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A Sustainable approach of Turning Potato Peel Waste towards Bioethanol Production using *Saccharomyces Cerevisiae*

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Abstract

The combustion of fossil fuels for energy production releases greenhouse gas. This contributes to the rise in temperature of the global atmosphere. The search for renewable energy sources has increased worldwide to mitigate this problem. A lot of potato peel waste is generated worldwide from households and food industries can be processed to produce bioethanol as a renewable energy. This study was focused on converting the PPW and Damaged Potatoes collected from the faculty canteen of University of Chittagong and local market to produce bioethanol. Acid and enzyme hydrolysis were carried out to convert the starch into glucose. Determination of ethanol produced from two different samples were identified by FTIR analysis. Ethanol were obtained from enzymatic hydrolysis of PPW and Damaged Potatoes were 5.89 gL^{-1} (1.05%) and 7.22 gL^{-1} (5.90%) respectively. The study suggests that both PPW and Damaged Potatoes can be used as the substrate for bioethanol production.

Keywords: Potato Peel Waste, Hydrolysis, *Saccharomyces cerevisiae*, Fermentation, Bioethanol.

Introduction

Biofuels are energy-rich compounds produced through biological processes or chemical alterations of the biomass of previously living plants and animals. The foremost producers of biofuels are photosynthetic bacteria, micro and macro algae and vascular land plants[1]. Biofuels are considered an alternative to fossil fuels[2] . They are grouped into four categories or generations. These generations of biofuels are produced from different types of feedstock. The first generation of biofuels is produced from traditional and non-traditional crop biomass such as sugar cane, corn, soybean etc. But it implicated competition for food, freshwater and land. Moreover, Deforestation and production costs are probable risk factors. To mitigate these issues, the second generation of biofuels is produced from non-food crop biomass, agriculture and forestry residues and high-yield crop biomass. To increase the biofuel yield in non-food crops they are genetically modified. Thus, improved non-food crops and algae are potential sources for third-generation of biofuels. The fourth generation of biofuels comes with a more improved yield of non-food crops and microbes. Different micro-organisms influence the growth of microalgae for fourth-generation biofuel production[3]. The world's largest source of energy is fossil fuels or hydrocarbon fuels[4]. They contribute to almost 90% of the total energy demand[5]. They are an essential part of modern life and commerce[6]. Their demand will continue to increase in the upcoming years to meet the demand for electricity in developing countries especially[5]. The consumption of fossil fuels increased by approximately 51% between 1995 and 2105. It is expected to increase by 18% more between 2015 to 2035[7]. They are the largest source of greenhouse gas emissions[8]. The only hope of solving global warming is to find alternative sources of cleaner energy[8].

The importance of environment-friendly and renewable sources of energy has been felt in the last few decades.

Moreover, climate change and global warming issues have added an extra dimension to the search for alternative sources of energy[9]. They emit negligible carbon during production which has minimum impact on global climate change. They are more beneficial than fossil fuels. They have better combustion efficiency and carbon sequestration which reduces greenhouse gas emissions. Moreover, the use of biofuel will decrease the dependency on fossil fuels. Agriculture and farming industries will develop further producing crops with increased yield. Crops with increased yield production will contribute to the development of agriculture and farming industries. Industrial investments will increase creating job opportunities[10]. Fruit wastes, also known as the bioethanol feedstock, becoming one of the richest sources of different fermentable sugars[11].

Fruit wastes, also known as the first generation of bioethanol feedstock, becoming one of the richest sources of different fermentable sugars[11]. Arumugam & Manikandan analyzed the composition of banana and mango fruits. As they sowed, the starch content of fruit pulp ranged from 0.507% to 0.632% and from 1.074% to 1.706% ranged in fruit peels. The starch content in raw potato peel wastes is 7.6% which is very much higher than fruit wastes. So, potato peels are considered a more potential source for bioethanol production.

Bangladesh is 7th among the top potato-producing countries in the world[12]. Potato is the second most produced crop in Bangladesh. Over 4.8 lakh hectares of land were brought under potato production[13]. The production of potatoes has exceeded 11 million tons against the 7.7million tons annual demand[14]. This causes the post-harvest loss of 25% of potatoes every year[14]. Because of this, the potato farmers lose at least Tk 2,500 crore every year. The government is encouraging potato export to mitigate this loss. However, the utilization of waste potatoes and potato peel wastes could be a more effective solution to this problem. This will not only prevent the post-harvest loss of potato farmers but also contribute to the country's increasing demand for renewable energy. In this study, potato peel wastes of different types of potatoes were collected to measure the amount of bioethanol from potato starch through fermentation by *Saccharomyces cerevisiae*. This work will be highlighted the potential of natural wastes for producing renewable energy sources.

Materials and Methods

Sample collection

The potato peel waste (PPW) was collected from the canteen of the Biological Sciences Faculty, University of Chittagong and Damaged potatoes were collected from the local market of Chittagong. Epiphytic yeast strains *Saccaromyces cerevisiae* was obtained from different sweet food sources like grapes and wasted bread were collected from the local market of Chittagong. Isolated strain was cultured in the Biochemistry and Molecular Biology laboratory by maintaining optimum temperature and P^{H}.

Sample preparation

Potato peels were dried overnight in an oven at 80°c and powdered using a grinder. Yeasts were collected by washing grape samples using NaCl saline solution (0.9% w/v). Serially diluted (1:10) in the same saline solution and plated on Malt Extract Agar (Sigma-Aldrich, Italy). Chloramphenicol was used to prevent bacterial contamination. Plates were incubated at 25^{0}C for 48h. The selection of colonies with different morphologies was randomly completed. To obtain pure isolates, single colonies were streaked on MA plates. Purification was repeated at least three times or until all the colonies on the streaked isolate had the same morphology[15,16]. Incubation at 30 ^{0}C and pH 6 were carried out for 84 hours for the culture of Yeast (*Saccharomyces cerevisiae)*. Conversion of fermentable sugar to ethanol was done through fermentation by *Saccharomyces cerevisiae.*

Moisture content determination

The potato mash was weighed and dried at 105 °C in an oven for 48 hours until the weight of the samples stabilized[17,18].

$$\text{Moisture content (\%)} = \frac{\text{weight of moisture}}{\text{weight of PPW}} \times 100$$

Determination of ash content

2 g of sample were taken in a crucible, and weight was recorded. The Triplicate of each sample was analyzed. Crucibles were placed in a muffle oven at 550 °C for 24 hours. Crucibles were cooled and taken out from the muffle furnace and placed into a desiccator to cool. The weight of the ashed sample and the weight of the crucible with ashed sample were measured[19,20].

$$\text{Ash content (\%)} = \frac{\text{weight of ash}}{\text{weight of PPW}} \times 100$$

Acid Hydrolysis

10 ml Hydrochloric acid (0.5%) was added to 240 g of the Damaged potato and potato peel powder. The mixtures were then autoclave at 121°C, 21psi for 15 min. 2L distilled water was added. The resulting mixture was placed in the water bath at a temperature of 75 °C for half an hour. Then, the hydrolyzed material was autoclaved at 121°C. Finally, the solution was filtered (45 μm, 4 Whatman GFD) and the pH was adjusted to 6.3 by sodium hydroxide[21,22].

Effect of yeast extracts at different concentrations

Different concentrations of yeast extracts ranged from (0, 2, 4, 5 and 8 g/L) and hydrolyzed PPW was added to the fermentation medium. The fermentation was carried out at 30 ^{0}C for 5 days with 3 mL of inoculums. The ethanol content in fermented samples was estimated every 24 hours and every yeast extract concentration was done in triplicates.

Fermentation using α-amylase and yeast

250 ml distilled water was added to 20 g of the potato peel waste (PPW) and autoclaved at 121 °C, 21 psi for 15 min. Commercial α-amylase (E.C.3.2.1.1) enzyme was added and the mixtures were placed in the water bath at 80°C temperature for 1 hour.

The hydrolyzed material was autoclaved at 121°C for 30 min. The solution was filtered with Whatman filter paper and pH was added to 6.5. Finally, 20 ml yeast liquid broth was added and fermentation was followed afterward for 7 days.

Effect of yeast extracts at different concentrations

Different concentrations of *Saccharomyces cerevisiae* (0, 1.5, 3, 4.5, 6 gL^{-1}) were added to vials containing 30 mL of fermentation medium. The samples were incubated.

Effect of fermentation time

The fermentation medium with a culture of *S. cerevisiae* as inoculum and the addition of yeast extract was incubated at 24 °C using a shaker incubator at 150 rpm for 5 and 7 days. Then, they were evaluated to determine their capacity to synthesize ethanol.

FTIR spectroscopy

Fourier -transform infrared spectroscopy was used to determine the presence of a functional group of alcohol bonds existing in the samples after the distillation process. Perkin Elmer spectrum 400 FTIR/ FT-FIR spectrometer with a region of 4000-400 cm^{-1} was used to evaluate the chemical structure of bioethanol from PPW.

Statistical analysis

All the analyses were performed in three independent experiments. The standard deviations as well as the arithmetic mean of the triplicate samples were estimated. Data were analyzed and compared by analysis of variance (Two factorial ANOVA). The probability level was fix at $p<0.05$.

Results and Discussion

Moisture and ash content of PPW

Determining the ash and moisture content is part of the proximate analysis for sample quality evaluation. PPW contained 10.11±0.03% and 3.85±0.02% moisture and ash content respectively (Table 1). The amount of moisture and ash obtained were higher and lower respectively than reported by Arapoglou, *et al*. 2010

(Arapoglou *et al.* 2010). This also refers to the suitability of PPW for microorganism growth and bioethanol production through acid or enzyme hydrolysis.

Table 1: Moisture content (%) and ash (%) of PPW.

Moisture and Ash content	Dry Weight (%)
Moisture content (%)	10.11±0.03
Ash (%)	3.85±0.02

Growth Curve Based on Temperature

Temperature influences the growth of yeast (*Saccharomyces cerevisiae*). The growth of yeast *(Saccharomyces cerevisiae*) at different temperatures is shown in Figure 1. The Optimum growth of *Saccharomyces cerevisiae* was observed between 30-35 ^{0}C. This corresponds to the study by Walsh, *et al.* 1977[23].

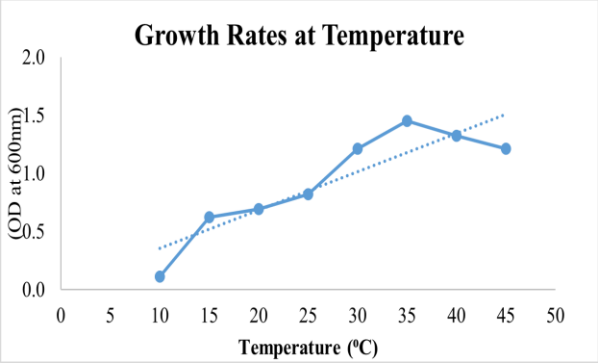

Figure 1. *Saccharomyces cerevisiae* growth curve based on temperature

Effect of P^{H} *on Saccharomyces cerevisiae growth*

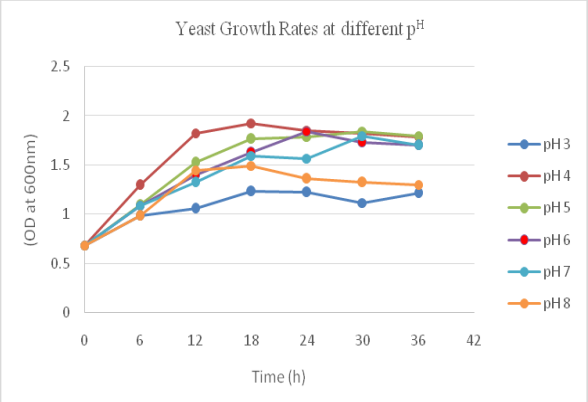

Figure 2. *Saccharomyces cerevisiae* growth curve in different P^{H}

P^{H} is an important parameter for the growth of *Saccharomyces cerevisiae* on a medium. Its growth regarding different P^{H} at different time intervals on PDA media is plotted in Figure 2. The figure shows that the growth of *Saccharomyces cerevisiae* is optimum between P^{H} 4 to 6. This is like the findings of Liu, *et al.* 2015[24].

FTIR analysis for ethanol

The O-H bond in alcohol absorbs at a higher wave number than it does in an acid - somewhere between 3230- 3550 cm^{-1}. This absorption would be at a higher number still if the alcohol isn't hydrogen bonded - for example, in the gas state. All the infrared spectra on this page are from liquids - so that possibility will never apply. FTIR analysis of ethanol produced from two different samples, Damaged potato (*Solanum tuberosum*) and potato peel waste (*Solanum tuberosum*)**.**

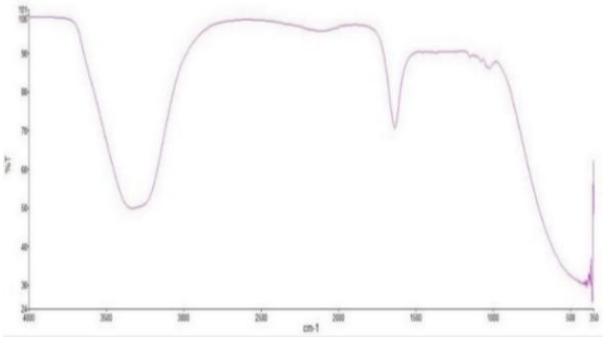

Figure 3(a). FTIR analysis for ethanol of damaged potato

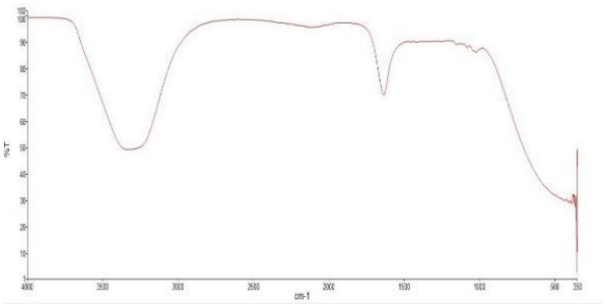

Figure 3(b). FTIR analysis for ethanol of potato peel waste

Ethanol determination by specific gravity

% v/v alcohol = (SG_2 - SG_1) / 0.0074

= (1.070-1.029)/0.0074

= 5.54%

Where,

SG1 is the initial specific gravity measurement

SG2 is the final specific gravity measurement

Fermentation at different time intervals

Ethanol production was measured after a 6-days incubation period and Incubation *Time (50-* 84 hrs). Fig 4 shows the amount of Ethanol production at different time intervals. Optimum ethanol production was recorded after 48 hours in both acid hydrolysis and enzymatic hydrolysis of the Damaged potato and PPW. Damaged potatoes produced 6.02 and 7.22 gL^{-1} ethanol from acid and enzymatic hydrolysis respectively. An amount of 2.02 and 5.89 gL^{-1} ethanol was produced from PPW using acid and enzymatic hydrolysis respectively.

Ethanol production from acidic hydrolysis and enzymatic hydrolysis

Damaged potato and PPW were hydrolyzed with HCl and Alpha-amylase. Later, they were fermented by *Saccharomyces cerevisiae* in a fermentation medium.

The hydrolysates produced ethanol which is shown in Table 2. The amount of ethanol produced by Alpha amylase hydrolysis of damaged potato (5.90%) was greater than that of PPW (1.05%). Acid hydrolysis of damaged potato and PPW also showed a greater amount of ethanol produced from damaged potato (5.56%) and less for PPW (0.95%). **Table 2:** Amount of Ethanol produced from Acid Hydrolysis and Alpha-amylase.

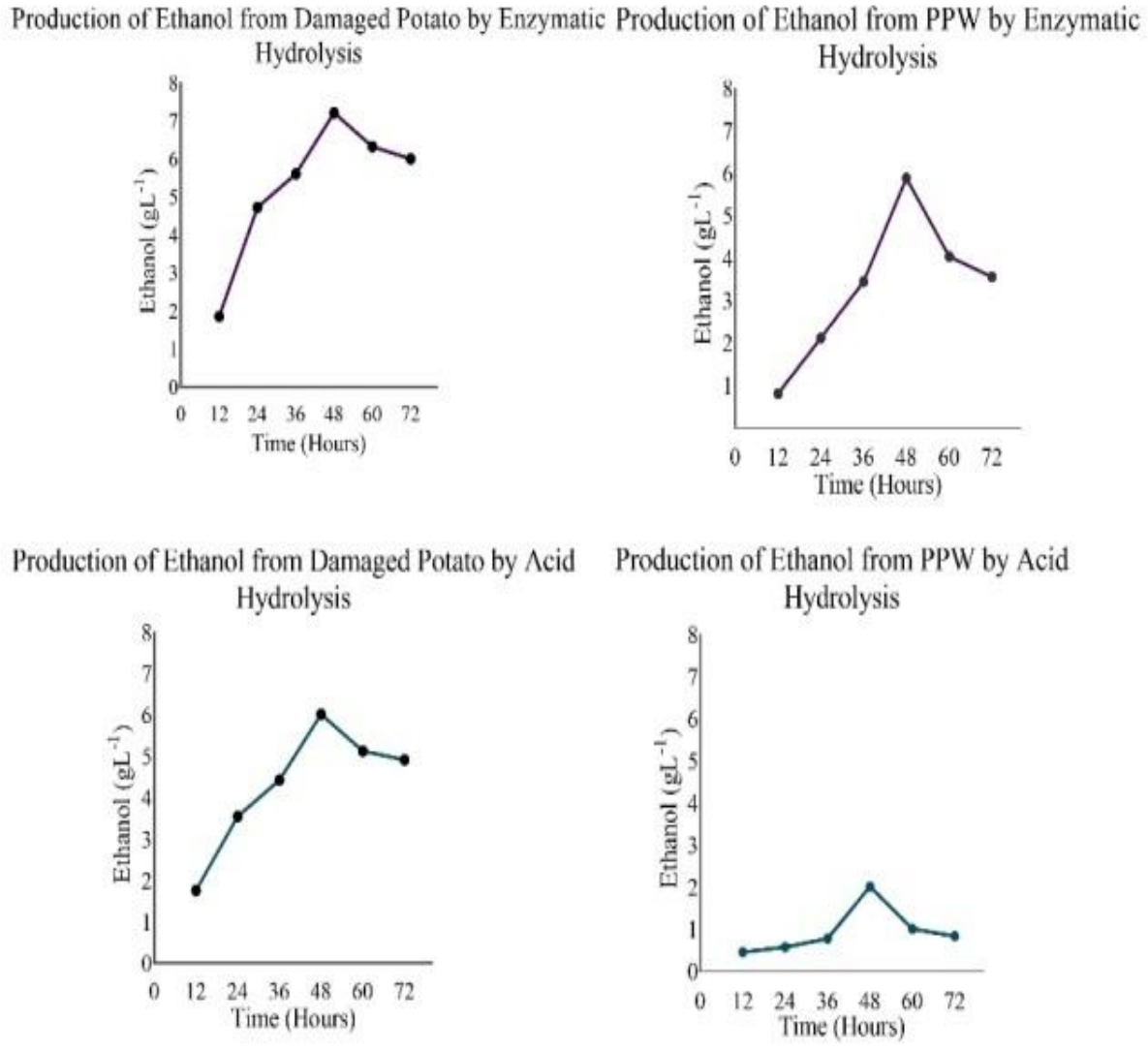

Figure 4. Production of Ethanol from Damaged Potato and PPW by Acid and Enzymatic Hydrolysis

No.	Sample	Amount of Ethanol	
		Acid Hydrolysis	Enzymatic Hydrolysis
1	Damaged potato	5.56±0.03	5.9±0.04
2	PPW	0.95±0.04	1.05±0.02

The amount of moisture and ash obtained from this study were higher and lower respectively than those reported by Arapoglou, *et al*. 2010. This refers to the suitability of PPW for microorganism growth and bioethanol production through acid or enzyme hydrolysis. The present study recorded 30-35^{0}C temperature for optimum growth of *Saccharomyces cerevisiae* which was similar to the results obtained by Walsh, *et al.* 1977. Another important parameter for optimum growth of *Saccharomyces cerevisiae* is P^H. This was between P^H 4-6. This is quite similar to the results obtained by Lin, *et al*. 2012 where optimum growth was recorded between P^H 4-5. The present study followed both the acid hydrolysis and enzymatic method for ethanol production. From both methods optimum ethanol production was reported after 48 hours. The time recorded for optimum ethanol production aligns with the results obtained by Lin, *et al*. 2012. Gosavi, *et al*. 2017 obtained 0.079%, 0.090%, 0.045% and 0.045% ethanol from sweet potato waste, pineapple, Indian chestnut and jackfruit respectively by following only the acid hydrolysis method. In comparison to the study by Gosavi, *et al.* 2017, the present study obtained a higher amount of ethanol (0.95%) from acid hydrolysis of PPW. The amount of ethanol obtained from enzymatic hydrolysis (1.05%) of PPW was even greater than that of acid hydrolysis. The findings from the present study show that the PPW of kitchens, cafeterias and households can be a potential source of bioethanol production. Moreover, considering the environmental impact and health hazards associated with acid hydrolysis, enzymatic hydrolysis can be considered a green method for bioethanol production regardless of its cost[25].

Conclusion

Potatoes are important sources of bioethanol and a large amount of potatoes are produced in Bangladesh every year. The food processing industries also produce a lot of PPW every year. The approach described here have an enormous potential for industrial-scale production of bioethanol because they are environmentally friendly, highly productive, of low cost, and easily manipulated. So, both damaged potatoes and PPW can be considered potential sources for bioethanol production. Further research is required to increase the yield of bioethanol in different potato species as well as PPW.

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Author Declaration

The authors declare that they have carried out the whole research work and the contents of the paper were not published before or submitted for publication or any other journal.

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