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Bioethanol Production from Various Lignocellulosic Residues - A Potential Sources

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Abstract

Bioethanol is a green fuel for transportation, environmentally sustainable resource. Nowadays, many countries use 10% ethanol with conventional petrol. The current bioethanol supply is insufficient to meet the fuel demand and thus it is necessary to seek different new resources for bioethanol production. Numerous lignocellulosic biomasses such as fruits, vegetables and agricultural residues have been identified and analyzed a sustainable resource for the production of bioethanol. This work explores the use ofvarious pulpresidues from different kinds of fruits separately, such as Ananas comosus (pineapple), Manilkara zapota (Chikoo), Citrus sinensis (orange), Vitis vinifera (grapes), Punica granatum (pomegranate), Citrus limetta (Mousami) and Mixed Fruit Extract (MFR) for bioethanol production. The collected fruitwaste is allowed for the fermentation process in the presence of yeast (Saccharomyces Cerevisiae) separately, resulting in the production of bioethanol. The ethanol yield during the fermentation process is monitored by an Abbs refractive instrument for every 24 hours, 48 hours, and 144 hours. It is observed that, after 144 hours (one week). The ethanol production is found to bemaximum, when Vitis vinifera (grape) pulp residue is used as raw material and it's yield is highest (67%)in comparison with the other fruit wastes. Further, it has been observed that, the bioethanol production is 65% , 57% in Punica granatum (pomegranate), Mixed Fruit Extract (MFR) respectively. Bioethanol quality is characterized by Fourier transform infrared spectroscopy (FTIR) and gas chromatography techniques.

Keywords: *Fruit waste, total sugar content, bioethanol, refractive index, FTIR, gas chromatography*

1. Introduction

The over consumption of fossil fuels has led to global warming and climate change. As a result, there is a drive to replace fossil fuels with clean, renewable fuels such as bioethanol and biodiesel. However, due to the rapid utilization of traditional fossil fuels and their unpredictable prices, promoting an alternative renewable energy source such as biomass conversion is necessary for national energy security. Ethanol, also known as ethyl alcohol (C_2H_5OH), is a clear, colorless, biodegradable liquid with low toxicity and low environmental impact. When ethanol is burned in the presence of air, carbon dioxide and water are produced. Ethanol is a high-octane gasoline that primarily replaces TEL. The fuel combination can also be oxygenated by blending ethanol with regular gasoline, resulting in complete combustion of the fuel and reducing harmful emissions. Ethanol fuel blends are most commonly used in the United States and other countries. The most preferred blend is 10% ethanol and 90% petrol (E10).

Lignocelluloses can be used as feedstock for bioethanol production, including fruit, vegetable waste, forest waste, agricultural residues and municipal solid waste (MSW). India ranks second in the world in terms of fruit and vegetable production, accounting for

10% and 14% of the total fruit and vegetable production respectively. Annual fruit and vegetable production in India is expected to exceed 243 million tons. [1]. Fruit and vegetable processing waste is rich in cellulose, hemicellulose, pectin, and minerals, but relatively low in lignin compared to agricultural waste such as corn, wheat straw, rice straw, etc. Fruit pulp residue consists of highly fermentable soluble sugars, cellulose, and hemicellulose. These chemical components in fruit trimmings can be an effective source for the synthesis of ethanol [2]. The disposal of fruit pulp residue can entail additional costs for processors,and the direct disposal to soil or waste can result in significant environmental pollution [3]. Converting plentiful fruit and vegetable processing waste (FRPW) into value-added goods is a viable and sustainable solution to minimizing pollution, promoting energy security and reducing greenhouse gas emissions [4]. Saccharomyces cerevisiae is considered to be the most important yeast strain for bioethanol production because of its ability to ferment a wide range of carbohydrates into ethanol. [5].

2. Materials and Methods

Raw material: the required raw materials of various fruit pulp waste are collected from the local fruits shops and yeast culture is collected from laboratory.

Chemical and reagents: the required chemicals and nutrients, both were purchased from Himedia, Sodium hydroxide, Sodium metabisulite and Hydrochloric acid were purchased from SD fine chemicals.

2.1 Extraction of juices

Various fruit pulp wastes (FPW), namely Ananascomosus (pineapple), Manilkarazapota (Chikoo), Citrus sinensis (orange), Vitisvinifera (grapes), Punicagranatum (pomegranate), Citrus limetta (Musami), and Mixed Fruit Extract (MFR), are collected from local fruit shops in Shimoga, Karnataka, INDIA. All known masses of fruit pulp waste(100 g) are washed and individually immersed in distilled water in a 1:1 ratio for 2 hours before being autoclaved at 120 ° C for 30 minutes. The fruit pulp residue is then squeezed to extract juice. The extracted fruit juices are filtered and collected separately in a presterile fermentation tank (Figure 1). The collected juice extract were allowed to cool at room temperature.

2.2 Inoculumsyeast culture preparation

The inoculum culture is prepared by inoculating 3% (w/v) yeast stock into a freshly prepared 200 ml sterilized nutrient agar broth and it is incubated for 24 hours at 28°C to 30°C before inoculating into the bioethanol production medium [9].

2.3 Bio ethanol Production from fruit pulp residue by Saccharomyces cerevisiae.

To ascertain the concentration of bioethanol in fruit pulp wastes. The FPW juice extracts are collected in a fermentation bottle (Figure 1). Before inoculation, pH of eachFPW medium is initially measured and adjusted to 5.0-6.0 by adding 0.1M H_2SO_4 and 0.1 M NaOH reagent drop by drop. Further, TheFPW medium is inoculated with 5% (v/v) inoculum and 2g of sodium Meta bisulfate (helps to avoid bacterial contamination) and allowed to ferment for 24 hours in a dark environment with frequent agitation. After 24 hours, 48 hours, and 144 hours of fermentation process, the fermented FPW juice media samples were collected for ethanol analysis.

Figure 1: Fermentation set up

Figure 2: Bioethanala distillation unit

2.4 Recovery of bioethanol by distillation method

Fractional distillation is a process for the separation of the ethanol-water racemic mixture. This method is based on the boiling points of the liquids. Since ethanol boils at a lower temperature (78.5°C) than water, it evaporates, leaving water in the distillation unit. After 144 hours (one week), all fermented fruit juices are filtered and sent to a distillation unit (Figur. 2) for bioethanol production. A series of distillations are carried out to produce a high concentration of bioethanol. Specific gravity, RI index, FTIR, and gas chromatography techniques were adopted to assess the quality of bioethanol.

3 Result and Discussion

Fruit and vegetable waste have a high concentration of fermentable carbohydrates, proteins, lipids, and dietary fiber, all of which serve as important carbon sources for microbial development. The disposal of processed fruit and vegetable scraps in municipal yards or direct disposal to the ground or landfills can pose significant environmental risks (7). Because there is a lack of sufficient infrastructure to manage such a large amount of biomass, as well as established commercial technology to convert waste into valuable by-products. The current study investigates the bioethanol concentrations produced from various fruit waste. The collected pulp residue is left to ferment in a fermentation system in the presence of S. cerevisiae (Figure. 1). During the fermentation, the ethanol concentration is determined by measuring the refractive index with an Abbe refractometer. The refractive index is a parameter used to measure the percentage of ethanol in ethanol-water mixture. The RI value of ethanol- water composition is as shown in Table 1.

3.1 Determination of Bioethanol in Fermented Fruit Extracts

The fruit juices obtained from the various fruit residues are fermented in a controlled manner. The bioethanol percentage has been determined by collecting and analyzing the sampling after 24, 48 and 144 hours of fermentation. Table 2 shows bioethanol yield after 24 hours, 48 hours and 144 hours fermentation (one week).

Table 1: RI of different concentrations of the water-ethanol mixture

Sl.No.	Absolute Ethanol in mL	Dis. water in mL	Refractive Index (RI)	Percentage of Ethanol (%)
1	0.5	9.5	1.334	5
2	1.0	9.0	1.336	10
3	1.5	8.5	1.338	15
4	2.0	8.0	1.339	20
5	2.5	7.5	1.340	25
6	3.0	7.0	1.344	30
7	3.5	6.5	1.346	35
8	4.0	6.0	1.348	40
9	4.5	5.5	1.349	45
10	5.0	5.0	1.352	50
11	5.5	4.5	1.354	55
12	6.0	4.0	1.356	60
13	6.5	3.5	1.356	65
14	7.0	3.0	1.358	70
15	7.5	2.5	1.360	75
16	8.0	2.0	1.361	80
17	8.5	1.5	1.362	85
18	9.0	1.0	1.363	90
19	9.5	0.5	1.364	95

From the presented in table 2, it has been observed that after 24 hours, the entire fruit extract medium would have a progressive increase in bioethanol production. During the fermentation process, it is found that with Vitisvinifera juice medium (Grapes), the production rate of bioethanol is10%with an RI of 1.336. Manilkarazapota (Chikoo), Citrus sinensis (Orange), Citrus limetta (mousami), and Mixed fruits extract (MFR) yields 7.5% bioethanol with a RI of 1.335. Furthermore, it has been observed that Ananascomosus (pineapple), Punicagranatum (pomegranate) juice generates a lower quantity of bioethanol, i.e., 5.5 % with RI 1.334.

Table 2: Percentage of bioethanol production after 24 hours. 48and 144 hours

Sl. No	Fruit reside waste	RI after 24 hrs	Bioethanol yield after 24 hrs	RI after 48 hrs	Bioethanol yield after 48 hrs	RI after 144 hrs	Bioethanol yield after 144 hrs
1	Ananascomosus (Pine apple)	1.334	5%	1.336	10%	1.338	15%
2	*Manilkarazapota (Chikoo)*	1.335	7.5%	1.337	12.5%	1.339	20%
3	Citrus sinensis(Orange)	1.335	7.5%	1.337	12.5%	1.337	12.5%
4	vitisvinifera (Grapes)	1.336	10%	1.340	25%	1.357	67.5%
5	Punicagranatum (promogranate)	1.334	5%	1.337	12.5%	1.356	65%
6	Citrus limetta(mousami)	1.335	7.5%	1.335	7.5%	1.337	12.5%
7	Mixed fruits extract(MFR)	1.335	7.5%	1.336	10%	1.355	57.5%

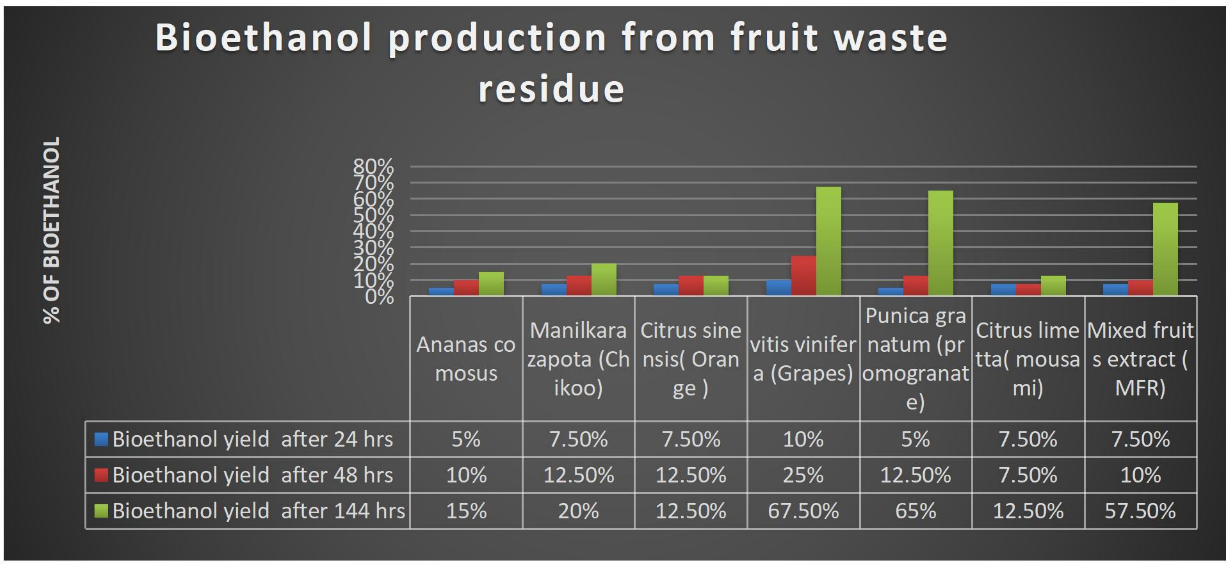
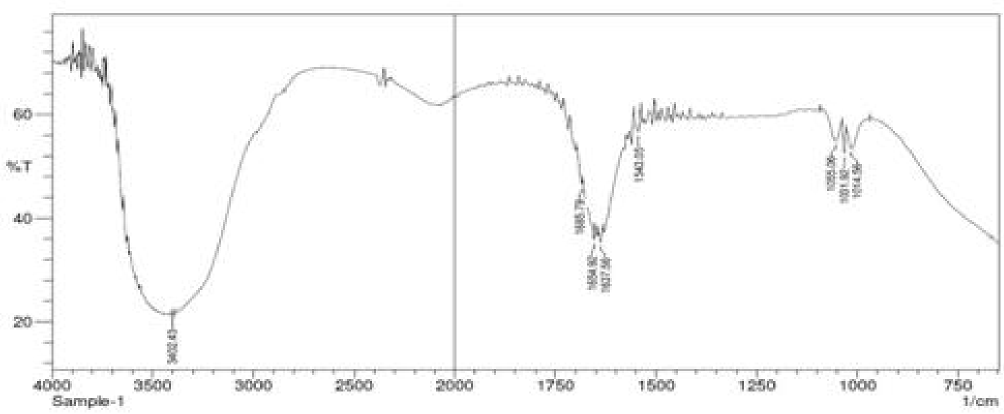


Figure 3: Bioethanol yield from various residues of pulp pulp



No.	Peak	Intensity
1	1014.56	53.451
2	1031.92	52.527
3	1055.06	54.867
4	1543.05	56.645
5	1637.56	35.344
6	1654.92	35.782
7	1685.79	46.61
8	3402.43	21.581

Figure 4: Infrared spectrum

After 48 hours of fermentation, it is observed that, in Punicagranatum (pomegranate) juice, the bioethanol production hasbeen increased from 5.5 % to 12.5 %. Furthermore, all fruit juice medium are allowedfor fermentation for about 144 hours (one week) by maintaining proper fermentation conditions.After 144 hours, there is a significant increase in percentage of bioethanol productionwith RI1.357 (67.0%). 1.356 (65.0%) and 1.355 (57%)in Vitisvinifera (grapes), Punicagranatum (pomegranate), and mixed fruit extract (MFR) juice medium respectively. The bioethanol yield remains the same after 48 hours in Ananascomosus (pineapple), Manilkarazapota (Chikoo), Citrus sinensis (orange), Citrus limetta (Mousami).

From the Figure 3, Vitisvinifera (grapes) juice medium, Punicagranatum (pomegranate) and mixed fruit extract (MFR) fruit residue extract produce a maximum bioethanol production of 67%, 65 %, and 57 % respectively. This is because of the high sugar content in juice extract (Table 3), which allows micro organisms to utilize it as a carbon source. Proper physicochemical qualities can increase trash ethanol production, and the residue left after fermentation can be used as fertilizer to help increase soil fertility.

Table 3. Sugar concentration in the fruit extract of the residue

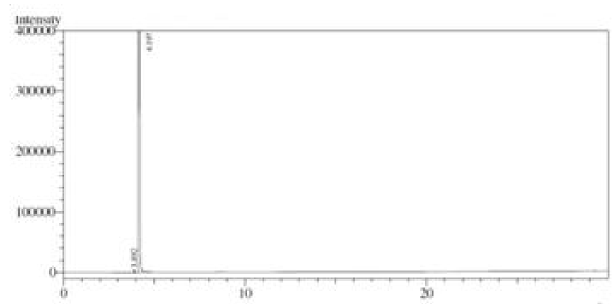
Sl. No.	PARTICULAR FRUITS	SUGAR %
1	Ananascomosus (Pine apple)	36.4%
2	*Manilkarazapota (Chikoo)*	41.44%
3	Citrus sinensis (Orange)	40.08%
4	vitisvinifera (Grapes)	46.6%
5	Punicagranatum (pomegranate)	42.72%
6	Citrus limetta (mousami)	39.84%
7	Mixed fruits extract(MFR)	41.52%

The Figure 4 reveals the IR spectral study of bioethanol from Vitisvinifera (Grapes). The stretching vibrations of bioethanol at 3402.43 cm-1 corresponds to the –OH group and intense absorption peaks at 1055-1014 cm-1. It corresponds to the -C-H group. The absorption peaks at 1685 cm-1 are due to the stretching of C=O. This confirms the structure of ethanol in the residual medium of distilled fermented fruit.

3.2 Characterization of Bioethanol by GasChromatography

The purity of distilled bioethanol was analyzed and subjected to GC analysis. The sample is tested in gas chromatography by passing through the column at a flow rate of 0.60 ml per min. With a pressure of 20.6 kPa, at 300 ° C. Nitrogen is used as a carrier gas during bioethanol analysis. Figure 5 shows the GC analysis report of bioethanol.

Figure 5 indicates the GC analysis data of bioethanol obtained after distillation. The intense peak at RT 4.1 minute shows ethanol separation in a distilled fermented fruit pulp residue extract. The obtained data is compared with standard chromatogram of ethanol and confirms that the presence of ethanol which is free from impurities.



Peak Table - Channel 1

Peak#	Ret.Time	Area	Height	Area%	Name
1	3.892	16411	5000	0.11	
2	4.197	15095745	3961325	99.89	
Total		15112156	3966325	100.00	

Figure 5: Gas chromatography data for bioethanol

4 Conclusions

In the current study, the production of bioethanol from fruit residue waste is explored as avalue addition. The collected fruit residue contains 20 to 30 % non-extractable carbohydrates and other nutritional sources and these carbon sources are easier for micro-organisms to proliferate in the medium. All residues of fruits, such as Ananascomosus (pineapple), Manilkarazapota (Chikoo), Citrus sinensis (orange), Vitisvinifera (grapes), Punicagranatum (pomegranate), Citrus limetta (Musami), and mixed fruits extract (MFR), were allowed to ferment in the presence of Saccharomyces cerevisiae(yeast),and the bioethanol yield was examined after 24 hours, 48 hours and 144 hours of fermentation.

The main observations are as follows.

1. All fruit pulp waste yield bioethanol after fermentation process. It is observed that the 10 % bioethanol yielded after 24 hour from the fruit pulp waste of Manilkarazapota (Chikoo), Citrus sinensis (Orange), Citrus limetta (mousami), 7.5% of bioethanol from Mixed fruits extract (MFR), Furthermore, it has been observed that 5.5 % of bioethanol from Ananascomosus (pineapple), Punicagranatum (pomegranate).

2. After 48 hours, there is gradual increase of 5% to 12.5 % bioethanol yield from Fruit pulp waste of Punicagranatum (pomegranate).

3. The result emphasizes that Vitisvinifera (Grapes), Punicagranatum (pomegranate) and mixed fruit extract (MFR) have a higher proportion of bioethanolafter 144 hours 67.0%. 65.0% and 57% respectively. Than other fruit residues.

4. This work has contributed to identifying many sources of fruit pulp residues that can be reused for bioethanol production at reasonable prices.

5. The bioethanol concentration can be integrated with proper physico-chemical reaction conditions.

6. Fermentation sludge is rich in nutritional sources and can therefore be used as agricultural manure.

7. In India, many tons of fruit pulp residues are disposed off without any treatment, which leads to the environment pollution. This work facilitates the conversion of agricultural and municipal waste into valuable by-products.

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