International Journal of Advanced Technology in Engineering and Science
Vol. No.3, Special Issue No. 01, November 2015
www.ijates.com
ijates
ISSN 2348 - 7550

AGRICULTURAL AND DOMESTIC REFUSE AS FEEDSTOCK FOR MICROBIAL PRODUCTION OF BIOETHANOL

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ABSTRACT

Rising global concern due to inflating fuel prices, climate change, oil supply, energy security and depleting reserves has roused interest in the production of fuels from renewable sources. The two most common types of biofuels in use today are bioethanol and biodiesel. These fruit and vegetable peels such as potato, banana and orange peels contain complex polysaccharides such as cellulose, starch, pectin etc. The energy harvested is due to recently fixated carbon through natural processes like photosynthesis in plants and micro algae that leads to carbohydrate hydrolysis into their monomeric forms for conversion to ethanol. A cocktail of commercial grade enzymes in combination with physical and chemical pretreatment methods was used for saccharification. The saccharified substrates were subjected to microbial fermentation with Saccharomyces cerevisiae. A high sugar to ethanol conversion yield of 46%, 49.9% and 50% of initial biomass was achieved for potato, banana and orange peels respectively. The final sugar concentration in the hydrolysates generated by this method was 5-10%. In order to economize the process, the hydrolysates were used to dilute cane molasses (60%) syrup to get a final concentration of 12.5% sugars. This method makes the process cost effective by eliminating the step of evaporation required to increase the sugar concentration in the hydrolysates. The result obtained ensures that the developed technology could be implemented to the existing alcohol plants/distilleries, augmenting their capacity and enhancing the economic feasibility of the process.

Keywords: Bioethanol, Fermentation, Saccharomyces cerevisiae, Fruit /Vegetable Peels, Distelleries.

I. INTRODUCTION

The principal sources of energy in today's era comprises of non-renewable resources whose unending demand and instability in fuel stock due to its rapid exhaustion has led to continuous price inflation of crude petroleum shifting the interest paradigmatically towards White biotechnology. White Biotechnology is the branch of Biotechnology that deals with production of fuels from renewable sources (Dellomonaco et.al, 2010). Biofuels derived from biomass have great potential for providing renewable and sustainable energy. Traditional feed stocks such as corn, food grains, sugarcane juice etc., can be used as biomass for production of biofuel; however feed stocks are used substantially for human and animal consumption, causing plausible social and economic barriers. On the other hand lignocellulosic refuse, can be used as biomass for the commercial production of bioethanol (Kumar.et.al, 2010).

Ethanol is used as fuel additive in gasoline or diesel to reduce the overall price of motor fuel. Benefits of the use of ethanol as motor fuel additive has encouraged Indian government to launch ethanol blending program, under which 5 % ethanol has to be blended with petrol and now it has set an indicative target of a minimum 20 % ethanol-blended petrol and diesel by 2017 (2002). Ethanol can be derived either by fermentation of sugar or by reaction of ethane with steam. "Bioethanol" is fuel produced by contemporary biological processes. Survey states that more than 30 % of the agro-wastes consist of fruits and vegetable peels which can serve as an affordable, renewable, cost efficient biomass for bio ethanol production. These agro-wastes are either dumped as municipal wastes or burnt, consequently adding to the greenhouse effect. Moreover the high cost involved in the production of bioethanol from lignocellulosic refuse, this technology has not yet been explored for commercial bioethanol production.

Previous studies on bioethanol production from potato, banana and orange wastes have shown that pretreatment (physical, chemical, biological) of the waste samples is required in order to make the raw material susceptible to enzymatic degradation (Sharma, 2007; Sirkar, 2008; Contreras, 2010). However, pretreatment of the samples leads to the accumulation of undesirable toxic byproducts. These toxic byproducts can lower the efficiency of fermentation resulting in a poor yield of bioethanol and making the process uneconomical (Okuda.et.al, 2008; Zang.et.al, 2008).

There are several advantages of using potato, banana and orange peels as biomass for bioethanol production. Potato is the second most popular staple food in the world and has become the fastest growing staple crop in India. Potato peel is usually avoidable during processing of potatoes. Potato peels contain sufficient quantities of starch, cellulose, hemicelluloses and fermentable sugars that can be used as ethanol feedstock (Arapoglou.et.al, 2010; Yamada.et.al, 2009). Similarly, banana being the second largest produced fruit after citrus, contributes about 16 % of the world's total fruit production (FAO, 2009). India is the largest producer of banana, contributing to 28 % of world's banana production in 2009 (FAO, 2009). Banana peels are a rich source of starch, protein, fats, lignin, pectin, cellulose and hemicelluloses, the percentage of which varies from green to mature bananas (Mohapatra, 2010). Citrus production is of particular interest because of its flavor and high content of vitamin C. Orange peels mainly consist of celluloses, hemicelluloses, pectin, minerals and orange oils. Development of more efficient methods for the production of ethanol from bio wastes would be a big leap towards commercialization of potato, banana and orange peels as biomass for bioethanol production. A number of microorganisms like *Zymomonas mobilis*, *E.coli* and *Saccharomyces cerevisiae* have been reported to ferment orange peels into ethanol along with the production of some toxic, inhibitory and undesirable byproducts which retard the growth of microorganisms during fermentation (Wilkins, 2008; Grohmann.et.al, 1994; Oberoi.et.al, 2010).

II. MATERIALS & METHODS

2.1 Strains and Raw Materials

All the chemicals used in this investigation were of analytical grade (Fischer, Qualigens) and commercial grade enzymes were obtained from M/s Maps (India) Ltd., Ahmedabad. The fruits and vegetable peels were collected from local processing units and vendors and were rapidly used for further studies. Strain of *Saccharomyces cerevisiae var. ellipsoideus* was procured from the institute. Moisture contents in the peels were determined by

drying 100g of peels at 80^{o} C till a constant weight was obtained (AOAC, 2000). Total carbohydrate was determined by method of Phenol Sulphuric Acid method (Fournier 2001) and reducing sugars were determined by method of DNSA (Miller, 1959). Alcohol contents were determined by GLC, Gas Liquid Chromatography (Umar, 2008).

2.2 Pre-Treatment and Saccharification of the Samples

2.2.1 Soaking and pre-treatment of samples

10g of samples were soaked in 100ml of distilled water, acid (1N HCl) or alkali (1N NaOH); heated at 90^{o}C in water bath for 3hrs and allowed to be cooled to room temperature.

2.2.2 Liquefaction

Starch gelatinization was performed by heating the samples to 70^{o}C. The suspensions of untreated and pre-treated samples were adjusted to pH 7.0 with acid or alkali before gelatinization. To all the above gelatinized samples, 0.10 ml (100 I.U) bacterial alpha-amylase was added and the samples were incubated at 40˚C for 1hr. The solution was heated slowly till it gets liquefied.

2.2.3 Saccharification

All the above liquefied samples were adjusted to pH 4.5 with 1N HCl and 0.10 ml, each of crude commercial enzymes containing alpha amylase (1000 I.U), glucoamylase (1000 I.U), cellulase (10 FPU), xylanase (100 I.U) and pectinase (75 I.U) were added individually as well as a cocktail; followed by incubation in water bath at 55˚C for 24 hrs for the saccharification.

2.2.4 Clarification of saccharified samples for analysis:

After making the final volume of the above mentioned hydrolysates to100.0 ml, a 10 ml portion was centrifuged at 10000 rpm at 5^{o} C for 10 min and the supernatant was collected for the sugar analysis.

2.2.5 Determination of hexoses in the hydrolysate:

Samples were diluted 100 times appropriately with distilled water and analyzed for sugar contents by DNSA method (Miller, 1959).

2.3 Fermentation of the Hydrolysates

Inoculum of the yeast strain was prepared in 50.0 ml of YPD (Yeast Peptone Dextrose) broth at 5.5pH and incubated at 25˚C for 48 hrs. The growth of yeast was observed microscopically as well as by taking optical density at 550 nm. The fermentation of the hydrolysate was done separately for all saccharified individual peel samples. A separate study was conducted to observe the fermentation on mixed peel hydrolysate samples. Also a trial was performed to ferment a solution containing cane molasses diluted with the mixed hydrolysate samples produced from peels to make a solution containing higher concentration of sugars.

The hydrolysates were enriched with 0.30% corn steep liquor and 0.10% of ammonium sulphate. The final pH was adjusted to 5.5 with 1N NaOH solution. A 5% inoculum was added to all the production flasks containing 500ml of above mentioned sterile production media, incubated at 25˚C for 72 hrs to allow the complete conversion of sugar into alcohol. The fermented samples were harvested and after making up the volume, a 10ml portion was centrifuged at 10,000 rpm at 5˚C for 10 min to remove the yeast cells and other solids present in the sample. These supernatant samples were used for determination of alcohol contents by GLC method and

residual sugars by DNSA method. Samples were analyzed for alcohol by Perkin-Elmer Clarus 500 gas chromatograph (GC) equipped with BAC1 column (of 15 m length and 2.5 x 10^{-6}m diameter) using Flame Ionization Detector (FID). Nitrogen is used as carrier gas and hydrogen is used for flame ionization.

The GC had been run in an isocratic mode with *Iso*-propanol being used as an internal standard. (Umar, 2008). The conversion yields of alcohol were calculated by the formula:

$$\text{Percent Alcohol} = \frac{\text{Ratio of Sample}}{\text{Ratio of Standard}} \times \frac{\text{Standard Dilution}}{\text{Sample Dilution}} \times 100$$

Where in,

$$\text{Ratio of Standard} = \frac{\text{Area of Standard Ethanol}}{\text{Area of Internal Standard n-propanol}}$$

$$\text{Ratio of Sample} = \frac{\text{Area of Sample}}{\text{Area of Internal Standard n-propanol}}$$

III. RESULTS AND DISCUSSION

3.1.1 Moisture Content

Samples were dried to calculate the moisture content and the solids contents of the sample. Drying is necessary to find out the moisture and total solid contents in the samples to optimize the saccharification process. For commercial process the wet peels as such can be used without drying to conserve the energy. The maximum solid contents were present in Orange peels followed by Potato and Banana peels (Table 1).

Table 1: Biochemical Characterization* of samples of peels

Sample of Peels	Solid contents	Moisture contents	Total Carbohydrates	Free Sugars
Potato	19.265	80.745	95.60	1.0
Banana	08.670	91.330	90.30	1.9
Orange	21.100	79.900	89.00	1.7

* Values in %

3.1.2 Total Carbohydrate content

Total carbohydrate content in the sample of peels gives the estimate of the possible sugar content. Higher the carbohydrate content greater is the proportion of sugar produced. In the total solids, major portion contributes to carbohydrates, followed by protein, ash and oil contents. The maximum amounts of carbohydrates were present in Potato followed by Orange and Banana peels (Table 2).

Table 2: Conversion of samples into Reducing sugar** after pretreatment with alkali and acid and enzymatic hydrolysis of untreated samples and pretreated samples with a cocktail* of enzymes.

Sample of Peels	Pretreatment in *Alkali* & *Acid*		Enzymatic hydrolysis (without pretreatment)	Enzymatic hydrolysis with Pretreatment in *Alkali* & *Acid*	
Potato	1.60	40.70	71.00	91.00	85.00
Banana	1.10	15.00	23.40	77.00	69.00
Orange	1.10	20.10	47.50	65.00	70.40

*Alfa amylase1000IU+ Glucoamylase 1000IU+ Cellulase10 FPU+ Pectinase 75IU+Xylanase 100IU ** Values in %

3.1.3 Pretreatments

Pretreatment is a necessary step to increase the rate of enzymatic saccharification. Pretreatments results in swelling of the organic matter present in the sample which increases the particle size, thus facilitates the accessibility of enzyme to substrate particle, resulting in enhancing the rate of hydrolysis by enzymes. (Mohammad J et.al, 2008). After the pretreatment with alkali the amount of sugar produced was very little in all the samples. On treatment with acid, a significant amount of sugar was produced due to the acidic hydrolysis of carbohydrates in peels. However, it was observed that most of the samples that were pretreated with alkali, resulted into overall better saccharification after enzymatic hydrolysis except in case of orange peels where acid pretreatment gave slightly better results (Table 3).

3.1.4 Enzymatic Saccharification

Enzymatic saccharification was carried out by using enzymes individually as well as with a cocktail of enzymes with untreated, acid pretreated and alkaline pretreated biomass samples. It was observed that the untreated samples resulted into poor saccharification yields which may be due to the inability of enzyme to reach and react with the whole substrate particles, thus only the part which was exposed to the enzyme could be hydrolyzed. In case of acid and alkaline pretreated samples, maximum saccharification value of 91 %, 77 % and 65% was achieved with Potato, Banana and Orange peels respectively using a cocktail of enzymes (Table 3).

REFERENCES

[1.] Arapoglou, D., Varzakas, T., Vlyssides, A., Israilides, C. 2010. Ethanol production from potato peel waste (PPW). Waste Manag. 30(10):1898-902

[2.] Banerjee, S., Mudliar, S., Sen, R., Giri, B., Satpute, D., Chakrabarti, T., Pandey, R. A., 2010. Commercializing lignocelulosic bioethanol: technology bottlenecks and possible remedies, Wiley Interscience, biofuels, bioprod, bioref, 4: 77 – 93, doi; 10.1002/ bbb.188.

[3.] Bidlack, J., Malone, M., and Benson, R., 1992. Molecular structure and component, (a) integration of secondary cell wall in plants. Proc. Okla. Acad. Sci. 72:51-56.

[4.] Blotlnitz, H.V., Curran, M.A., 2007. A review of assessments conducted on bioethanol as a transportation fuel from a net energy, greenhousegas and environmental life cycle perspective, Journal of cleaner production 15, 607- 609.

[5.] Contreras, A., Díaz, G., Gallardo. L., Loaiza, R., 2009. Energy ratio analysis of genetically-optimized potato for ethanol production in the Chilean market. Spanish Journal of Agricultural Research 2010 8(3), 559-569.

[6.] Dellomonaco, C., Fava, F., Gonzalez, R., 2010. The path to next generation biofuels: success and challenges in the era of synthetic biology, Review Microbial cell factories.9 (3):1186/1475–2859–9–3, 9:3doi.10. http://www.microbialcellfactories.com/content/9/1/3

[7.] Dhabekar, A., Chandak, A., 2010. Utilization of banana peels and beet waste for alcohol production. Asiatic J. Biotech. Res. 2010; 01: 8-13.

[8.] Fournier, E., 2001.Current Protocols in Food Analytical Chemistry E1.1.1-E1.1.8

[9.] FAO Food and agricultural organization, Geneva, 2009. www.fao.org/production/faostat.

[10.] Fox, D., Gray, P., Dunn, N., Marsden, W., 1987. Factors affecting the enzymic susceptibility of alkali and acid pretreated sugarcane bagasse. J. Chem. Tech. Biotechnol. 40: 117-132.

[11.] Grohmann, K., Baldwin E.A., Buslig, B.S., 1994. Production of ethanol from enzymatically hydrolyzed orange peels by yeast Saccharomyces cerevisiae. Appl.Biochem.Biotechnol, 45-46: 315-327.

[12.] Grohmann, K., Randall, G. C., Buslig, B.S., 1996. Fermentation of orange peels hydrolysates by ethanologenic E.coli, effects of nutritional supplements. Applied Biochem and Biotech, 57-58, 383-388.

[13.] Haagensen, F. Ahring, B. K. 2005. Enzymatic hydrolysis & glucose fermentation of wet oxidized sugarcane baggase & rice straw for bioethanol production.2005. Risϕ- R- 1517(EN) 184-95.

[14.] Kumar, S., Singh, S. P., Mishra, I.M., Adhikari, D. K., 2010. Bioethanol Production from lignocellulosic biomass. IPPTA. J. Vol.22, No.3, 143-150 doi link July – sept. , India.

[15.] Manikandan, K., Saravanan, V., Viruthagiri, T., 2008. Kinetic studies on ethanol production from banana peel waste using mutant strain of Saccharomyces cerevisiae. Indian J. Biotech 7: 83-88.

[16.] Miller, G. L., 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar - Anal. Chem., 31 (3), 426–428.

[17.] Mohapatra, D., Mishra, S., Sutar, N., 2010. Banana and its by-products utilization: An Overview. Journal of Scientific and Industrial Research, Vol: 69, 323-329.

[18.] Okuda, N., Soneura, M., Ninomiya, K., Katakura, Y., Shioya, S., 2008. Biological detoxification of waste housewood hydrolysate using Ureibacillus thermo sphaericus for Bioethanol Production. J Biosci Bioeng 106 (2): 128 – 33, assessed on doi

[19.] Oberoi, H.S., Vadalani. P.V., Madt, R.L., Saida, L., Jithama, P., 2010. Ethanol production from orange peels: two stage hydrolysis and fermentation studies using optimized parameters through experimental design. J. Agri. Food Chem. 58 (6) 3422-3429.

[20.] Sharma, N., Kalra, K.L., Oberoi .H.S., Bansal, S., 2007. Optimization of fermentation parameters for production of ethanol from kinnow waste and banana peels by simultaneous saccharification and fermentation. Indian J. Microbiol 47 (4) 310-316.

[21.] Sirkar, A., Das, R., Chowdhury, S., Sahu, S.J., 2008. An Experimental Study and Mathematical Modelling of Ethanol Production from Banana Peels by Hydrolysis and Fermentation, IE (I) Journal 88:4-10

[22.] The gazette of India: Extraordinary [part i- sec. i] Ministry of Petroleum and Natural Gas Resolution New Delhi, 3rd September, 2002 No. P-45018/28/2000-C. C. –

[23.] Taherzadeh, M., Karimi, k., 2008. Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. Int. J. Mol. Sci. 9, 1621-1651; DOI: 10.3390/ijms9091621

[24.] Tiwari, H.K., Marwaha, S.S., Rupal, K., 1986. Ethanol from banana peels. Agri.Wastes: 16 (2), 135-146.

[25.] Umar, M., Saleemi, A.R., Faheem, M., Qaiser, S., 2008. Heterogeneous Kinetics and Residue Curve Map Determination for Ethyl tert-Butyl Ether Synthesis via Reactive Distillation Using Ion Exchange Resin Catalysts. Braunschweig, B., Joulia. X., (Editors) 18th European Symposium on Computer Aided Process Engineering – ESCAPE 18, Department of Chemical Engineering, University of Engineering & Technology Lahore, 54890, Pakistan, 2008 Elsevier B.V.

[26.] Wilkins, M.R., 2008. Effect of orange peel oil on ethanol production by Zymomonas mobilis. Biomass and Bioenergy. 33: (3), 538-541.

[27.] Yamada, S., Shinomiya, N., Ohaba, K., Shekikawa, M., Oda, Y., 2009. Enzymatic hydrolysis and ethanol production of by-products from potato processing plant. FSTR 15: 653-658. Food Sci. Technol. Res.,15(6), 653 – 658, 2009

[28.] Zang, B., Von Keitz, M., Valentas, K., 2008. Maximizing the liquid fuel yield via a biorefining process. Biotechnology Bioeng 101 (5): 903 – 12. Assessed doi

[29.] Leif J Jönsson1*, Björn Alriksson2 and Nils-Olof Nilvebrant32013. Bioconversion of lignocellulose: inhibitors and detoxification.Biotechnology for Biofuels. 6:16.