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PRODUCTION OF BIOETHANOL FROM AGRICULTURAL WASTES USING JUICE ISOLATE

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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 wastes 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.

Key words: Bioethanol, Fossil Fuels, Fermentation, Agricultural Wastes

INTRODUCTION

To minimizing the consumption of petroleum, ethanol or bioethanol can be used as the alternative fuel to petrol, or in mixture with petrol. Bioethanol produced through the processing of organic matters; either by using the waste products or by crops which are grown specifically for the purpose of making ethanol **[1]**. It can also be produced from wide variety of raw materials. The processing material belongs to three main categories; sucrose enriched organic matters, such as sugarcane, starchy supplemented materials as corn comprising polysaccharides that is hydrolysed to attain sugars suitable for fermentation and lignocellulosic biomass comprising a complex of numerous polysaccharides which can be break down into similar process in fermentable sugars **[2]**. Lignocellulosic encompassing biomass is generally in use and belongs to "second generation". This method is in the growth stage and has not been in generally use **[3]**.

Basically ethyl alcohol or ethanol is biodegradable and a colorless liquid; it is low in toxicity & also a source of environment pollution when splits. It produces carbon dioxide and water after burning **[4,5]**. Ethanol is an high octane fuel and acts as an substitute of lead which is an octane enhancer in petrol. The mixture of petrol and ethanol oxidise the fuel mixtures **[6]**.

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 **[7]**. 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 **[8]**. 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 **[9,10]**.

METHODOLOGY

Sample collection:

Samples were collected in sample specimen bag from local juice corners from Lucknow**[11]**.

Isolation and purification of bacteria:

The fermented juice samples were serially diluted and then cultures isolated after spreading the diluted samples in sterilized nutrient agar plates. The mixed cultures were purified by streak plate method after selecting the cultures on the basis of colony morphology **[12]**.

Screening by fermentation of bacterial sample:

The cultures were screened by alcohol testing followed by fermenting the agricultural wastes **[13]**.

Alcohol testing:

1 ml of sample and 4 ml of distilled water was added. Then 1 ml 1% potassium dichromate ($K_2Cr_2O_7$), 1 ml 1% potassium iodide (KI), and 1 ml 1% starch, was added to it. After the completion of incubating period at room temperature for 10 min, the absorbance was taken at 540 nm and by observation of the change in colour during the reactions **[14]**.

Identification of bacteria:

For identification, Gram staining and few other biochemical tests were performed based on Bergy's manual **[15]**.

Fermentation and 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 **[16,17]**.

RESULTS

Sample collection:

The juice samples were collected from five different location of Lucknow, as mentioned in Table 1. The collected samples were allowed for the fermentation to obtain the desired cultures.

Isolation and purification of cultures:

Bacterial cultures were isolated from fermented juice samples using serial dilution, spread plate and streak plate method as shown in figure 1.

Table 1: The juice samples collected from respective juice corners from different locations of Lucknow.

S no	Sample	Juice corners
S1	Pomegranate Juice	 Vibhuti Khand
S2	Orange Juice	 Indira Nagar
S3	Sweet Lemon Juice	 Polytechnic Chauraha
S4	Sugarcane Juice	 Charbagh Railway Station
S5	Grape Juice	 Vinamra Khand

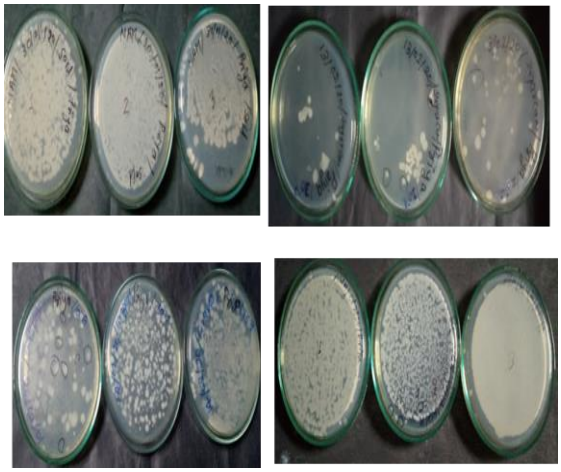


Figure 1: Bacterial cultures on agar plates after the serial dilution and spreading.

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

Screening by fermentation and alcohol testing of bacterial sample:

On the basis of alcohol testing from 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 double distilled water as blank, as shown in table 2, figure 3, 4.

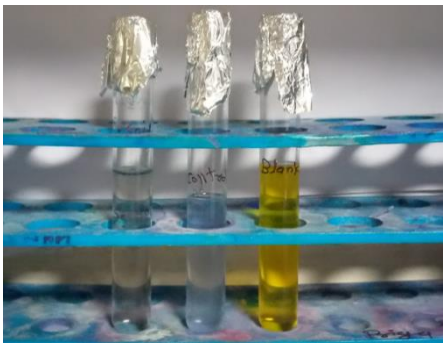


Figure 3: Samples for the screening of alcohol presence.

Table 2: 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

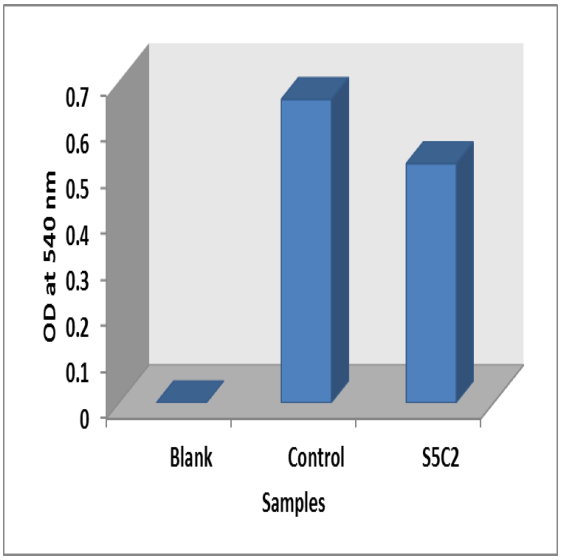


Figure 4: Graphical representation of the bioethanol production from respective samples.

Selection of production media for bioethanol production:

The production media was selected on the basis of the growth of the selected culture S_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 as presented in table 3 and figure 5.

Table 3: 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

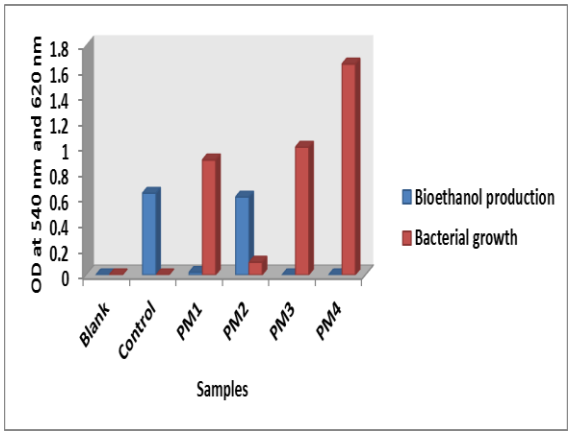


Figure 5: Selection of production media on the basis of maximum bioethanol production and minimum bacterial growth.

Fermentation and distillation of bioethanol:

The culture S_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.

Estimation of bioethanol:

The estimation was carried out by alcohol testing. Where pure ethanol shown 0.67 absorbance and the crude product as well distilled product represents 0.62 and 0.59 absorbance at 540 nm as mentioned in **table 4, figure 6.**

Table 4: 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**

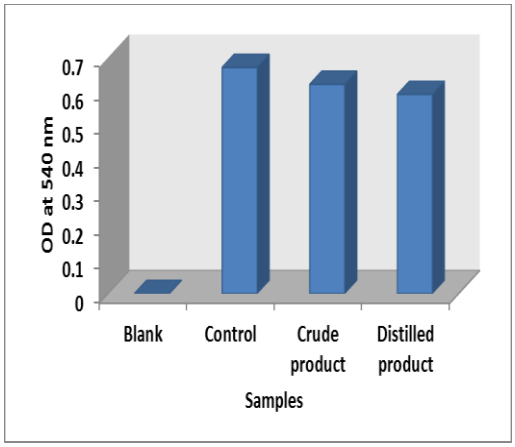
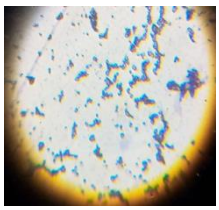
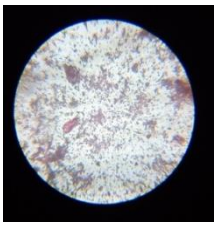
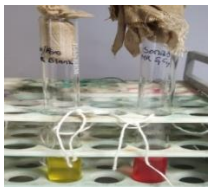


Figure 6: Graphical representation of estimation of crude and distilled bioethanol.

Biochemical characterization of S_5C_2:

Table 5: Strain identification of the culture S_5C_2

S no.	Tests	Remarks
1	Gram's staining	Positive, *Streptococcus* 
2	Endospore staining	Negative 
3	Catalase test	Positive 
4	Indole test	Negative
5	Mannitol test	Positive 

DISCUSSION 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 1 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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