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BIOETHANOL PRODUCTION FROM WASTES USING JUICE ISOLATES

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ABSTRACT

Bioethanol production from renewable sources to be used in transference is now a growing demand globally due to continuous exhaustion of fossil fuels, political and economic disasters and continuously increasing alarm on environmental safety. Raw materials such as juices from different sources were selected for the isolation of the bacteria, for the fermentation of agricultural waste and production of bioethanol. This work will examine bioethanol production from natural sugar containing juices gained from some energy crops such as sugarcane, fruits etc., that are the most attractive choice because of their cost-effectiveness and feasibility to use. The batch fermentation was engaged in the production of bioethanol from these sugar juices as well as the agricultural wastes.

Keywords: Energy, Juices, Bioethanol, Renewable Sources, Fossil Fuels

INTRODUCTION

Ethanol is a fuel that can be used as an alternative to petrol, or in mixture with petrol to reduce the amount petroleum consumption. Bioethanol Ethanol is produced through the processing of organic matter, either waste products or crops are grown specifically for the purpose of making ethanol.

Bioethanol can be produced from a wide variety of raw materials. They fall into three main categories. Materials containing large amounts of sucrose can be fermented, such as sugarcane; starchy material as corn containing polysaccharides, which may be hydrolysed to obtain sugars suitable for fermentation; and Lignocellulosic biomass containing a complex of several polysaccharides that can break down in similar manner down in fermentable sugars Lignocellulosic biomass is used often called “second generation”. This technique is in the development stage and has not yet been widely used.

Ethanol or ethyl alcohol is a clear colourless liquid; is biodegradable, low in toxicity and causes little environment pollution when split. Ethanol burns to produce carbon dioxide and water. Ethanol is a high octane fuel and has replaced lead as an octane enhancer in petrol.

By mixing ethanol with petrol we can also oxidize the fuel mixture, so it burns completely and reduce pollution emissions.

Ethanol fuel blends sold in the United States 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.

Bioethanol is an alcohol made by microbial fermentation from carbohydrates in sugar-starch bearing plants such as corn, sugarcane, sweet sorbet or lignocellulosic biomass. Bioethanol production includes three processes pre-treatment to separate hemicellulose and lignin from cellulose hydrolysis cellulose to obtain fermentable sugars and fermentation to convert sugars into ethanol. Followed by distillation to separate and purify the ethanol.

Ethanol (CH_3CH_2OH) is an alcohol, a group of chemical compounds whose molecules are the hydroxyl group (-OH) , attached to one carbon atom, melts at -114.1^{O}C, boils at 78.5CC, and has a density of 0.789g/ml at 20^{O}C.

The word alcohol derives from the Arabic *alkuhul* which refers to the fine powder pf antimony used as eye makeup alcohol originally referred to any powder, but medieval chemists later applied the term to refined products of distillation, and this led current usage. Ethanol inaccurate with water and in proportions of most organic solvents.it is useful for many substances and as a solvent in making perfumes, paints, lacquer and explosives. It can be oxidized first to form acetaldehyde and then acetic acid, it can be dehydrated to make ether. Alcoholic solutions of non-volatile materials are called tinctures, if it is volatile; the solution is called spirit.

MATERIALS AND METHODOLOGY

Samples collection:

The different juices were collected from the local juice corners of different location of Lucknow.

Isolation of bacteria from samples:

The juices were collected from juice corners and then allowed to ferment. Further the fermented juice was serially diluted in 0.85% NaCl solutions. Further, diluted samples were spread on nutrient agar plates and then pure culture plates were prepared by selecting the cultures on the basis of different morphological parameters.

Screening of isolates for bioethanol production:

Initially the pure cultures were inoculated in sterilized waste product, sugarcane baggage and allowed for fermentation at 37°C for 1 week and then crude form of alcohol was extracted by centrifugation at 10,000 RPM for 15 min.

Alcohol testing was carried out by adding starch (1%), $K_2Cr_2O_7$ (1%), KI (1%) to the crude sample and then compared with 100% pure ethanol.

Strain identification of isolates:

Various biochemical tests such as grams staining, endospores staining, glucose fermentation test, mannitol test etc. as were performed and strains were identified by using Bergy's manual.

Study of growth parameters of isolates:

The cultures were inoculated in sterilized medium and then the absorbance were taken at 620 nm in UV-Vis spectrophotometer at different time intervals.

Effect of pH & temperature on growth of isolates:

The cultures were treated at different temperatures and different pH to observe the effect of these parameters on the growth of isolates.

Fermentation & distillation of bioethanol:

The sterilized selected media was prepared by adding the sugarcane bagasse's for the batch fermentation. Further the culture was inoculated and incubated at 37^0C for 7 days at shaker incubator. Once the fermentation completed the distillation was carried for the purification of bioethanol and then the alcohol estimation was carried out for testing the presence of the alcohol.

RESULTS

Collection of juice samples:

The juice samples were collected from different locations of Lucknow.

Table 1: Collected Juice samples

S.No	Sample Name
1.	 Orange juice
2.	 Grape juice
3.	 Sugar cane juice

Bacterial isolation by serial dilution:

Total 25 bacterial cultures were isolated from the all five fermented juice samples using serial dilution and spread plate method. These cultures were selected on the basis of their different morphological characteristics.

Figure 1: Bacterial cultures on agar plates after the serial dilution and spreading.

Bacterial purification:

Bacterial purification was done using the streak plate method by streaking the selected cultures in Petri plates.

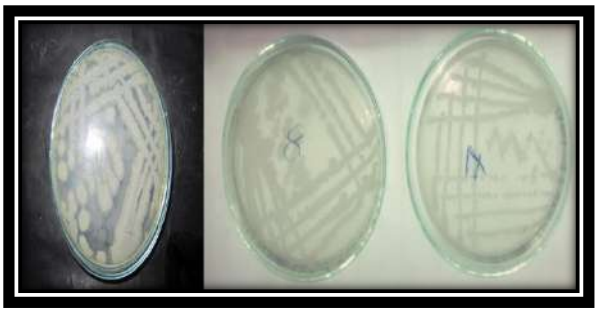


Figure 2: Few pure bacterial culture in agar plates after streaking.

Colony morphology

The cultures obtained from different samples were differentiated based on their morphology. The morphology of all the selected colonies is given in the table below.

Table 2: The morphology of all the selected colonies.

Culture name	Shape	Margin	Elevation	Pigmentation	Surface	Texture	Opacity
C1	Circular	Entire	Flat	White	Rough	Hard	Opaque
C2	Spindle	Discrete	Raised	Off-white	Smooth	Soft	Translucent
C3	Circular	Discrete	Flat	Off-white	Smooth	Soft	Opaque
C4	Rhizoidal	Curled	Convex	White	Rough	Hard	Opaque
C5	Circular	Discrete	Flat	Off white	Rough	Gummy	Opaque
C6	Circular	Entire	Raised	Yellowish	Smooth	Hard	Translucent
C7	Irregular	Lobate	Convex	Off white	Smooth	Soft	Opaque
C8	Spindle	Discrete	Raised	White	Rough	Hard	Opaque
C9	Circular	Discrete	Flat	Off-white	Smooth	Soft	Opaque
C10	Spindle	Discrete	Raised	White	Rough	Hard	Opaque
C11	Punctiform	Lobate	Pulmonete	Green	Smooth	Soft	Opaque
C12	Circular	Discrete	Flat	White	Rough	Hard	Opaque
C13	Irregular	Entire	Flat	Off white	Rough	Soft	Opaque
C14	Circular	Lobate	Convex	Yellowish	Convex	Soft	Opaque
C15	Circular	Lobate	Flat	Off white	Smooth	Gummy	Opaque
C16	Irregular	Lobate	Raised	Off white	Convex	Hard	Opaque
C17	Circular	Curled	Raised	Off white	Convex	Hard	Opaque
C18	Circular	Lobate	Flat	Yellowish	Smooth	Soft	Opaque
C19	Circular	Curled	Raised	White	Smooth	Soft	Opaque
C20	Circular	Curled	Raised	White	Smooth	Soft	Opaque
C21	Irregular	Entire	Convex	Off-white	Convex	Soft	Opaque
C22	Circular	Lobate	Pulmonate	Green	Convex	Hard	Opaque
C23	Irregular	Lobate	Flat	Off-white	Rough	Gummy	Opaque
C24	Circular	Entire	Convex	Yellowish	Convex	Soft	Opaque
C25	Circular	Lobate	Flat	Off white	Smooth	Gummy	Opaque

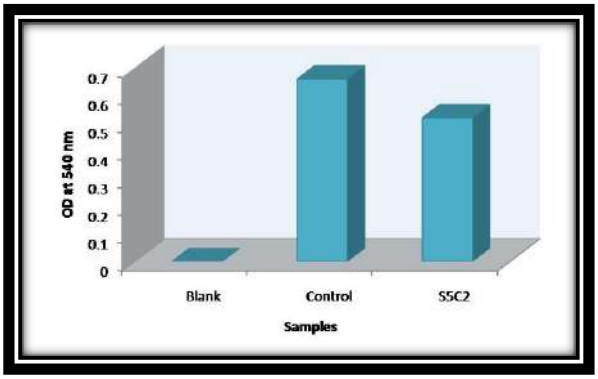
Screening by fermentation and alcohol testing of bacterial sample:

On the basis of alcohol testingfrom all the isolated cultures after the fermentation, sample S_5C_2 selected as best culture for the production of bioethanol and remaining cultures were not able to produce alcohol. Where, ethanol is used as the control and distilled water as blank.

Figure 4: Samples for the screening of alcohol presence.

Table 6: Screening of juice isolates for bioethanol production

Sample	OD at 540 nm	OD at 620 nm
Blank	0.00	0.00
Control	0.66	0.00
S_5C_2	0.52	0.09



Graph 1: Representation of the bioethanol production from respective samples.

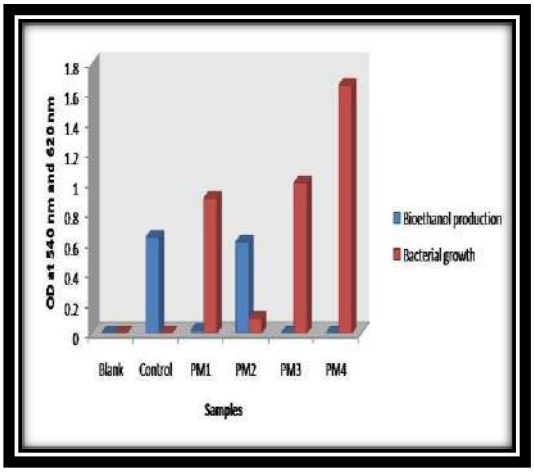
Selection of production media for bioethanol production-:

The production media was selected on the basis of the growth of the selected cultureS_5C_2 and the production of bioethanol as a by-product after fermentation of sugarcane bagasse.

Where, production media 2 was selected as a best for fermentation process, due to minimum bacterial growth and maximum bioethanol production.

Table 7: Selection of the production media.

Sample	OD at 540 nm	OD at 620 nm
Blank	0.00	0.00
Control	0.64	0.00
PM1	0.02	0.90
PM2	**0.61**	**0.10**
PM3	0	1.00
PM4	0	1.65



Graph 2: Selection of production media on the basis of maximum bioethanol production and minimum bacterial growth.

Fermentation and distillation of bioethanol:

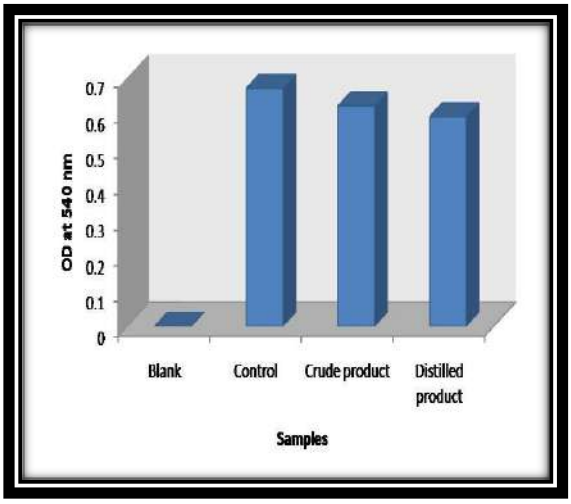
The cultureS_5C_2 was inoculated in the sterilized fermentative media and incubated at 37^0C for a week, after that the fermentative samples were filtered and then distillation process were carried out.

Figure 7: Batch fermentation for bioethanol production.

Estimation of bioethanol:

Table 8: Estimation of crude and distilled product of bioethanol.

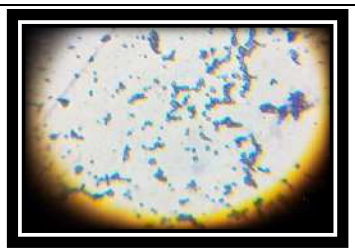
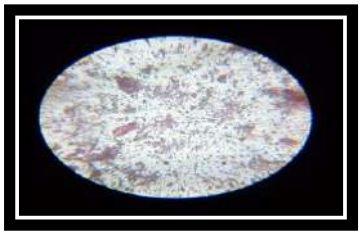
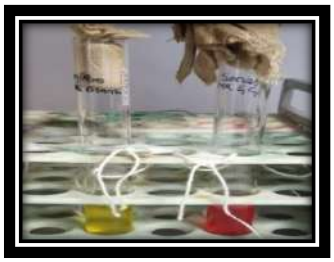
Sample	OD at 540 nm
Blank	0.00
Control	0.67
Crude product	0.62
Distilled product	**0.59**



Graph 3: Graphical representation of estimation of crude and distilled bioethanol.

Biochemical characterization of S_5C_2:

Table 9: Results of Biochemical tests

Tests	Remarks
Gram's staining	 Positive, *Streptococcus*
Endospore staining	Negative 
Catalase test	 Positive
Indole test	Negative
Mannitol test	Positive 

Effect of temperature and pH on growth of isolates

The isolates growth at different temperature range on solid media plates to check the effect of temperature on the growth. The selected temperatures were 4°C, 37°C and 60°C & pH4, pH7 and pH11. The maximum growth was found at 37°C and pH 7. The results are shown below.

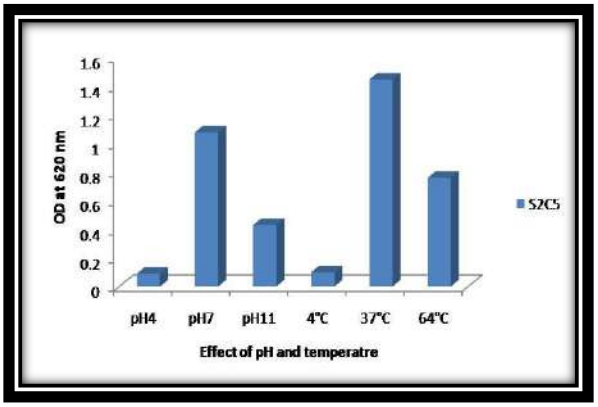


Figure : Effect of pH and temperature on culture growth.

DISCUSSIONS AND CONCLUSION

Five different sources were selected for the isolation of the desired bacteria such as, pomegranate juice, orange juice, sweet lemon juice, grape juice, sugarcane juice.

These juices were naturally rich in sugar content; hence it can also be used for the fermentation for biofuel, bioethanol production. Initially the juice samples were serially diluted in 0.85% NaCl solution (normal saline) further the sample from the dilution factor 10^{-8}, 10^{-9}, 10^{-10} were spread over sterilized nutrient agar plates and the twenty seven bacterial cultures were shortlisted on the basis of morphological parameters.

The selected cultures were screened for the production of bioethanol by fermenting the juices by using these isolates. After performing the ethanol estimation it was found that only culture 2 shows the best property for the production of bioethanol as mentioned in table and figure. Although present industrial fermentation for fuel bio-ethanol production employs two types of feedstocks such as free sugars containing juice, free fermentable sugars and starch is more economic than starch feedstocks as the former can straight be used in fermentation without any previous treatment.

However, good yield is depends rather on the collection of microorganisms and fermentation methods and procedures as well as the encouragement of several factors. In addition, selection and development of different potential genetic varieties of juice producing crops will also enhance the commercial bioethanol production.

Several technological progresses have already been investigated but most of them are still confined to the laboratory.

Therefore, a comprehensive economic and process analysis is required to develop an industrially suitable production strategy that will solve our energy crisis by producing more ethanol in a stable way.

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