



Steam Explosion as the Pretreatment Method for Ethanol Production from Orange Peel Waste

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ABSTRACT

Citrus fruits world production is estimated to be over 88 million tons per year, and oranges alone account for about 55% of such amount. Orange peel waste (OPW) is the solid residue of membranes, cores, juice sacs, etc which represents over 50% of the processed fruits. Its disposal is of great concern from the environmental point of view. Due to the large availability and composition rich in the present investigation was focused on the production of ethanol from OPW which will be an economic, environmental friendly and efficient approach. Strains of *Aspergillus niger* and *Saccharomyces cerevisiae* was used for hydrolysis and fermentation of pretreated orange waste, respectively. Spectrometry scanning electron microscopy (SEM) and Fourier transformation infrared (FTIR) were used to characterize the sample. Sugar percentage was found to be 3.64 in pretreated sample after 24 hours, which is responsible for the yield of ethanol (6.17 v/v). The results indicated that orange peel can be used as a good low cost alternative feedstock for ethanol production.

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INTRODUCTION

Demand and price of global non-renewable energy resources like natural gas and oil increases day by day. Hence, the search for alternative sources has been attracting worldwide interest [1]. Major countries in the world are facing serious challenges to manage such issues associated with conventional energy sources [2]. In such scenario, alternative and renewable sources of energy gained huge attention from governments of many countries across the world to protect the environment as well as to supply energy needs while reducing dependency on fossil fuels [3]. Worldwide production of ethanol has been estimated around 51,000 million liters [4]. Renewable resources such as fuel ethanol provide numerous benefits in terms of environmental protection, economic benefit and national energy security. Various raw materials can be utilized for ethanol fermentation. In order to select the cost effective process, lignocellulosic biomass is another option for ethanol fermentation. But lignocellulosic biomass [5] (wood, straw) has to undergo

the most complicated pretreatment process prior to fermentation which includes removal of lignin followed by the hydrolysis of cellulose [6]. One alternative method to utilize this waste is to produce fuel grade ethanol as it contains sugars and polysaccharides. It has been reported that the exploitation of these resources may provide a sustainable, efficient, cost-effective, convenient and safe energy supply at a local, regional and national level [7, 8]. Hence, an attempt was made to apply steam explosion for the pretreatment of orange peel waste (OPW) and production of ethanol, as it contains cellulose, hemicellulose and pectin. Extensive work has been done by enzymatic hydrolysis to liquefy the OPW and maximize monomer sugar content. These sugars can be subsequently or simultaneously converted into ethanol by fermentation. As limonene is an inhibitor of yeasts, removal of limonene is a critical step in the production of ethanol from OPW.

MATERIAL AND METHODS

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Dried orange peel waste (OPW) was collected from the local market, washed properly with distilled water for removing adhered soil and other particles and shade dried for about 7 to 10 days. The dried samples material was then pulverized into fine powder using hammer mill and stored in airtight container for further experimental work as shown in Figure 1. The chemical composition of the raw material was determined using the standard laboratory analytical procedures for biomass analysis provided by the National Renewable Energy Laboratory (Colorado). About five grams of OPW was weighed and pretreatment using steam explosion system was carried out at 150°C for 60 minutes. In this procedure the limonene present in the sample was removed. The sample was filtered using Whatman filter paper (0.001mm) and dried at 60°C for overnight.



Figure 1. Pulverized sample of OPW

Pure strains of *Aspergillus niger* and *Saccharomyces cerevisiae* were procured from CTCRI, Bhubaneswar, India and were maintained on PDA and YPD, respectively. 1.5g of each samples (treated as well as untreated) was taken in 250ml flasks containing 150 ml of sodium acetate buffer (50mM, pH 5) to which 0.5ml of the extracellular enzyme extract of *Aspergillus niger* was added and incubated at 50°C for 24 hours. Sugar content was analyzed using DNS method before and after the incubation period. Enzymatic hydrolysis of OPW was performed by re-suspending the solid residues obtained from the pretreatment. After 24 hrs of incubation, one ml of yeast extract (*Saccharomyces cerevisiae*) was inoculated in both control and treated sample at 28°C for 72 hours on a shaker at 120rpm. Ethanol was estimated using UV-visible spectrophotometer (Systonics-2203) at 600nm from the distilled sample by adding dichromate reagent. The functional group of synthesized orange sample was identified using FTIR (JASCO FTIR-420, Japan FTIR). Scanning electron microscope (SEM) analysis was carried out using (Joel Model-JSM 6510) machine. Thin films of the sample were prepared on a carboncoated copper grid by just adding the slight amount of the sample on the grid.

RESULTS AND DISCUSSION

Bioethanol production from orange peel waste using steam explosion pretreatment was studied in this work. In OPW, cellulose amount (17.6%) was found to be higher as compared to untreated sample (10%). Similar results were also shown by [9] according to which the cellulose amount of OPW was 21.2%. The hemicellulose percentage was found to be 9.6 and 7% in OPW and untreated one, respectively. Similar result was also observed in literature [10]. But in case of pectin (%), no significant difference was observed in treated and untreated one as shown in Figure 2

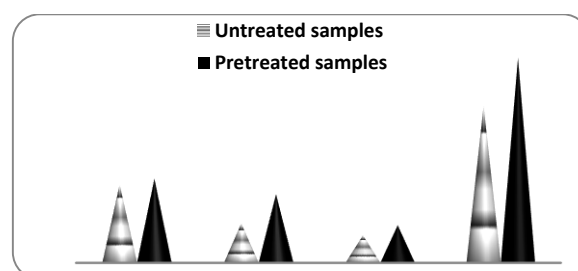


Figure 2. Chemical composition of orange peel waste

During the present investigation, limonene was removed from the OPW sample at optimal temperature of 150 °C in one hour. In the hydrolysis, maximum sugar percentage was found to be 3.64% in pretreated sample after 24 hours. Ethanol yield (v/v) in pretreated samples was 6.17 as compared to untreated i.e. 1.27 as represented in Figure 3.

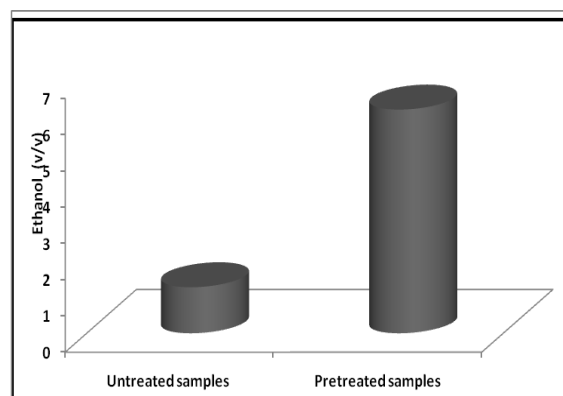


Figure 3. Ethanol yield by OPW

SEM Analysis

Scanning electron microscopy has been used to study the morphology of orange peel waste in each treatment as shown in Figure 4 (a-d).

Fourier transform infrared spectroscopy (FTIR) spectroscopy is widely used to identify the functional groups in complex organic mixtures and to compare the similarities between the substances. In the present study,

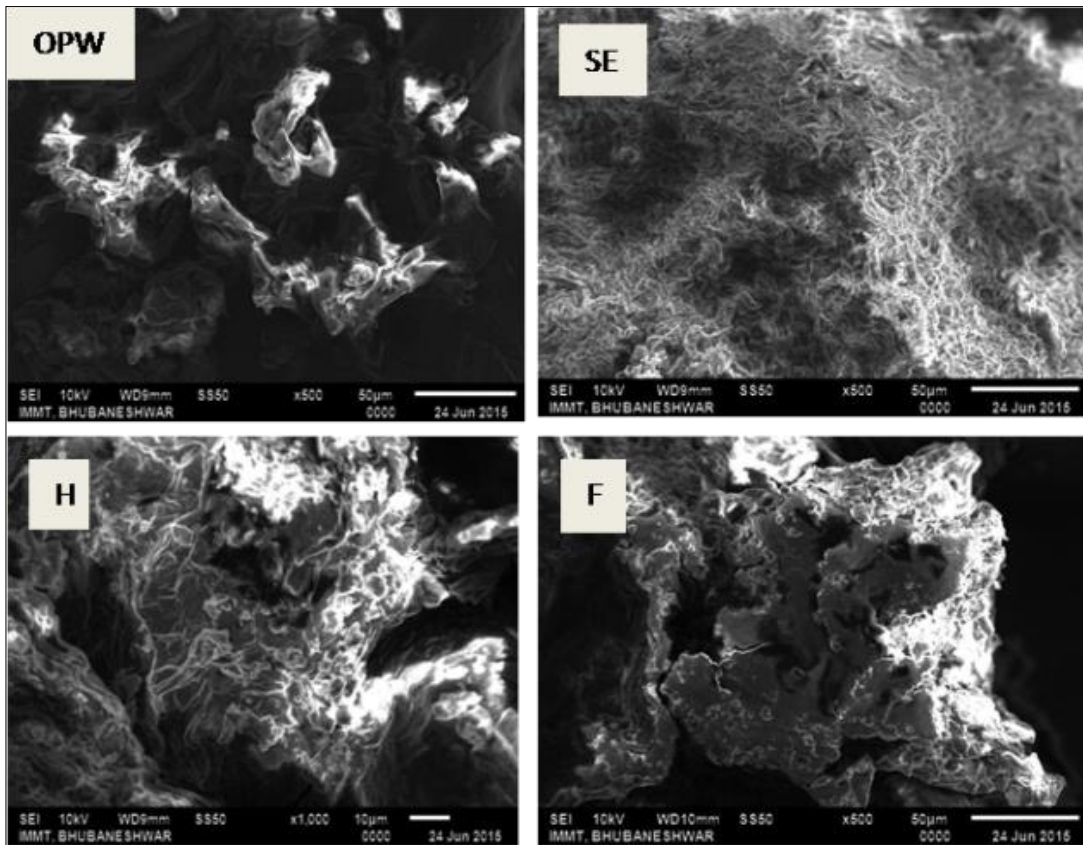


Figure 4. OPW-Orange Peel waste; SE – Steam Explosion; H- Hydrolysis; F- Fermentation FTIR Analysis

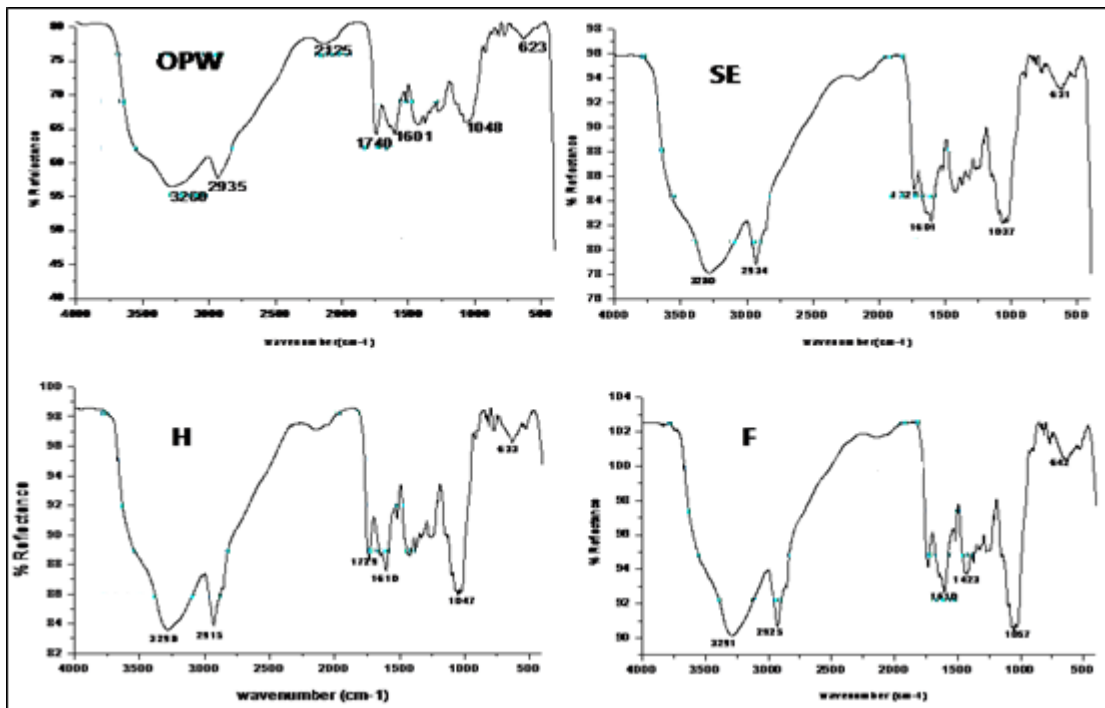


Figure 5. OPW-Orange Peel waste; SE – Steam Explosion; H- Hydrolysis; F- Fermentation

the FTIR spectra of the orange peel waste before and after each treatment was carried out as shown in Figure 5(a-d). The OPW was found to contain cellulose, hemicelluloses, pectin, etc. Total dry matter, sugar and limonene content are critical to ethanol yield and the final viscosity of fermented OPW. Operating temperature in a pretreatment step is essential for the removal of limonene as the vapor pressure is strongly dependent on temperature [11] studied the economics of d-limonene recovery and found the optimal temperature for recovering limonene by steam stripping was in the range of 110–132 °C and also reported that the vapor pressure of limonene increased dramatically above 140 °C. The enzymatic hydrolysis of the orange peels was together with a preliminary cultivation on the obtained hydrolyzate the final part in the present work. Several studies dealing with simultaneous saccharification and fermentation were carried out by several workers [12]. Results from the preliminary cultivation on enzymatic hydrolyzed orange peel showed that the maximum ethanol concentration and yield was obtained after 24 h as shown in Figure 3. Scanning electron microscopy (SEM) and Fourier transform infrared analysis (FTIR) analysis of native and enzyme pre-treated biomass was done to study physical and chemical changes. The peels have an irregular and porous surface. Similar results were also observed by other workers [13]. The FTIR result showed that most of the bands are common after every treatment such as C-H, H-C=O: C-H, C-C, C-OH and C-Br. Ethanol yield (v/v) of OPW samples was found to be 6.17 as compared to untreated i.e. 1.27.

CONCLUSIONS

Results of this study revealed that pretreatment of the substrate are necessary for obtaining of more sugars as a result of enzymatic hydrolysis. The sugars produced by enzymatic hydrolysis were readily converted into ethanol by using *S. cerevisiae* in fermentation at 30 °C for four days of incubation. Orange peel waste is found to be a very effective substrate for ethanol production and this might be helpful for scaling up and make the process cost effective.

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Persian Abstract

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چکیده

تولید جهانی مرکبات بیش از ۸۸ میلیون در سال تخمین زده شده است و تولید پرتقال به تنهایی حدود ۵۵٪ از این مقدار می باشد. تفاله ی پرتقال (OPW) مثل باقی مانده های غشا جامد، هسته و غیره بیش از ۵۰ درصد از میوه های فراوری شده را تشکیل می دهد که دفع آن از نقطه نظر زیست محیطی بسیار نگران کننده می باشد. با توجه به در دسترس بودن و ترکیب غنی این مواد، پژوهش حاضر بر تولید اتانول از OPW تمرکز کرده است که یک رویکرد اقتصادی، دوستدار محیط زیست و کارآمد می باشد. گونه ای از قارچ *Saccharomyces cerevisiae* و *Aspergillus niger* برای هیدرولیز و تخمیر تفاله های پرتقال پیش تیمار شده و از SEM و FTIR برای تعیین خصوصیات نمونه استفاده گردید. درصد قند در نمونه پیش تیمار شده پس از ۲۴ ساعت، ۳۶۴ مشاهده شد، که مسئول بازده اتانول می باشد (۶,۱۷ / V). نتایج نشان داد که پوست پرتقال می تواند به عنوان یک خوراک کم هزینه، جایگزین خوبی برای تولید اتانول باشد.