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Production of biofuel from fruits and vegetable wastes

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Abstract
Production of biofuel from fruits and vegetable waste has been carried out with the singular aim of converting the waste to useful material. The Bioethanol was produced by using fruit waste like Indian water chestnut, sweet potato, jackfruit and pineapple. To achieve this, the conversion of fruits and vegetable wastes were respectively carried out via acid hydrolysis, which yielded fermentable sugar. A pure culture of *Saccharomyces cerevisiae* was used to carry out the fermentation process. It was seen that after fermentation, pineapple waste produced 0.090% (0.90 mg/ml) ethanol, sweet potato waste produced 0.079% (0.79 mg/ml) ethanol, Indian water chestnut waste produced 0.045% (0.45ml/ml) ethanol whereas jackfruit waste produced 0.045% (0.45 mg/ml) ethanol. The study considered the enormous amount of fruits and vegetable wastes thrown away daily in every household, and therefore embarked upon the conversion of these wastes into a useful product, ethanol.

Key words: fruits and vegetable wastes, biofuel, fermentation, conversion, distillation

1. Introduction
Aegle marmelos (L) Corr. Family Rutaceae commonly known as *Bael* is one of the most important medicinal plant as well as a fruit plant throughout the tropical countries. All parts of *Aegle marmelos*, such as the roots, bark, leaves, flowers, fruits Fossil fuels immensely contribute to environmental pollution, degradation and also enhance greenhouse gas emission leading to depletion of ozone layer (Rabah *et al.* 2014). The burning of fossil fuels at the current rate is likely to create an environmental crisis globally through the generation of carbon (IV) oxide (CO_2), methane (CH_4), and a significant quantity of nitrous oxides. Most of these harmful gases are formed due to incomplete combustion of fossil fuels. As a result of this, there is a growing international quest for an alternative energy source. Ethanol produced from biomass through fermentation contains 35% O_2 that may result in a more complete combustion of fuel and thus reduces tailpipe emissions (Chandel *et al*. 2007) [3].

Biofuel is a renewable source of energy and hence can be used as an alternative to conventional fossil fuels. It burns up to 75% cleaner than fossil fuels. Use of agricultural waste having no economic value for biofuel production gives a better way of efficiently utilizing agricultural land. Sugarcane molasses, groundnut shells, rice husks, straw, corncobs, etc. are being studied as substrates for biofuel production. Biofuel is produced through contemporary biological processes, such as agriculture and anaerobic digestion, rather than a fuel produced by geological processes. Biofuel are a wide range of fuels which are derived from biomass or plant matter and animal waste as well as organic waste materials. Biofuels are also derived from industrial and municipal waste, and forestry / agricultural residues (Oniya *et al*. 2014) [14].

Bioethanol is produced from any fodder crop which contains simple sugar in abundance or their polymers (Hughes *et al.* 2009) [8]. The polymers like starch and cellulose are broken down into simple sugars through chemical hydrolysis or enzymatic hydrolysis (saccharification), and then converted by fermentation process to ethanol and carbon dioxide (Jamai *et al.*, 2007) [9], in saccharification, process starch is converted into simple sugar(monosaccharide) using microorganism or enzymes such as glucoamylase and α-amylase (Shapouri *et al*., 2004) [17].

Yeast, fungi, certain microalgae and genetically modified microorganisms are used as feedstock for biofuel production (Gohel *et al.*, 2013) [5]. The yeast, *Saccharomyces cerevisiae*, produces ethanol by fermentation of glucose. But it is unable to ferment pentose sugars. Sufficient biomass of yeast can be produced using fermentors and other advantages include smaller area for production as compared to plants, easy extraction method and ability to grow on a wide variety of media (Gohel *et al.*, 2013) [5].

The present study was aimed at converting wastes Indian water chestnuts, sweet potato, pineapple and jackfruit to a valuable product, ethanol fuel via acid hydrolysis. In this study, Indian water chestnut, sweet potato, pineapple and jackfruit waste were selected, as the fruits are rich in carbohydrates, as compared to others. They are easily available in a large amount to produce Bioethanol compared to other waste. The non edible parts of this fruits are used as waste like its outer cover, crown of pineapple and hard covering of Indian water chestnut.

2. Materials and methods
Raw material for fermentation
Fruit/vegetable wastes from Indian water chestnuts, sweet potato, pineapple and jackfruit were used. The selection of fruits waste was done due to their availability. The outer covering of water chestnuts, skin peels of sweet potatoes, the leafy shoots of pine apple and the fruit waste of jackfruits were collected from the fruit market and household waste. The collected waste was washed with the tap water. The obtained material was cut into small pieces, kept in a tray and allowed to oven dried for 24 to 48 hrs to remove the moisture. Oven dried fruits waste was powdered using a blender and stored in an air tight container.

Organisms used
Saccharomyces cerevisiae (Baker's yeast) was used for fermentation. Selection of given yeast was done due to their ability to produce high yield of alcohol by fermenting sugars. The yeast granules were grown for 24-48 hrs at room temperature in Sabouraud's broth. The broth was streaked on sterile Sabouraud's agar plates. Pure cultures of *Saccharomyces cerevisiae* were maintained on Sabouraud's agar slants. Regular subculturing was carried out in 1- 2 weeks. 24- 48 hr grown cultures were used for the study.

Acid hydrolysis
The fine powder of the fruit waste was subjected to acid hydrolysis using sulphuric acid (20ml) of various concentrations *i.e.* 2%, 4%, 6%, 8%, and 10%. The mixture was autoclaved at 121˚C, 15psi for 20 min and further cooled to room temperature. The hydrolysate was filtered to remove the residue. The hydrolysate was neutralized using Sodium hydroxide. The total amount of total carbohydrates, glucose and xylose present in filtrates was checked, which helped to narrow down the concentration of H_2SO_4 at which best hydrolysis was obtained. From the standard graph, the total carbohydrates derived from Indian water chestnut waste, sweet potato waste, pineapple waste and jackfruit waste using various concentration of H_2SO_4 were extrapolated.

Estimation of total carbohydrates
Total amount of carbohydrates present was estimated by Phenol-Sulphuric acid method using glucose as a standard having a concentration of 1 mg/ml and the colorimetric reading was taken at 490nm (Sadashivam, 2004) [16].

Estimation of glucose and xylose
The amount of glucose present in the neutralized hydrolysate was estimated by DNSA method and the colorimetric reading was taken at 540nm (Sadashivam, 2004) [16]. Estimation of Xylose present was carried out by Phloroglucinol assay and the colorimetric reading was taken at 540nm (Ayudhya *et al.*, 2007) [2]. Glucose and xylose sugars were used as standards having a concentration of 1mg/ml and 0.5 mg/ml resp.

Detoxification of hydrolysate
The obtained filtrates from acid hydrolysis were heated at 60 °C. Sodium hydroxide was added till the pH reached 9.0 - 9.5. For the detoxification of harmful materials in the hydrolysate, calcium hydroxide powder was added till the pH reached 10.0. Using Whatman filter paper 1, the mixture was filtered to remove insoluble residues and the supernatant was collected (Ayudhya *et al*., 2007) [2]. The amount of Xylose and Glucose was determined by Phloroglucinol assay and DNSA assay respectively.

Preparation of Fermentation medium
1.6g of Peptone was added to the neutralized hydrolysate (pH 5.6) obtained. It was autoclaved at 121˚C, 15 psi for 15 mins and used as fermentation medium. The amount of glucose was estimated by DNSA method.

Fermentation process
Pure culture of *S. cerevisiae* was inoculated into the sterile Sabouraud broth. Flasks were incubated at room temperature and kept at both static and shaker conditions. The cell density of the *S. cerevisiae* suspension was checked using Haemocytometer count. 2ml of culture (0.529×10^6cells/mm^3) was then added to the fermentation medium and allowed to ferment. Aliquots were removed at different time intervals and subjected to distillation. Distilled samples were subjected to ethanol estimation by Dichromate method (Zimmermann, 1963). A standard set of tubes containing 0.2 – 1.0 mg/ml alcohol was run and from the standard graph, the total alcohol content in distillate was estimated.

Minimum inhibitory concentration (MIC) determination of alcohol and sugars
MIC of alcohol: Minimum Inhibitory Concentration of alcohol for *S.cerevisiae* was found by using 30% alcohol by tube turbidity method. A standard concentration of 30% alcohol solution was made is sterile Sabouraud broth. The diluent was plain sterile Sabouraud broth. The tubes were incubated at 37^0C for 24 hrs. The tubes were checked for visible growth (Mazzola *et al.,* 2001).

MIC of glucose and xylose: Minimum Inhibitory Concentration of glucose for *S.cerevisiae* was found by using 80% glucose by tube turbidity method. A standard concentration of 80% glucose and 50% xylose solution was made is sterile Sabouraud broth. The diluent was plain sterile Sabouraud broth. The tubes were incubated at 37^0C for 24 hrs. The tubes were checked for visible growth (Mazzola *et al.*, 2001).

3. Result and discussion
The selection of fruit wastes like Indian water chestnut, sweet potato, pineapple and jackfruit was selected on the basis of the carbohydrate amount of the fruit. The selection of organism *Saccharomyces cerevisiae* is done as it is ability to convert sugar into ethanol by fermentation process. A pure culture of *Saccharomyces cerevisiae* was isolated. A monochrome staining was performed to check for its purity and the growth characteristics were studied. Pure cultures of *Saccharomyces cerevisiae* were maintained on Sabouraud agar slants. Regular subculturing was carried out in 1- 2 weeks. 24- 48 hr gown cultures were used for the study.

MIC of Alcohol, Glucose and Xylose
Saccharomyces cerevisiae could tolerate up to 18% alcohol, 50% glucose and 30%xylose. MIC range for Alcohol, glucose and xylose for *Saccharomyces cerevisiae* was found to lie in the range of 18.1% - 21% alcohol, 51% - 60% glucose and 30% - 35% xylose.

Estimation of total carbohydrates, glucose and xylose
The total amount of carbohydrates obtained after hydrolysis of 1 gram of Indian water chestnut waste, sweet potato waste, pineapple waste and jackfruit waste powder was estimated using Phenol sulfuric acid assay. The hydrolysis was carried out using 20 ml of various concentrations of sulphuric acid – 2%, 4%, 6%, 8% and 10% H_2SO_4 respectively.
The total amount of carbohydrates in 2% acid hydrolysate was found to be highest after the acid hydrolysis step (Figure 1). Therefore further studies were carried out using 2% H_2SO_4 for hydrolysis of the given fruits waste.

For further study, 5 grams of Indian water chestnut waste, sweet potato waste, pineapple waste and jackfruit waste powder was hydrolysed using 100 ml of 2% of H_2SO_4. The hydrolysate obtained after acid hydrolysis contained Glucose and Xylose as the two main fermentable sugars which were further quantified by DNSA and Phloroglucinol methods respectively.

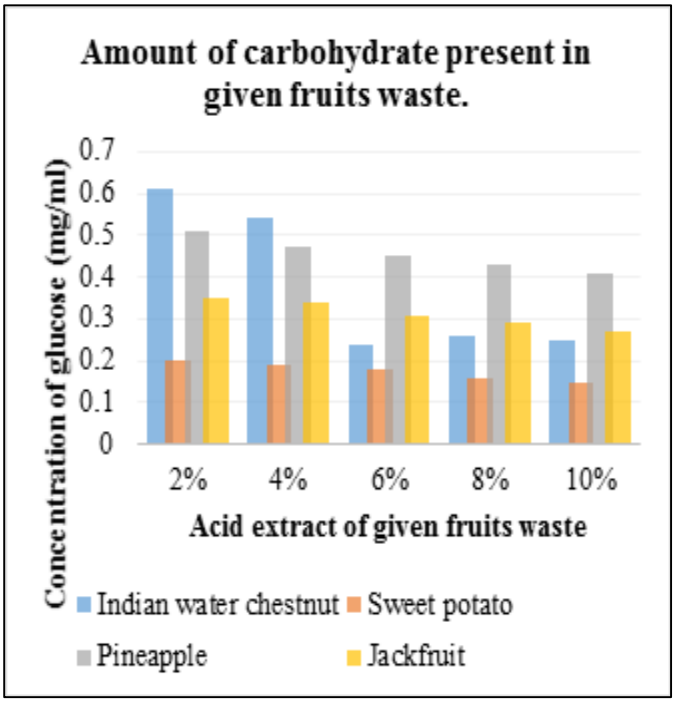

Fig 1: Total Carbohydrates present in wastes after acid hydrolysis.

The acid hydrolysate which was obtained after step 1, step 2 and step 3 of the methodology was subjected to DNSA assay to find the total glucose (Figure 2) and total xylose (Figure 3) content. Prior to DNSA method, the pH of the medium was checked and made alkaline if required.

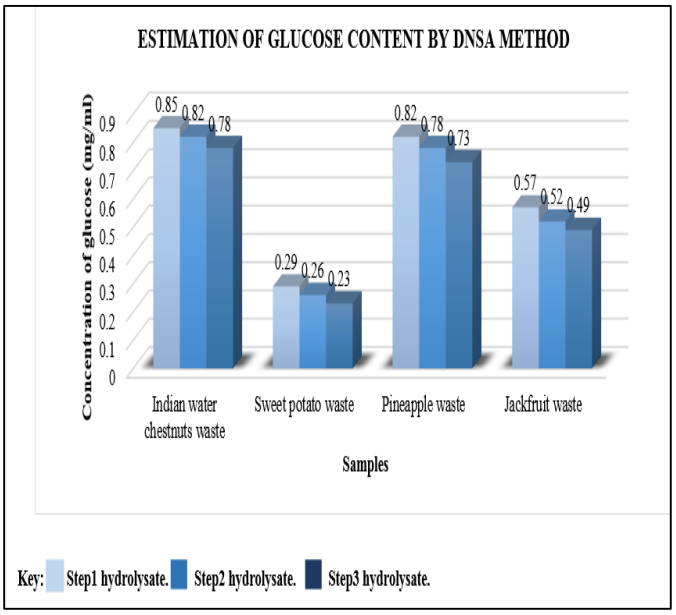

Fig 2: Estimation of glucose content using DNSA assay.

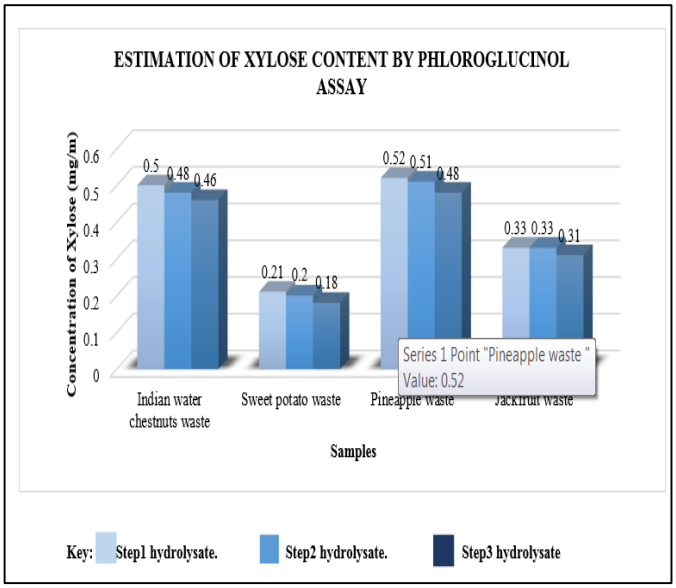

Fig 3: Estimation of Xylose content using Phloroglucinol assay

Detoxification step was carried out to check if it imparts any benefit in terms of cell growth in the Indian water chestnut waste, sweet potato waste, pineapple waste and jackfruit waste medium or whether it impedes the growth of yeast.

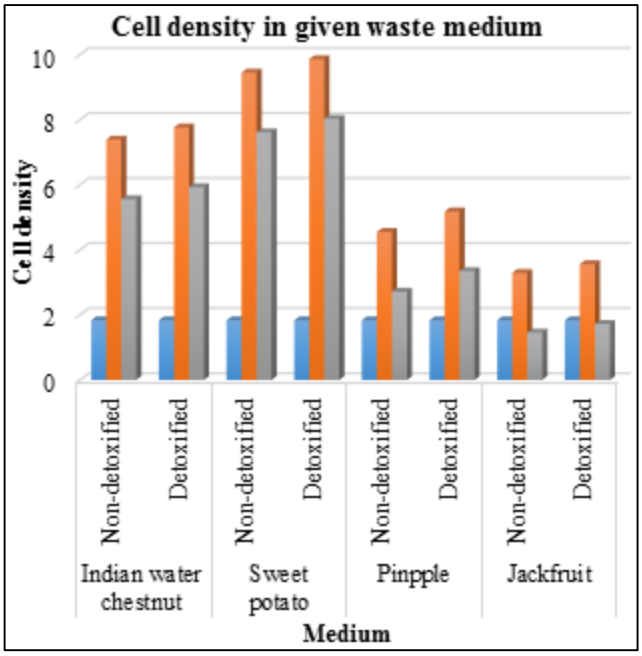

Fig 4: Cell density in given fruits waste medium

It was found that there was a no increase in cell density (cells/ml) of yeast inoculated in the non-detoxified Indian water chestnut waste, sweet potato waste, pineapple waste and jackfruit waste medium as compared to the cell density (cells/ml) seen in the detoxified Indian water chestnut waste, sweet potato waste, pineapple waste and jackfruit waste medium after 48 hours of incubation. There was a steady increase in cell density (Figure 4). Therefore this detoxification step was found to be necessary as it was seen that the non-detoxified Indian water chestnut waste, sweet potato waste, pineapple waste and jackfruit waste medium

impedes the growth of yeast and will thereby also affect the ability of the yeast to ferment the sugars to alcohol.

It is also seen that furfural, a by-product of xylose degradation, was also generated as a consequence of acid hydrolysis (Ackerson *et al.*, 1981) [1]. The rate of degradation depends on temperature and concentration of Sulfuric acid (Gonzales *et al.*, 1986) [6]. Therefore, over liming with $Ca(OH)_2$ and heating at high temperature are required for removal or reduction of volatile compounds (*e.g.* Furfural and phenol), acetic acid and tannic acid, which is generally resulting in better fermentability of the hydrolysate (Martinez *et al.*, 2000) [12].

Estimation of alcohol content by Dichromate assay

The amount of alcohol produced by the *Saccharomyces cerevisiae* in the fermentation broth was found out by Dichromate method.

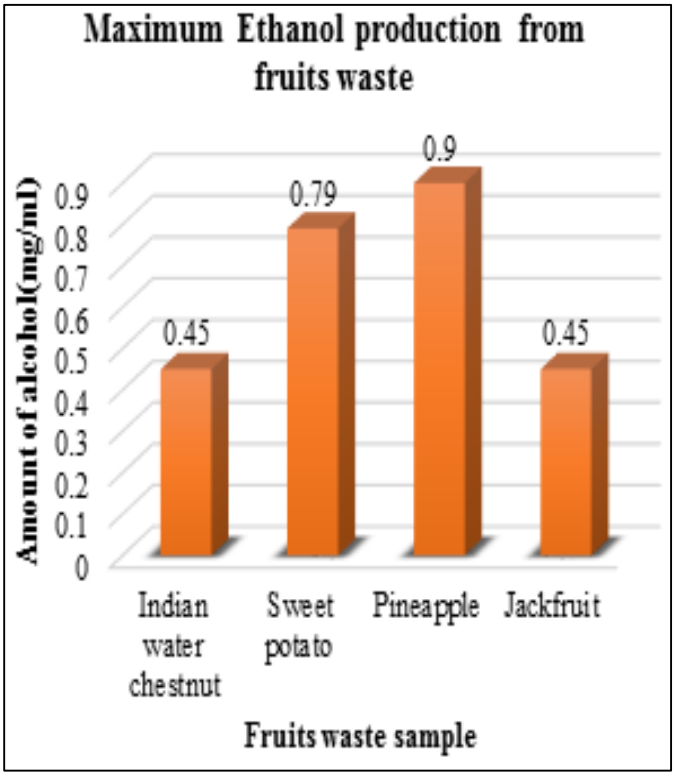

Fig 5: Maximum ethanol produced from the given fruits waste

It was seen that after fermentation using *Saccharomyces cerevisiae*, pineapple waste produced 0.090% (0.90 mg/ml) ethanol, sweet potato waste produced 0.079% (0.79 mg/ml) ethanol, Indian water chestnut waste produced 0.045% (0.45 mg/ml) ethanol whereas jackfruit waste produced 0.045% (0.45 mg/ml) ethanol (Figure 5). The amount of ethanol produced was comparatively less in comparison to the work carried out by Hossain *et al.* (2008), Kumar *et al.* (2015) [10] and Kumoro *et al.* (2012) [11].

The fermentation process was carried out for 11 days. It was observed that the alcohol production was highest on day 3 for Indian water chestnut waste (0.45 mg/ml) and Jackfruit waste (0.45 mg/ml) resp. whereas maximum alcohol production was seen on day 5 when sweet potato waste (0.78 mg/ml) and pineapple waste (0.9 mg/ml) was used. There was steady decrease in the alcohol production as the days proceded indicating the glucose level decreasing (Figure 6).

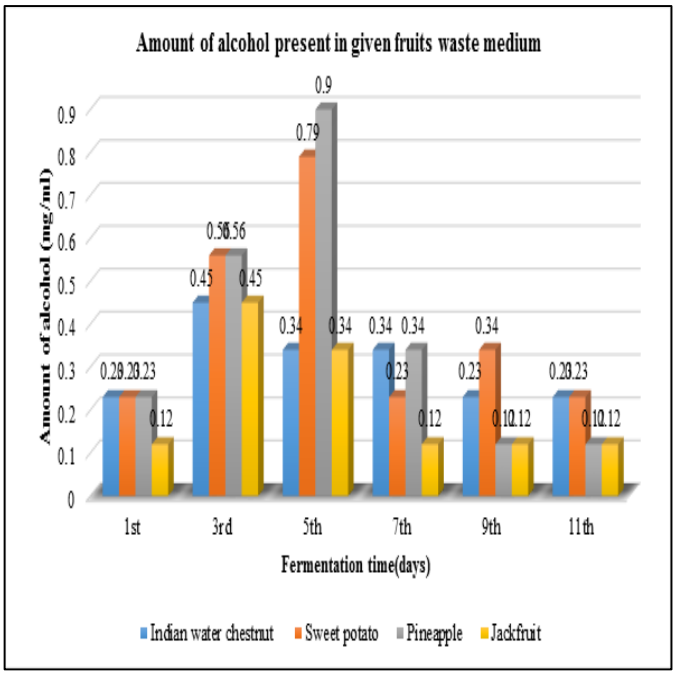

Fig 6: Alcohol content in given fruits waste produced by *Saccharomyces cerevisiae***.**

Hossain *et al.*, (2010) [7] reported rotten pineapple produce 8.7% ethanol. Kumar *et al.*, (2015) [10] used raw material as sweet potato for the production of ethanol and the production as found to be 0.15%. Kumoro *et al.*, (2012) [11] used jackfruit juice and the ethanol production was seen to be 12.13%. This vast variation may due to the fewer amounts of fermentable sugars present in wastes of pineapple, sweet potatoes, Indian water chestnut and the jackfruit in comparison with the whole fruit or fruit juices which were used.

4. Conclusion

India is amongst top five bioethanol producing countries (Demirbas, 2009). Currently bioethanol is produced from alcoholic fermentation of molasses or simple sugars, which are produced from crops generating starch or sugar.The bioethanol was produced by using fruit waste like Indian water chestnut, sweet potato, jackfruit and pineapple with the help of *Saccharomyces cerevisiae*. Maximum amount of bioethanol was obtained from the pineapple waste and then sweet potato waste *i.e.* 0.090% and 0.079% respectively whereas bioethanol obtained from the Indian water chestnut waste and the jackfruit waste was 0.045%. The method used is a simple, reliable process for economical bioconversion of given fruit waste to alcohol. The waste part of fruit is easily available at large amount and they obtained at free of cost compare to the whole fruit which help to reduce the cost of biofuel. Further standardization of the process needs to be carried out for optimum bioethanol production.

5. Acknowledgment

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