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High Biomass Sorghum as a Potential Raw Material for Biohydrogen Production: A Preliminary Evaluation

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Abstract

Six high biomass sorghum lines (IS 22868, IS 27206, IS 15957, IS 16529, ICSV 93046 and CSH 22SS were evaluated for their potential as a substrate material for biohydrogen production by anaerobic fermentation using methanogens deactivated cow dung based mixed microbial consortia. The data revealed that all selected high biomass sorghum lines differed significantly for candidate biomass traits as well as for biomass composition. The high biomass lines, IS 27206 and IS 22868 recorded higher stalk and stover yield compared to others. Least biomass yield (stalk and stover) was noticed with ICSV 93046. The lignin content is low in IS 27206 and IS 15957. Highest biohydrogen yield was observed in IS 27206 followed by IS 22868 and ICSV 93046. The lignin content is negatively correlated with biohydrogen production.

Key words: Anaerobic fermentation, Biohydrogen production, High biomass sorghum, Lignin.

Introduction

Industrial development and urbanization is always associated with increased utilization of energy. This is in addition to dwindling petroleum reserves and increasing green house gas (GHG) emissions which are the major concerns of future energy needs which stress upon development of alternative and environment-friendly energy resources/technologies. International Energy Out look 2011, projects 85% growth in energy consumption by non OECD nations while 18% accounts for OECD economies by 2035, taking 2008 levels as base (1). In most of the developed and developing countries energy consumption is expected to rise by 30% within next two decades (2). The biomass research and development technical advisory committee estimated that one billion dry tonnes of biomass would be required only to replace the petroleum consumption. The United States Department of Agriculture (USDA) states that the United States could potentially produce more than 1.3 billion dry tons annually, exceeding the amount needed to replace 30% of the country's oil consumption (2).

Among different biofuel compounds, hydrogen gas is gaining edge over other such as bioethanol or biobutanol or methane mainly due to it's a high energy (122 KJ/g) content and as clean fuel (3). Hydrogen can be produced by steam reforming of hydrocarbons and coal gasification. However, hydrogen production by anaerobic fermentation from renewable resources is a more promising technology over several other alternatives. In accordance with sustainable development and waste minimization issues, biological hydrogen production, known as "green technology", has received considerable attention in recent years.

Use of renewable and widely distributed cellulosic feed stock is considered as the effective for energy production as agricultural land alone could produce one billion tons of dry biomass annually without disrupting food and feed demands (4). Though several agro industrial materials and energy crops have been the subject of research, efforts have been on development of short duration, widely distributed and uncompetitive lignocellulosic biomass feed stock with altered structural composition. Of late, sorghum bicolor (L.) moench has emerged as the best option over switch-grass (*Panicum virgatum* L.), *Miscanthus* and other feed stock materials because of its wide adaptation in semi-arid and arid environments and its utility for food, feed, fodder and fuel (5). However, so far, sorghum has gained little importance as an energy crop compared to corn and switchgrass. Yet this has a greater yield potential as biofuel feedstock and it is estimated that corn stover, switch grass and sorghum can produce 3.14, 15.6 and 29.12 harvestable dry tons ha^{-1}, respectively (6) and subjectable for further increase to produce 33.6 to 44.8 harvestable dry tons ha^{-1} through genetic improvement.

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India has recently identified several high biomass sorghum varieties under the ongoing biomass sorghum development program funded by Ministry of New and Renewable Energy (MNRE), Government of India. The objective of the present investigation is to understand the chemical nature of improved high biomass sorghum materials and their potential for their use as a raw material for fermentative biohydrogen production using anaerobic mixed consortia.

Materials and Methods

High biomass sorghum lines: The ICRISAT gene bank has the largest number of sorghum germplasm accessions of over 39600. A total of 412 lines (368 germplasm accessions and 44 high biomass lines of breeding program) were screened for biomass traits in 2010-11 at ICRISAT and 38 promising lines were identified for high biomass. Among them, the top six high biomass lines (IS 22868, IS 27206, CSH 22SS, IS 15957, ICSV 93046 and IS 16529) were evaluated for agronomic traits following randomized complete block design with 4 rows each of 4 m length with 75 cm × 15 cm crop geometry during rainy season of 2011. Recommended crop management practices were followed. Data from internal two rows were considered for plot yield calculation. The data on plant height (m), inter-node diameter (mm), number of internodes and stalk yield were recorded at physiological maturity while stover yield were recorded at 15% moisture level. Plot yield data were converted to kg ha^{-1} using the plot size as factor. The variety ICSV 93046 was proven commercialized sweet sorghum variety developed at ICRISAT while CSH 22SS was the first sweet sorghum hybrid from Directorate of Sorghum Research released in India for commercial cultivation and used as a national check (7) while other lies were germplasm accessions identified at ICRISAT for high biomass.

Statistical Analysis: General linear model (GLM) was used for analysis of variance (ANOVA) and to calculate significant differences among improved varieties using SAS software (SAS Institute Inc., 1991). GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) software version 2.0 (8) was used for simple linear regression analysis between traits. The statistical significance of the differences between the means was estimated by the least significant difference and all significant results were reported at the $P \leq 0.05$ levels.

Inoculum development for biohydrogen production: Inoculum for H_2-production was developed according to Prakasham *et al.,* (9). Briefly, hydrogen-producing mixed consortia that originated from buffalo dung compost was collected in Hyderabad city, Andhra Pradesh, India and the hydrogenotrophic methanogens were deactivated by heat treatment for 30 min at 100° C. The developed inoculum was stored under anaerobic environment for further use.

Anaerobic fermentation: Fermentation experiments were performed according to Prakasham *et al.*, (9) in 250 ml serum vials as batch reactors consisting of methanogens deactivated inoculum (15%) – 15 ml, 5 g *bmr* sorghum stover material, 15 ml of nutrient stock solution (prepared using the following composition (in g/L) NH_4Cl – 0.5, KH_2PO4 – 0.25, K_2HPO_4 – 0.25, $MgCl_2.6H_2O$ – 0.3, $FeCl_3$ – 0.025, $NiSO_4$ – 0.016, $CoCl_2$ – 0.025, $ZnCl_2$ – 0.0115, $CuCl_2$ – 0.0105, $CaCl_2$ – 0.005 and $MnCl_2$ – 0.015). The final working volume of 150 ml was made up with distilled water. These flasks were deoxygenated with nitrogen gas for the development of an anaerobic environment. These flasks were incubated at 37 ± 1 °C in an orbital shaker with a rotation speed of 100 rpm to provide better mixing of the substrates. The volume of biogas produced was determined using glass syringes of 5–50 ml. All the experiments were performed in triplicates and the average values were reported.

Chemical Analysis: The analysis of sorghum samples for cellulose, hemicellulose and lignin content was done by Tappi (10) and permanganate oxidation method (11) in duplicates. The hydrogen gas measured as a percentage of the total volume was determined using a 100% hydrogen standard with gas chromatograph (GC, Agilent 4890D) equipped with a thermal conductivity detector (TCD) and 6 feet stainless column packed with Porapak Q (80/100 mesh). The operational temperatures of the injection port, the oven and the detector were 100 °C, 80 °C and 150 °C, respectively. Nitrogen gas at a flow rate of 20 ml/min was used as the carrier.

Results and Discussion

In order to have suitable sorghum varieties with improved traits for biomass production, six different high biomass varieties of sorghum (IS 22868, IS 27206, CSH 22SS, IS 155957, ICSV 930461 and IS 16529) were either developed or identified by ICRISAT, Patancheru, Hyderabad. These lines were evaluated for their agronomic traits as well as screened for production of biohydrogen by anaerobic fermentative mixed bacterial consortium.

The six high biomass sorghum lines were characterized in terms of candiate agronomic traits. The ANOVA revealed significant differences for the agronomic traits studied *i.e.* plant height (m), internode diameter (mm), number of internodes, fresh stalk yield (t ha^{-1}) and stover yield (t ha^{-1}) among the entries in the

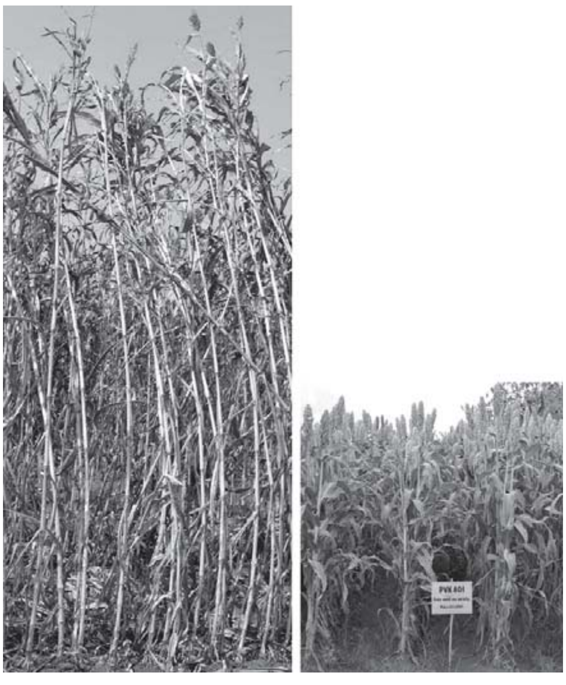

Fig. 1. Comparative view of high biomass sorghum line (IS 27206) and grain sorghum line (PVK 801)

study. The entry IS 27206 (6.2 m) was the tallest (Fig 1) followed by IS 16529 and IS 15957 (Table - 1). The same entry, IS 27206 recorded highest average internode diameter (22.38 mm), fresh stalk yield (90.5 t ha^{-1}) and stover yield (54.5 t ha^{-1}). The next best lines for biomass yield were IS 16529 (52.1 t ha^{-1}) and IS 15957 (44.3 t ha^{-1}). The association analysis revealed that plant height (0.93), internode diameter (0.98) and fresh stalk yield (0.98) were positively correlated with stover yield or dried biomass. Therefore breeding efforts need to be intensified to improve plant height and internode diameter to genetically enhance biomass yield.

To characterize further, all high biomass sorghum lines have been analyzed for their structural composition. It is evident from the Table - 2 that all developed high biomass sorghum lines have shown cellulose content in the range of 27-52% while the hemicellulose percentage ranging from 17-23% followed by lignin composition in the range of 6.2-8.1%. This observed variations in major components of biomass i.e., cellulose, hemicelluloses and lignin do suggest that all selected high biomass sorghum lines are genetically different and show significant genetic variability in agronomic traits (Table - 1) and structural component composition (Table - 2). Lignin is considered as one of important structural chemical component which is responsible for providing the strength to plant in addition to protection from the microbial attack (9, 12-14). Less lignin content was observed in the lines, IS 27206 and IS 15957. Critical analysis

Table 1. Mean performance of high biomass sorghum lines for agronomic characteristics

Genotype	Plant height (m)	Inte-rnode diameter (mm)	Number of Internodes	Fresh stalk yield (t ha^{-1})	Stover yield (t ha^{-1})
ICSV 93046	3.9	16.75	14.5	53.6	29.9
IS 15957	5.9	21.01	14.6	71.6	44.3
IS 16529	6.1	21.94	15.1	82.7	52.1
IS 22868	5.8	20.35	13.2	74.4	43.8
IS 27206	6.2	22.38	13.8	90.5	54.5
CSH 22 SS	4.2	18.86	14.4	60.7	35.7
LSD (P<0.05)	0.8	1.90	0.8	9.3	6.4

Table 2. Chemical composition of developed high biomass sorghum varieties

High biomass sorghum line	Chemical composition (%)		
High biomass sorghum line	Cellulose	Hemicellulose	Lignin
IS 22868	45±0.353	20±0.367	6.6±0.226
IS 27206	52±0.077	17±0.042	6.2±0.226
CSH 22SS	27±0.410	21±0.268	6.6±0.403
IS 15957	33±0.494	18±0.487	6.2±0.197
ICSV 93046	49±0.113	19±0.042	7.1±0.091
IS 16529	37±0.282	23±0.254	8.1±0.247

further indicated that holocellulose content differed from 48 to 69% depending on the line further suggesting the high biomass sorghum lines differ in their genetic constituents and biomass production during their life cycle.

Biochemical conversion of lignocellulosic biomass to biofuel require a process that involves in removal of lignin component, popularly termed as pretreatment of biomass, which is subsequently followed by a breakdown of the complex structural carbohydrates chains to fermentable sugars either by enzymatic or by chemical methods (15). The resulting sugars can then be converted to desired product by fermentation using specialized microbial flora and conditions. One of the major challenges in this biomass to biofuel conversion technology is associated with complexity of converting biomass though the efficiency of this conversion process is dependent on a host of factors which can convert the sugar chains more or less accessible to microbial strains for further metabolism associated synthesis of biofuels. Understanding these factors and manipulating them to enhance the accessibility of convertible sugars is an active area of research (16, 17). In fact, it is generally understood that higher content and easier accessibility of these complex chains of sugars are important, since these establish the optimized biofuel yield. In this context, sorghum compares well with corn stover and other herbaceous and energy biomass crops. Another major challenge in biohydrogen production by dark anaerobic fermentation is to improve the rate and the yield of hydrogen production for an economic process. Hence, biological and engineering studies must be concentrated on these issues. Since, raw material cost is another concern in biohydrogen fermentations and the above high biomass sorghum lines showed high fresh stock and stover yield, these lines were evaluated for biohydrogen production by anaerobic fermentation process using enriched hydrogen producing microbial consortia.

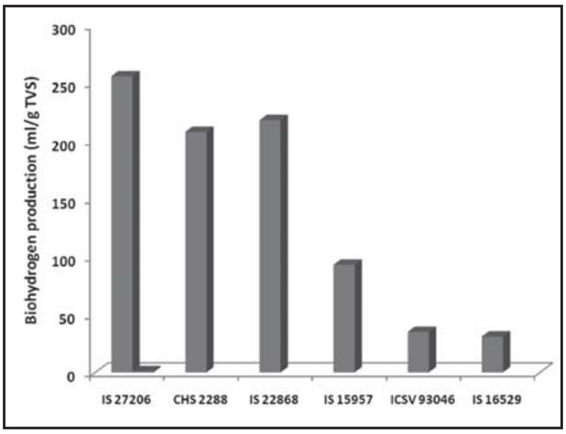

Fig. 2. Anaerobic biohydrogen production using high biomass sorghum lines as substrate material

Biohydrogen production differed with each of the high biomass sorghum lines (Fig 2). Highest biohydrogen yield was noticed with IS 27206 sorghum line followed by IS 16529 and IS 22868. The least production was observed with IS 16529 line. Such biohydrogen variation may be attributed to difference in chemical composition of the lines especially with respect to cellulose and lignin content as noticed in table - 1 and table - 2. This is because, cellulose is the major component of biomass material which upon enzymatic hydrolysis yields fermentable sugars then could be used as raw material for biohydrogen production by enriched biohydrogen producing microbial consortia developed from cow-dung as reported earlier by (16). Comparative evaluation of biohydrogen yield with respect lignin content do suggested that there is little variation in lignin content with the lines of IS 27206 and IS 15957 (Table - 2) however, a large variation in biohydrogen production values were noticed. A maximum of 240 ml of hydrogen/g TVS was observed with high biomass sorghum line IS 27206 whereas IS 15967 supported only > 100 ml of hydrogen/g TVS (Fig 2). In addition, the lines ICSV 93046 and IS 16529 consist of 7.1 and 8.1% of lignin, respective also showed lower production of biohydrogen (>75 ml of hydrogen/ g TVS) (Fig 2). This data suggest that only cellulose and hemicelluloses are mainly responsible for biohydrogen production and whose digestion play significant role in overall product yield by anaerobic mixed biohydrogen consortia. Similar nature of linkage has been observed by Prakasham *et al.,* (9) and reported negative association of lignin content with biohydrogen production by anaerobic fermentation with mixed anaerobic consortia as inoculum when brown-mid rib sorghum was used as substrate material. The above results inidicate exploitable variation for hydrogen production in high biomass sorghum lines in terms of composition of biomass and fermentation conditions particularly pH.

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