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REVIEW ARTICLE

Development of Lignocellulosic substrate for Bioethanol production

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ABSTRACT
Bio-ethanol from lignocellulosic biomass is one of the important alternatives being considered due to the easy adaptability of this fuel to existing engines and because this is a cleaner fuel with higher octane rating than gasoline Sorghum feedstock is good cellulosic substrate for bio-ethanol production. Cellulose is most abundant polymer in the world .Cellulose makes a large fraction of the plant dry weight, being typically in the range of 35-50%.Lignocellulosic biomass is considered as the only foreseeable feasible and sustainable resource for renewable fuel; but the lingo-cellulosic ethanol commercialization is largely limited due to the lack of easily digestible substrate or cost effective processing technologies and cost of enzymes. Cellulosic ethanol is a biofuel produced from wood, grasses, or the non-edible parts of plants.It is a type of biofuel produced from lignocelluloses, a structural material which makes most of the biomass of plant.
Key words:*Biomass ,Lignocellulose, bioethanol,feedstock*

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INTRODUCTION

India is a country with a positive outlook towards renewable energy technologies and committed to the use of renewable sources to supplement its energy requirements. The country is one among the few nations to have a separate ministry for renewable energy which address the development of biofuels along with other renewable energy sources. Indian distilleries use molasses as the feedstock for ethanol production and the annual supply of molasses is sufficient only for producing approximately 2.7 billion liters of ethanol. Of this, only a minor share is available for fuel use [1,2]. Also the prices of molasses ethanol can soar once the current government subsidies on sugar are lifted and this would mean that other raw materials such as grains or lignocellulosic biomass has to be used for fuel ethanol production.

With a huge population to feed and limited land availability, the nation needs to develop bio-ethanol technologies which use biomass feedstock that does not have food or feed value. For ethanol production; feedstock availability, its variability and sustainability are the main issues to be addressed. Technologies for biomass to ethanol conversion are also under various stages of development. Various bottlenecks in such technologies include the pre-treatment of biomass, enzymatic saccharification of the pretreated biomass, and fermentation of the hexose and pentose sugars released by hydrolysis and saccharification [4]. Each of these problems requires substantial R&D efforts for improved efficiency and process economics

In the U.S. corn is by far the largest carbohydrate source used to produce ethanol. But for a wide range of reasons, scientists are looking at alternative feedstock's. Sorghum has been identified as a promising candidate both in the U.S. and in the developing world. The U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA) are both strongly committed to expanding the role of biomass as an energy source. In particular, they support biomass fuels and products as a way to reduce the need for oil and gas imports; to support the growth of agriculture, forestry, and rural economies; and to foster major new domestic industries biorefineries making a variety of fuels, chemicals, and other products.

RELEVANCE OF RESEARCH IN RELATION TO INDIA

India is a fast growing economy with an inherent increase in demand for energy. With a huge population and limited land resources, the nation is looking for alternative renewable fuels to support the pace of growth. The demand for liquid transportation fuels is constantly increasing and bio-ethanol might be one of the most potent solutions to the problem. India is one of the largest producers of ethanol and currently all commercial ethanol production in the country uses molasses as feedstock. However, most of it is consumed for application in liquor and chemical industries and the surplus availability can barely support the current demand created by a mandatory 5% blending of ethanol in gasoline implemented in several states. One of the major difficulties that would be faced by bio-ethanol technology developers as well as future entrepreneurs will be the choice of feedstock. Though India generates a huge amount of biomass residues as agro-and forest residues, the only feasible feedstock among these would be the crop residues due to problems in collection and logistics. Even in the case of crop residues, the availability is limited due to the use of a major fraction of it as feed and fuel in rural areas. The residues from major agricultural crops like rice wheat and sugar cane are mostly consumed in as fodder or as raw material for competing industries like paper, and less than 10% are available in surplus.

CELL WALL COMPOSITION

Cellulose :

Cellulose is organic compound with chemical formula $(C_6 H_{10} O_5)n$,a polysaccharide consisting of linear chain of several β (1-4) linked D-glucose units. Cellulose is structural component of primary cell wall in plants. Cellulose is the most abundant organic polymer on the earth.

Hemi cellulose:

A hemi-celluloses also known as polyose is any of severalheteropolymers, such as arabinoxylans present along with cellulose in all plant cell wall. Hemicellulose is amorphous structure with little strength. It is easily hydrolysed by base or acid and hemicellulase enzymes.

Lignin:

Lignin is an organic material constitutes lignified elements of plants and wood .It is the second most abundant renewable carbon source on earth. About 40 to 50 million ton of lignin produced per annum worldwide.

CHARACTERIZATION OF CELLULOSE HEMICELLULOSES AND LIGNIN

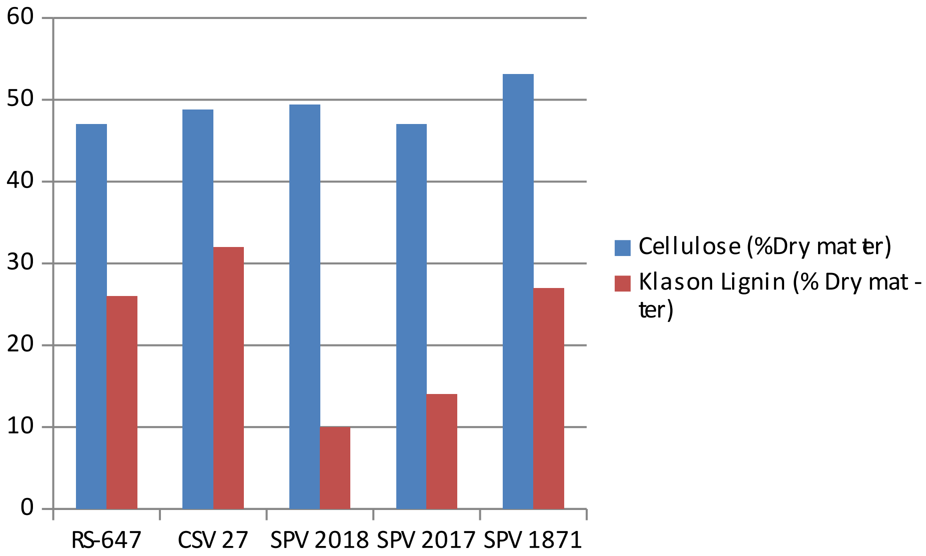
Material and Method

Extractive-free biomass sample ground to 40 mesh.Measure moisture content.0.175 oven dried grams weighed in digestion tubes.Add 1.5 ml 72% Sulfuric acid and stir with glass rod. Placed in water bath at 30 ºC for 1 hour (stirred few times). Add 42 ml DI water (3% sulfuric acid) and place in autoclave set at 121 ºC for 1 hour autoclave set at 121 ºC for 1 hour.Take out and cool in ice/water bath.Filter with porcelain crucibles fitted with glass fiber Filter with porcelain crucibles fitted with glass fiber G8 filters.Filter preparation Filter preparation.Washed and dried in 105 ºC oven.Solid is Klason lignin. Liquid is acid soluble lignin measured using UV-spectro photometer at 205 nm.

RESULTS

Table1

Sorghum genotypes	Cellulose (%Dry matter)	Hemicellulose (% Dry matter)	Alpha cellulose (% Dry matter)	Holocellulose (%Dry matter)	Klason Lignin (% Dry matter)
RS-647	47.0	14.2	45.2	61	26
SPV 1870	48.8	20.2	46.8	69	32
SPV 2018	49.4	24.6	47.5	74	10
SPV 2017	47.0	19.0	45.8	66	14
SPV 1871	53.1	24.9	45.9	79	27



Graph1: showing Cellulose% and Klason Lignin % in Sorghum genotypes

Fiber analysis of promising sorghum genotypes

At present, researchers are more interested in sugars-based ethanol (first generation ethanol)which can also be used as food source (examples are corn,sorghum and sugarcane) to non food based ethanol (second generation ethanol) such as lignocellulosic biomass and as a consequence limit the competition between fuels and food production. Field crops are one of the best sources of renewable energy which can be used as feed-stock for biofuels production.

Sorghum is a multipurpose crop with the potential to achieve sustainable biofuel production, human food and animal feed products. Typical first generation biofuels are sugarcane ethanol, starch-based or "corn" ethanol and biodiesel. Sugar rich crops especially those that yield multiple end products, are promising. Second-generation ethanol requires lignocellulosic plant residue.Lignin is the important supportive polymer for stand ability of plant. Plant with low lignin content lodge easily. Optimization of lignin content is one of the primary objective in developing plant substrate for bio-ethanol. Effective lignocellulosic bioenergy conversion requires appropriate pretreatment to liberate the plant cell wall polysaccharides (cellulose and hemi-cellulose) from its lignin coating .The major difficulty in converting biomass to bio-fuels is high pretreatment costs for removal of lignin, and high cost of enzymes used for saccharification. Sorghum biomass especially low lignin brown mid-rib sorghum is good substrate for extraction of sugars and converting it into ethanol. Diversity in sorghum genotypes can be exploited for ethanol production. Forage sorghum developed and bred to contain the BMR gene,has less lignin and will be good substrate for cellulolytic enzymes. Less lignin ensures the plants are softer and easier to apply pretreatment methods. Significant differences were observed in bmr mutants for stover yield plant height,stover yield nitrogen content,invitro organic matter digestibility(IVOMD) and acid ditergent lignin content(ADL)(9).Brown midrib (BMR) mutant plants evidence theimportance of genetic selection to improve the digestibility of forages. In studies with mutant plants, it was observed that, despite their lower agronomic value,the genotype had lower content of lignin and celluloseand higher digestibility, intake and productivity per animal (8). For neutral detergent fiber (NDF) and acid detergent fiber (ADF) there were no differences between genotypes, the mean values were 58.08 and 35.28%,respectively (10).

In present study sorghum genotypes RS647 (bloomless), CSV27 (dual purpose) ,SPV2017 (bmr), SPV2018 (bmr) and SPV 1871 were characterize for cellulose, hemi-cellulose and lignin content. Cellulose % ranged from 47.0 to 53.1, hemi-cellulose from14.2 to 24.6 %.SPV 2017 and SPV 2018 has reduced lignin percent 10 and 14 respectively (Table1). Cellulose content in high biomass sorghum lines ranged from (27-52%) while hemicellulose (17-23%) (7). Graph shows lowest lignin content in SPV 2018 followed by SPV 2017.CSV 27 and SPV 1871 has high lignin and cellulose content.

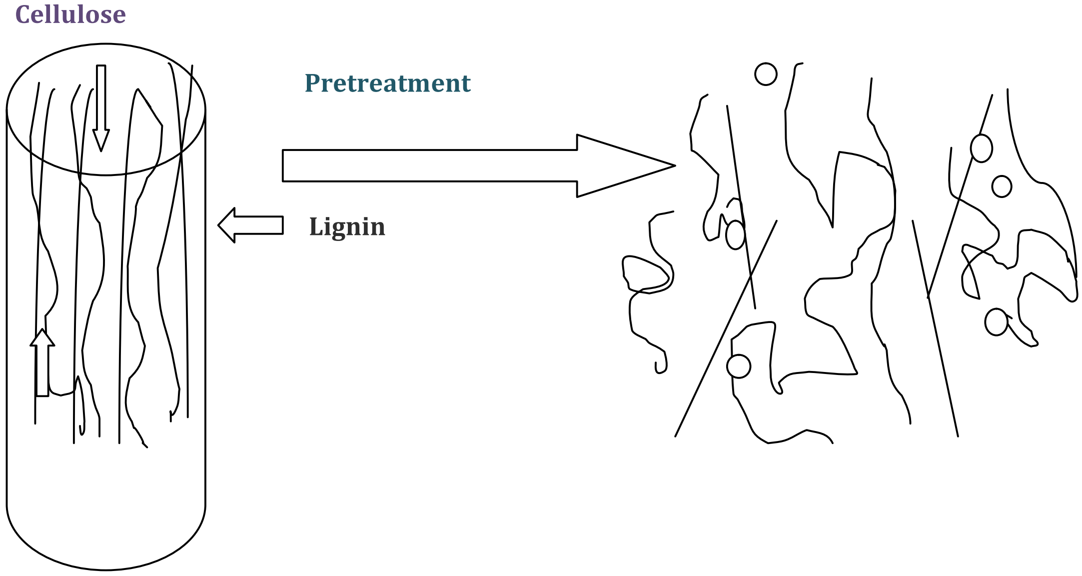
Table 2: **Comparison of lignin contents (%w/w,)**

Genotype	Klason Lignin	ADL
Bmr 12/bmr6	10.6	1.09
Bmr-6	12.4	2.16
Bmr-12	12.7	2.03
Wild type	14.6	2.92

ADL:Acid detergent lignin
Adapted from Michael Cotta and Bruce Michael Cotta and Bruce Dien USDA - ARS - National Center for Agricultural Utilization Research

Processing of Lignocellulosic Biomass

Processing of cellulosic biomass to produce biofuels requires that feedstock has to be harvested; besides the choice of the feedstock and the growing and harvesting techniques, suitable to make them produce biomass that is easier to process. Second, the biomass is pretreated thermo-chemically to remove lignin, solubilize hemicellulose and make the cellulose more accessible.Enzymatic hydrolysis that converts the cellulose to sugar. The enzymes that are needed for this step are then added to the biomass. Fourth, the sugars are fermented to ethanol by microorganisms and the ethanol is removed from the bioreactor. Additionally, the ethanol is usually distilled after the extraction, because the ethanol needs to be highly concentrated in order to be used as fuel additive. In



Hemicellulose

Ethanol yield determined by accessibility of cellulases to cellulose

order to get an efficient process some of the waste material can be reutilized in the process. Lignin for example can be burnt to generate electricity and heat for the process, but there are also technologies under development that would allow converting it to higher value products. To minimize the fresh water consumption it can be reutilized, but the problem is that dissolved inhibitory substances might accumulate and interfere with the process. So the positive effects of water reuse and the increased energy and material use due additional detoxification steps have to be balanced.

There are five stages to produce ethanol from lignocellulosic material.

1. Pretreatment to make lignocellulosic material amenable to hydrolysis.
2. Breaking of cellulose into sugars
3. Separation of sugar solution from lignin
4. Microbial fermentation of sugar solution
5. Distillation to produce 95%pure alcohol
6. Dehydration to bring ethanol over up to 99.5

Pretreatment methods are not well established in rice bran,wheat straw.On the other hand special type of sorghum i.e bmr sorghum can be used in introgression breeding program to develop low lignin cultivars suitable for pretreatment methods. Among all methods available for the pretreatment of lignocelluloses, acidic and alkaline treatments have been proven to have practical advantages.

Reduced lignin concentration in lingo-cellulosic biomass can increase forage digestibility and saccharification yields of biomass for bioenergy. In sorghum (Sorghum bicolor (L.) Moench) and several other C4 grasses, brown midrib (bmr) mutants have been shown to reduce lignin concentration. The bmr-12 near-isolines generally had lowest stover lignin content and highest fiber digestibility,bmr-6 was intermediate, and wild-type counterparts had highest lignin content and lowest fiber digestibility.(8).

Brown midrib sorghum

Wild relatives of sorghum species and land races are good source of genetic variation.Brown midrib sorghum Sudan grass could be exploited to create varieties with low lignin and high biomass through introgression breeding.Forage sorghum developed and bred to contain the BMR gene,has less lignin and will be good substrate for cellulolytic enzymes.Less lignin ensures the plants are softer and easier to apply pretreatment methods.

A class of low lignin mutants that were discovered in maize [first identified in Minnesota](6)

Biomass energy has the potential to greatly reduce our greenhouse gas emissions. Biomass creates about the same amount of carbon dioxide as fossil fuels, but every time a new plant grows, carbon dioxide is actually removed from the atmosphere. The net emission of CO2 will be zero as long as plants continue to be replenished for biomass energy purposes.

These energy crops, such as fast-growing trees and grasses, are called biomass feed-stocks. The use of biomass feed-stocks can also help increase profits for the agricultural industry. Agro-industrial biomass comprised on lignocellulosic waste is an inexpensive and renewable

FUTURE PROSPECTS

Sorghum cultivars with reduced lignin can pave a better way to increase second generation cellulosic ethanol pro-duction as compared with other crop residues and also improve process economics targeting higher conversion efficiency. Reduced lignin content will be highly beneficial for improving biomass conversion yield. Globally research efforts are focused on utilizing plant cell walls as renewable resources for the energy production, chemical precursors, and fuels; however, the traditional uses of plant cell walls as fodder and fiber also remain important. A brown midrib sorghum has high digestibility than other sorghum genotypes. BMR derivatives offers tremendous flexibility to producers. Apart from pasture forage, these bmr genotypes is exceptionally palatable. There is a rise in demand for renewable energy sources for biofuels. To meet the demand, there is a need to develop sorghum cultivars that produce high stalk yield per unit time, input energy and land areas in different agro-climatic areas. Future research should also address the optimization of sorghum as an energy crop through exploration of the available genetic resources through plant breeding.

It is prime important that not only scientific, but also sociopolitical and climatic aspects have to be considered. It is not sufficient to develop efficient and affordable processing techniques to get sustainable fuels. Also the production of the biofuel feedstock must be sustainable and should not compete with food and other more basic requirements of society. However, ethanol that is produced from lignocellulosic biomass can solve the current conflict between food and fuel production that emerged with the first generation biofuels and can make a contribution to renewable energy production.

CONCLUSION

In recent years, introduction of sorghum plants containing the bmr gene generated much interest because plants with this trait have lower lignin concentrations than conventional types. The BMR mutant of forage sorghum contained substantially less cell wall content than other sorghum types and resulted in greater fiber digestibility. The enhanced cell wall digestibility from BMR sorghum improved milk yield of mid – lactation dairy cows at grazing. The reduced levels of lignin increase the bioconversion efficiency of biomass and reduce the cost of production of biofuels. The bmr 6 mutation in sorghum encodes cinnamyl alcohol dehydrogenase 2 (CAD2). Sorghum bmr12 locus encodes orthologous caffeic O-methyltransferase(COMT).Caffeic O methyl transferase(COMT) are members of an evolutionary conserved O-methyltransferase family, whose function in lignin biosynthesis has been documented in both monocots and dicots.

It can be concluded that the bmr sorghum bagasse is a remarkable feedstock for ethanol production regarding to its easy cultivation and favor properties as well as high glucan fraction. It is a potential energy crop in nearly all temperate, subtropical, and tropical climates. It produces sugars juice, grains with high starch content, and bagasse. The bagasse is usually used for energy production by incineration. Significant differences exist in sorghum cultivars for the amount of lignin reduction and its impact upon quality traits. Identifying tall bmr derivatives which has high biomass and low lignin is important for bioethanol industry. Lignocellulosic biomass is considered a future alternative for the agricultural products that are currently used as raw material for bioethanol production, because it is more abundant and less expensive than food crops, especially when waste streams are used. Furthermore, the use of lignocellulosic biomass is more attractive in terms of energy balances and emissions.

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