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International Journal of Innovative Research in Technology, Science & Engineering (IJIRTSE)
ISSN: 2395-5619, Volume – 2, Issue – 6. June 2016

PRODUCTION OF BIO-ETHANOL FROM SUGARCANE JUICE USING SACCHAROMYCES CEREVISIAE

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ABSTRACT

In developing countries like India, we are in need to find an alternative and Environmental Effective fuel. The factors such as population explosion, growth of industries, higher number of automobiles has resulted in energy crisi. This has increased dependence on conventional fuel such as gasoline, which in turn causes more pollution. The knowledge on effects of global warming, Green House Effect, Environmental Degradation, Ozone depletion has pushed the people to move towards an alternative and a clean fuel. The ethanol produced from agricultural stocks satisfies the above condition and hence will be a effective replacement. Fermentation of glucose by yeast results in ethanol production. *Saccharomyces Cerevisiae (Yeast)* is the cheapest strain available for the conversion of Bio-mass substrate. In the present study, it is used for Bio-ethanol production from Sugarcane Juice. In this study, Fermentation of Sugarcane juice is done to produce a mixture, from which Ethanol is effectively obtained through distillation. Comparative study between two samples, that is heated sugarcane juice and normal one is done. The effect of heat on the fermentation sample is studied and percentage of ethanol produced from each sample is compared. Obtained Ethanol is mixed with gasoline and can be used as fuel. The effective Gasoline-ethanol ratio is obtained by trail and error method and is tested for working.

INTRODUCTION

Nowadays, the petroleum products are running out of race due to unbalanced relation between supply and demand besides air pollution of sources. The hike in petrol cost is mainly due to shortage of resources which leads to search for alternate fuel to replace fossil fuels. An eco-friendly bio-ethanol is one such alternate fuel that can be used in unmodified petrol engines mixing with gasoline. Combustion of ethanol results in relatively low emission of volatile organic compounds, carbon monoxide and nitrogen oxides. The emission and toxicity of ethanol are lower than those of fossil fuels such as petroleum, diesel etc.

The problem such as increase in population, energy crisis has increased the need of an alternate fuel. The developing countries face the problem of energy crisis which could be satisfied by bio-fuel such as ethanol. By the birth of 21th century the thought of protecting the environment and promotion of green environment has grown among the citizen of the world. The phenomenon such as greenhouse effect, global warming, ozone layer depletion has made the people to take more interest on pollution free environment. The need of controlling pollution has been ensured by different countries of the world.

Several earth summits and environment conferences have been conducted by United Nations in order to control the pollution emission by various countries. On such was conducted in Copenhagen, Brazil where several resolutions regarding toxic gases has been made.

Sustainable development is a development attained by satisfying the needs of present generation without affecting the need of future generation. Hence in order to attain this we have to find an alternate fuel replacing the conventional petrol and kerosene. Bio-ethanol is one such alternate fuel. **ETHANOL:**

Ethanol is a clear, colourless liquid with a characteristic agreeable odour. In dilute aqueous solution, it has a sweet flavour but is more concentrated solutions it has a burning taste. Ethanol (C_2H_5OH) is an alcohol, group of chemical compounds whose molecules contain a hydroxyl group (-OH) bonded to a carbon atom.

The word alcohol derives from Arabic alcohol, which devotes a fine powder of antimony produced by distilling antimony and used as an eye make up. Alcohol originally referred to any fine powder, but medieval alchemists later applied the form to the refined products of distillation and this led to the current usage.

Ethanol has been made since ancient times by the fermentation of sugars. All beverage ethanol and more than half of industrial ethanol is still made by this process. Simple sugars are the raw material. Zymase, anzymase from yeast changes the simple sugars into ethanol and carbon dioxide. The fermentation reaction represented by the simple equation.

$$\mathbf{C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2}$$

Some countries are either producing or using ethanol in large quantities or are providing incentives to expand ethanol production and use Brazil and Sweden are using large quantities of ethanol as a fuel. Some Canadians provinces promote ethanol use as a fuel by offering subsidies of up to 45 cents per gallon of ethanol.

Ethanol is the common solvent, produced by fermentation technology. Yeast cultures are carrying out ethanol fermentation on sugars in the absence of oxygen. So we can observe the growth of yeast in different parameters such as different carbon sources, various pH and different temperatures. Best culture was identified and isolated. This yeast strain was inoculated into different juices from sugar beet, sugar cane and potato. Yeast extract sucrose broth were prepared sterilized and then yeast inoculated into this broth. This broth was incubated at 25^0 for 3 days. Optical density was noted at 560 nm.

Ethanol is the colourless liquid at room temperature. It has slight fruity odour. Ethanol completely miscible in water. It's density less than water. It is neutral, it shows negative result for acid test. It is very flammable burns with pale blue flame. It has the melting point at (-114.1^0C). It is boiling point 78.5^0C. Density 0.789 g/ml at 20^0C.

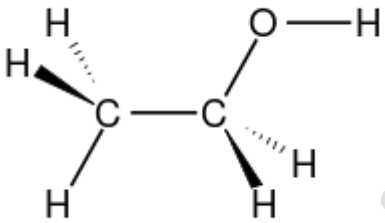
Ethanol fuel is ethanol (ethyl alcohol), the same type of alcohol found in alcoholic beverages. It is most often used as a motor fuel, mainly as a biofuel additive for gasoline. World ethanol production for transport fuel tripled between 2000 and 2007 from 17 billion to more than 52 billion litres. From 2007 to 2008, the share of ethanol in global gasoline type fuel use increased from 3.7% to 5.4%. In 2011 worldwide ethanol fuel production reached 22.36 billion U.S. liquid gallons (bg) (84.6 billion liters), with the United States as the top producer with 13.9 bg (52.6 billion liters), accounting for 62.2% of global production, followed by Brazil with 5.6 bg (21.1 billion liters). Ethanol fuel has a "gasoline gallon equivalency" (GGE) value of 1.5 US gallons (5.7 L), which means 1.5 gallons of ethanol produces the energy of one gallon of gasoline.

Ethanol fuel is widely used in Brazil and in the United States, and together both countries were responsible for 87.1% of the world's ethanol fuel production in 2011. Most cars on the road today in the U.S. can run on blendsof up to 10% ethanol, and ethanol represented 10% of the U.S. gasoline fuel supply derived from domestic sources in 2011. Since 1976 the Brazilian government has made it mandatory to blend ethanol with gasoline, and since 2007 the legal blend is around 25% ethanol and 75% gasoline (E25). By December 2011 Brazil had a fleet of 14.8 million flex-fuel automobiles and light trucks and 1.5 million flex-fuel motorcycles that regularly use neat ethanol fuel (known as E100).

Bioethanol is a form of quasi-renewable energy that can be produced from agricultural feedstocks. It can be made from very common crops such as sugar cane, potato, cassava and corn. There has been considerable debate about how useful bioethanol is in replacing gasoline. Concerns about its production and use relate to increased food prices due to the large amount of arable land required for crops as well as the energy and pollution balance of the whole cycle of ethanol production, especially from corn. Recent developments with cellulosic ethanol production and commercialization may allay some of these concern.

CHEMICAL NATURE OF ETHANOL:

The chemical formula of ethanol is C_2H_5OH. It is formed when the glucose is broken down by the action of yeast. It is the simple carboxylic compound with two carbon molecules next to methanol.



$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

USES OF ETHANOL:

- Blended with gasoline, Ethanol can be used as the fuels in vehicles.
- Same petrol engine can be used for the ethanol.
- Alternative for kerosene in household activities.
- Used in spirit lamps.
- It acts as the antifreeze agent.

ETHANOL IN INDIA:

- **Fast growing economy:**

India is the Second largest super power in Asia. It is one of the fast growing economy in the world. The high population is the major threat to the India's economy. This could be satisfied by finding an alternate fuel. Ethanol is an effective alternate fuel. This could boost the economy and would never deter the rate of growth. The effective using of eco-friendly alternate fuel such as ethanol would help india to attain the super power status within a decade.

- **Agricultural country:**

India is blessed with perennial rivers and fertile northern plains. The fertility of soil, monsoon distribution and suitable temperature makes it a best agricultural country. The green revolution took place in 1970's as made us self-sufficient. India could now meet its own needs and even export agricultural materials to other countries. This quality of India, could be made useful in mass production of sugarcane.

- **More energy dependent:**

In the recent decades, India had developed in all the three sectors of the economy. At the same time, the population of the country has also increased at almost equal rate. This has increased India's dependent on energy a lot. Thus, the need for alternate fuel as also grown up.

- **Sugarcane – a perennial crop:**

Sugarcane can be cultivated throughout the year in all seasons. It can be cultivated in all parts of India.The humidity, temperature conditions, soil type, availability of water has made India the best country for cultivating sugarcane.

- **Ethanol blending program:**
  i) 2001 : Government permitted adding ethanol in petrol. Pilot project in Uttar Pradesh.
  ii) 2006 : 5% ethanol blended petrol (EBP) began in most states, except JK and North East.
  iii) 2008 : National biofuel policy. Now oil companies required to blend atleast 5% ethanol with petrol. But project mostly #EPICFAIL. Most companies not blending more than 2% ethanol, because ethanol not easily available reasonable price.
- **SumitraChoudhary Committee:**

Government of India in 2012 has appointed a committee under SumitraChoudhary for meeting the energy crisis. The recommendations of the committee are as follows.

i) India need to find the alternate fuel by 2015.
ii) The alternate fuel must be eco-friendly and must release pollutants than the conventional gasoline.
iii) Ethanol may be the effective alternate fuel, as it burns cleanly and releases very less greenhouse gases.
iv) 20% of ethanol blending should be done by the year 2017.

Modi Sarkar, since assuming office barely a week, has announced a serious of initiatives from its election manifesto. After a decade of lethargy, it is a pleasant change to see the new Indian government working at a brisk pace. While the sense of purpose is appreciated, the proposal by the Minister for Transport, Highways, and Shipping, NitinGadkari, to introduce ethanol into India's petrol is worrisome.

Ethanol is an alcohol fuel that is added to petrol in some countries to lower cost to the consumer, reduce consumption of oil, lessen the environmental impact of transportation. Mixed fuels are rated by the amount of ethanol contained; for example, common blends are E10, which contains 10% ethanol, and E15, which contains 15% alcohol. Another common blend is E85, also known as flex fuel, in which the ethanol ranges from 51% in winters to 83% in summers.

Ethanol blends do not affect performance of the vehicle in terms of torque or horsepower but they do lower the mileage of the vehicle up to 25%. This is because ethanol has lower energy content than petrol; a higher blend therefore means lower mileage. It is possible for ethanol blends to be cheaper per litre but costlier per kilometre.

Maize is the most common source of ethanol in the United States and sugarcane in Brazil, who, between them, are responsible for almost 90% of the world's production of ethanol. Other sources such as agricultural feedstock, sugar beet, switch grass, potatoes, cassava, barley, and sunflower are also available but require more processing. The energy balance - the difference between the energy ethanol gives and the energy required to produce ethanol—for maize ethanol is marginally positive but the data is controversial. Hence ethanol from sugarcane will be an effective one.

In addition, though ethanol reduces air particulate pollution, its production results in fairly high greenhouse gas emissions and negates any the environmental benefit of the shift from fossil fuel. Although the figures are still being debated, several studies have indicated that ethanol might release more GHG over its total lifecycle than petrol by two and a half times.

The biggest concern with the addition of ethanol to India's fuel supply is that it will take up scarce land set aside for grazing and other crops to grow fuel. With the demand likely to be huge, there is bound to be a shift in land use from food crops to fuel crops. Agriculture will become even more intensive. Without adequate reforms and education in the agricultural sector to increase productivity, the shift to fuel crops will result in food price inflation. The savings from reduced oil imports will be spent instead on subsidies and and higher food prices.

Despite the questionable benefits of ethanol-blended petrol, both the United States and Brazil support their ethanol habit with enormous government subsidies to the sector. NarendraModi's idea of smart cities and bullet train could be achieved in near future with the help of alternate fuel such as ethanol.

LITERATURE REVIEW

1. *Mancilha et al., 1984; Converti et al., 1985; Gregory et al., 1984; Shigum et al., 1987; Torres et al., 1986 and Tobias et al., 1983.*Improvements in ethanol fermentation have been focused on taking up of renewed interest in research works in several areas such as use improved mutant strains yeast development from cheaper sources, use of cheaper source raw materials, optimum reactor design, better nutrients for optimum cell growth etc.

2. *S.Cerevisiae Cen PK*: Growth on glucose was not significantly affected in pho 13 Delta mutants. The rates of specific Xylose uptake were approximately. 5 times higher in the deletion strain than in the parental strain when growing on glucose-Xylose mixtures and up to 10 fold higher when growing on xylose along. (Van vleet JH *et al*., 2007)

3. *Atiyeh H, Duvnjak Z., (2001)* produce the ethanol and enriched fructose syrups from a synthetic medium with various sucrose concentrations using the mutant saccharomyces cerevisiae ATCC 36858 was investigated Ethanol yields were in the range of 72 to 85% of theoretical value when sucrose concentrations were above 81 g/l. The volumetric ethanol productivity was 2.23g ethanol per 1 hour in a medium containing 216 g/l sucrose.

4. *Kasemts K et al., 2007:*Obtained steady state values of the glycerol to ethanol formation ratio (0.1 molmol(-1) corresponding to those predicated from the stoichiometric model of fermentative yeast growth showed that the complete repression of respiration was obtained in auxostat culture and that the model is suitable for calculation of Y(ATP) and Q(ATP) values for the aerobic fermentative growth. Increase in tryptone concentration resulted in an increase in the specific growth rate from 0.44 to 0.62 h (-1) and Y (ATP) from 12.5 to 18.5 mol ATP g dwt (-1).

5. *Ishtar snoek Is Y de SteeH (2007)* says that yeast is not able to synthensize sterols and unsaturated fatty acids. The composition of the cell wall and cell membrane show major difference when aerobic and anaerobic cells are compared many more factors play a role anaerobic condition is suitable for alcohol production.

6. *Kong QX et al., (2006):*To improve ethanol production in saccharomyces cerevisiae two yeast strains were constructed in the mutant KAM.3 the FPSI gene which encodes a channel protein responsible for glycerol export was deleted. The mutant KAM.11 has the GLTI Gene (encoding glutamate synthase) placed under the PGK1 while having the FPS 1 deletion. Growth rate and biomass concentration remained virtually unchanged with the mutant KAM-11, compared to that of the parent. Over expression of GLT1 by the PGK 1 promoter along with FPS1 deletion resulted in a 14% higher ethanol.

7. *Cot M. et al., 2007*: Production phase uncoupled to growth, the extent of which was critical for high level ethanol production as the level of production was found to be highly variable, on this high variability by means of a detailed physiological analysis of yeast cells in two fed-batch fermentations showing the most extreme behaviour.

8. *Da Silva Filho.E.A., et al.,:*The population stability depends on the use of well-adapted strain that can fir to a particular industrial plant. This stability helps to keep high level ethanol yield and it is absolutely required when intending to use recombinant strains. Yeast strains have been previously isolated from different distilleries in Northeast Brazil and clustered in genetic strains by PCR-fingerprinting. Selection of a novel Saccharomyces cerevisiae strain by its high dominance in the yeast population. The new strain, JPI strain, presented practically the same fermentative capacity and stress tolerance like the most used commercial strains, with advantages of being highly adapted to different industrial units in Northeast Brazil that used sugar cane juice as substrate.

9. *Alves SL Jr.et al.,:*Characterize the kinetics of yeast cell growth, sugar consumption and ethanol production during maltose or maltotriose utilization by several S.cerevisiae yeast strains. While both high-and low affinity transport activities were responsible for maltose uptake from the medium, in the case of maltotriose the only low-affinity (Km 36+/-2) transport activity was mediated by the AGTI permease. In conclusion the AGTI transporter is required for efficient maltotriose fermentation by S.cerevisiae yeasts, highlighting the importance of this permease for breeding and / or selection programs aimed at improving sluggish maltotriose fermentations.

10. *Silton et al., 1983:*The rapid depletion of world petroleum reserves and it is rising prices day by day, new sources of hydrocarbons must be found to supply our chemical and energy needs. Alcohol fermentation offers promising alternative as it can be produced from various sources of raw materials.

11. *Shiyum et al., 1987, Coverti et al., 1985*: Alcoholic fermentation is the study of yeast strains able to utilize sugar solutions more concentrated than those generally fermented in usual practice. The limits of ethanol tolerance if the yeast STRAIN.

12. *Ingledew 1993; Novozymes 2002:*The fermentations are carried out as a simultaneous saccharification and fermentation (SSF), where glucoamylase and Yeast (saccharomyces cerevisaie) are added simultaneously. Some plants have a separate pre-Saccharification step, where glucoamylase is allowed to function at 55-65^{0}C for 2-4 h before cooling down to the fermentation temperature (30-35^{0}C) after which the yeast is added.

13. *Oliveria VA, et al., (2007):*Saccharomyces cerevisiae strains from Brazil, were isolated and characterized aiming at the selection of starter yeasts to be used in the production of cachaca, the Brazilian sugar cane spirit. The methodology established took into account the screening for biochemical traits desirable in a yeast cachaca producer, such as no H2S production, high tolerance to

ethanol and high temperatures, high fermentative capacity, and the abilities to flocculate and to produce myocins. Furthermore the yeasts were exposed to drugs such as 5, 5', 5" – trifluro – D, L – leucine and cerulenin to isolate those that potentially overproduce higher alcohols and esters.

SCOPE:

- Bio-ethanol is produced from Sugarcane, Corn, Sugar beet, Potato etc., Hence it's an efficient source of Energy.
- It has less impact over the Environmental Degradation.
- In developing Countries like India, bio-ethanol would be an efficient fuel medium in the near future.
- Release of Greenhouse gases is less in case of bio-ethanol and Global warming can be controlled.
- In developing countries like India, green revolution would help in enormous production of ethanol.

OBJECTIVE:

- To produce bio-ethanol from sugarcane juice using Baker's Yeast.
- To distil the ethanol out of the mixture, by difference between the boiling point of alcohol and water.
- Effective ethanol extraction from the mixture.
- To test the presence of bio-ethanol in the mixture produced.
- To run a gasoline engine using the correct blending ratio of ethanol-gasoline mixture.

MATERIALS AND METHODS

Ethanol production is done through fermentation process, the fermentation of sugarcane juice by baker's yeast (*SaccharomycesCerevisiae).*

SUGARCANE JUICE:

Sugarcane juice is obtained by crushing the sugarcane. It consist of water, glucose and other sugar compounds. Sugarcane is one of the most important cash crops capable of producing high bio-mass yielding energy in the form of sugar, power and alcohol. India has the largest area of sugarcane, 4.23 million hectare and yielding about 299 Million tonnes of sugarcane. Sugarcane being a perennial crop, can be produced throughout the year. Agriculturally self-sufficient and Fast growing economies, such as India, which are more energy dependent can make use of this ethanol to meet their needs.

Fig.1 ; Sugarcane juice

Creation of ethanol starts with photosynthesis causing a feedstock, such as sugar cane or a grain such as maize (corn), to grow. These feedstock's are processed into ethanol.

In India, sugarcane is grown almost in all areas especially in Northen states of Punjab, Haryana, Himachal Pradesh, Rajasthan, UP. It is called "KabbinaHalu or Kabbina rasa" in Kannada, "oosacharas or gannekaras" in Marathi. It is mostly used as a beverage by mixing with pineapple or lime or ginger. Sugarcane juice is heated and converted into syrup which can be used as sweetener. In the foreign countries like Brazil ethanol is produced from the sugarcane molasses. In India sugarcane molasses cannot be used as it is less effective in our climatic conditions. Hence sugarcane juice is used for ethanol production.

Saccharomyces Cerevisiae (Yeast):-

Kindgom: Fungi

Phylum: Ascomycota

Subphylum: Saccharomycotina

Class: Saccharomnycetes

Order: Saccharomycetales

Family :Saccharomycetacea

Genus :*Saccharomyces*

Species: *cerevisiae*

ETYMOLOGY:

"Saccharomyces" derives from Latinized Greek and means "sugar-mold" or "sugar-fungus", *Saccharo* being the combining from "sugar" and *myces* being fungus. *Cerevisiae*comes from Latin and means of "beer". Other names from the organism are.,*S. cerevisiae*short form of the scientific name.

Saccharomyces Cerevisiae is a species of yeast. It is perhaps the most useful yeast, having been instrumental to winemaking, baking and brewing since ancient times.

Fig.2 ;*SaccharomycesCerevisiae (Yeast)*

It is believed to have been originally isolated from skin of grapes. It is one of the most intensively studie eukaryotic model organisms in molecular and cell biology, much like Echerichia coli as the model bacterium. It is the micro-organism behind the most common type of fermentation. S. *cerevisiae*cells are round to ovoid, 5-10 micrometer in diameter. It reproduces by a division process known as budding. The optimum temperature for growth of *S. Cerevisiae*is 30-35°c.

COMMERCIAL APPLICATION:

- *S. Cerevisiae*is used in brewing beer.
- It is used in baking.
- It is used in aquaria.

METHODOLOGY:

The production of bio-ethanol involves two main process, namely Fermentation and Distillation.

1. FERMENTATION:

During ethanol fermentation, glucose and other sugars in the corn (or sugarcane or other crops) are converted into ethanol and carbon dioxide.

$$\mathbf{C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 + heat}$$

Like any fermentation reaction, the fermentation is not 100% selective, and other side products such acetic acid, glycols and many other products are formed to a considerable extent and need to be removed during the purification of the ethanol. The fermentation takes place in aqueous solution and the resulting solution after fermentation has an ethanol content of around 15%. The ethanol is subsequently isolated and purified by a combination of adsorption and distillation techniques. The purification is very energy intensive.

During combustion ethanol reacts with oxygen to produce carbon dioxide, water, and heat:

$$\mathbf{C_2H_5OH + 3\ O_2 \rightarrow 2\ CO_2 + 3\ H_2O + heat}$$

Starch and cellulose molecules are strings of glucose molecules. It is also possible to generate ethanol out of cellulosic materials. That, however, requires a pretreatment that splits the cellulose into glycose molecules and other sugars that subsequently can be fermented. The resulting product is called cellulosicethanol, indicating its source.

Ethanol may also be produced industrially from ethene (ethylene), by hydrolysis of the double bond in the presence of catalysts and high temperature.

$$\mathbf{C_2H_4 + H_2O \rightarrow C_2H_5OH}$$

International Journal of Innovative Research in Technology, Science & Engineering (IJIRTSE)
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By far the largest fraction of the global ethanol production, however, is produced by fermentation.

Alcoholic fermentation, also referred to as ethanol fermentation, is a biological process in which elements such as glucose, fructose and sucrose are converted into cellular energy and thereby produce ethanol and carbon dioxide as metabolic waste products. Because yeasts perform this conversion in the absence of oxygen, alcoholic fermentation is considered an anaerobic process.

Anaerobic fermentation is carried out for the ethanol production. The fermentation is carried out in closed container leaving a small way for the carbon di oxide that evolves during the process. This anaerobic condition provides necessary condition for effective ethanol production.

SAMPLE – 1: Sugarcane juice and yeast:

In this sample, fermentation is carried out in a normal sugarcane juice. The fresh sugarcane juice obtained from the cane is collected in a fermentation flask. The yeast is added in a very smaller amount, say 2-3 pinch of yeast for 250ml of sugarcane juice.

The flask is closed with the rubber stopper and a small way for carbon dioxide is left. It is kept undisturbed for a period of 5-7 days for the effective production of ethanol. The carbon dioxide escapes out and sample consisting of water and ethanol is obtained after the fermentation period (as shown in figure 3)

Fig.3 ;Sugarcane juice and yeast

SAMPLE – 2: Heated sugarcane juice and yeast:

In this sample, the heated sugarcane juice is used. The sugarcane juice is heated at the temperature of 1000^{o}c for 5-10min. It is observed that, there is about 10-12% decrease in volume of sugarcane juice. This sample is fermented by adding few pinches of yeast.

Fig.4 Heating of Sugarcane juice

The flask is closed same as that of the previous case and the fermentation is carried out for 5-7 days. It is kept undisturbed till the fermentation period. The heating of the sugarcane juice will result in the loss of the water content . This could increase the sugar concentration in the solution. It is tried in order to check whether heating has any stimulation in the fermentation process or not.Till now the fermentation is carried out only with the normal sugarcane juice (as shown in the fig.5)

Fig.4.5 Heated sugarcane juice with yeast

Both the beer factories and the ethanol factories carry out fermentation with unheated sample. This heating of sugarcane juice and carrying out the fermentation is innovative part of this project.

Fig .6.Fermentation samples.

2.DISTILLATION:

The process of separating the component substances from a liquid mixture by evaporation and condensation. It may result essentially complete separation or partial separation that increases the concentration of selected components. Here ethanol is effectively extracted from the ethanol- water mixture.

Distillation is a process of separating the component substance from a liquid mixture by selective evaporation and condensation. Distillation may result in essentially complete separation or it may be a partial separation that increases the concentration of selected components of the mixture. In either case the process exploits differences in the volatility of mixture's components. In industrial chemistry, distillation is a unit operation of practically universal importance, but it is a physical separation process and not a chemical reaction.

Distillation of fermented products produces distilled beverages with a high alcohol content, or separates out other fermentation products of commercial value.

An installation for distillation, especially of alcohol, is a distillery. The distillation equipment is a still.

DESCRIPTION OF APPARATUS:

- **Heating furnace:**

The sample is heated using this furnace. The chamber consist of a heating unit and a flask holding unit. The fermentation sample after 5-7 days is taken in the flask and is heated at a temperature of 100^{o}c.

- **Condensation unit:**The sample when heated vaporises. The external water supply is given to the chamber. This condenses the vapour produced. The external water supply is given through pipes.
- **Collection unit:**

The vapour is condensed and gets collected in a beaker kept at the end. The collection of liquid is based on the boiling points of various liquids pres0ent in the sample.

The two samples namely sample-1 and sample-2 are distilled separately and the solution is collected. At first, the sample is heated at a temperature of 100^{o}c where water is collected with the

alcohol for the pure alcohol to be obtained further distillation is carried out by heating at the temperature of 70-80°c. The solution collected is marked as S-1 and S-2 respectively.

Sample-1 Sample-2

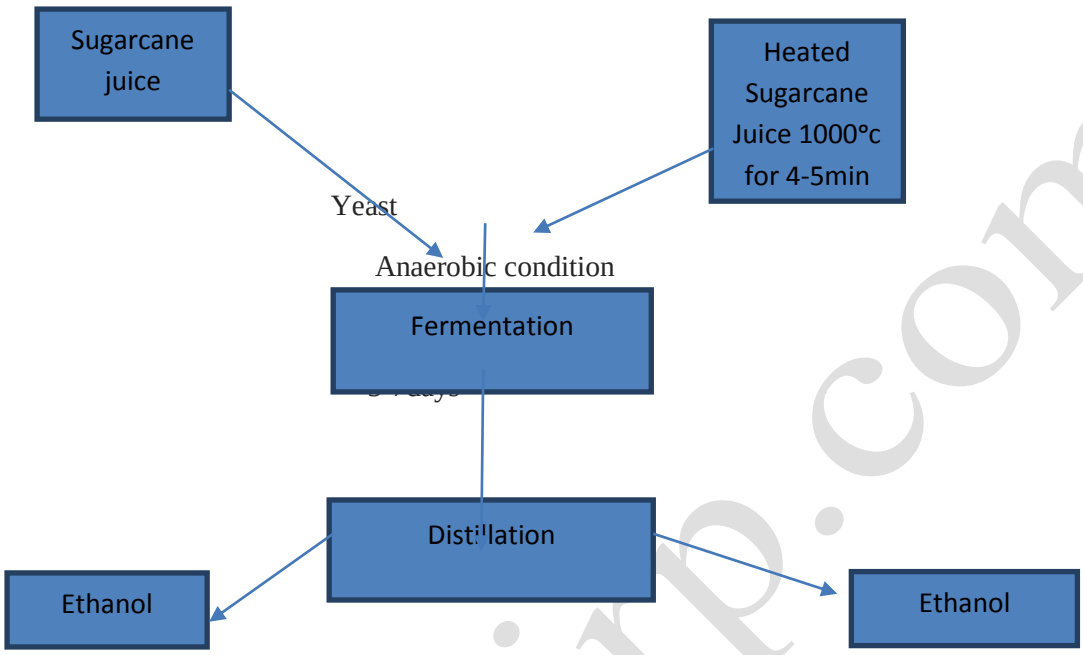

Fig.7. Distillation of sample – 1

The sample gets vaporized when heated and condensed by external water supply. It gets collected as a liquid in the beaker.

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Fig.8. Sample-1 after distillation.

Fig.9. Distillation of sample – 2.

The sample gets vaporized when heated and condensed by external water supply. It gets collected as a liquid in the beaker.

Fig.10. Sample-2 after distillation.

RESULT AND DISCUSSION

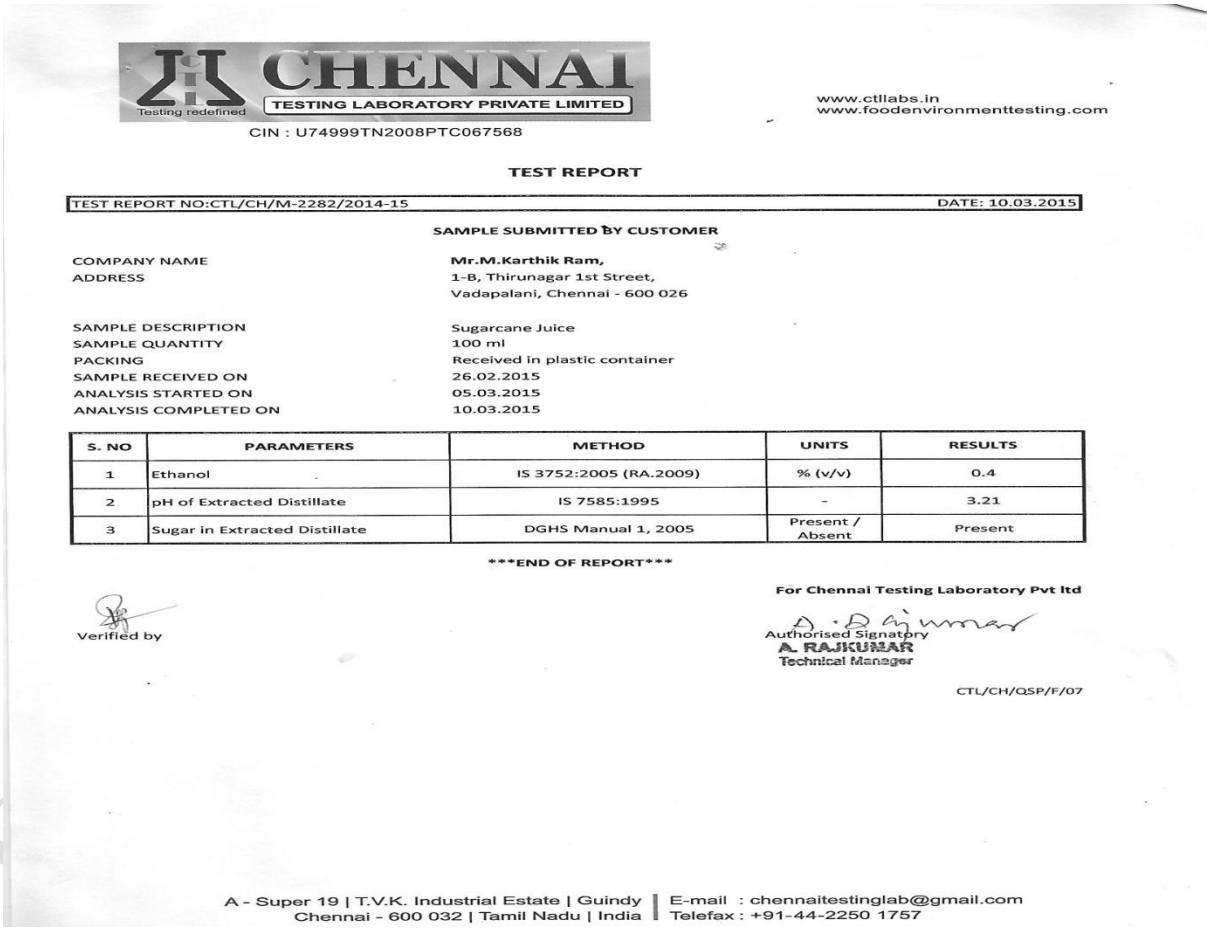
COMPARISION OF ETHANOL PRODUCTION IN SAMPLES:

After distillation, the ethanol is collected out of fermented sugarcane juice. It is observed that heated sample i.e. S-2 has produced more ethanol than the sample S-1. This ensures that heating of the sugarcane juice before fermentation has an effect on the alcohol production. This when tested has proved that there is an enormous increase in alcohol concentration when sample is heated. It is from 0.4% to 9.2%. This is the best part of the project where a new thinking of heating the sample has provided the best result in alcohol production.

The sample obtained from a fermentation of normal sugarcane juice and the heated sugarcane juice is taken. This sample is given to Chennai testing laboratory private limited centre, Guindy for studying the amount of ethanol formed and its characteristics.

The characteristics such as pH, sugar concentration and the quantity of ethanol is studied through various experiments in the centre.

The report confirming this result has been attached below.



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TESTING LABORATORY PRIVATE LIMITED
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www.foodenvironmenttesting.com

TEST REPORT

TEST REPORT NO:CTL/CH/M-2282/2014-15 DATE: 10.03.2015

SAMPLE SUBMITTED BY CUSTOMER

COMPANY NAME	**Mr.M.Karthik Ram,**
ADDRESS	1-B, Thirunagar 1st Street, Vadapalani, Chennai - 600 026
SAMPLE DESCRIPTION	Sugarcane Juice
SAMPLE QUANTITY	100 ml
PACKING	Received in plastic container
SAMPLE RECEIVED ON	26.02.2015
ANALYSIS STARTED ON	05.03.2015
ANALYSIS COMPLETED ON	10.03.2015

S. NO	PARAMETERS	METHOD	UNITS	RESULTS
1	Ethanol	IS 3752:2005 (RA.2009)	% (v/v)	0.4
2	pH of Extracted Distillate	IS 7585:1995	-	3.21
3	Sugar in Extracted Distillate	DGHS Manual 1, 2005	Present / Absent	Present

END OF REPORT

For Chennai Testing Laboratory Pvt ltd

Verified by

Authorised Signatory
A. RAJKUMAR
Technical Manager

CTL/CH/QSP/F/07

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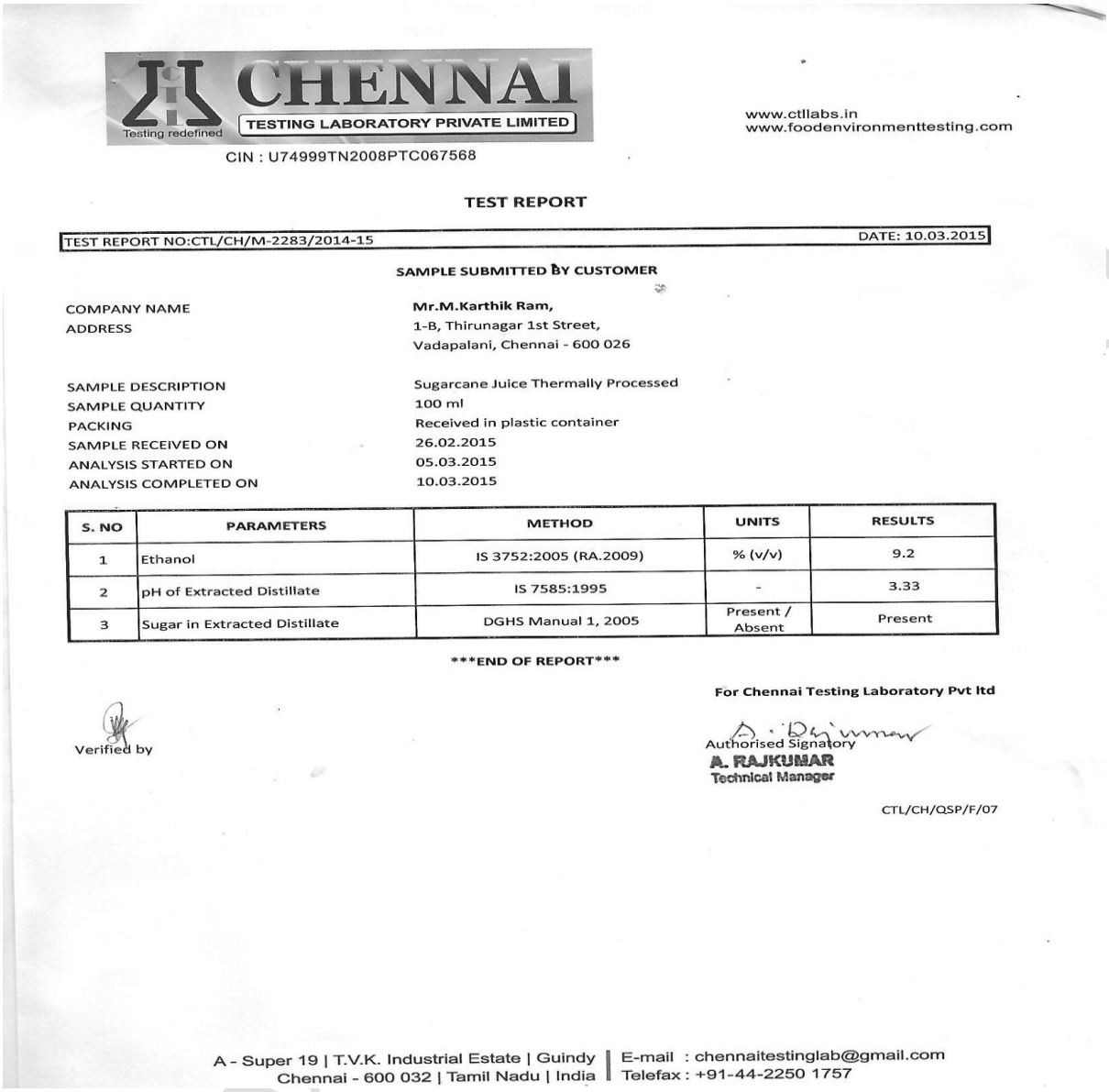

CHENNAI
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CIN : U74999TN2008PTC067568

TEST REPORT

TEST REPORT NO:CTL/CH/M-2283/2014-15 DATE: 10.03.2015

SAMPLE SUBMITTED BY CUSTOMER

COMPANY NAME	**Mr.M.Karthik Ram,**
ADDRESS	1-B, Thirunagar 1st Street, Vadapalani, Chennai - 600 026
SAMPLE DESCRIPTION	Sugarcane Juice Thermally Processed
SAMPLE QUANTITY	100 ml
PACKING	Received in plastic container
SAMPLE RECEIVED ON	26.02.2015
ANALYSIS STARTED ON	05.03.2015
ANALYSIS COMPLETED ON	10.03.2015

S. NO	PARAMETERS	METHOD	UNITS	RESULTS
1	Ethanol	IS 3752:2005 (RA.2009)	% (v/v)	9.2
2	pH of Extracted Distillate	IS 7585:1995	-	3.33
3	Sugar in Extracted Distillate	DGHS Manual 1, 2005	Present / Absent	Present

END OF REPORT

For Chennai Testing Laboratory Pvt ltd

Verified by

Authorised Signatory
A. RAJKUMAR
Technical Manager

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This heating of sample before fermentation increases the alcohol production. This idea would be very useful in case of mass scale production of ethanol. It increase the ethanol production about 9times. Till now ethanol has been produced from the normal sugarcane juice. This innovative idea can be very useful in the near future where mass scale of ethanol production is expected to happen.

TEST FOR ETHANOL:

The presence of ethanol in the both S-1 and S-2 is tested by following two tests.

ESTERIFICATION TEST:

Esterification is a chemical reaction used for making esters. In this carboxylic acids when combined with alcohol in the presence of conc. Sulphuric acid it forms the ester with fruity odour. The samples present in S-1 and S-2 is tested for this reaction. A few ml of sample from S-1 is taken in a beaker a

few drops of conc. Sulphuric acid is added to it, after adding few amount of carboxylic acid the fruity odour is evolved. This confirms the presence of alcohol in the solution.

The same test is repeated with the sample from S-2 and desirable result is obtained. Hence the presence of alcohol is tested in both S-1 and S-2.

5.2.2. TEST WITH POTASSIUM DICHROMATE:

A few amount of sample is taken from the sample S-1 and a few pinches of potassium dichromate is added to it. The solution is well stirred and it results in the formation of pink coloured solution then few drops of conc. Sulphuric acid is added. This results in the change of colour from pink colour to green colour. This confirms the presence of ethanol in the solution.

The same test is repeated with the sample taken from sample S-2. The presence of ethanol is tested from the both the samples S-1 and S-2.

CHARACTERISTICS OF ETHANOL:

- **COLOUR:**
  The ethanol obtained from both the samples is colourless.
- **pH:**
  The pH of the both the samples is taken through pH meter. The pH of the ethanol obtained from the fermentation of normal sugarcane juice is 3.21. The pH of the ethanol obtained from the fermentation of heated sugarcane juice is 3.33.

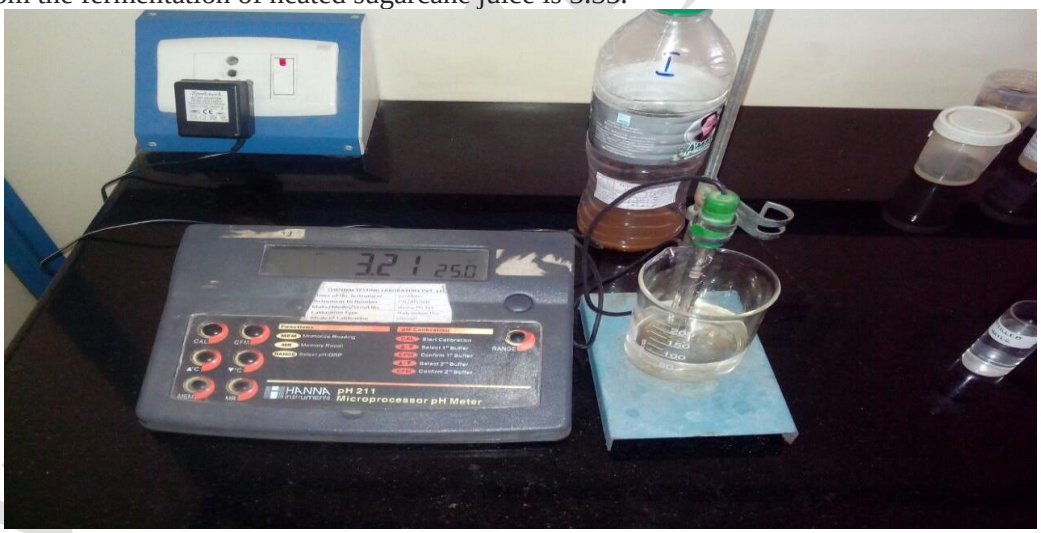

Fig.11 pH of the sample S-1

The above figure shows the pH of the sample S-1.

Fig.12; pH of Sample S-2

The above figure shows the pH of Sample S-2.

- **SUGAR CONCENTRATION:**
  The presence of sugar is tested in both sample S-1 and S-2.
  The study of characteristics shows the following gives observations.

  - The ethanol is a colourless solution.
  - The pH of the compound increases with increase in the temperature.
  - The concentration of sugar in the ethanol is tested to be in small amount.
  - It is highly volatile liquid and gives chilling effect when touched with hand
  - It vaporises easily and also undergoes oxidation when kept opened for large period of time.
  - It smells like an alcohol.

APPLICATIONS

EFFECTIVE ETHANOL – GASOLINE RATIO FOR FUEL:

The ethanol obtained through the fermentation could be blended with gasoline and can be used as an effective fuel. The blending of about 10-15% is allowed in order to run the currently available gasoline engine.

The effective mixture is identified by means of trial and error method. The four samples namely petrol, E100 (100% ethanol), E95 (95% ethanol and 5% gasoline), E90 (90% ethanol and 10% of gasoline), E85(85% of ethanol and 15% of gasoline) is prepared.

The cotton plug is immersed in all the above samples and fumes are collected by burning them (as shown in fig 5.3 & 5.4). The colour of the fumes collected in each sample is noted. It is found that the E85 produces clean fumes when compared to the other mixtures. The E85 is effective substituent for the conventional gasoline.

E85:

E85 is an abbreviation for an ethanol fuel blend of 85% denatured ethanol fuel and 15% gasoline or other hydrocarbon by volume, although the exact ratio of fuel ethanol to hydrocarbon can vary considerably while still carrying the E85 label. The ethanol content is adjusted according to the local

climate to maximize engine performance. ASTM 5798 specifies the allowable fuel ethanol content in E85 as ranging from 51% to 83%. E85 is commonly used by flexible-fuel vehicles (FFV) in the United States and Europe. In the United States, government subsidies of ethanol in general and E85 in particular have encouraged a growing infrastructure for the retail sale of E85, especially in corn growing states in the Midwest.

Fig.13 Preparation of E85 mixture.

One purported advantage of E85 over conventional gasoline is a reduction in tailpipe emissions, if one disregards the fact that E85 increases the emissions of acetaldehyde from vehicles. Additionally, there is a greater potential for localized production of ethanol in agricultural areas and utilization of waste materials. Also, a diversification of fuel sources reduces dependence on any one particular type of fuel and may increase stability of supply.

When environmentalists concern themselves with emissions from combustion engines there are four primary types of pollutants scientists study. These emissions are hydrocarbons (HC), oxides of ni-trogen (NO_x), carbon monoxide (CO) and carbon dioxide (CO_2). Because E85 is predominantly ethanol the tailpipe emissions are much different than that of regular gasoline.

E85 consumes more fuel in FFV when the vehicle uses the same compression ratio for both E85 and gasoline, because of its lower air–fuel ratio and lower heating value.

Fig.14; E85 mixture.

Use of gasoline in an engine with a high enough compression ratio to use E85 efficiently would likely result in catastrophic failure due to premature fuel ignition, as the octane rating of gasoline is not high enough to withstand the greater compression ratios in use in an engine specifically designed to run on E85. Using E85 in a gasoline engine has the drawback of achieving lower fuel economy, as more fuel is needed per unit air (stoichiometric ratio) to run the engine in comparison with gasoline. The additional ethanol required for a stoichiometric fuel ratio helps compensate for lack of energy provided by ethanol's lower heating value (LHV), which is lower than the LHV of gasoline.

Currently, Brazil, USA are the leading producers and consumers of E85. In Brazil, 40-80% of the vehicles run through the E85. This E85 releases very less reduces and burns completely than gasoline. The greenhouse gases released due to the combustion of E85 is less when compared to gasoline. Thus the threats of global warming, melting of ice could be brought into control by large scale replacement of gasoline with ethanol.

Fig.15;Fumes from burning of petrol.

In India, the government came with the idea of replacing the conventional gasoline with an alternate fuel and suggested ethanol could be a fine replacement. About 45% of ethanol produced in the country is consumed as alcohol and remaining 40% is used in chemical industries.

Fig.16; Fumes from burning of E85 mixture.

In 2014, the environment minister of India, Mr. NITHIN GATKARI emphasized the need to replace gasoline with ethanol. The ideas of eco-friendly vehicles and buses were to be achieved in the near future. As an initiation, a bus which runs in 100% ethanol is introduced in Nagpur.

In the United States, the price of E85 must be much lower than gasoline. E85 was at least 20% less expensive in most areas, as recently as 2011. However as of March 2012, the difference in the retail price between E85 and gasoline is 15% or less in the vast majority of the United States. E85 also gets less MPG, at least in FFV. In one test, a Chevy Tahoe FFV averaged 18 MPG [U.S. gallons]

for gasoline and 13 MPG for E85, or 28% fewer MPG than gasoline. In that test, the cost of gas averaged $3.42, while the cost for E85 averaged $3.09, or 90% of the cost of gasoline.

RUNNING OF GASOLINE ENGINE WITH E85 MIXTURE:

Fig.17;Addition of E85 to engine.

Fig. 18. Fumes collected from engine running with E85.

CONCLUSION

In the 21st century, the major problems faced by the world is population explosion, energy crisis, environment degradation and faster rate depletion of fossil fuels such as coal and petroleum etc., thus the need of an alternate and eco-friendly fuel has been stressed around the world. The bio-fuels such as ethanol which can be produced in economical means, causing less pollution could be an effective replacement in the long term visionary.

From the above study, the following conclusions can be drawn.

1. The ethanol can be obtained by the fermentation of sugarcane juice and the yeast.

2. The heating of sugarcane sample before fermentation would result in 9 times increase in the ethanol production.
3. The effective ethanol gasoline mixture is 85% ethanol and 15% gasoline. It is denoted as E85. This could be the best substitution for the conventional gasoline.
4. E85 burns completely in the air and leaves back less residues. The percentage of greenhouse gases released is very much lower when compared to the conventional gasoline.
5. In developing countries, ethanol could have an effective impact on their economy.

As ethanol is produced from sugarcane, it gives rise to the food vs fuel conflict. However, green revolution, BT in agriculture could solve this issue. Many fast growing economies such as brazil succeeded in this. Hence India, is no longer away from becoming an "bio-ethanol hub". Increasing the dependence on ethanol would make India, a super power within some years. Rome cannot be built in a day, it needs time and clever minds.

Fig.7.1. (a)

Fig.7.1.(b)

India's first green bus which runs in 100% ethanol – Nagpur

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