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Studies on efficient utilization of fruit peel waste for Bio-Ethanol production

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Abstract
The current work is a basic study on utilization of fruit peels of banana, muskmelon, pineapple and water melonas lignocellulosic sources for the production of bioethanol. Fruit peels contain good amounts of cellulose and hemicellulose which can be pretreated to release fermentable sugars. Efforts were directed towards understanding critical factors influencing substrate loading, acid hydrolysis and fermentation parameters. Various concentrations of substrate (10, 15 and 25%), acid treatment (0.5 and 1%) and Incubation time(60-180 min) were the factors considered for pretreatment optimization. It was observed that the reducing sugar content was highest in the peels treated with 0.5% acid with an incubation period of 1 hour at 60^0C.The highest yields of ethanol were observed in samples at 10% substrate concentration after about 7days.

Keywords: Fruit peels, Bioethanol, pretreatment, acid hydrolysis, Lignocellulosic biomass

Introduction

Today's critical challenge is to sustainably opt for clean energy technologies which is going to aid multidimensional growth and progress, the focus being lesser dependence on non-renewable energy sources and improvement of global climate. Overreliance on fossil fuels in industry for fast economic growth has led to severe depletion in the quality of earth's environment [Baruah *et al*, 2018] [1]. Therefore there is an immediate need to switch over to noninvasive, resilient and efficient energy systems which will help mitigate emissions and also sustain high energy generation, conversions and utilizations [Walker *et al*, 2011; Oliva *et al*, 2017] [2, 3]. Hence there arises a primary obligation to understand the details of large scale clean technology applications with respect to use of low carbon emission energy sources, their socio-economic feasibility and the challenges to overcome in adopting newer energy systems [Behera *et al*, 2014; Chen *et al*, 2017] [4, 5]. As per the Kyoto Protocol [UNFCC 1998] and its subsequent Doha amendment [UNFCC, 2012] [12], the major concern to target in the current world scenario has been to limit, reduce or eliminate emissions in the form of Green House gases (GHG) from the Earth's atmosphere so as to avoid drastic impacts to climate [Hanaki *et al*., 2018] [8].

That rampant use fossil fuels like coal, oil and natural gases have contributed significantly in GHG emission has been documented [Oliva *et al*., 2017] [3]. So it is mandatory to opt for an environmentally safe biofuel with lesser emissions and costs [Sarkar *et al*, 2012, Wang *et al*, 1999] [9, 10]. Bioenergy forms like bioethanol, a second generation biofuel that can be efficiently obtained from lignocellulosic biomasses of plant wastes serve as an alternate and clean fuel source [Hossain *et al*., 2008; 2013] [11]. Since lignocellulosic wastes comprise a renewable and sustainable resource for the production of bioethanol, studies on the various methodologies involved in their efficient utilization for higher yields of bioethanol needs to be prioritized [Menon *et al*., 2012; Meng *et al*., 2014] [12, 13].

Lignocellulosic materials are agrowastes made of polymeric carbohydrates consisting of cellulose (30-45%) and hemicelluloses (20-30%) in close association with lignin (15-20%)and acetyl groups that are highly resistant to hydrolysis [Zhao *et al*, 2017; Kumar *et al*, 2017; Zoghlami A *et al*, 2019] [14, 16, 32]. Since these form the major sources for second generation biofuels, pretreatment becomes a prerequisite to modify the carbohydrate polymers and convert them into fermentable sugars [Guerriero *et al*., 2015] [32]. Pretreatment approaches basically aim at working on factors like lignin based cell wall structures, available surface area, crystalline nature of cellulose and extent of carbohydrate polymerization [Myat *et al*, 2015] [18]. Different types of lignocellulosic biomasses are available *viz*. agriculture wastes generated from crop residues, fruits and vegetables, agricultural and forest residues and each of these biomasses require various types of pretreatments depending on their complex physical structure andchemical composition. Hence there arises a need for various strategized pretreatments like mechanical milling and grinding, pyrolysis, high pressure steaming, acid or alkaline hydrolysis, hydrogen peroxide treatment, organic sovlent based treatment or biological treatments [Baruah *et al*, 2018] [1]. The major aim of all the pretreatment methods is to convert the cellulose content in the lignocellulosic biomasses into glucose which can be further fermented into bioethanol [Kumari *et al*., 2018] [1].

Low cost, sustainable production of bioethanol from renewable sources currently serves two purposes, *viz*., a) to target negative carbon emission biofuels which will make us less reliable on petroleum and b) manufacture of massive volumes of sanitizers (Since ethanol/bioethanol is a proven germicide and a sanitizing agent). Since all protocols pertaining to Covid pandemic management require exhaustive use of sanitizers it becomes imperative for the research fraternity to assert for efficient bioethanol protocols.

The current work is therefore targeted on utilization of fruit peels as a lignocellulosic source for production of bioethanol. Fruit wastes are mainly generated through domestic consumption, industrial production of processed foods, bruising/over-ripening of fruits and lack of storage during post harvest stages [Anderson *et al*., 2008; Hu *et al*, 2011] [20]. Fruit peels in particular are rich in sugars; do not have any commercial importance and are often dumped with municipal waste in landfills [Oberoi *et al*., 2010; Sanchez-Orozco *et al*., 2012] [22]. Hence their utility as a second generation feedstock helps in waste management as well as a source to generate bioethanol which is of commercial importance [Mtui, 2009, Ayala *et al*., 2017, 2021] [24, 26]. These usually contain cellulose, hemicellulose lignin and pectin are serve as good raw materials to obtain fermentable sugars [Oberoi *et al*, 2010] [22]. However, as discussed earlier, it is necessary to optimize the pretreatment methods to enhance the availability of fermentable sugars from these raw materials for better yields of bioethanol. The current research focus at studying various parameters such as substrate loading, conditions for acid hydrolysis and fermentation settings required for enhanced bioethanol yield from various fruit wastes.

Materials and Methods

Sample collection and processing

The fruit wastes of banana, muskmelon, pineapple and watermelon were collected from the local fruit markets and juice outlets of Mysore, INDIA. The fruit peel samples were collected into ziplock polythene bags. Before processing the fruit peels were cut into small pieces of 1-2 cm for easy handling and oven dried60^{0}C for about 18-24 hours. The dried peels were pulverizedby milling in a mechanical grinder, sieved in 1mm mesh size and stored in air tight polythene covers at room temperature (**Figure. 1**)

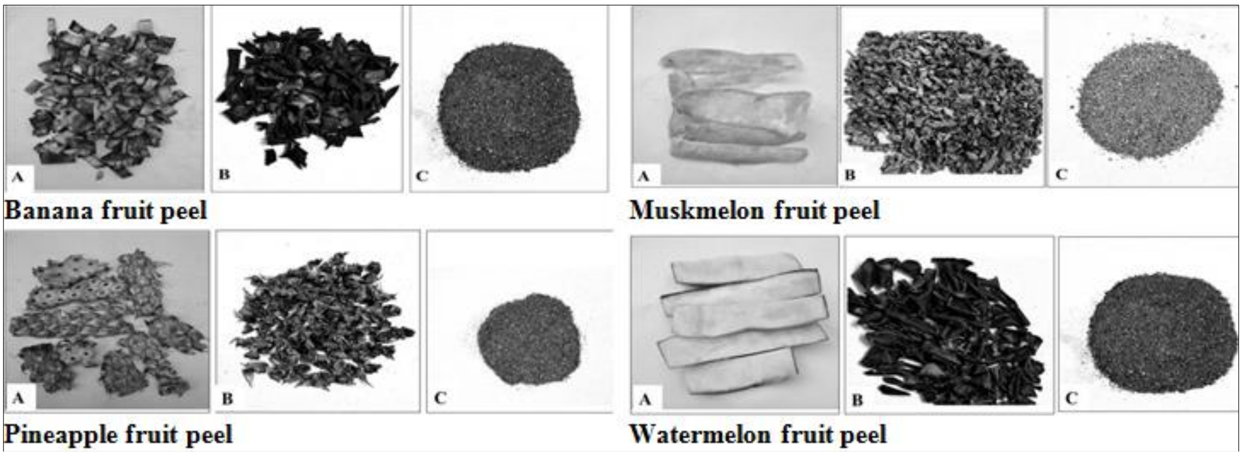

Fig 1: Processing of fruit peels samples; A) Fresh, B) Dried, C) Powdered

Pretreatment-acid hydrolysis of fruit wastes

To confirm the optimum time and acid concentration for acidification, different dried fruit peels (banana, muskmelon, pineapple and watermelon wastes) were subjected to acid hydrolysis separately by treating them with various concentrations of sulphuric acid (0, 0.5 and 1%) and soaked at three different time intervals *viz*. 0, 60 and 180 minutes. Post acid treatment, they were autoclaved at 121 ^{0}C for 15 minutes and allowed to cool at room temperature. Samples were drawn for the analysis of total sugars and reducing sugars content.

Solid – Liquid ratio

Three different substrate concentrations were taken in separate conical flasks. 200 ml of distilled water was added to each conical flask. These were pre-treated with 0.5% concentrated sulphuric acid for 60 min followed by autoclaving at 121℃ for 15 minutes followed inoculation of yeast for alcohol fermentation.

Estimation of total sugar/carbohydratecontent

The total sugar/carbohydrate content in the sample was estimated by phenol sulphuric acid method [Dubios *et al*., 1956] and the yellow brown colored hydroxymethyl furfural-phenol complex was spectroscopically measured at 490nm.

A calibration curve was generated for the above spectroscopic assay and was used to determine the total sugar content in the dried fruit peel samples.

Estimation of reducing sugar content

3,5-Dinitrosalicylic acid (DNSA) method was used to estimate the total reducing sugars content in the dried fruit peels with D-glucose being used as a standard[Miller,1959].

A calibration curve was plotted for the above spectroscopic anlysis and was used to determine the reducing sugar content in the dried fruit peel samples.

Preparation of Inoculum

About 1 ml of yeast (*S. cerevaciae*) inoculum was taken and inoculated aseptically in to Yeast extract dextrosebroth (YPD broth) and incubated in a rotatory shaker at 150 rpm for 24 hours at 37^{0}C. 5 ml of this culture was bulked to 1000 ml of YPD broth and again kept in the rotatory shaker under same conditions with constant colorimetric monitoringat 600nm. When the OD value of the culture reached 1.0(1x10^{8}cells/ml), an aliquote of this culture (2ml/100 ml) was used as an inoculum.

Inoculation and Fermentation

The pretreated fruitpeels in the flasks were sterilized and inoculated with 2ml of the above inoculums and left undisturbed for a lab scale batch fermentation that was carried out at 37^{0}C and on a rotatory shaker incubator (120-150rpm). Samples were drawn on the 7, 14 and 21 day for spectrophotometric estimation of alcohol by Chromic acid method [Caputi *et al*., 1968] [29].

Distillation of Alcohol

The fermented fruit peels were subjected to distillation at a temperature of 60°C. The condensed alcohol was collected, measured and stored in air tight glass bottles under dark conditions.

Results and Discussion

Pre-treatment is an important step for the biochemical conversion of lignocellulosic biomass into bio-ethanol by converting them into simple sugars. In the current experimental setup, sulphuric acid is used for hydrolysis. Reducing sugar concentrations were found to increase with acid treatment at 0.5 %. It was evident from the results that further increase in acid content not shown much effect on reducing sugar content in all the studied (Table.1)

Table 1: Total and Reducing sugar concentrations(before and after pretreatment with 0.5% and 1% Sulphuric acid)

Fruits waste samples	Concentrations of sulphuric acid in %					
	0		0.5		1	
	TS(mg/g)	RS (mg/g)	TS (mg/g)	RS (mg/g)	TS (mg/g)	RS (mg/g)
Banana	7.7	4.6	7.6	6.5	7.5	6.4
Muskmelon	7.8	3.8	7.7	5.0	7.8	5.1
Pineapple	8.1	4.5	7.9	5.8	7.92	5.85
watermelon	5.2	2.8	5.1	3.9	5.0	3.93
TS: total sugar, RS: reducing sugar						

While optimizing time of treatment, 0.5 % acid was used at three time intervals (0, 60 and 180 min). From the results observed, with an increase in incubation time (at 60 min) acid hydrolysis was taken place and obtained higher reducing sugar content with the control (at time 0). Further increase is not shown much effect on hydrolysis. (Table.2). Higher concentration and also higher incubation time has not shown much difference in the yield of reducing sugars comparatively. The same trend is observed in all the cases, hence selected for further studies.

Table 2: Effect of time intervals used for pretreatment of fruit wastes

Fruits waste samples	Time intervals in min					
	0		60		180	
	TS (mg/g)	RS (mg/g)	TS (mg/g)	RS (mg/g)	TS (mg/g)	RS (mg/g)
Banana	7.8	4.4	7.7	6.4	8.0	6.6
Muskmelon	8.0	3.5	7.8	5.3	7.82	5.4
Pineapple	8.2	4.6	7.8	5.4	7.85	5.6
watermelon	5.1	2.6	5.35	4.1	5.5	4.3
TS: total sugar, RS; reducing sugar						

The results obtained were evident that pretreatment with sulphuric acid was found to be a good option for hydrolysis at 0.5 % and incubation time of 60 min. The pretreatment has shown above 50 % more reducing sugar content compared with untreated samples in all the studied cases **(Table.3)**

Table 3: Effect of acid pretreatments on fruit waste

Fruit waste samples	Non-acidified		Acidified	
	Total sugar (mg/g)	Reducing sugar (mg/g)	Total sugar (mg/g)	Reducing sugar (mg/g)
Banana	8.2	5.4	7.6	5.5
Muskmelon	7.8	4.2	7.7	5
Pineapple	8.8	5.7	7.9	5.8
Watermelon	5.2	3.8	5.1	3.9

Fermentation was carried out using 3 varied substrate concentrations with optimized pretreatment conditions. Fermented samples were analyzed for the alcohol content after 7, 14 and 21 days. The study was successful in producing alcohol in all cases with varied yield of alcohol with respect to sample, solid liquid ratio and fermentation period. Alcohol was produced significantly during initial time period i.e., 7th day in all the studied samples. It is observed that when compared to solid content (substrate concentration), with increase in substrate concentration, alcohol content also increased. However, the increase is not much favorable (Table. 4).

Table 4: Alcohol production by different fruit wastes samples

Fruit waste samples	Substrate Concentration (%)	Total percentage of alcohol		
		7th day	14th day	21st day
Banana	5	5.4	5.2	4.8
	10	5.8	6.2	6.8
	15	6.8	7.8	7.9
Muskmelon	5	2.8	3.2	3.1
	10	3.2	3.6	4.2
	15	4.4	4.8	5.0
Pineapple	5	5.2	5.6	5.6
	10	6.6	7.2	7.6
	15	7.4	7.8	8.0
Watermelon	5	3.0	3.6	4.0
	10	4.0	5.2	5.8
	15	4.6	5.8	6.5

In case of lower solid content (5 % substrate) alcohol produce was only upto 7th day, later on it was remained almost the same (not significant improvement) in concentration was observed. In case of 10 and 15 % substrate concentration, the alcohol production was still seen up to 21st day. However, the increase is insignificant. The study indicate that the solid liquid ratio is also has an important role in production of alcohol. Compared to the higher concentrations, lower solid – liquid ratio is more profitable in terms of ethanol production and also the time management. Among the studied samples pine apple and banana wastes have been shown higher yields of bioethanol due to the presence of more carbohydrate contents. Overall studies are efficiently seen in utilizing the waste material to the value addition to the product.

Acid pre-treatment is often used for high yield of simple-sugars from lignocellulosic biomass through hydrolysis which is generally used through concentrated or diluted acids [Mohanty and Abdullah, 2016] [30]. Strong acids such as sulphuric acid, phosphoric acid and hydrochloric acid have been widely used for treating lignocellulosic biomass because of powerful action on cellulose hydrolysis and no enzymes are needed subsequent to the acid hydrolysis [Harmsen *et al*., 2010] [31]. The same is confirmed in the current study also. The efficacy of the treatments have been found to be directly proportional to the concentration of total and reducing sugars estimated post acid hydrolysis of the fruit peels samples, where simple sugar content is directly related to the bioethnol production.

Conclusions

The current study explores the utilization of fruit wastes as a source for the bioethanol production. Optimization of pretreatment methods followed by fermentation using *S. cerevaciae* was carried out. The results indicate that, the pretreatment helped to enhance the availability of free sugars due to acid hydrolysis and fermentation for conversion of sugar to alcohol. Since the substrates used here are the low cost waste materials incurred during fruit processing, this methodology may help in waste utilization process.

Conflict of Interest: None

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