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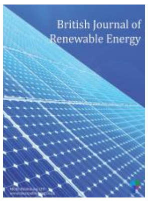

Productivity and Bioethanol Potentials of Wild Sorghum (*Sorghum arundinaceum*)

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Abstract:

Wild sorghum, *Sorghum arundinaceum* is found in fresh water swamps in the Niger Delta and have remained untapped. This study evaluated the productivity and bioethanol potentials of the grass. Ten plots were randomly selected along Amassoma-Yenagoa road, Bayelsa state and triplicate samples were harvested. The samples were milled and resultant bagasse dried. A productivity of 16.85 - 33.67 tonnes/ha of the grass was obtained. The physico-chemistry of the extract ranged from 4.533 – 4.853 (pH), 107.372 – 186.672 µS/cm (conductivity), 1.0090 – 1.0237 (specific gravity), 4.300 – 9.066% (sugar) and 2.666 – 5.533% (ethanol). These physicochemical characteristics of the grass extract suggest that it can be used for sugar and ethanol production and the bagasse for power generation.

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1. Introduction

Nigeria has joined countries searching for efficient and alternative energy resources to the depleting fossil resources. Typically, energy is a vital and strategic tool needed to attain minimum quality of life [1]. Biomass has enormous potential in the production of alternative energy. Biomass-based energy resources of Nigeria include bioelectricity via gasification technology [2, 3], and biofuel including biodiesel via esterification and transesterification and bioethanol through saccharification. Bioethanol is a potential substitute to fossil-based gasoline due to similarities in their physicochemical properties. Presently, Brazil and USA are the World largest producers of ethanol accounting for about 65% of global production, mainly from sugar cane (Brazil) and corn (USA) [4].

In Nigeria, feedstocks that have been approved for bioethanol production are cassava and sugar cane and to lesser extent sweet sorghum. Ohimain [5] reported that between 2007 and 2009 twenty (20) bioethanol plants was proposed to be handled by both government and private sector. Of these, 10, 8 and 2 are using cassava, sugarcane and sweet sorghum as feedstocks respectively. Till date, most of the factory did not attain the target aim. Some end up at conception, planning, engineering/design, procurement, installation and construction stage. Some of the associated challenges that led to the closure and non actualization of the projects aim is attributed to shortage of feedstocks, changes in government policies along with regime change.

Nigeria is the largest producer of cassava accounting for about 54 million metric tonnes [6]. Nigeria has a population of about 170 million and cassava is a major staple food widely utilized in several form including gari, fufu etc. Recently, the government of Nigeria has approved 40% high quality cassava flour into bread making, exceeding the 5 – 10% announced by previous political administration (1999 – 2010). This is to reduce the money spent on the importation of wheat that is used for the bread making conventionally. Bread is a major food consumed by nearly every ethnic nationality. Sugarcane on the other hand, is basically used in the production of sugar mostly. The food application of these selected feedstocks has significantly affected its efficiency and effectiveness for bioethanol production.

However, for the bioethanol industry to be sustainable, efficient and effective, lignocellulosic feedstocks including grasses has to be incorporated as feedstock. In Nigeria, some inedible grasses have shown potentials for bioethanol production. Some of these grasses include Elephant grass (*Pennisetum purpureum*) [7], wild sorghum (*Sorghum arundinaceum*). These grasses are highly productive and have remained unutilized.

Sorghum is annual plant that generally belongs to poaceae family with several species. Monteiro et al. [8] described sorghum as group of annual herbaceous C4 species with only one species – *Sorghum bicolor* (L.) Moench with three different subspecies. *Sorghum bicolor* L. Moench has ethanol productivity of 3.9 – 4.7 t/ha [4]. Sorghum is a C4 grass with high photosynthetic effectiveness and water utilization. Typically, C4 plants have the potentials to produce high dry matter. Sorghum are found in Africa, Asia, and Central America especially in semi-arid tropical and dry temperate countries. Sorghum grows under varying range of soil and climatic condition including minimal germination temperature (8 - 10ºC), soil with heavy clay or sand content with pH of 4.5 – 8.5 [4].

Monospecific stand of *Sorghum arundinaceum* is found close to fresh water bodies in Bayelsa, Rivers and Delta states in the Niger Delta wetland region of Nigeria. *Sorghum arundinaceum* is facultative wetland plant (i.e having an estimated probability of 67 – 99% and 1 – 33% occurrence in wetland and non-wetland condition respectively). *Sorghum arundinaceum* have a life span of 4 – 6 months (April - September) under Nigeria climate. The plant usually resurfaces in April of the following year in the same area, despite all human interference such as burning in quest of food production. The plant could read 2.0 – 4.0 meters tall with adventitious root used for adaptation. When dried, the plant is basically used for lighting by fishermen in most coastal region of Bayelsa state, Nigeria. This lighten properties suggest that the plant has high calorific value. In other West African country like Ghana, *Sorghum arundinaceum* is used as livestock feed [9]. Typically, information about the productivity and bioethanol potentials of *Sorghum arundinaceum* is scares in literature.

Therefore, in line with the national policy on bioethanol production from locally sourced feedstock so as to create jobs and boost rural and agricultural development [10], we hereby evaluate the productivity and bioethanol potentials of wild sorghum (*Sorghum arundinaceum*) found in Nigerian environment.

2. Materials and Methods

2.1. Plant Identification

Sorghum morphology and physiology has been documented by Kimber et al. [11], Mahmood [12], Ng'uni [13], Upadhyaya et al. [14]. *Sorghum bicolor* is widely dominant among African. Four wild races of *Sorghum bicolor* exist including *Sorghum arundinaceum*, *Sorghum virgatum*, *Sorghum aethiopicum* and *Sorghum verticilliflorum* [11, 14]. *Sorghum arundinaceum* is a wild relative of grain sorghum with grassy appearance and many tillers, open panicles and small grain that shatter from the head [15]. *Sorghum arundinaceum* is sexually compatible with grain sorghum and has the same chromosome number [15]. Based on the characteristics previously described by Kimber et al. [11], Mahmood [12], Ng'uni [13], Upadhyaya et al. [14], Jordan et al. [15], the *Sorghum arundinaceum* was identified.

2.2. Field Sampling

Mono specific stands of matured wild sorghum grass were randomly selected at Wilberforce Island and its environs along Yenagoa-Amassoma road, Bayelsa State, Nigeria. At each sampling plot, a 1 x 1m^2 quadrant was launched and all the wild sorghum biomass within the quadrant was harvested to ground level and packaged in jute bags [7]. This process was carried out in triplicate.

2.3. Productivity

The resultant wet weight of the wild sorghum samples was measured using weighing balance (Spring Dial) in each plot. The grass content of each bag was cut into smaller pieces using machete and quantitatively re-packaged in a bag [7]. Hydraulic jack was used to extract the liquid extract of the grass, hence leaving a solid wet residue called wild sorghum grass bagasse. The wet bagasse was oven-dried at 80°C to a constant weight according to the methods of the Association of Official Analytical Chemist [16]. The percentage dry matter was calculated by multiplying the ratio of dry matter to fresh weight by 100 [17]. From these calculations, the productivity in tones/ha was established [7].

2.4. Physicochemistry of the Wild Sorghum Extracts

Measuring volumetric cylinder was used to determine the volume of the grass extract and physicochemical parameters such as specific gravity, sugar and alcohol content was analyzed based on the method previously described by Ohimain et al. [7, 18].

2.4.1. Specific Gravity

Specific gravity (SG) bottles with glass stoppers were filled to the brim and closed. All spillage on the body of the bottle was cleaned. The weight of the bottle was measured with analytical balance (Metler Toledo) and the SG was mathematically computed;

$$\text{SG} = \frac{(\text{Mass of SG bottle} + \text{samples}) - \text{Mass of the empty bottle}}{\text{Volume of SG bottle}}$$

2.4.2. % Alcohol content

The $K_2Cr_2O_7$ method was employed for the determination of % ethanol. An alcohol standard curve was prepared by diluting a 98% - 100% absolute ethanol, to give a series of standards, 20% - 80%. From each of these standard solutions, 1ml of alcohol was added into a test tube and 5ml of 0.1M $K_2Cr_2O_7$ was added and incubated for 30minutes at room temperature. The spectrophotometer (Jenway 650 UV/VIS) was set up at a wavelength of 540nm. The blank used in this case was 1ml of distilled water in a test tube and 5ml of 0.1M $K_2Cr_2O_7$ added and incubated at room temperature for 30 minutes. This was used to zero the spectrophotometer, and absorption values were then taken, the curve obtained was linear. The samples were also treated in the same manner and their absorbances were measured. A standard graph of absorbance versus alcohol percentage was drawn, and alcohol percentage values were calculated by extrapolation from the curve.

2.4.3. % Sugar

Potassium ferricyanate in the presence of NaOH was used to determine the percentage sugar. 1 ml of the filtered sample was put into a test tube followed by the addition of 5 ml of 0.1, potassium ferricyanate solution and 1 ml of 2M NaOH solution. The test tubes were then placed in a water bath at 100^{O}C and incubated for 10 – 15 min until the greenish yellow colour developed. A standard 100% sugar solution was prepared as the stock sugar solution from D-glucose crystals by weighing 100 g of glucose into 100ml volumetric flask and making up to the mark with distilled water. By using the $M_1V_1 = M_2V_2$ relationship, various dilutions ranging from 20% - 80% were created. Using the same procedure as that of the samples, the standard glucose solution was treated. The spectrophotometer (Jenway 650 UV/VIS) was set at 420 nm after incubation. Absorbance values were taken and a calibration curve was drawn. The percentage of sugar was determined by extrapolation from the standard curve.

2.4.4. pH and Conductivity

The pH and conductivity was determined in-situ according to the scheme of Ademoroti [19] using pH meter (HANNA HI 9820) and conductivity (Hach's CO 150).

2.4.5. Yeast Counts

Wild sorghum extract was serially diluted and plated on Sabouraud dextrose agar containing 0.05 mg/ml chloramphenicol for yeast counts. The yeast was identified based on morphological, cultural guide provided by Nwachukwu et al. [20].

2.5. Statistical Analysis

The data were analyzed using SPSS version 16. Descriptive statistics (mean and standard error) was carried out, and analysis of variance (ANOVA) was established and Duncan Multiple Range Test was used for mean separation at p= 0.05.

3. Results and Discussions

Table 1 present the productivity of the wild sorghum. The wet weight, %dry weight, grass extract volume and dry matter yield ranged from 161.91 – 229.90 tonnes/ha, 10.61 – 14.45 tonnes/ha, 1633.30 – 33062.14 litres/ha and 16.85 - 33.67 tonnes/ha respectively, being significantly different ($P<0.05$) among the different plots. The variation that exists on the productivity of the wild sorghum in this study could be attributed to the age of the plant as well as their sizes. The wet weight, % dry weight, grass extract volume in tonnes/ha were significantly higher than the yield reported from elephant grass by Ohimain et al. [7]. The dry matter yield is higher than other grasses grown in Nigeria without fertilizer application. Ohimain et al. [7] reported dry matter yield of elephant grass in the range of 7.00 – 11.33 tonnes/ha. But the result of this study was comparable to results of previous report on elephant grass under non-Nigerian environment. Lewandowski et al. [21] reported dry matter yield of elephant grass in the range of 22.0 – 31.0 tonnes ha/year. Again, the biomass yield from this study is higher that estimated referential biomass yield of 15 – 20t/ha of dry matter for sweet sorghum [4]. Wild sorghum being a seasonal plant could be harvested once in a year under Nigeria setting. Due to the fact that wild sorghum are naturally close to fresh water swamps with the application of fertilizer the energy ratio could be high. Again, due to lack of pesticides, and fertilizer application wild sorghum is environmentally friendly.

The physico-chemistry of the wild sorghum extract are presented in Table 2. The physicochemical characteristics ranged from 4.53 – 4.85 (pH), 107.37 – 186.67 µS/cm (electrical conductivity), 1.009 – 1.024 (specific gravity), 4.30 – 9.07% (sugar) and 2.67 – 5.53% (ethanol). There was significant difference ($P<0.05$) in each of the physicochemical parameters in the different plots. This finding of this study has superior ethanol properties than previous report on other lignocellulosic C4 feedstock. Ohimain et al. [7] studied bioethanol potentials of elephant grass and reported the physicochemical properties as 1.56 – 1.60 (specific gravity), 2.59 – 4.47% (sugar), 1.36 – 2.85% (alcohol). Based on the sugar and ethanol concentration, the variation could be attributed to conversion of initial sugar to ethanol by spontaneous inoculation of *Saccharomyces cerevisiae* during extraction processes [7]. Again, the age of the plant could influence the sugar and ethanol content of the extracts. The pH of the grass extract suggests that significant fermentation have occurred during processing stage. This is because pH of sap reduces with corresponding increase in fermentation period [20]. The specific gravity is higher than 0.79 meant for actual value for ethanol. The high specific gravity is an indication of water in the ethanol. Basically, ethanol from lignocellulosic feedstock contains significant volume of water. The yeast population ranged from 0.830 – 6.766 x10^7 cfu/ml being significantly different (P<0.05) in the entire plot (Table 2). The population of yeast found in this study was slightly higher than order of 10^6 cells/ml, reported from elephant grass extract by Ohimain et al. [7] and within the ranged found in raphia palm sap as reported by Ohimain et al. [18]. Basically, the infestation of microorganisms on sugary extracts enhances the proliferation of yeast and bacteria for the conversion of the sugar found in the extracts into ethanol through spontaneous process [22, 23]. This suggests that *Saccharomyces cerevisiae* are colonizers of sugary extracts. The alcohol content of the grass extract is lesser than the range 5.8 – 8.8% that could be produced by yeast [24].

Table 1: Productivity of wild sorghum

Plots, #	Wet weight, tonne/ha	Dry weight, tonne/ha	% Dry weight	Grass extract Volume, Litres/ha
A	188.47±3.53b	24.67±1.45b	13.55±0.58bc	18168.40±29.71b
B	161.91±1.20a	16.85±0.49a	10.61±0.19a	16331.30±64.00a
C	211.91±8.83d	28.95±0.88d	13.62±0.20bc	28164.42±20.73c
D	218.36±5.82d	30.44±2.60de	13.88±0.88cd	29122.04±69.80d
E	243.23±8.87f	33.67±1.50f	13.78±0.12bcd	33062.14±12.10f
F	222.40±1.15e	31.32±2.17e	14.07±0.73cd	30613.40±14.95e
G	208.24±1.53d	26.71±0.32c	13.15±0.10b	28061.24±98.00c
H	221.13±5.65e	31.04±3.62e	13.95±0.99cd	30547.01±34.37e
I	196.13±1.86c	26.30±2.71c	13.89±0.87cd	18172.14±34.78b
J	229.90±3.81e	32.14±5.12ef	14.45±0.21d	30431.24±18.85e

Same letter along the column is not significantly different (P>0.05) according to Duncan Multiple Range Test statistics; mean± standard error (n=3)

Table 2: Physico-chemistry and yeast count of the wild sorghum extracts

Plots,#	pH	Conductivity	Specific gravity	Alcohol, %	Sugar, %	Yeast count, cfu/ml x 10^7
A	4.73±0.12bc	175.23±3.71def	1.009±0.00a	2.67±0.15g	9.07±0.12e	0.913±0.145g
B	4.75±0.32bc	107.37±1.76a	1.012±0.02ab	5.23±0.88bc	4.77±0.88c	6.766±0.491e
C	4.85±0.31b	171.33±8.82de	1.017±0.01bcd	3.10±0.58b	7.50±0.12d	0.830±0.346f
D	4.73±0.42bc	189.80±3.511g	1.018±0.00bcde	5.08±0.02b	4.20±0.15a	4.533±0.273d
E	4.73±0.93bc	131.33±8.81bc	1.018±0.01bcde	5.10±0.06b	4.67±0.88bc	1.666±0.088ab
F	4.53±0.12a	182.93±4.40efg	1.023±0.03de	5.15±0.06b	4.30±0.06ab	2.433±0.186b
G	4.74±0.76bc	165.00±5.77d	1.021±0.01de	5.53±0.88e	4.40±0.21abc	4.400±0.458cd
H	4.62±0.35ab	172.90±5.77de	1.024±0.03e	5.25±0.58bcd	4.33±0.09ab	1.466±1.453a
I	4.60±0.57fg	186.67±4.05fg	1.019±0.03cde	5.47±0.44de	4.67±0.03bc	4.400±0.200ed
J	4.82±0.28c	150.00±4.16c	1.014±0.02abc	5.39±0.03cde	4.47±0.09abc	3.533±0.120c

Same letter along the column is not significantly different (P>0.05) according to Duncan Multiple Range Test statistics; mean± standard error (n=3)

4. Conclusion

Wild sorghum is an invasive wild plant in fresh water swamp in the Niger Delta region of Nigeria especially in Delta, Bayelsa and Rivers states. They basically constitute nusiance to farmers in the region, where they have to be eliminated via manual labour (spending substantial amount of money) and or the use of herbicides (which could contaminate the environment). In search of high efficient and sustainable feedstock for the automotive industry, we evaluated the productivity and bioethanol potentials of wild sorghum. Results show that the extract from wild sorghum could be distilled and used as ethanol, while the baggase could be used as fuel to generate electricity via steam cycle. The conversion of wild sorghum to bioethanol has environmental, social and economic benefits.

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