 13: Production of glucose by xylanase enzyme from Bacillus thuringensis In this the more amount of glucose is produced by grass in heat treatment.

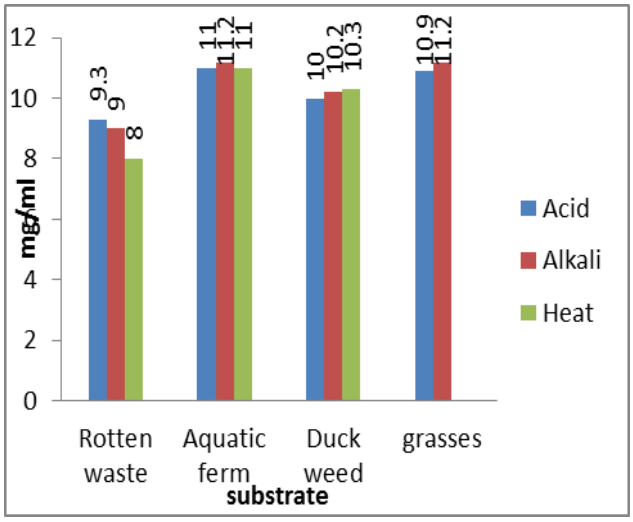

Fig 14: Production of glucose by xylanase enzyme from Klebsiella pneumonia In this the more amount of glucose is produced by aquatic ferm in acid treatment.

3.1.3. DUCK WEED

Table 5: Estimation of reducing sugar.

Microbes	Amount of reducing sugar (mg/ml)
Clostridium – Acid	0.5
Clostridium – Alkali	0.9
Clostridium – hot water	0.3
Bacillus thuringeinsis – Acid	0.8
Bacillus thuringeinsis – Alk	0.3
Bacillus thuringeinsis – hot water	0.3
Klebsiella – Acid	0.6
Klebsiella – Alkali	0.8
Klebsiella – hot water	0.4

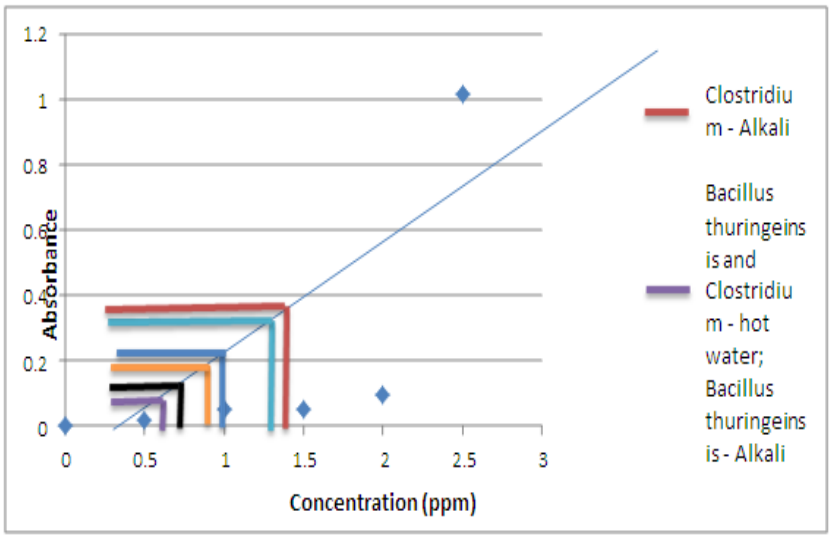

Fig 15: Determination of reducing sugars.

3.1.4. GARDEN GRASSES

Table 6: Estimation of reducing sugar.

Microbes	Amount of reducing sugar (mg/ml)
Clostridium – Acid	1.3
Clostridium – Alkali	2.1
Clostridium – hot water	0.9
Bacillus thuringeinsis – Acid	0.8
Bacillus thuringeinsis – Alkali	1.0
Bacillus thuringeinsis – hot water	0.5
Klebsiella – Acid	0.8
Klebsiella – Alkali	0.9
Klebsiella – hot water	0.5

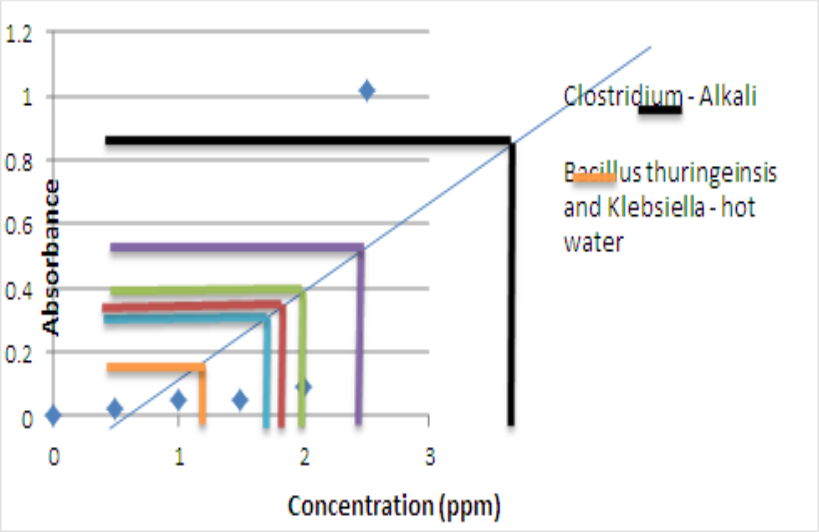

Fig 16: Determination of reducing sugar.

Fig 17: Media for fermentation.

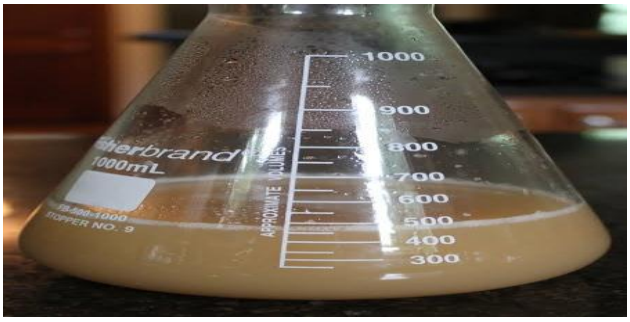

Fig 18: yeast added medium.

Fig 19: 1 week old fermentation.

3.2. Result of ethanol production

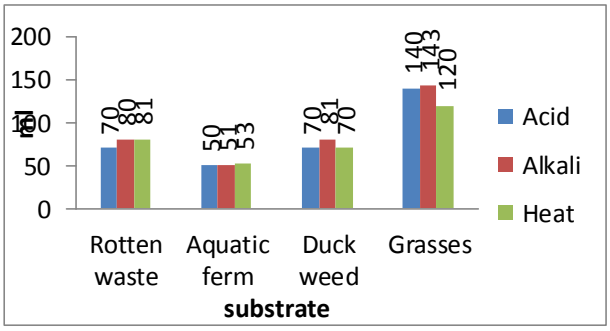

Fig 20: Bio ethanol by Bacillus thuringensis. The more amount of bio ethanol was produced by grass which undergone alkali pretreatment.

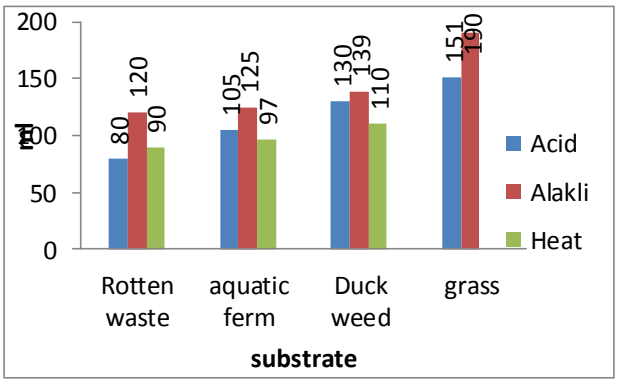

Fig 21: Bioethanol by *clostridium thermocellum*. The more amount of bio ethanol was produced by grass which undergone alkali pretreatment.

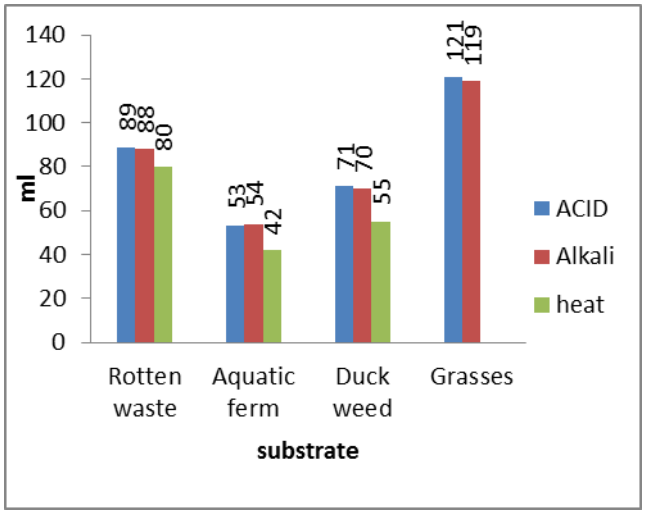

Fig 22: Bioethanol by from Klebsiella pneumonia The more amount of bio ethanol was produced by grass which undergone acid pretreatment.

3.3. PURIFICATION RESULT

Fig 23: The purification of bio-ethanol using activated Carbon. The fig in the left is before purification and fig in the right is after purification.[6] The specific gravity of the ethanol changes from 0.85 to 0.81 ,which shows Removal of impurities from it.

Fig 24: The purified Bioethanol by distillation Process is shown in the figure.

3.3.1. Ceric ammonium nitrate test

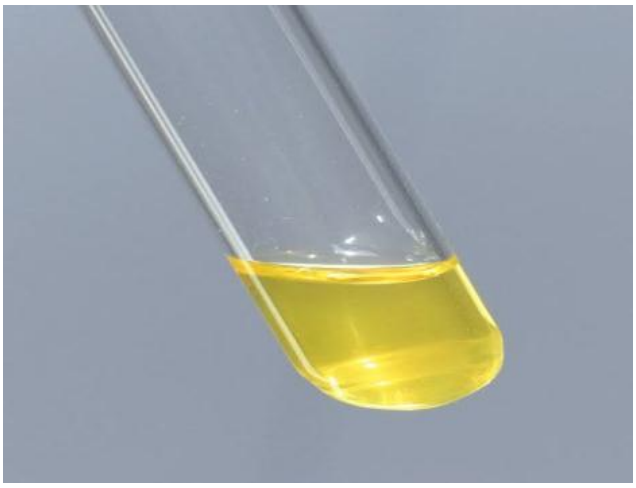

Fig 25: The Ceric ammonium nitrate solution Before adding ethanol

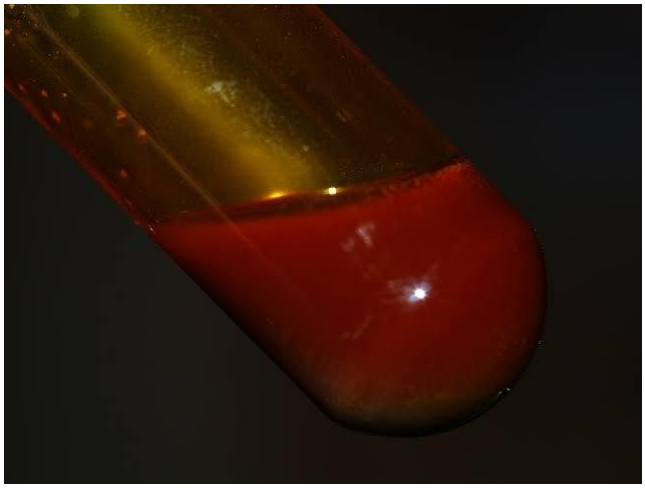

Fig 26: The ceric ammonium nitrate solution after adding ethanol .It confirm the presents of ethanol in the solution.

3.3.2. Specific gravity test

Fig 27: The specific gravity of the ethanol is observed by hydrometer and it is noted to be 0.85 at 25°C.

3.3.3. Burning test

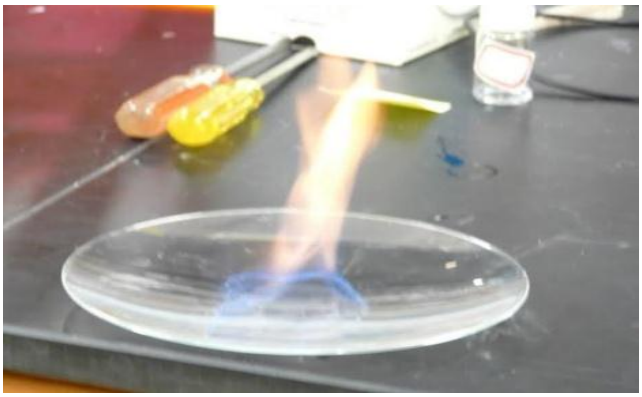

Fig 28: The blue flame without smoke was observed.

4. DISCUSSION

- Ethanol is very important because of its use as a biofuel.
- It is an renewable energy which can be produced from the waste materials. which can reduce the amount of waste produced by humans.
- It reduces pollution.
- It can be use in normal petrol engine.
- The production of bio ethanol was high in grass and in other the production was comparatively same or less.

5. CONCLUSION

The current ethanol production, purification, and analysis techniques have been reviewed. Ethanol is produced from various kinds of substrates. The substrates used for ethanol production vary by different countries due to their different farming conditions. From the environmental stand point, utilization of lignocellulosic biomass for ethanol production is being studied more intentionally. Ethanol is purified almost only by distillation in the industry. Although distillation is one of the most effective liquid-liquid separation techniques, it contains some critical disadvantage, cost and limitation on separation of volatile organic compounds. Many purification techniques of water and wastewater are expected to be applied to ethanol purification as well. Ozonation could degrade impurities. Activated carbon could remove impurities without adsorbing ethanol, and gas stripping could simply remove high volatile compounds without any heating.

6. REFERENCES

1. WALKER, G.M. 125th Anniversary Review: Fuel alcohol: Current production and future challenges. Journal of the Institute of Brewing, 2011; 117(1): 3-22.
2. African Journal of Plant Science a `Studies on cellulose degrading bacteria in tea garden soils'was done by A. Balamurugan*, R. Jayanthi, P. Nepolean, R. Vidhya Pallavi and R. Premkumar in from Plant Pathology Division, UPASI Tea Research Institute, Valparai, Coimbatore, 2011; 642: 127. Tamil Nadu, India.
3. Bioethanol from Lignocellulosic Biomass: Current Findings Determine Research Priorities done by Qian Kang, Lise Appels, Tianwei Tan and Raf Dewil from Lab of Bioprocess, College of Life Science and Technology, Beijing University of Chemical Technolog.
4. Pelagia Research Library Advances in Applied Science Research, Bioethanol production from weed plant (Cyperus rotundus) by enzymatic hydrolysis was done by Nitesh Kumar, Jai Prakash Singh, Ravi Ranjan, Subathra Devi C. and Mohana Srinivasan V. from School of Bio Sciences and Technology, VIT University. 2013.
5. ZAH, R.; BÖNI, H.; GAUCH, M.; HISCHIER, R.; LEHMANN, M. and WÄGER, P. Life Cycle Assessment of Energy Products: Environmental Impacts Assessments of Biofuels. Executive Summary Report, EmpaTechnology and Society Lab, Switzerland, 2007; 20.
6. WU, Z. and LEE, Y.Y. Nonisothermal simultaneous saccharification and fermentation for direct conversion of lignocellulosic biomass to ethanol. Applied Biochemistry and Biotechnology, 1998; 70-72(1): 479-492.
7. Kim S. and Dale B.E.: Global potential bioethanol production from wasted crops and crop residues. Biomass and Bioenergy, 2004; 26: 361-375.
8. Murphy J.D. and McCarthy K.: Ethanol production from energy crops and wastes for use as a transport fuel in Ireland. Appl. Energy, 2005; 82: 148-166.
9. Demirbas A.: Bioethanol from cellulosic materials: A renewable motor fuel from biomass. Energy Sources, 2005; 27(4): 327-337.
10. Paszner L.: Bioethanol - Fuel of the future. Pulp and Paper Canada, 2006; 107(4): 26-29.
11. Azizov A. Sh.: The obtaining of fuel bioethanol. Appl. Solar Energy, 2006; 42(3): 55-56.
12. Ryan L., Convery F. and Ferreira S.: Stimulating the use of biofuels in the European Union: Implications for climate change policy. Energy Policy, 2006; 34: 3184-3194.

13. Bioethanol Production from Waste Office Paper in International Scientific Journal Journal of Environmental Science by R. Maceiras, V. Alfonsín Centro Universitario de la Defensa, Escuela Naval Militar, Plaza de España, 2: 36920. Marín, Spain.
14. WANG, L.; TEMPLER, R. and MURPHY, R.J. A Life Cycle Assessment (LCA) comparison of three management options for waste papers: Bioethanol production, recycling and incineration with energy recovery. Bioresource Technology, 2012; 120: 89-98.
15. WILKIE, A.C.; RIEDESEL, K.J. and OWENS, J.M. Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. Biomass and Bioenergy, 2000; 19(2): 63-102.
16. Berg C.: Worldethanol production 2001. The distillery and Bioethanol Network. Available at http://www.distill.com/world_ethanol_production.htm
17. ZHAO, X.; CHENG, K. and LIU, D. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. Applied Microbiology and Biotechnology, 2009; 82(5): 815-827.
18. Alice Jeng from University of Oklahoma Chemical Engineering, Brazil, had done a research about “PRETREATMENT METHODS FOR BIOETHANOL PRODUCTION” in his paper which was published in International Research Journal of Environment Sciences, 2012.
19. ZHI-FU, G.; CHAN, A.W. and MINNS, D.E. Life cycle assessment of bio-ethanol derived from cellulose. The International Journal Life Cycle Assessment, 2003; 8(3): 137-141.