

Bioethanol production from weed plant (*Cyperus rotundus*) by enzymatic hydrolysis

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ABSTRACT

*Bioethanol is considered to be an alternative of non renewable fuel sources like diesel and petrol. Nowadays in many countries, Bioethanol is blended with gasoline in fixed proportion and then used as fuel. Bioethanol is produced by fermentation of carbohydrate. In this investigation, *Cyperus rotundus* (weed plant) which contains around 20 to 22% of carbohydrate is used for production of bioethanol. To produce bioethanol, submerged fermentation of weed plant was carried out by *Aspergillus niger* and *saccharomyces cereviceae*, *Aspergillus niger* produced cellulase enzyme which converted cellulose into monomeric carbohydrate unit, subsequently *Saccharomyces cereviceae* was inoculated for conversion of monomeric carbohydrate into ethanol. Hydrolyzed sugar from biomass was estimated by DNSA method. Total 22 % of biomass was converted into simple monomeric carbohydrate. Amount of ethanol produced from biomass is estimated by potassium dichromate method, it was found to be that 40 % of simple monomeric carbohydrate was converted into bioethanol.*

Keywords: *Aspergillus niger*, Bioethanol, *Cyperus rotundus*, Enzymatic hydrolysis.

INTRODUCTION

Amount of petrochemical like diesel and petrol are limited, these are nonrenewable energy resources due to these reason the whole world is under shade of energy crisis and looking for alternative source of fuel. Many countries have been using Biofuel as alternative to petrochemicals like diesel and petrol. Bioethanol is a biofuel which is blended with gasoline in fixed proportion and used as alternative to fuel like diesel and petrol. Maximum of 20 % bioethanol can be blended with gasoline to be used as alternative fuel source in same carbonator engine. This technique has been adopted in Brazil and USA. Bioethanol is renewable source of energy which is commonly produced by fermentation of carbohydrate. Nowadays research is going on all over world for production of bioethanol from biomass. Biomass such as cellulose, animal fat, etc is used for production of ethanol. Main sources of biomass which is converted into ethanol are sugarcane, corn, wheat bran, cassava, sweet potato etc. These are used for ethanol production but they are mainly used for food source and if these sources will be used for the ethanol production, the whole world is going to face food crisis as world population is increasing rapidly. To prevent the world from fuel crisis and food crisis, research has been focused on production of biofuel from waste biomass and plant sources which are not for food purpose like waste generated in sugar mill, chemical pulp generated in paper industry and weeds plant etc. The aim of our investigation was to produce ethanol from weed plant *Cyperus rotundus*, which is generally found in tropical and subtropical region and considered as most troublesome weed which can be found anywhere at the bank of water bodies, in the field with crops plant, it's a versatile nature makes easy to cultivate. In South India people generally cultivate this weed for animal feed but this weed plant also contains some pharmaceutical property. It belongs to the family *Cyperaceae*. It is rich in carbohydrate and fat. The physicochemical composition of *Cyperus rotundus* on dry matter basis is moisture (24%), fat (29%), Crude protein (9%), Ash (2%), crude fiber (12%) and carbohydrate (21%) (Oseni M et al, 2011).

MATERIALS AND METHODS

Sample collection and pretreatment

Cyperus rotundus plant was collected from crop field near to Vellore city. Plant was cut into pieces of 2-3 cm and dried for 4 days, subsequently oven dried at 70 °C for 6 hours and then ground into fine powder using high speed blender.



Figure 1: Collection of *Cyperus rotundus* plant from crop field and conversion into powder form.

Formation of slurry and liquefaction

50 grams of dry weed powder were mixed with 200 ml of distilled water. Whole mixture was heated at 90°C for 45 min in hot water bath to liquefy.

Inoculum preparation

Aspergillus niger and *Saccharomyces cereviceae* were grown on petriplate containing Sabouraud dextrose agar, Cell from single well isolated colony were inoculated in conical flask containing 50 ml of 3% SDA media for preparing spore suspension. *Aspergillus niger* culture was kept at the room temperature for 36 hours, while *saccharomyces cereviceae* culture was kept on incubator shaker at 37° C for 18 hour prior to inoculation.

Enzymatic hydrolysis

To convert cellulose into simple monomeric carbohydrate 6 ml of *Aspergillus niger* spore suspension were inoculated in 200 ml of slurry and kept for 4 days in an incubator shaker at 100 RPM at 37°C for continuous shaking.

Estimation of reducing sugar

Liquefied slurry was transferred into falcon tube and centrifuged at 10,000 rpm for 10 min. Supernatant were transferred to fresh tube and reducing sugars were estimated by DNSA reagent (Dinitrosalicylic acid) as described by Miller (1959). DNSA reagent is prepared by dissolving 1g of dinitrosalicylic acid, 200 mg crystalline phenol and 50 mg of sodium sulfite in 100 ml of 1% NaOH. After addition of DNSA reagent to the standard solution and test solution as shown in Table 1 below, whole reaction mixture was incubated in boiling water bath for 15 min followed by addition of 40% of Rochelle salt in solution to stabilize color of reaction mixture. Subsequently observance was measured at 510 nm. The whole set of experiment was performed in triplicate.

Table 1: Reducing sugar estimation by DNSA method

| S.No. | Concentration(µg/ml) | Volume(µl) | Water(ml) | DNSA(ml) | Rochelle salt(ml) | O.D at 510 nm |
|-------|----------------------|------------|-----------|----------|-------------------|---------------|
| 1 | 0.00 | 0.000 | 3.000 | 3.00 | 1.00 | 0.000 |
| 2 | 200 | 600 | 2.400 | 3.00 | 1.00 | 0.310 |
| 3 | 400 | 1200 | 1.800 | 3.00 | 1.00 | 0.610 |
| 4 | 600 | 1800 | 1.200 | 3.00 | 1.00 | 0.910 |
| 5 | 800 | 2400 | 0.600 | 3.00 | 1.00 | 1.050 |
| 6 | 1000 | 3000 | 0.000 | 3.00 | 1.00 | 1.250 |
| 7 | Test sample | 10 | 2.990 | 3.00 | 1.00 | 0.715 |

Fermentation

Fresh *Saccharomyces cereviceae* culture was inoculated into liquefied slurry. Conical flask was sealed completely to induce an anaerobic condition for efficient fermentation. Monomeric Sugar units were converted into Bioethanol by action of mixture of enzyme produced by *Saccharomyces cereviceae*.

Ethanol estimation

Amount of ethanol produced by fermentation was estimated by potassium dichromate method. Initially 10 ml of liquor was centrifuged at 10,000 rpm for 30 min at 4°C. One gram of potassium dichromate was dissolved in 100 ml of 5M sulphuric acid solution, prepared chromic acid reagent was added to standard as well as test sample according to table 2 followed by 10 min of water bath at 90 °C and addition of 40 % Rochelle salt solution. Absorbance was measured at 600 nm.

Table 2: Potassium dichromate method for estimation of Bioethanol

| S.No. | Ethanol(ml) | D. water(ml) | Chromic acid(ml) | Rochelle salt(ml) | O.D at 600 (nm) |
|-------|-------------------|--------------|------------------|-------------------|-----------------|
| 1. | 0.00 | 10.00 | 3 | 1 | 0 |
| 2. | 0.20 | 9.80 | 3 | 1 | 0.119 |
| 3. | 0.40 | 9.60 | 3 | 1 | 0.111 |
| 4. | 0.60 | 9.40 | 3 | 1 | 0.172 |
| 5. | 0.80 | 9.20 | 3 | 1 | 0.19 |
| 6. | 1.00 | 9.00 | 3 | 1 | 0.26 |
| 7. | Test sample(10ml) | 0.00 | 3 | 1 | 0.112 |

RESULTS AND DISCUSSION

Recently, due to shortage in natural resources like petrochemical and natural gas Scientist have been trying to develop new renewable energy resources. As it is mentioned earlier in study that nowadays bioethanol is used as alternative to petrochemical, due to this reason many researchers have tried to find out various sources for production of Bioethanol, biomass is one of the resources for bioethanol. Various technique has been used for conversion of biomass into ethanol like acid treatment of biomass before fermentation, Enzymatic hydrolysis etc. In this study *Cyprus rotundus* (Weed plant) were selected for study, which contains 21% carbohydrate of total biomass on dry weight basis. In this study pretreated biomass of *Cyperus rotundus* was inoculated with *Aspergillus niger* for enzymatic hydrolysis. It produces cellulase enzyme which hydrolyzed polysaccharides into simple sugar. Result of sugar estimation in hydrolyzed solution revealed that 22 % of total biomass was converted into simple sugar by enzymatic hydrolysis.

Figure 2: Standard graph derived from Table1 which is used to calculate amount of monomeric carbohydrate produced.

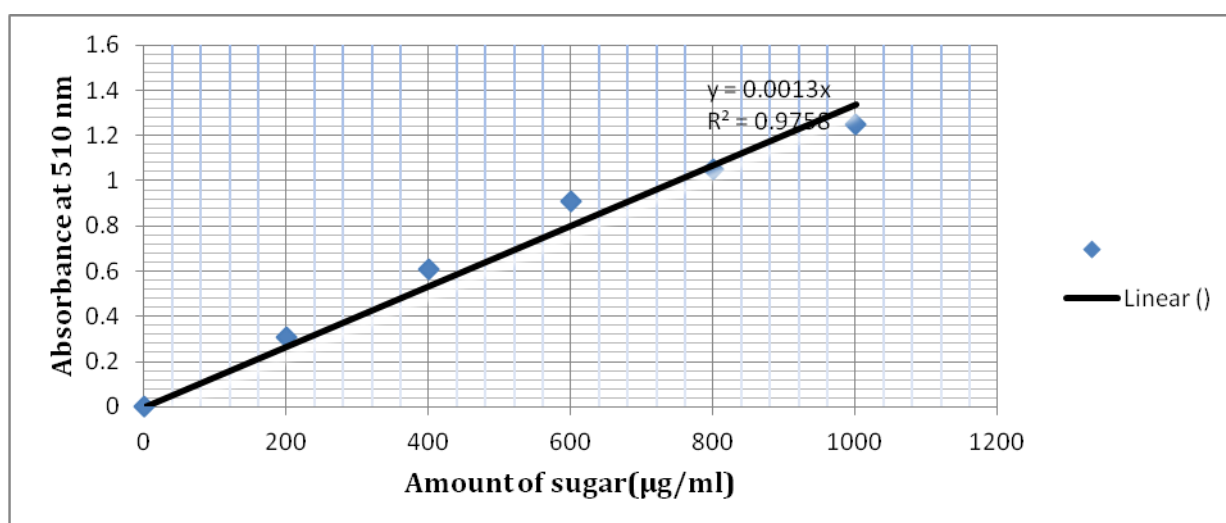
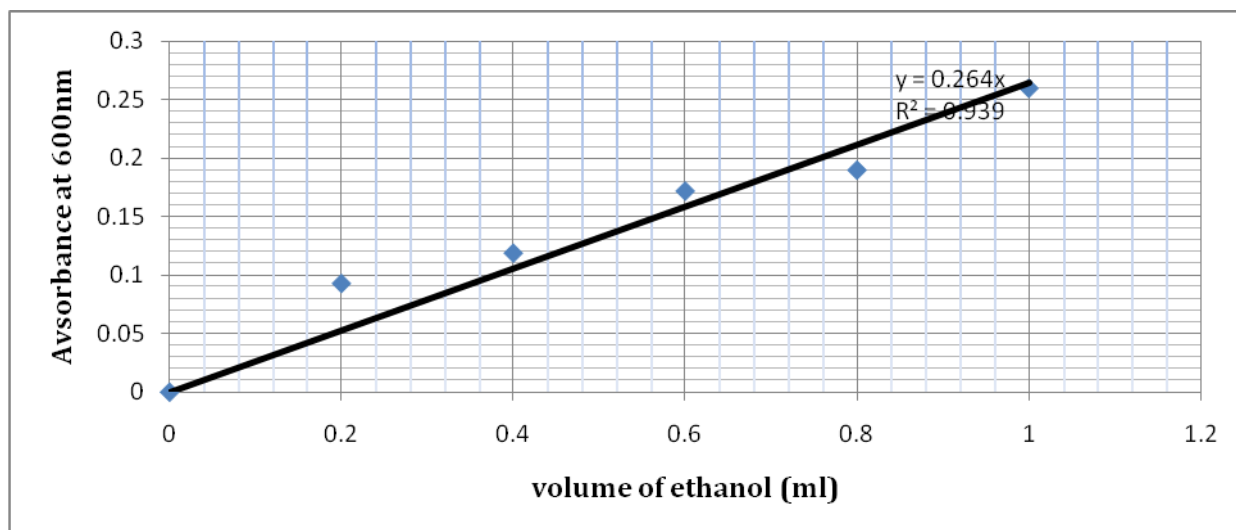


Figure 3: Standard graph derived from Table2 which is used to estimate amount of ethanol produced by fermentation.



The enzymatically hydrolyzed slurry of *Cyperus rotundus* biomass was directly used as media for ethanol fermentation. *Saccharomyces cereviceae* was inoculated in hydrolyzed juices to carry out fermentation. Amount of ethanol produced by fermentation is estimated by potassium dichromate method. Result of this investigation revealed that 40 % of simple monomeric carbohydrate was converted into bioethanol. Conversion of sugar into ethanol mainly depends on types of yeast strain used for fermentation. Efficiency of conversion of carbohydrate into ethanol by yeast cell mainly depend on acidity of medium and availability of oxygen, so to obtain higher efficiency optimum acidic condition should be maintained throughout the experiment. During this study acidity of the medium was maintained 4.5. Most of the countries are using crops like corn, sugarcane, potato, etc to produce bioethanol. But in many countries like India, china and other country which has high population density, it is not possible to use crop plant or potato for fuel resources because chances of facing food crisis will increase. In these situations weed plants like *Cyperus rotundus* can be good alternative sources for ethanol production.

CONCLUSION

Fuel crisis is biggest problem in the world. It motivates researchers to find out a new and low cost source for ethanol production. In this study, *Cyperus rotundus* was used for ethanol production. During this study it was found that sufficient amount of ethanol can be produced from this weeds plant. There are many other weeds plants are available which has high carbohydrate content so it may serve as good alternative resource for ethanol production. Since research in this area is very rudimentary so further investigation is required to optimize condition for efficient production of ethanol from weed plants and waste biomass.

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