"Unlocking the Potential of Cellulosic Biomass: Bioethanol Production Strategies"

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ABSTRACT:

Ethanol is one of the most widely used liquid biofuels in the world. The move from sugar-based production into second-generation, lignocellulosic-based production has been of interest due to an abundance of these non-edible raw materials. Bioethanol is one of the alternatives to conventional fossil fuels. In the present demand for renewable and sustainable sources of energy to overcome the burden of the world energy crisis, grasses present exciting options. The grasses were analyzed for their potentiality as alternative energy crops for cellulosic ethanol production by biological process. In this study, we aim to evaluate the ethanol production potential from the grass and to suggest a production process based on the results obtained from the study. The composition of grasses varies with species, but in general, it consists of lignin (18%), hemicelluloses (25-30%), and cellulose (29-50%). The process includes two main steps. First, lignocelluloses must be pretreated to remove lignin and enhance the penetration of hydrolysis agents without chemical destruction of cellulose and hemicelluloses. Second, the pretreated material is converted to bioethanol by hydrolysis and fermentation. The samples were initially pretreated with acid, enzymatic, and natural degradation methods. It was followed by the estimation of sugars and the subsequent bioethanol production ranged from 35-89 gm/l.

Key Words: Bioethanol, Lignocellulosic, Natural degradation, Acid Pre-treatment

INTRODUCTION

For thousands of years in history, alcohol production from natural agricultural products with high starch and sugar contents such as fruits, grains, sugarcane, etc has been widely known. Microbial cultures are allowed to grow on these carbohydrate-rich materials to convert these sugars and starches to ethanol via their metabolism. The present times attract biofuels from around the world owing to their environmental friendliness. They are renewable as a neutral carbon source, that does not break the balance in the atmosphere's air contents leading to global warming. They are among the most feasible ways to counteract human dependency on traditional fossil fuels.

The current scenario of global warming has been undeniably posing a worldwide threat, due to the increase of greenhouse gases. One way of approach to this problem is the application of alternative energy. Thus, it is necessary to search for sources of renewable energy such as solar, wind, hydraulic, geothermal, and tide energy. On the other hand, the biofuels from biomass can also represent a promising type of energy source. Bioethanol is now considered an interesting biofuel and has been attracting attention since it can be directly used in place of benzene or diesel. It can be applied in the form of a mixture with benzene called gasohol or blended with diesel called diesohol. Bioethanol can be produced from biomass such as starch, sugar, and lignocellulosic materials. Previous studies revealed that the cheaper and more suitable one for the production of ethanol can be derived from corncobs, grasses, starch, sweet sorghum, etc. However, the use of starch and sugar from sweet sorghum might lead to the problem of food crisis. Therefore lignocellulosic biomass is considered the best and cheap source for bioethanol production.

Grasses such as lignocellulose biomass are promising feedstocks for renewable bioethanol production, since these raw materials have high productivity, require low agricultural inputs, have positive environmental impacts, are easy to process, and do not compete with food crops. However, bioethanol production from grass biomass requires efficient pre-treatment, enzymatic hydrolysis, and microbial fermentation processes which vary with the types of grass species and the microorganisms used. Pretreatment is an important process for the delignification of lignocellulose biomass and is dependent on the type of lignin present in the biomass and the degradation pathway employed for the removal of the specific type of lignin. Further, enzymatic hydrolysis converts the cellulose and hemicellulose into monomers, making it feasible for the fermenting microorganisms to convert it into bioethanol where the use of improved strain and biomass can yield higher ethanol on an industrial scale.

The objective of this research was to select the type of grasses suitable as feedstocks for Bioethanol production by biological processes. In the natural degradation method, the cellulosic materials are degraded to fermentable sugars for ethanol production in a single step. The obtained sugar is continuously utilized by Saccharomyces cerevisiae (yeast) in co-fermentation for ethanol production.

MATERIALS AND METHODS:

Samples: 6 Different samples of grasses were collected from the Telangana region viz.,

S.No	Sample
1	Dry grass
2	Bagasse
3	Rice husk
4	Green grass
5	Galugu,
6	Sorghum

The polysaccharides, cellulose, and hemicelluloses are intimately associated with lignin in the plant cell wall, the lignin component acts as a physical barrier and must be removed to make the carbohydrates available for further hydrolysis process. Therefore, pretreatment is a necessary process for the utilization of lingo-cellulosic materials to obtain an ultimately high degree of fermentable sugars.

ACID PRE-TREATMENT:

250g of chopped cellulosic material was suspended in 1% sulfuric acid solution in a ratio of 1:10(w/v). The mixtures were incubated in a hot water bath for 20min at 80°C and the sample is pressed on a cheesecloth the juice of the sample is obtained and tested for reducing sugar content colorimetrically by the DNS reagent method according to Miller(1959). Dilute-acid hydrolysis has been successfully developed for the pretreatment of lignocellulosic materials. Sulfuric acid at concentrations usually below 4% wt, has been of the most interest in such studies as it is inexpensive and effective

NATURAL DEGRADATION PROCESS:

The different cellulosic samples were chopped to marble size and 50ml of water is added to 250g of each sample, they were autoclaved at 121°C temperature for 15 minutes at 15lbs pressure. The mixture turns into a semisolid form, and the sugar content is estimated by the DNS reagent method, It is allowed for co-fermentation with Saccharomyces cerevisiae for 7 consecutive days at 35-40° C temperature with agitation. A greater benefit of this method is it is economical, environmentally friendly, it is low energy requirement and has mild operation conditions.

ENZYMATIC METHOD:

The samples were treated with amylase, lipase, and a combination of both. Hydrolysis in a batch fashion is generally characterized by a limited logarithmic phase, associated with the rapid release of soluble sugars, followed by a declining rate of sugar production as the reaction proceeds. Substrate concentration is one of the main factors that affect the yield and initial rate of enzymatic hydrolysis. At low substrate levels, an increase in substrate concentration normally increases the yield and reaction rate of the hydrolysis.p H and temperature are also great contributing factors. The enzymatic hydrolysis breaks down cellulosic material into simpler sugars which are then fermented with yeast to produce ethanol.

MICROBIAL FERMENTATION

Yeast cells (Saccharomyces cerevisiae) were precultured aerobically in YPD medium at 30°C for 48 hours, harvested by centrifugation for 5 minutes, and then washed twice with distilled water. The cells were then resuspended in a 20 mL YP medium containing each sample. Ethanol fermentation proceeded for 7 consecutive days at 35-40° C temperature with agitation in closed bottles, each equipped with a siliconized tube and check valve.

ETHANOL ESTIMATION:

The above-fermented wash is distilled and the distillate collected was tested for ethanol concentration (table-3) by potassium dichromate method (Yoswanthana and Phuriphipat 2010)

RESULTS AND DISCUSSION

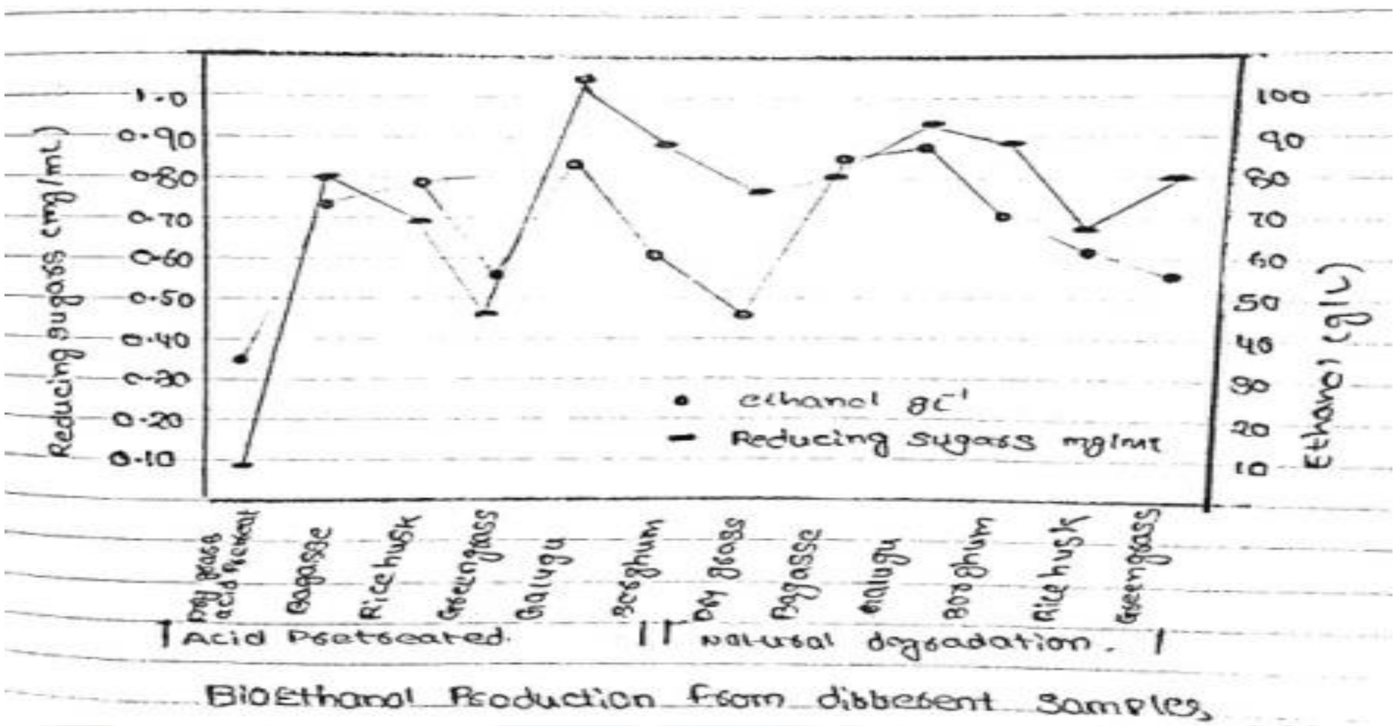
The composition of grasses varies with species, but in general, it consists of 18% of lignin, 25-3 % hemicelluloses, and 29-50% cellulose which makes it an alternative fuel source. 6 Different cellulosic samples were taken for ethanol production, they were variously degraded with acid, enzymatic, and natural degradation pretreatment methods. Hydrolysis of the main components cellulose and hemicelluloses into glucose and xylose as the substrates of fermentation is necessarily required for the production of ethanol. In our study the sugar concentration ranged from 0.10 -1.2mg/ml and the ethanol concentration ranged from 35-89gm/l with various pre-treatments (table 1).

In the present study acid pre-treatment did not show any significant improvement in either sugar or ethanol production. The conflicted preliminary results in the acid pretreatment may be due to certain chemicals present in the grasses such as tannins and silica. The higher amounts of monosaccharides and ethanol were obtained by natural degradation which is compression under high pressure. We have used amylase and lipase enzymes individually and in combination for ethanol production. The use of enzymes by default and using natural degradation has improved the production of bioethanol substantially. The highest ethanol concentration was 89 g/l from the local variety Galugu followed by Bagasse at 82 g/l. Seasonal availability of bagasse makes Galugu the best suitable resource. Sorghum being a food crop there was a search for non-food alternatives like Galugu. The higher amounts of sugar and ethanol were obtained from GALUGU by natural degradation method & co-fermentation, so we conclude that galugue is found to be an efficient and economical source for Bioethanol production.

S.NO	Sample	Sugar conc. In AP(mg/ml)	Sugar conc. in ND(mg/ml)	Ethanol conc. in AP(gm/l)	Ethanol conc in ND(gm/l)
1	Dry grass	0.10	0.78	35	45
2	Bagasse	0.80	0.79	72	82
3	Rice Husk	0.68	0.67	79	62
4	Green grass	0.45	0.81	56	57
5	Galugu	1.12	0.95	82	89
6	Sorghum	0.85	0.89	60	70

Table showing different sugar and ethanol concentrations, AP=acid pretreatment

ND=natural degradation



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