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Bioethanol production from local fruit waste and its optimization

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Bioethanol has been a focus of researchers as alternate green fuel. Agro residues could be a promising resource for bioechanol production. In this study, we explored the potential of these fruit wastes to produce total reducing sugars (TRS), pentose sugars (PS) and bioethanol.. For this purpose, fruit wastes (pineapple, cashew fruit and plantain peels) were taken as a substrate with the use of microorganism *Saccharomyces cerevisiae*. The conversion of fruits wastes were carried out via acid hydrolysis, which yielded fermentable sugar. The results showed that the ethanol production rate through fermentation of fruit waste yields was optimal at pH 5.5, temperature 32°C, specific gravity 0.865 and a concentration of about 6.10%. For viability of producing ethanol in large quantities and reasonable costs, we did optimization of various physicochemical parameters viz. pH, temperature and specific gravity. The waste materials after fermentation serve as soil fertilizer.

Keywords: Cashew fruit, Pentose sugars, Pineapple, Plantain peels, Total reducing sugars

Rapid rise in world population and industrialization has lead to increased universal energy demand. The standard sources of this energy are fossil fuels such as petroleum, nuclear, coal, natural gas and hydropower. According to the International Energy Agency, 80% of the world energy utilization is based on oil, coal and natural gas. The world oil demand is proposed to increase by 1.6% each year[1-2]. Biofuel is a renewable source of energy and hence can be used as an alternative to conventional fossil fuels. Fruit wastes rich in reducing sugars are interesting feed stocks for the production of first generation bioethanol[3]. Biofuels are also derived from industrial, municipal waste, and forestry/agricultural residues[4]. The availability of these non-renewable energy resources will decline as a result of the increase in energy demand[5].

In Asia, fruits and vegetable wastes alone account for 37% of the total agricultural waste[6]. Even after consumption, considerable fruit waste is generated during fruit storage and industrial processing. Bioethanol is regarded superior to gasoline fuel[7], and the least expensive and easily available raw material for the production of bioethanol is the fruit waste[8].

Ethanol production using microbial fermentation consists of three major steps. Making a fermentable sugar solution is the basic step. Fermenting the sugars to form ethanol in the solution is the second step, done under special microbial conditions. Separating and purifying ethanol from the solution is the final step, which is usually achieved by distillation technique[9-14].

The most well-known and commercially significant yeasts that have been primarily used for bioethanol production are related species and strains of *Saccharaomyces cerevisiae*. These organisms have long been utilized to ferment the sugars of rice wheat, barley and corn to produce alcoholic beverages and in the backing industry. One yeast can ferment approximately its own weight of glucose per hour .One of the challenges involved in saccharification of the cellulosic component is the high enzyme costs and to a lesser extent, the loss of some of the hemicellulosic sugars during pretreatment[15]. In this study, we made an attempt to improve ethanol production by maturation process from mix source of diverse fruits pineapples, cashew and plantain peel. Further, we analyzed the effect of various parameters like pH, temperature, specific gravity on concentration of ethanol.

Materials and Methods

Preparation of substrates-

The fruit wastes (pineapple, cashew fruit and plantain peels) were collected from a local juice outlets of Konni town in Pathanamthitta District, Kerala. The samples were collected in clean zip-lock bags and stored in refrigerator for further experiments. The raw material was weighed and cut into small chunks of approximately half inch size

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using a sharp knife and homogenized in a mixer. About 100 g of the homogenate was taken in a sterile glass beaker (Borosil make) and 500 mL of luke warm sterile distilled water was added. The mixture was thoroughly agitated in the mixer and the juice was extracted by filtering through a clean muslin cloth.

Immobilization of yeast

Five grams of commercially available instant dry yeast (food grade) was mixed with 10 mL of lukewarm water for activation, and was made into fine slurry using a glass rod. The yeast slurry was mixed with 10 mL 4% (w/v) sodium alginate prepared in sterile double deionized water. This slurry was extruded as discrete droplets using a 1 ml micropipette rather than continuous stream so as to form beads of 0.5 mm diameter into a beaker containing 100 ml of 0.1M $CaCl_2$ solution. The beads thus formed were kept undisturbed in the 0.1M $CaCl_2$ solution for 30 minutes for hardening. These beads were rinsed tvwice in sterile deionized water and stored in test tubes closed with cling film at 4℃.

Estimation of reducing sugars in fruit juice

Fruit juices contain a wide variety of reducing sugars notably glucose and fructose. The amount of reducing sugars is a vital parameter in the production of ethanol as glucose serves as the substrate for the enzyme zymase for conversion into ethanol. The amount of reducing sugar was estimated colorimetrically at 540 nm using Di nitro Salicyclic acid (DNS) method. Maltose @ 2 mg/mL was used as the standard[16].

Fermentation of fruit juice and determination of ethanol

About 100 mL of the extract of each pineapple, plantain and cashew apple were transferred into three 500 mL sterile conical flasks. The juice was then sterilized in a pressure cooker (Conditions: 121℃, 15 lbs for 20 min). Upon cooling, the fruit juices in the respective flasks were inoculated with 50 immobilized beads and kept undisturbed. After seven days of fermentation 10 mL of sample was withdrawn with a sterile glass pipette for the measurement of specific gravity and hence percentage of alcohol was determined. Determination of percentage of alcohol (ethanol) by specific gravity method provides an approximate method. It assumes the difference between the specific gravity before and after the fermentation. This is solely due to the conversion of sugars into alcohol after fermentation. The percentage of alcohol is determined by the following formula.

$$\% \left(\frac{v}{v}\right) alcohol = \frac{SG1 - SG2}{0.0074}$$

SG1 and SG2 are the specific gravities before and after fermentation, respectively.

Estimation of specific gravity

Specific gravity is the ratio of the density of a substance to the density of a reference substance (pure water having density 1.0 g/cm^3). The values of specific gravity of the fruit juice before and after fermentation reflects the amount of ethanol formed. Specific gravity of the fruit juice is measured with a method in the bottle is weighed empty and then filled with the liquid whose specific gravity is to be found, and then reweighed. The difference in weight is divided by the weight of an equal volume of water to give the specific gravity of the liquid.

Effect of Temperature on ethanol production

Temperature plays a major role in the production of ethanol, since the rate of alcoholic fermentation increases with the increase in temperature. To optimize the fermentation temperature, the fermentation was carried out at temperature ranges between 25 and 40^{o}C. About 100 mL of fruit juice (pineapple, cashew fruit and plantain peels) were kept in respective temperatures for seven days of incubation. After the fermentation process the concentration of ethanol produced by each fruit juice was determined.

Effect of pH on ethanol production

The P^H value has significant influence on alcoholic fermentation. To optimize the pH value of the fermentation process, the pH value ranges from 4 to 8 (pH 4, 5, 6,7,8) were used. About 100 mL of fruit juices (pineapple, Cashew fruit and plantain peels) were kept in respective pH values for seven days of incubation. After the incubation process the specific gravity and concentration of ethanol were determined.

Results and Discussion

Effect of specific gravity on concentration of ethanol

Specific gravity is used to measure the sugar content. As the fermentation progressed, the specific gravity is considerably decreased and reached a value of 0.865 at 36 h and remained constant. The decrease in specific gravity is clear indication of sugar fermentation and resulting in ethanol production. The specific gravity reaching a constant value after incubation period is the indication of the end of fermentation.

Effect of specific gravity on concentration of ethanol in pineapple peel, cashew fruit and ripe plantain peel showed that the maximum yield of ethanol was obtained at specific gravity 0.865, 0.785 and 0.972, respectively (Table 1).

Effect of specific gravity on concentration of ethanol in cashew fruit showed that as the specificity increases, concentration of ethanol produced increases up to 6.30 for a concentration of 0.785, and gradually reduces to a concentration of 2.60 at specific gravity 0.986. For in ripe plantain peel, there was a peak in concentration of ethanol production at specific gravity 0.74 to 0.97 which gradually reduced to 2.9 at specific gravity 1.31.

Effect of temperature on concentration of ethanol in fruit juices

The effect of temperature on concentration of ethanol in pineapple, cashew fruit and ripe plantain peel showed the maximum yield of ethanol from the pineapple and cashew fruit waste samples was at 32°C while the ripe plantain peel gave maximium yield at 34°C (Fig. 1). In pineapple peel, a peak in ethanol concentration could be observed at 28°C to 32°C which gradually reduced at 36°C. Similarly in cashew fruit, we observed a rise in concentration from 4.30 to 6.28 at 28°C to 32°C and it reduced to 3.20 at 36°C. In ripe plantain peel, it showed initially a reduction in concentration at 28°C to 30°C which increased to 34°C (maximum) and then reduced to 36°C.

Table 1 — Effect of specific gravity on concentration of ethanol in pineapple peel, cashew fruit and ripe plantain peel

pineapple peel		cashew fruit		ripe plantain peel	
Specific gravity (SG 2-SG 1)	Conc. (%)	Specific gravity (SG 2-SG 1)	Conc. (%)	Specific gravity (SG 2-SG 1)	Conc. (%)
0.886	4.60	0.986	2.60	1.310	2.9
0.878	5.40	0.878	3.90	1.260	3.4
0.865	6.10	0.785	6.30	1.100	4.3
0.856	4.30	0.756	5.10	0.972	5.6
0.851	3.60	0.712	4.10	0.741	3.8

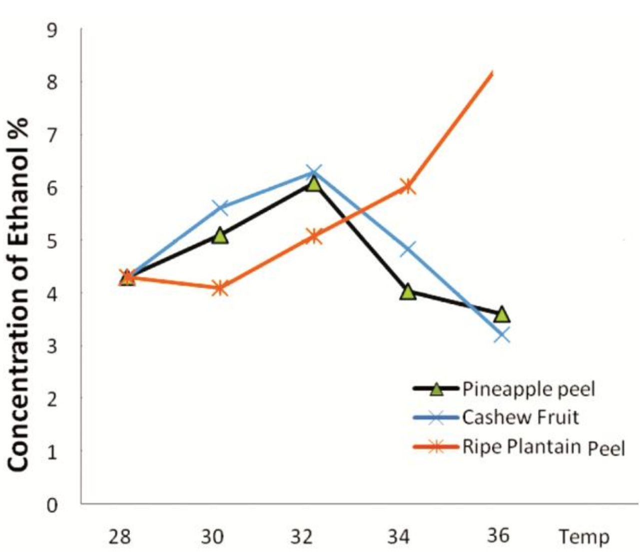

Fig. 1 — Effect of temperature on concentration of ethanol in (A) pineapple peel; (B) cashew fruit; and (C) ripe plantain peel

Effect of pH on concentration of ethanol in fruit juices

Effect of pH on concentration of ethanol in all the three samples, pineapple peel, cashew fruit waste and ripe plantain peel revealed that the maximum yield of ethanol was obtained at a pH of 6 and minimum at 8 (Table 2). There was a gradual rice in concentration from pH 4 to 6 which got reduced at pH 7 and 8. In ripe plantain peel, the concentration of ethanol gradually increased from a pH 4 to 6 up to a concentration of 6.60 and decreased to 3.9 at pH 7 and then increased slightly to 4.02 at pH 8. Effect of pH on the concentration of ethanol showed the maximum yield of ethanol from plantain peel at a pH of 6 and minimum at 8, approximately close to the constant value of ethanol (Table 2). The results showed that increase in pH from 4.0 to 5.0 yields an increase in alcohol concentration, productivity as well as efficiency. The optimum pH range for *S. cerevicea* strain HAU-1 was found to be between pH 4.5-5.0[17].

Table 2 — Effect of pH on concentration and specific gravity of ethanol in pineapple peel, cashew fruit and ripe plantain peel

pH	SG-1 (Before Fermentation)	SG-2 (After Fermentation)	SG2-SG1	Conc._SG2-SG1 0.0074
pineapple peel				
4	11.62	11.58	0.034	4.60
5	11.08	11.04	0.039	5.40
6	11.36	11.31	0.047	6.40
7	10.98	10.94	0.031	4.30
8	10.76	10.73	0.029	4.02
cashew fruit				
4	10.37	10.460	0.041	5.60
5	10.06	10.092	0.032	4.40
6	10.28	10.327	0.047	6.40
7	09.82	09.851	0.031	4.20
8	09.76	09.789	0.029	4.02
ripe plantain peel				
4	10.39	10.425	0.035	4.80
5	10.28	10.319	0.039	5.40
6	10.16	10.208	0.048	6.60
7	10.07	10.098	0.028	3.90
8	09.97	09.999	0.029	4.02

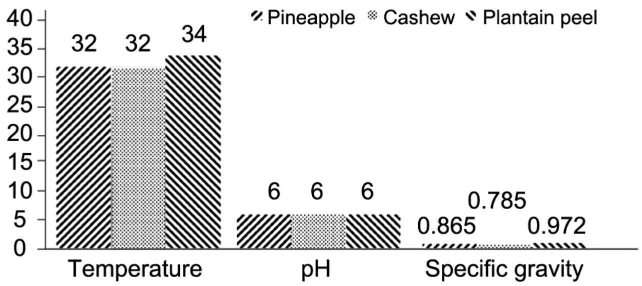

Fig. 2 — Effect of concentration of ethanol on temperature, pH and specific gravity of studied species

Effect of concentration of ethanol on temperature, pH and specific gravity of fruit juices

From this study, it is clear that the maximum yield of ethanol was obtained at temperature 32°C; pH 6 and specific gravity 0.865 in pineapple, temperature 32°C; pH 6 and specific gravity 0.785 Cashew fruit and temperature 34°C; pH 6 and specific gravity 0.972 in ripe plantain peel in which is appropriately close the constant value of ethanol. Fig. 2 shows the effect of concentration of ethanol on temperature, P^H and specific gravity of studied species. This study reveals the increase in ethanol production and optimization of various physiochemical parameters. This process is environment friendly and the leftover residues after fermentation can be disposed in the soil acting as a fertilizer for the soil.

Conclusion

In our present study we observed that the cashew fruit, pineapple peel, ripe plantation peel have ability to produce reducing sugar and it could be fermented using immobilized yeast at different temperature, pH and specific gravity. The results showed that the maximum yield of ethanol was obtained at temperature 32°C; pH 6 and specific gravity 0.865 in pineapple, temperature 32°C; pH 6 and specific gravity 0.785 in cashew fruit and temperature 34°C; pH 6 and specific gravity 0.972 in ripe plantain peel. Since the fermentation conditions are optimized, this procedure may be used for large scale production of bioethanol from fruit wastes.

References

1 Demirbas A, Biofuels sources, biofuel policy, biofuel economy and global biofuel projections. *Energy Convers Manag*e, 49 (2008) 2106.

2 Demirbas A, Political, economical and environmental impacts of biofuels: a review. *Appl Energy*, 86 (2009) 108.

3 Joshi VK & Sndhu DK, Preparation and evaluation of an animal feed byproduct produced by solid state fermentation of apple pomace. *Bioresour Technol*, 56 (1996) 251.

4 Oniya C, A potential crop in (the) People's of Republic of China. *Appl Energy*, 86 (2014) S95.

5 Alonso-Pippo W, Luengo CA, and Alberteris LA, Practical implementation of liquid biofuels: the transferability of the Brazilian experiences. *Energy Policy*, 60 (2013)70.

6 Demirbas A, Competitive liquid biofuels from biomass. *Appl Energy*, 88 (2011) 17.

7 Jones AM, Thomas KC & Inglew WM, Ethanolic fermentation of molasses and sugarcane juice using very high gravity technology. *J Agric Chem*., 42 (1994) 1242

8 Demirbas A, Waste management, waste resource facilities and waste conversion processes. *Energy Convers Manage*, 52 (2011) 1280.

9 Kun LY, *Microbial biotechnology: principles and application*. (World Scientific Publishing Co. Pvt. Ltd, Singapore), 2003.

10 International Energy Agency. World energy outlook. (2011) [Retrieved 2013 Nov 8]. http://www.iea.org/publications/freepublications/publication/WEO2011_ WEB.pdf

11 Fuel Grohmann, Ethanol after 25 years. *Trends Biotechnol,* 17 (1999) 482.

12 Sharma KD & Hudson D, The potential viability of biomass ethanol as a renewable fuel source: a discussion. (Staff Report, 003; Starkville, Mississipi State University, USA), 2007.

13 Itelima. Acceleration of high gravity yeast fermentation by acetaldehyde addition. *Biotechnol Lett,* 24 (2013) 891.

14 Kanokphormsangkhark. Optimization of fermentation temperature and mash specific gravity for fuel alcohol production. *Cereal Chem*, 76 (1999) 82.
Bothast RJ & Schlicher MA, Biotechnological processes for conversion of corn into ethanol. *Appl Microbiol Biotechnol*, 67 (2011) 19.

15 Miller GL, Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem,* 31 (1959) 426.

16 Yadav AN, Dilbaghi & Sharma; Pretreatment of sugarcane molasses for ethanol production by east, *Indian J Microbiol*, 37 (1997) 37.