RESEARCH PAPER | Microbiology | Volume : 4 | Issue : 12 | Dec 2014 | ISSN - 2249-555X

In-Vitro Multi Enzymatic Degradation Process of Agrowastes in the Production of Bioethanol

KEYWORDS: Aspergillus niger, Trichoderma viride, bioethanol, Sacharomyces .sp, Fermentation.

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ABSTRACT *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. Cellulase was used for the hydrolysis of cellulose and hemicellulose and amylase is used for the hydrolysis of starch present in the raw materials. Fermentation of the hydrolyzed samples was done using Sacharomyces .sp. 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.*

INTRODUCTION

In view of continuously rising petroleum costs and dependence upon fossil fuel resources, considerable attention has been focused on alternative energy resources. Production of ethanol or ethyl alcohol (CH_3CH_2OH) from biomass is one way to reduce both the consumption of crude oil and environmental pollution. Ethanol is a desirable fuel additive because it allows fuel to burn more cleanly and lowers Green house 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 (Cheremisinoff, 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 chemical properties of the components of lignocellulosics make them a substrate of enormous biotechnological value (Malherbe and Cloete, 2003). Lignocellulose is the major structural component of woody plants and non-woody plants such as grass and represents a major source of renewable organic matter. Lignocellulose consists of lignin, hemicellulose and cellulose, (Fengel and Wegener, 1989; Eaton and Hale, 1993). Cellulases and hemicellulases have numerous applications and biotechnological potential for various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture (Wong and Saddler, 1992; Bhat, 2000; Sun and Cheng, 2002; Beaucheminet *al.*, 2003). The saccharification process of cellulose waste relies on participation of cellulolytic organisms and their cellulase enzymes (Beguin and Aubert, 1994; Singh and Hayashi, 1994; Lynd *et al.*, 2002).

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 collected from rural areas of Bangalore.

Enzyme Production:

Enzyme such as amylase and cellulase were produced by *Aspergillus niger* and *Trichoderma viride* isolated from soil samples and identified based on colony and conidial morphology. They were grown in potato dextrose broth incubated at room temperature for seven days

Enzyme Assay:

After incubation, fermented 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).

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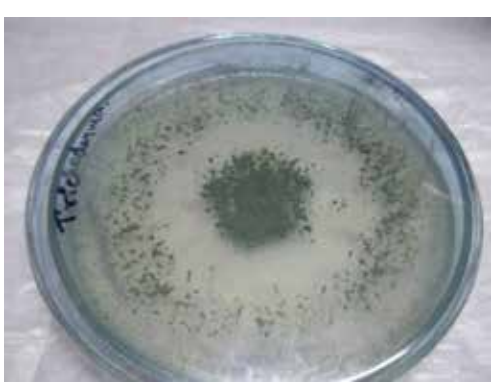
Enzymatic Hydrolysis:
10% of biomass was boiled in distilled water. Incase of cassava, fruit pulp, rice extract, sweet potato, potato, sugar cane beet and rice straw samples are chopped, boiled and filtered. Extract was then sterilized, after sterilization 5% of enzymes was added for hydrolysis and incubated for 3 hours at 37°C. In case of saw dust, coconut pith, groundnut mill waste, rice husk, leaf litter, wood bark, maize husk and waste paper (25 gm) in 250ml distilled water are boiled as a whole and kept for sterilization. After sterilization 5% of enzymes was added for hydrolysis at 37 °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.

Distillation:
The Primary distillation was carried in a distillation flask at 80°C (boiling point of ethanol) and fraction collected and the percentage of ethanol was determined by Specific Gravity Method.

RESULT AND DISCUSSION
The enzyme, amylase and cellulase were produced by *Aspergillus niger* and *Trichoderma viride* Figure-1 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°C for 3 hours of incubation and reaction was arrested by incubating at 4°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°C and amount of ethanol produced was tabulated (Table 1 and Fig 2). 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.



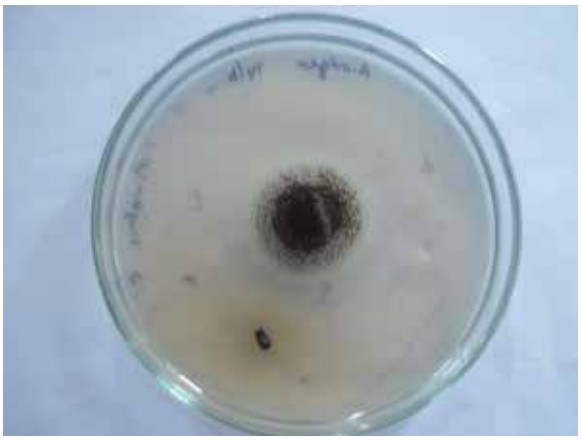


Figure-1 Trichoderma and Aspergillus isolates

Sl.No	Sample	Volum of extract before distillation in mL	Volum 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	boiles 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	coconut pith	250	245	5
13	groundnut waste	250	231	19
14	leaf litter	250	243	7
15	maize husk	250	231	19

Table 1: Volume of ethanol produced from different raw materials

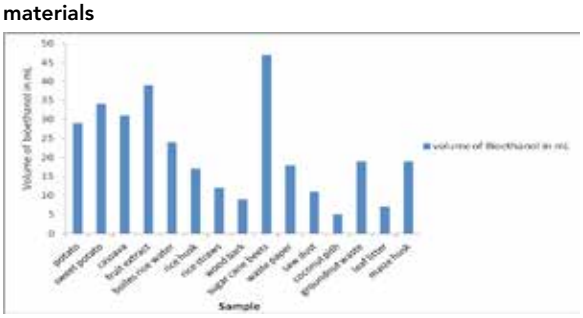


Fig 2: Volume of ethanol produced from different raw materials

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S.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	boiles 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	coconut pith	0.9736
13	groundnut waste	0.9441
14	leaf litter	0.9121
15	maize husk	0.9724

Table 2: Ethanol Determination by Specific Gravity Method

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)

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