Sugar Production Potentials of Some Sweet Sorghum Hybrids Cultivated in Heavy Metals Polluted Soil

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***Abstract*—Biomass samples from field trials of seven sweet sorghum hybrids cultivated in heavy metals polluted area where harvested and the main production features where quantified. Sugars were harvested from sorghum stalks by pressing the juice and by water extraction of sugars from resulted bagasse. Sugars production was quantified (glucose by enzymatic assay and total reducing sugars by DNS assay). The sorghum hybrids evaluated in this work accumulated high quantities of biomass in 140 days of cultivation (up to 33 tons D.M. ha^{-1}). Up to 270 mg g^{-1} of juice was extracted from the fresh biomass containing up to 85 mg ml^{-1} reducing sugars. By water extraction, low concentration sugars solutions are obtained, but higher yields reported to sorghum biomass comparing with juice pressing. the first three best potentials in terms of sugar production per hectare are hybrids F135ST, Sugargraze II and Sugargraze. In conclusion, Sweet sorghum [*Sorghum bicolor* (L) Moench] can be successfully cultivated in heavy metals polluted area to produce biomass and sugars for industrial purpose that can be used to produce biofuels and other biochemicals. Cultivation of the seven sorghum hybrids in tested conditions for a period longer than 140 days (up to 170 days) does not increase the sugar production potentials, by contrary, in the most of cases the yielding potentials decreases.**

***Index Terms*—sweet sorghum hybrids, sugar production, polluted soil**

I. Introduction

The finite character and high prices of fossil fuels, energy independence, and the environmental concerns related to the use of fossil fuels, such as greenhouse gas emissions and climate change, have pushed the global community towards the development of new, renewable energy sources [1], [2]. Bioethanol has become very attractive as a substitute for fossil based fuel. The USA and Brazil, the two largest bioethanol producers, made tremendous progress in producing bioethanol from corn, respectively sugarcane [3]. Even so, other sustainability issues arise in the form of the food vs. fuel debate, or the matter of high quantities of waste remaining after producing first generation biofuels. In this regard, an alternative solution is bioethanol production from crops cultivated in polluted areas. In a bio - refinery concept, sugars obtained from sugar crops can be used as feedstock to obtain chemical bio – products, offering an alternative not only for the energy industry but also for the chemical industry [4]. *Sorghum bicolor* (L.) Moench, is one of the most investigated energy crops [5] due to a variety of advantages that it presents over sugarcane and starchy raw materials. Both sweet and grain varieties produce high yields, even under a wide range of environmental conditions, it exhibits high tolerance to draught, can be cultivated on marginal lands and has low input requirements [6]-[8]. Last, but not least sorghum is one of the most variable plant in terms of genetic resources, making breeding and development of new cultivars, adapted to different climate zones around the globe, easier [9]. Sorghum cultures have been previously applied in phytoextraction experiments in in polluted areas [10]-[12]. Our research results indicated sweet sorghum as a good alternative to use heavy metals polluted land for fuel bioethanol production [13]. In this regard, the aim of this study was to evaluate the potential of sugars production of several sweet sorghum hybrids cultivated in heavy metals polluted area. The main aim of the sugars obtained in such areas is to be used in biorefineries for biofuels and other biochemical production.

II. Materials and Methods

Experimental plots consisting of Sweet sorghum hybrids were cultivated in Copsa Mica area, Romania. In this area, the soil is polluted by a nonferous metals smelter processing metal sulfides until 2009. Soil loading with heavy metals, at the level 0 - 20cm, was previously reported [14] and the main four pollutants found in the soil samples are: Zn, Pb, Cu and Cd in average

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concentrations of 530.00, 460 .00, 24.50 and 10.10mg kg^{-1} respectively.

Sorghum plots where located at approximately 7 km distance from the smelter ($40^0$42'46"N, $74^0$0'21"W).

A. Biological Material

Seven varieties of sorghum [*Sorghum bicolor* L. Moench], with different origins, were taken into study in the present research:

1. *Sorghum bicolor x sudaneze* var. Jumbo
2. *Sorghum bicolor x* sweet sorghum var. Sugargraze
3. *Sorghum bicolor x sudaneze x* sweet sorghum var. Sugargraze II
4. *Sorghum bicolor* convar. *saccharatum* var. F135ST (produced in Romania)
5. *Sorghum bicolor* var. Super Sile
6. *Sorghum bicolor* x sudaneze var. Freya
7. *Sorghum bicolor* var. Bulldozer

After harvesting, the fresh biomass was transported and processed for subsequent analysis. Morpho-physiologic characteristics (plant height, stem weight, biomass production/ha) as well as biochemical indicators (dry matter content, reducing sugars concentration) were measured for at least 45 plants from each sorghum variety.

B. Sugar Harvesting by Pressing

Aerial part of the plants (stems, leaves and panicles) where harvested from the experimental plots. Leaves and panicles were removed and stems were milled using a Retsch SM100 laboratory mill to 2cm theoretical length. The fresh biomass obtained after milling was pressed using a stainless steel/aluminium hand press (Ferrari Group, Italy). The sorghum juice obtained was frozen until analysis. To evaluate the sweetness of the juice, we analysed the total soluble extract (degree Brix), total reducing sugars and glucose. As sucrose is easily hydrolysed into glucose and fructose, we assumed that by analysing total reducing sugars content in the juice, we find the main sugar contents of the sorghum juice. Moreover, by subtracting the value of glucose concentration (G) from the value of total reducing sugars concentration (RS), we can find the concentration of the other reducing sugars in the sorghum juice (F). The spent biomass resulted after pressing is hereinafter referred as bagasse.

C. Sugar Harvesting by Water Extraction from Bagasse

Sorghum stems are can have different water contents, depending of sorghum variety, phenophase, climatic conditions. Consequently, the quantity of juice harvested by pressing sorghum stems varies depending on sorghum humidity. More humid biomass yields more juice, as the dryer sorghum stems yields no juice. The sugars that could not be harvested by pressing, were extracted from sorghum bagasse by water solubilization. The extraction method applied in this work was performed according to Brugnoli *et al.* (1988) with slight modifications. Samples consisting of 5g of finely milled biomass mixed with 50ml distilled water were microwaved for 1 min at 800 watts (for stopping enzymatic activities). After cooling, the samples were incubated for two hours at 37.8 ℃, under constant stirring (150 rpm). The resulted liquid was filtered using filter paper and frozen until analysis. We followed the same parameters as in sorghum juice: Brix, concentration in reducing sugars and glucose.

D. Analytical Methods

Dry matter content was determined using the NREL Laboratory Analytical Procedures [15]. Total soluble solids were measured as degrees Brix using a refractometer (Hanna Instruments, Germany). To determine the total sugars, samples harvested from sorghum juice and water extraction were centrifuged to remove solids and supernatant was analyzed by DNS method, according to IUPAC standards [16].

Glucose concentration in both extracts was determined using a reaction kit for quantitative determination of glucose in liquids applying GOD-PAP enzymatic method. Glucose is determined after enzymatic oxidation in the presence of glucose oxidase (GOD). The formed hydrogen peroxide reacts under catalysis of peroxidase (PAP) with phenol and 4-aminoantipyrine to form a red violet quinoneimine dye as indicator.

III. Results and Discussions

A. Biomass Production

The most intensive growth of sorghum cultures was noticed starting July, when the air and soil temperatures reached the optimal values for these plant species. The plants growth occurred mainly during the last sixty days of cultivation (in the months of July and August). According to other researches, the harvest of sorghum biomass is recommended after 140-150 days of cultivation [17]-[19]. In this work we study the accumulation of sugars in sorghum stems in the indicated period (sampling in September, after 140 days of cultivation) and after the first frost of October (sampling after 170 days of cultivation). In September, the first samples were harvested and measurements regarding plants height, dry mater and stem weight are presented in Table I. Regarding the stage of development in the seven sorghum varieties, six of them formed panicles containing seeds in low percentage, while Jumbo variety either did not formed panicles, or the panicles did not emerge from the upper bud.

The most important parameter in our study is the stems weight, as there the sugars are accumulated. Regarding this aspect, data in Table II indicate hybrid F135ST as the top producer of biomass, reaching 800 grams of biomass / stem, followed by Sugargraze hybrids. If we report the mass values obtained for individual plants per hectare of culture by multiplying individual measured values with the seeding density, we obtained productions in the range of 32 and 106 tones / hectare for fresh matter and in the range of 10 and 34 tones / hectare for dry mater. The obtained productions are in the limits indicated by seeds producers.

TABLE I. AVERAGE PRODUCTION OF BIOMASS IN SELECTED SORGHUM VARIETIES AFTER 140 DAYS OF CULTIVATION

Sorghum variety	Plant height (cm)	Stem weight (g)	Stem dry matter content (%)	Production (t/ha)*	
				Stems (fresh biomass)	Stems (dry matter)
Sugargraze	205	690	30.39	103.5	31.45
Sugargraze II	274	625	30.88	97.75	28.95
Jumbo	240	432	32.48	64.80	21.05
Super Sile	202	222	32.25	33.30	10.74
Freya	294	214	30.49	32.10	9.79
Bulldozer	356	452	31.76	67.80	21.53
F135ST	292	802	28.17	120.30	33.89

*Calculated by multiplying the individual stems weight with the number of plants according to the seeding dosage (15.000 plants / ha)

TABLE II. AVERAGE PRODUCTION OF BIOMASS IN SELECTED SORGHUM VARIETIES AFTER 170 DAYS OF CULTIVATION

Sorghum variety	Plant height (cm)	Stem weight (g)	Stem dry matter content (%)	Production (t/ha)*	
				Stems (fresh biomass)	Stems (dry matter)
Sugargraze	290	584	35.53	87.6	31.12
Sugargraze II	279	517	37.21	77.55	28.86
Jumbo	240	419	34.66	62.85	21.78
Super Sile	216	314	32.55	47.10	15.33
Freya	305	172	39.6	25.8	10.22
Bulldozer	411	405	35.5	60.75	21.57
F135ST	310	708	32.45	106.20	34.46

*Calculated by multiplying the individual stems weight with the number of plants according to the seeding dosage (15.000 plants / ha)

B. Sugar Extraction

After fragmenting the sorghum stems and obtaining fragments comparable with billets obtained in sugar cane harvesting, the fragments where milled with Retch laboratory mill in two rounds: first milling without the mesh and the second milling using a 2cm mesh. The milled biomass was pressed with laboratory press. The juice and bagasse were harvested; samples were collected and frozen until analysis.

The highest quantities of juice were extracted from Sugargraze. Sugargraze II. Jumbo and F135ST (Table III). These hybrids accumulated the highest concentrations of reducing sugars in juice harvested from the stems as well.

The highest quantities of juice were extracted from Sugargraze. Sugargraze II. Jumbo and F135ST (Table III). These hybrids accumulated the highest concentrations of reducing sugars in juice harvested from the stems as well.

Regarding sugar harvesting by water extraction (Table IV), Sugargraze II, F135ST and Buldozer revealed best results, in the range of 52 – 67mg/g fresh biomass. Anyway, by this procedure solutions containing low concentration of sugar are obtained (between 4 and 8 g/l reducing sugars) comparing with pressing, juice containing high-concentration of sugars is obtained in the range of 80g/l.

Samples harvested in October (after 170 days of cultivation) indicate mature plants, leaves spotted with rusty areas and frequent dried leaves. Excepting Jumbo hybrid, where panicles are very rare (around 20%), all other hybrids developed panicles and the seeds are mature. Frequency of ramifications is between 5 and 12 per plant, some hybrids (Super Sile and F135ST having rare ramifications on the main stem. Generally, the consistency of the stems is harder comparing with the phase of 150 days of cultivation and in all hybrids the core of the stem is more dehydrated. Low quantities of juice or even no juice was extracted by pressing the sorghum stems in this phase (Table V).

TABLE III. SUGAR PRODUCTION BY PRESSING OUT JUICE AFTER 140 DAYS OF CULTIVATION

Sorghum variety	Juice extracted from biomass (g/g)	°Brix in juice	Concentration of reducing sugars in juice (g/l)	Concentration of glucose (g/l)	Concentration of other reducing sugars in juice (g/l)	Bagasse dry matter content (%)
S	J	B	RS	G	F	BG
Sugargraze	0.27	11.6	64.58	16.26	48.32	27.98
Sugargraze II	0.22	15.8	84.52	14.35	70.17	30.50
Jumbo	0.23	8.6	70.61	15.37	55.24	28.18
Super Sile	0.09	17.2	22.13	10.00	12.13	29.59
Freya	0.02	11	39.68	15.37	24.31	29.90
Bulldozer	0.07	11	59.45	15.10	44.35	29.69
F135ST	0.21	14.1	36.88	14.33	22.55	24.94

TABLE IV. CUMULATED SUGAR PRODUCTION BY PRESSING AND WATER EXTRACTION AFTER 140 DAYS OF CULTIVATION

Sorghum variety	Reducing sugars from extracted juice (mg/g biomass)	Reducing sugars from water extract (mg/g biomass)	Total quantity of reducing sugars (mg/g biomass)	Reducing sugars from extracted juice (kg/ha)	Reducing sugars from water extract (kg/ha)	Total quantity of reducing sugars (kg/ha)
1	2	3	4	5	6	7
Sugargraze	17.44	35.11	52.55	1541	3103	4644
Sugargraze II	18.59	67.61	86.2	1448	5265	6713
Jumbo	16.24	18.13	34.37	988	1103	2091
Super Sile	1.99	42.78	44.77	66	1424	1490
Freya	0.79	17.5	18.29	25	562	587
Bulldozer	4.16	52.13	56.29	282	3535	3817
F135ST	7.74	55.75	63.49	868	6254	7122

Regarding water extraction (Table VI), we found that in some hybrids higher quantities of sugars were extracted after 170 days by water extraction comparing with the previous phase, especially in Sugargraze II hybrid, where the yields of reducing sugars reached 80 mg/g biomass. Although by water extraction the sugar yields are higher reported to biomass, it is obvious that the main procedure for sugar extraction from sorghum stems should be juice harvesting by pressing because solutions with high concentration of sugars are harvested this way.

Data obtained in our research indicate important differences between sugars yield potentials in different sorghum hybrids. Although the tested hybrids are all indicated as specially obtained for biomass/forage production, some of them can be successfully used for sugars production.

Data in columns RS in Table III and Table V indicate high concentrations of reducing sugars in juice harvested by pressing from hybrids Sugargraze, Sugargraze II and Jumbo.

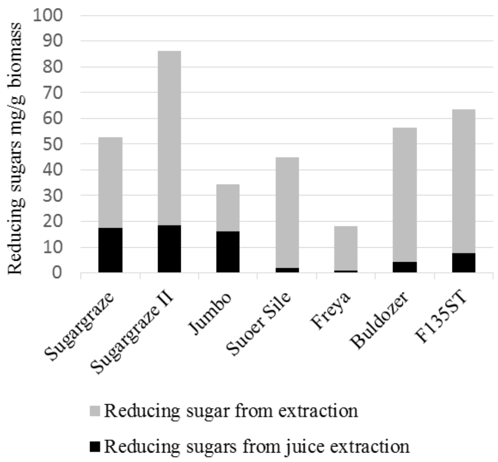

Figure 1. Sugars yields potentials reported to fresh biomass of sorghum stems. Extraction by pressing and water extraction after 140 days.

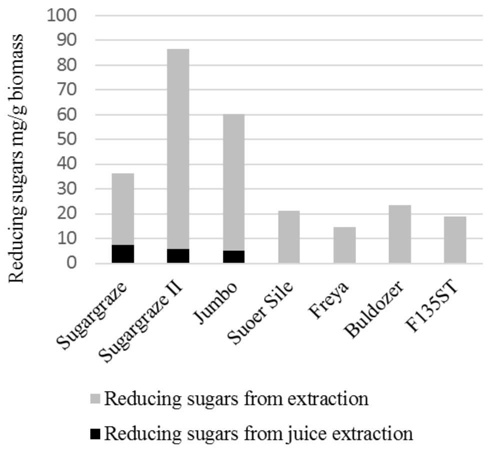

Figure 2. Sugars yields potentials reported to fresh biomass of sorghum stems. Extraction by pressing and water extraction after 170 days.

Overall yields are generally higher in samples harvested after 140 days of cultivation comparing with samples harvested after the first frost in autumn. The sugars yield potentials reported to biomass are comparatively presented in Fig. 1 and Fig. 2. Hybrids Sugargraze II, F135ST, Bulldozer and Sugargraze express high production potentials.

In the first period of cultivation, when the sugars yield obtained by pressing are higher than in the next period of sampling. By multiplying the sugars yields potentials reported to biomass with the biomass production potentials reported in Table I and Table II, we obtained the sugar production potentials reported in kilograms per hectare (data comparatively presented in Fig. 3 and Fig. 4).

It is obvious that the first three best potentials in terms of sugar production per hectare are hybrids F135ST, Sugargraze II and Sugargraze, in the range of 4500 – 7000kg/ha.

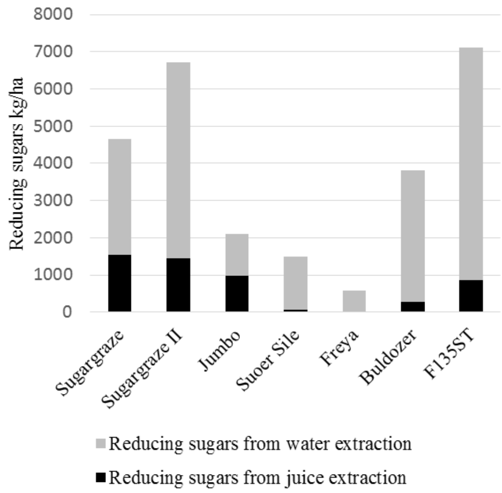

Figure 3. Sugars yields potentials reported to land surface of sorghum crops. Extraction by pressing and water extraction after 140 days.

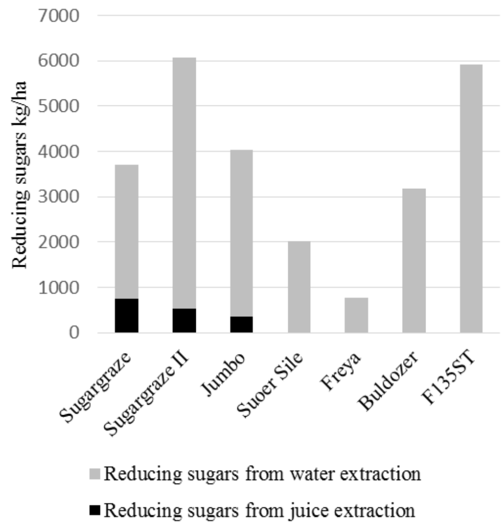

Figure 4. Sugars yields potentials reported to land surface of sorghum crops. Extraction by pressing and water extraction after 170 days.

These sugars obtained in polluted area can be used in biorefinery to produce biofuels and other biochemical, as described in our previous research [13], and bagasse can be used as co-substrate in anaerobic digestion for biogas production. In the same previous work, data indicate that the metal concentrations in sorghum juice obtained in the same polluted area are below the established maximal thresholds; therefore, the sorghum juice can be used in food industry as well.

Regarding the extraction of sugars from sweet sorghum stems, it is recommended to combine pressing with solubilization to fulfil production potentials indicated in this study. Further research focused on ensiling sorghum biomass combined with sugars preservation and extraction are currently carried out by our team.

TABLE V. SUGAR PRODUCTION BY PRESSING OUT JUICE AFTER 170 DAYS OF CULTIVATION

Sorghum variety	Juice extracted from biomass (g/g)	ºBrix in juice	Concentration of reducing sugars in juice (g/l)	Concentration of glucose (g/l)	Concentration of other reducing sugars in juice (g/l)	Bagasse dry matter content (%)
S	J	B	RS	G	F	BG
Sugargraze	0.168	10.6	43.66	1.08	42.58	26.02
Sugargraze II	0.120	10.6	46.67	1.55	45.12	27.58
Jumbo	0.098	8.3	52.42	0.94	51.48	29.59
Super Sile	0	0	0	0	0	32.55
Freya	0	0	0	0	0	39.60
Bulldozer	0	0	0	0	0	35.50
F135ST	0	0	0	0	0	32.45

TABLE VI. CUMULATED SUGAR PRODUCTION BY PRESSING AND WATER EXTRACTION AFTER 170 DAYS

Sorghum variety	Reducing sugars from extracted juice (mg/g biomass)	Reducing sugars from water extract (mg/g biomass)	Total quantity of reducing sugars (mg/g biomass)	Reducing sugars from extracted juice (kg/ha)	Reducing sugars from water extract (kg/ha)	Total quantity of reducing sugars (kg/ha)
1	2	3	4	5	6	7
Sugargraze	7.33	28.96	36.29	750	2965	3715
Sugargraze II	5.6	60.75	66.35	524	5551	6075
Jumbo	5.14	55.01	60.15	345	3690	4035
Super Sile	0	21.14	21.14	0	2013	2013
Freya	0	14.61	14.61	0	763	763
Bulldozer	0	23.57	23.57	0	3171	3171
F135ST	0	18.94	18.94	0	5917	5917

IV. CONCLUSION

Sweet sorghum [*Sorghum bicolor* (L) Moench] can be successfully cultivated in heavy metals polluted area to produce biomass and sugars for industrial purpose.

Sugar production potentials of different sweet sorghum hybrids differ and potential yields can have highly different values depending on the type of sorghum hybrids.

Hybrid F135ST obtained locally, in Romania, have been found to have the highest sugar production potential reported to the area of cultivated land, after 140 days of cultivation, followed by (imported) hybrids Sugargraze II and Sugargraze.

Cultivation of the seven sorghum hybrids in tested conditions for a period longer than 140 days (up to 170 days) does not increase the sugar production potentials, by contrary, in the most of cases the yielding potentials decreases.

Sugar extraction can be carried out by direct pressing of milled biomass in the first period of cultivation, when humidity of the plants is higher. Nevertheless, extraction technologies combining solubilization and pressing should be applied to extract the sugars contained in sweet sorghum stems.

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