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Original Research Article

In-vitro Enzymatic activity in the production of Bioethanol using Agrowastes

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A B S T R A C T

Bioethanol is a domestically produced liquid fuel made from renewable plant resources known as biomass. Ethanol is also an effective tool for reducing air toxics that comes from the transportation sectors and is presently the alternative to the depleting resources. In this present work, bioethanol was produced by using 15 ready and cheaply available agricultural raw materials. The biomass used are cassava, fruit pulp, rice extract, sweet potato, potato, sugar cane beet, saw dust, coconut pith, groundnut waste, rice straw, rice husk, leaf litter, wood bark, maize husk and waste paper. To produce ethanol from biomass two key processes were followed, first the starch or hemicellulose and cellulose portions of the biomass were broken down in to simple sugars through a process called saccharification. Second, the sugars are fermented to produce ethanol. Two enzymes were used for the hydrolysis of the biomass namely amylase from *Aspergillus niger* and cellulase from *Trichoderma viride*. Fermentation of the hydrolyzed samples was done using *Sacharomyces.s*p. The fermented product was purified by primary distillation process at 80°C and the fraction is collected. The ethanol is then determined by specific gravity method. Further studies can be done to yield more amount of ethanol by optimizing the activity of enzymes over biomass, incubation temperature, and incubation time and also secondary distillation after primary distillation.

Keywords

Aspergillus niger, *Trichoderma viride*, Bioethanol, *Sacharomyces cerevaciae*, Fermentation.

Introduction

Ethanol is a desirable fuel additive because it allows fuel to burn more cleanly and lowers greenhouse gas emissions. It is cost-effective to blend ethanol into gasoline in view of high crude oil prices in recent years (Louime and Uckelmann, 2008).The idea to use ethanol as a source of energy is not new. The oldest evidence about alcohol used as an engine fuel comes from 1899. Between the world wars about 4 million cars used gasoline blended with 25% volume of ethanol (Chereminisoff, 1979). Several methods have been identified to produce bioethanol. Bioethanol can be synthesized from cellulose and hemicellulose that originates from the many sources of biomass (Cheng et al., 2007). Bioethanol is one form of renewable energy source that is fast gaining foot hold as potential fuel to power automotive engine. Microscopic yeast cells break down the starch and water, creating the so called Bioethanol and carbondioxide as end products. Bioethanol burns to produce carbondioxide and water in complete combustion, a process akin to gasoline (Mohammad, 1999). In an earlier study (Taherzadeh, 1999), physiological effects of inhibitors on ethanol from lignocellulosic materials and fermentation strategies was comprehensively investigated. Yeast based fermentation, for example, has yielded ethanol from sugar or crops. The production of ethanol by fermentation of sugar has already been commercially established, but innovative studies could bring improvements to reactors and separation systems. To produce ethanol from lignocellosic materials, it is essential to hydrolyze it before fermentation at the demonstration and industrial level. Enzymatic hydrolysis is still at an early stage, requiring substantial fundamental research (e.g., for increased yields) (Kucuk and Demirbas, 1997). The purposes of this study are to identify the types of biomass that can be used to produce bioethanol and evaluate biomass-to-ethanol opportunities and to investigate the sugars from both the cellulose and hemicelluloses to bioethanol via fermentation.

Materials and Methods

Samples

Cheaply available agricultural raw materials such as cassava, fruit pulp, rice extract, sweet potato, potato, sugar cane beet, saw dust, coconut pith, groundnut waste, rice straw, rice husk, leaf litter, wood bark, maize husk and waste paper were used in this study.

Enzyme Production and Assay

Enzyme such as amylase and cellulase were produced by *Aspergillus niger* and *Trichoderma viride* in potato dextrose broth incubated at room temperature for seven days. After incubation, the media was filtered and centrifuged, supernatant was collected for enzyme assay. Amylase was assayed by starch plate method and cellulase was assayed by DNS method (Dinitrosalicylic acid).

Two Step Enzymatic Hydrolysis

10% of biomass was boiled in distilled water. Incase of cassava, fruit pulp, rice extract, sweet potato, potato, sugar cane beet and rice straw samples are chopped, boiled and filtered. Extract was then sterilized, after sterilization 5% of enzymes was added for hydrolysis and incubated for 3 hours at 37 ^{o}C.

In case of saw dust, coconut pith, groundnut mill waste, rice husk, leaf litter, wood bark, maize husk and waste paper, 25 gm of the sample was taken in 250ml distilled water and boiled as a whole and kept for sterilization. After sterilization 5% of enzymes was added for hydrolysis at 37 ^{o} C for 3 hours of incubation and filtered aseptically.

Fermentation

Hydrolyzed and filtered extracts were fermented using *Sacharomyces s*p for seven days of incubation at room temperature in rotary shaker. 3% of the yeast inoculum was added to the substrate for fermentation to convert simple sugars to alcohol.

Primary distillation was carried in rotary vacuum flask at 80 ^{o} C (boiling point of ethanol) and fraction was collected. Ethanol was determined by specific gravity method and potassium dichromate method.

Results and Discussion

The bioconversion of waste to useable energy is also a part of utilization of waste, as by burning solid fuel for heat, by fermenting plant matter to produce fuel, as ethanol, or by bacterial decomposition of organic waste to produce methanol (Okonko et al., 2009)

Bioethanol can be synthesized from cellulose and hemicellulose that originate from many sources of biomass. Current studies focused on the production of bioethanol from oil palm waste using *Sacharomyces cerevisie* as fermentation agent. Result obtained indicates that, as the concentration of glucose increases ethanol concentration also increased. Highest ethanol yield obtained in this work with a concentration of 15mg/ml of glucose was 13.8% (w/w) (Cheng et al., 2007)

In the present study raw materials such as cassava, fruit pulp, rice extract, sweet potato, potato, sugar cane beet, saw dust, coconut pith, groundnut mill waste, rice straw, rice husk, leaf litter, wood bark, maize husk and waste paper were collected in and around Bangalore, Karnataka, India.

Cellulose is degraded by enzymes know as cellulases that are able to hydrolyze the cellulose polymer to its monomer, the sugar glucose, that is naturally fermented to ethanol by the yeast *Saccharomyces cerevisiae*. Therefore, this biocatalyst is central for the biomass ethanol technology (Sjostrom, 1993).

The enzyme, amylase and cellulase were produced by *Aspergillus niger* and *Trichoderma viride* is determined in the present study, assay of amylase was done in starch agar plate method result zone of inhibition which indicates presence of amylase and cellulase activity was determined by DNS method. These crude enzymes are used for two step enzymatic hydrolysis of biomass.

In the hydrolysis process, few extract of biomass and whole biomass were treated with 5% of the crude enzymes and incubated at 37^{o}C for 3 hours of incubation and reaction was arrested by incubating at 4^{o}C for 15 minutes. Further studies like purification of the crude enzymes and optimization parameters may give better result for degradation of starch or hemicellulose and cellulose present in the biomass.

Fermentation was carried out for the hydrolyzed samples using *Sacharomyces. s*p at room temperature for 7 days of incubation. Primary distillation of the fermented samples was carried out in Rotary vacuum evaporator at 80^{o}C and amount of ethanol produced was tabulated (Table 1 and Fig 1). Produced ethanol is determined by Specific gravity method (Table 2)

The specific gravity of absolute ethanol is 0.79, specific gravity of ethanol produced by biomass is given in table 2, and by secondary distillation we can produce better form of bioethanol. Further studies by optimizing certain parameters and by proceeding secondary distillation we may produce pure form of ethanol using cheap raw materials and other sources.

Table.1 Volume of Ethanol produced from different raw materials

Sr. No.	Sample	Volume of extract before distillation in ml	Volume of extract after distillation in ml	Volume of Bioethanol in ml
1	Potato	250	221	29
2	Sweet Potato	250	216	34
3	Cassava	250	219	31
4	Fruit Extract	250	211	39
5	Boiled rice water	250	226	24
6	Rice Husk	250	233	17
7	Rice Straws	250	238	12
8	Wood bark	250	241	9
9	Sugar cane beets	250	203	47
10	Waste paper	250	232	18
11	Saw dust	250	239	11
12	Cocunut pith	250	245	5
13	Groundnut waste	250	231	19
14	Leasf litter	250	243	7
15	Maize husk	250	231	19

Figure.1 Graphical representation of Volume of Bioethanol produced

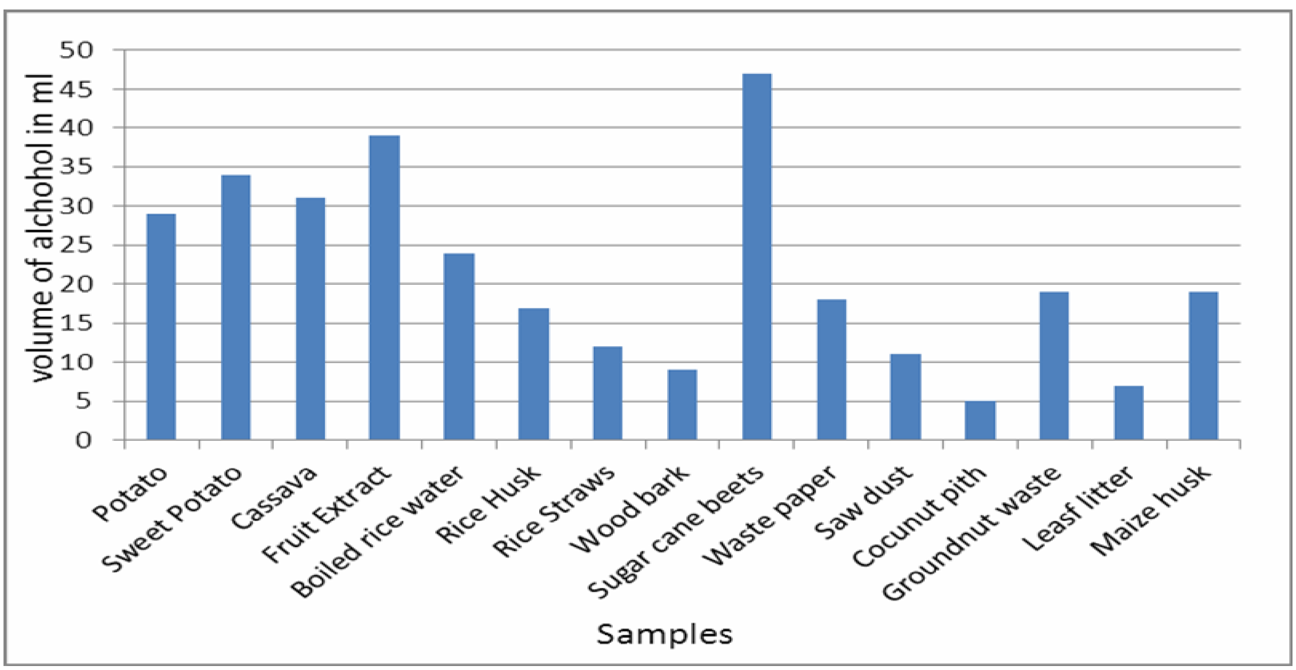

Table.2 Ethanol Determination by Specific Gravity Method

Sr. No.	Sample	Specific Gravity of Ethanol
1	Potato	0.9645
2	Sweet Potato	0.9429
3	Cassava	0.9441
4	Fruit Extract	0.9321
5	Boiled rice water	0.9598
6	Rice Husk	0.9426
7	Rice Straws	0.9557
8	Wood bark	0.9692
9	Sugar cane beets	0.8418
10	Waste paper	0.9817
11	Saw dust	0.9231
12	Cocunut pith	0.9736
13	Groundnut waste	0.9441
14	Leasf litter	0.9121
15	Maize husk	0.9724

Bioethanol was produced from various agricultural raw material using two-step enzymatic process, viz. enzyme hydrolysis (amylase and cellulose) followed by fermentation. Fermentation with *Sacharomyces*.sp was done in anaerobic condition. Result showed that, specific gravity of ethanol (0.8418) obtained from sugarcane beet is very close to the specific gravity of absolute ethanol (0.79) than the other samples. Further work will be carried out to evaluate the economic potential of this process.

References

Cheng, C.K., Hajar H. Hani and Ku Syahidah Ku Ismail, 2007. Production of Bioethanol from Oil Palm Empty Fruit Bunch. *ICoSM*: 69-72.

Chereminisoff, N.P, 1979. Gasohol for Energy Production. *Ann Arbor Sci*. Pub: 12

Iheanyi Omezuruike Okonko., Ogunnusi., Tolulope Adeola., Fajobi Enobong Aloysius., Adejoye., Oluseyi Damilola and Ogunjobi Adeniyi Adewale, 2009. Utilization of Food Wastes for Sustainable Development. *EJEAFChe.*, 8:263-286

Kligtik, M. M. and A. Demirbas, 1997. Biomass conversion processes. *Energy Convers. Mgmt.*, 38:151-165.

Louime, C. and H. Uckelmann, 2008. Cellulosic ethanol: Securing the planet future energy needs. *Int. J.Mol. Sci*., 9: 838-841.

Mohammad J. Taherzadeh, 1999. Ethanol from Lignocellulose: Physiological Effect of Inhibitors. Chalmers University of Technology

Sjostrom, E. 1993. Wood chemistry Fundamentals and applications, 2ed., academic Press Inc., London. Soderstrom, J., L. Pilcher, M. Galbe and G. Zacchi, 2003. Two-step steam pretreatment of softwood by dilute H_2SO_4 impregnation for ethanol production. *Biomass and Bioenergy*., 24: 475-486.

Taherzadeh, M. J. 1999. *Ethanol from Lignocellulose: Physiological Effects of Inhibitors and Fermentation Strategies.* Thesis for the Degree of Doctor of Philosophy, Department of Chemical Reaction Engineering, Chalmers University of Technology, Goteborg, Sweden.