ISSN 0975-6299

Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

BIO TECHNOLOGY

USE OF FRUIT BIOMASS PEEL RESIDUE FOR ETHANOL PRODUCTION

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ABSTRACT

Ethanol made biologically from cellulosic biomass, including agricultural and forestry residues, portions of municipal waste being widely recognized as a unique transportation fuel with powerful economic, environmental and strategic attributes. Approximately, 1 kg of residue is produced for each kilogram of grains harvested. In this study, we demonstrate that the fruit biomass peel residue could be used to produce fuel grade ethanol. A chemical pre-treatment process using alkaline peroxide or acid hydrolysis was applied to remove lignin, which acts as physical barrier to cellulolytic enzymes. *Aspergillus niger* was used in the experiment. The pre-treatment process effectively removed lignin. Ethanol production in the culture sample was monitored using high performance liquid chromatography. The results indicate that ethanol can be made from the fruit biomass peel residue. The fermentation system needs to be optimized further to scale up the process for large-scale production.

KEYWORDS

Ethanol, *Aspergillus niger*, Cellulose, Lignin, Acid hydrolysis

INTRODUCTION

Ethanol is one of the most important renewable fuels contributing to the reduction of negative environmental impacts generated by the worldwide utilization of fossil fuels.
Production of fuel alcohol from cellulosic feedstock is of growing interest worldwide. Cellulosic biomass is an abundant renewable resource on earth and includes various agricultural residues [1]. Some of these agricultural residues such as straw, cornhusk, and sugarcane residue represent an abundant, inexpensive, and readily available source of renewable lignocellulosic biomass [2]. At the present time, this readily available biomass is considered as a waste and is disposed of through agricultural burning after harvest. Sugar production is a major industry in south Louisiana, and for the past two hundred years sugarcane farming has been a vital component of Louisiana's economy and culture. As of 2004, there were 461,738 acres of sugarcane grown by 718 producers within 24 Louisiana parishes. Approximately 424,799 acres were harvested for sugar, producing a total of 1,174,028 tons of sugar .Every year after sugarcane is harvested, farmers typically reduce residue by open air burning. This is a cost-effective way to remove the fibrous content that would otherwise significantly reduce milling efficiency and decrease profits, as well as to clear residue from the Weld that hinders farming [3].The open air burning practice not only affects the quality of air but also the quality of life to those who live in the area. Smoke from open air burning contains respirable particles that are less than 10μ in size [4]. National air quality standards have been set for particulate matter that is equal to or less than 10 μ. Studies have suggested that populations exposed to particles of that nature suffer from asthma and bronchitis in addition to pulmonary morbidity and mortality [5]. One alternative to open air burning is the production of ethanol from sugarcane residue. Ethanol is a clean burning, renewable resource that can be produced from fermented cellulosic biomass [6]

In many parts of the world, demand for ethanol as an alternative fuel source has steadily increased [7] due to efforts in decreasing the overall amount of greenhouse gases emitted into the atmosphere [8], dwindling fossil fuel resources [6] and increased gasoline prices. Since 1970s, it has become clear that availability of domestic natural gas and petroleum cannot meet the growing demand for these energy sources. Therefore, there has been serious concern for developing renewable energy sources in an effort to ease the severity of the expected shortage. One possibility is the conversion of waste or grown organic matter into liquid and gaseous fuels. Currently, the United States produces approximately three billion gallons of ethanol from corn annually [9].While ethanol can be produced from fermented agricultural products, which are abundant renewable resources found world-wide [1], there are major limitations to efficient ethanol production from agricultural residues. These limitations include the close physical and chemical associations between lignin and plant cell wall polysaccharides, together with cellulose crystallinity [10] . Lignin forms a protective shield around cellulose and hemicellulose, protecting the polysaccharides from enzymatic degradation. To convert the biomass into ethanol, the cellulose must be readily available for cellulose enzymes [1]. Thus, by removing the lignin, the cellulose becomes vulnerable to enzymes and allows the yeast to convert the glucose into ethanol during

fermentation [11]. Therefore, a pretreatment must be applied to degrade the lignin in the sugarcane residue, decrease cellulose crystallinity, and increase the surface area for enzymatic activity[1].

The current study was initiated to determine the optimal pretreatment conditions for high efficiency ethanol production from the fruit biomass peel residue. The residue was subjected to alkaline hydrogen peroxide pretreatments and sulfuric acid pretreatments, followed by three weeks of fermentation using the *Aspergillus niger*. The results indicated that ethanol can be made from the fruit biomass peel residue.

MATERIALS AND METHODS

(i) Microorganism Collection

The Fungi, *A.niger* were isolated from fruit pulp wastage by using Serial dilution plate technique method [12]and maintained on Czapek (DOX) agar medium, Nigam (1995) cells were harvested in the late exponential growth phase [13] and concentrated and the studies further carried in our laboratory.

(ii) Raw material (substrate)

Fruit biomass peels were collected from a local market were chopped into small pieces and dried in an oven at 65^0C for 48hr.The dried substrate was powdered with an electric grinder to a mash size of 40, packed in polyethylene bags and stored at room temperature.

(iii) Alkaline pretreatment

The purpose of the alkaline pretreatment was delignification. The removal of lignin is necessary for cellulose to become readily available for the enzymes, which permit the yeast to convert the glucose into ethanol [11]. The amount of weight lost following chemical pretreatment of residue was due to lignin removal [11] .Greater weight loss equals more lignin loss. The percent weight lost was used to compare pretreatment effects on lignin removal. Delignification was tested by soaking each residue in various concentrations (0%, 2%, 4%, and 6%) of household hydrogen peroxide at various pHs (8, 11.5, and 13), for various time intervals (8, 24, and 48 h). Fruit biomass peel residue was collected from local market chopped into small pieces and dried in an oven at 65^0C for 48hr. Five grams of dry fruit biomass peel residue was weighed and placed in beakers. Subsequently, three, 1% H_2O_2 solutions were made. The pH of separate 1% H_2O_2 solutions was adjusted to 8, 11.5, or 13 by adding sodium hydroxide (NaOH) tablets. Enough of each treatment solution was added to the beakers to submerge the fruit biomass peel residue, and allowed to soak for 8, 24, or 48 h. This experiment was repeated for H_2O_2 concentrations of 0%, 2%, and 4%. Deionized (DI) water was substituted for H_2O_2 for the 0% treatment level. In addition, a DI water control was conducted without adjusting the pH. Each H_2O_2, pH and time treatment combination was repeated four times. After the allotted amount of time for soaking, the residue was removed from the solutions by filtering through a piece of cheesecloth. The residue was then triple rinsed for 30min in DI water and oven dried at 100 °C for approximately 10 h. Finally, the residue was reweighed. The weight difference is equivalent to the amount of lignin removed. Upon conclusion of the alkaline pretreatments, analysis of variance was used to determine the pretreatment conditions that removed the most lignin. The best pretreatment was then used for further fermentation experiments. Fermentation experiment was conducted using the *Aspergillus niger*. The pretreated fruit biomass peel was used in the fermentation experiment. The pretreated residue was placed into anaerobic bottles containing 100mL of sterile tap water and 5% (v/v) of the

Aspergillus niger. This experiment was run in duplicates along with duplicate controls without pH adjustment. Samples were taken on days 0, 6, 12, 18, and 24 with a 5mL syringe. Samples were microcentrifuged at 10,000 rpm for 5min and the supernatant was used to monitor ethanol production using HPLC analysis.

(iv) Acid pretreatment

The purpose of acid hydrolysis was to remove lignin from the fruit biomass peel residue, which hinders enzymatic hydrolysis of cellulose for ethanol fermentation. Dilute sulfuric acid (H_2SO_4) concentrations (0.0, 0.2, 0.4, and 0.6M) were used in this pretreatment. For the acid hydrolysis pretreatment, approximately 5 g of dry fruit biomass peel residue was placed into anaerobic bottles containing 100mL of DI water and 0.2M H_2SO_4 and allowed to soak for 24 h. The bottles were subsequently autoclaved and allowed to cool before 5% (v/v) of the *Aspergillus niger* was added. Samples were taken on days 0, 6, 12, 18, and 24 using a 5mL syringe, microcentrifuged at 10,000 rpm for 6min and transferred to HPLC vials. Ethanol production was monitored using HPLC analysis. This experiment was repeated using 0.0, 0.4, and 0.6M H_2SO_4, and each treatment had three replicates.

(v) Analytical techniques

Ethanol production was analyzed by high performance liquid chromatography (HPLC) on a Varian Pro Star Autosampler Model 410 liquid chromatograph equipped with two solvent pumps, a model 210 programmable multiwavelength detector set at 210nm, a data module, and a model 320 system controller. The mobile phase was 0.0025N H_2SO_4. Aliquots of 10 µL were injected into an organic acid column (Varian organic acid column, Cat#SN 035061) at 22 °C. The flow rate of the mobile phase was 0.6mL/min, and the analysis was done under isocratic mode. Quantification of ethanol was done by using standard ethanol.

(vi) Statistical analysis

Data were subjected to analysis of variance (ANOVA) followed by a Turkey *post hoc* range test (*p*60.05) [14]

RESULTS AND DISCUSSION

1. Alkaline pretreatment:

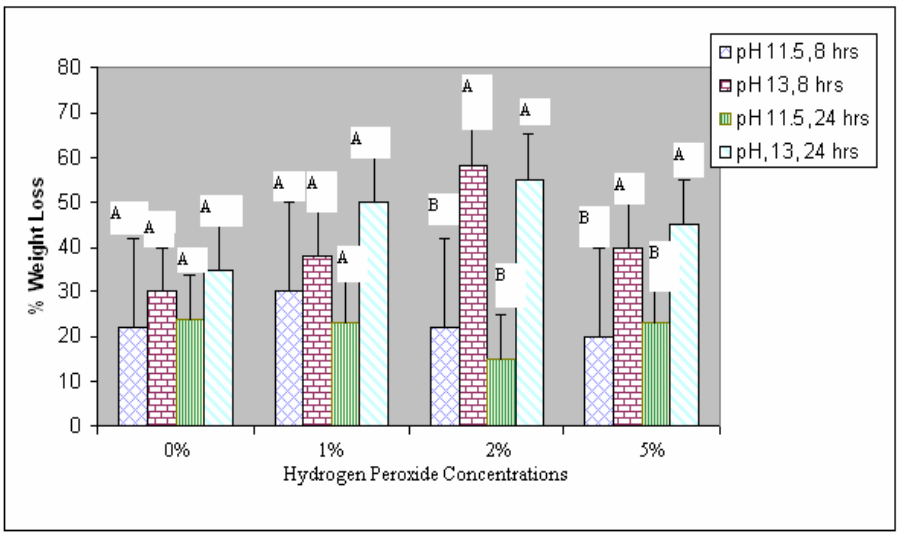
The alkaline pretreatment of 2% H_2O_2 (pH 13) soaked for 8 h removed the most lignin in fruit biomass peel compared to other treatment combinations (Graph 1 and 2).Therefore, this treatment was chosen for fermentation of the fruit biomass peel. Treatment combinations consisting of pH 8 or soaking for 48 h were not significant (*p*D0.9) (data not shown).

Lignocellulosic biomass cannot be saccharified by enzymes to high yields without a pretreatment, mainly because the lignin in fruit cell walls forms a barrier against enzymatic attack [15]. An ideal pretreatment would reduce the lignin content and crystallinity of the cellulose and increase the surface area [1]. Lignin is degraded in nature by various organisms, but the mechanism of natural degradation is largely unknown. It is thought that oxidants such as H_2O_2 may play an important role. Under certain conditions, H_2O_2 is known to react with lignin and has been widely used to bleach high-lignin pulps [10], recently reported that under suitable conditions, H_2O_2 will delignify wheat straw and other crop residues to a point where the cellulose can be enzymatically converted to glucose with near quantitative yield. According to Gould and Freer (1984), H_2O_2 treated lignocellulosic materials can be rapidly fermented to ethanol with greater than 90% efficiency in the presence of cellulase. In the present study, fruit biomass peel residue pretreated with 2% H_2O_2 at a pH of 13 soaked for 8 h (Graph 1 and Graph 2) removed

58.90% of the total weight of the sample; therefore, removing more lignin than any other pretreated sample.

Graph : 1

Alkaline pretreatment

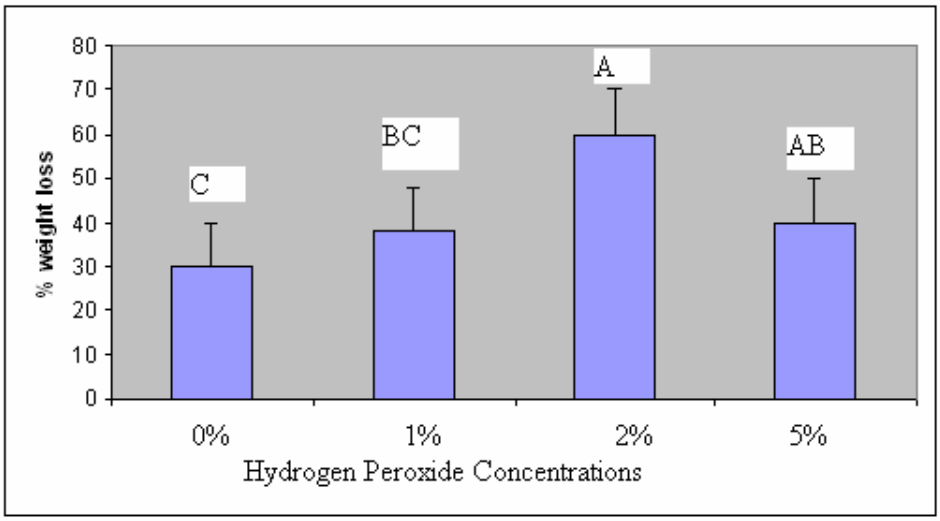


Graph 1

Mean (± standard deviation) percent weight loss from fruit biomass peel after soaking in different H_2O_2 concentrations for 8 or 24 h at a pH of 11.5 or 13. Means denoted by the same letter are not significantly different from each other within treatments.

Graph : 2

Alkaline pretreatment



Graph 2

Mean (± standard deviation) percent weight loss from fruit biomass peel after soaking in different H_2O_2 concentrations for 8 h at a pH of 13.Means denoted with same letter are not significantly different from each other.

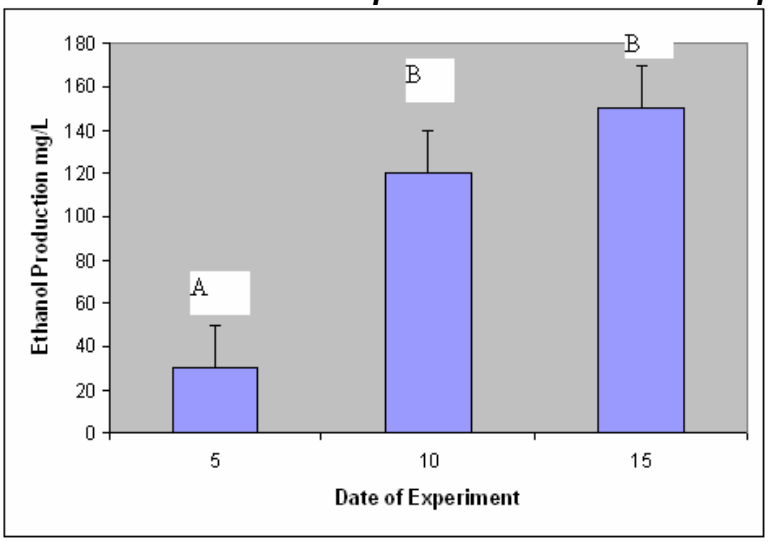
These results are similar to those concluded from other research. Maximum delignification of wheat straw occurred at a pH of 11.5 or higher and the increase in saccharification efficiency was nearly complete after eight hours at room temperature [2]. Krishna and Chowdary (2000) concluded that alkaline peroxide pretreatments were effective in providing fractionation of the hemicellulose and lignin components and resulted in efficient hydrolysis in linn leaves. In another study [10] wheat straw treated for several hours at room temperature with 1% H_2O_2 at a pH of 11.5 released slightly more than onehalf of its lignin as water-soluble degradation products. They found that increased concentrations of H_2O_2, more alkaline pH, or repeated H_2O_2 treatments did not alter the total amount of lignin solubilized. However, based upon the present research, increased pH levels did remove more lignin than lower pHs. Furthermore, one study concluded that in the absence of H_2O_2 only a very small fraction of the lignin present in the straw was released [10]. Another report also obtained similar results in research conducted on sugarcane residue [3].Pretreatments soaked in 1% H_2O_2 at a pH of 11.5 for 8 h at room temperature removed 40% of lignin.

Fruit biomass peel pretreated in 2% H_2O_2 (pH 13) for 8 h was subjected to fermentation. Fruit biomass peel was fermented for 15 days and sampled every five days. Optimal fermentation conditions for pretreated Fruit biomass peel residue at 2% H_2O_2, pH 13, 8 h was determined to occur on day 10, producing a mean of 120 mg/L (Graph 3).

Results from this research were slightly higher than those reports produced[16], where ethanol production was 118mg/L. However, another report was found to be alkaline pretreated corn cobs, corn husks, and corn stalks produced ethanol with an overall 90% efficiency [2], while kenaf and oak shavings produced enhanced ethanol yields, although significantly below the theoretical maximum. It must be noted that, mainly cellulase enzyme added prior for fermentation [2]

Graph 3

Optimal fermentation conditions for pretreated Fruit biomass peel residue



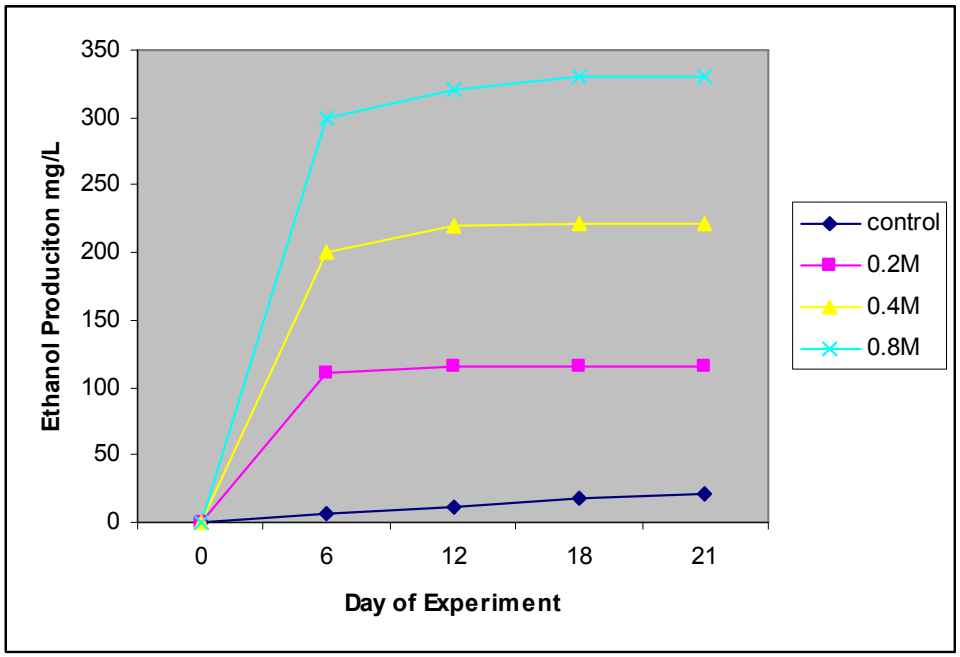
Graph 3

Mean (± standard deviation) ethanol production (mg/L) from fermentation of alkaline pretreated fruit biomass peel residue, 2% H2O2 (pH 13) 8 h for a 5, 10, and 15 day fermentation period. Means represented with the same letter are not significantly different from each other.

(ii) Acid hydrolysis:

The Fruit biomass peel acid treatment of 0.8M H_2SO_4, fermenting for 12 days produced more ethanol than any other treatment combination up to day 12. Fermentation for more than 12 days did not increase ethanol production (Graph 4). For acid hydrolysis, the optimal concentration of H_2SO_4 was 0.8M H_2SO_4. Results for The Fruit biomass peel show that fermenting for 12 days was the most efficient acid hydrolysis treatment for ethanol production, producing 330mg/L ethanol (Graph 4). In acid hydrolyzed experiments of waste cotton carried out [6]. 0.2mol/L H_2SO_4 was the optimal acid treatment, producing 13.2 g/L of ethanol in 24 h.

Graph 4
Acid hydrolysis



Graph 4
Mean ethanol production (mg/L; ± standard deviation) from fruit biomass peel subjected to different concentrations of acid hydrolysis over time.

After comparing alkaline H_2O_2 and H_2SO_4 acid treatments, it was shown that acid hydrolysis produced the most ethanol from the residue. More ethanol was produced from fruit biomass peel when treated with 0.8M H_2SO_4 for 15 days compared to alkaline pretreated residue at 2% H_2O_2 (pH 13) 8 h fermented for 10 days (Graph 5).

Graph 5

Comparing alkaline H_2O_2 and H_2SO_4 acid treatments

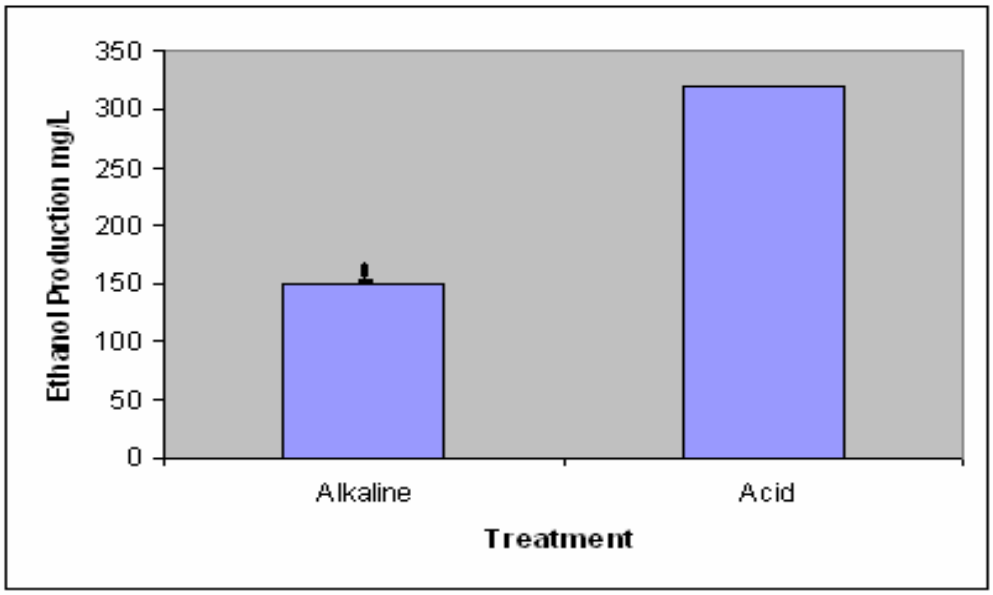


Figure 5

Mean (± standard deviation) ethanol production (mg/L) from acid (0.8M H2SO4) fruit biomass peel residue for 15 days and alkaline (2% H_2O_2 (pH 13) 8 h) pretreated residue fermented for 10 days. Asterisk denotes a significant difference at p D0.05.

This preliminary study showed that ethanol production from fruit biomass peel residue is possible. Lignin prevents the degradation of cellulose mainly by acting as a physical barrier between the cellulolytic enzyme and its substrate. Consequently, the rate and extent of enzymatic cellulose degradation in lignocellulosic materials is inversely related to the lignin content [10] with maximum cellulose degradation occurring only after 50% or more of the lignin has been removed. In this study, we achieved a significant removal lignin from the sugarcane residue, which resulted in higher production of ethanol. Further research is needed to optimize the conditions for maximum production of ethanol from fruit biomass peel residue.

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