



Assessment of quantities and composition of corn stover in Ghana and their conversion into bioethanol



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ARTICLE INFO

Article history:

Received 4 September 2020

Revised 13 January 2021

Accepted 19 February 2021

Editor: DR B Gyampoh

Keywords:

Renewable energy

Feedstock

Corn stover

Bioethanol

ABSTRACT

Corn stover (CS) has currently received great attention worldwide as a potential feedstock for the production of bioethanol as alternative renewable fuel or additive to gasoline. This work evaluates the quantities and chemical characteristics of the CS available in Ghana and their conversion into bioethanol. The average quantity of CS production in Ghana per year was estimated to be 1.77×10^6 t. The cellulose, hemicellulose, and lignin contents of CS obtained from *Pioneer 30 W 40*, a local maize variety, were found to be 37.72 %, 20.62 %, and 30.50 %, respectively. Alkali pretreatment and enzymatic hydrolysis of the CS yielded a reducing sugar concentration of 157.96 mg/ml within 24 h and an ethanol yield of 0.045 l/kg. It was estimated that 40 % of the average annual CS production has the potential of yielding 140.855×10^6 l of bioethanol.

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Introduction

Bioethanol is a renewable fuel that serves as an octane enhancer or oxygenated additive or alternative fuel to gasoline [7]. It is presently produced from food sources such as sugar crops (sugarcane, sugar beets, and sweet sorghum) and starchy crops (corn, cassava, and wheat). These sugar and starchy crops (feedstock) chemically consist of polymers of fermentable sugars (especially glucose) which can be easily hydrolyzed and fermented to ethanol using fermenting yeast [2]. The current major consumption of ethanol is in the transportation sector, where fuels consisting of ethanol and gasoline, usually termed 'gasohol' are used in vehicles to reduce vehicular emissions of greenhouse gases [10,42]. E10 and E85 are ethanol-gasoline blend fuels containing 10 % and 85 % ethanol respectively. Notable countries such as Brazil and the USA have set blending mandates and policies to encourage the use of gasohol. Brazil, the second-highest producer of ethanol, has an ethanol blending mandate up to E27 whereas the USA, the highest producer of ethanol, has available E10, E15, and even E85 fuels at fuel stations [29,33]. Other mandates by different countries are reported by Timilsina and Shrestha [43].

Factors driving countries to set up and implement bioethanol blending mandates and policies are rising demand for energy (due to increasing human population and advancement in technology), national energy security, environmental concerns, and desire to increase farm income [8]. Bioethanol is required in substantial amounts to be able to sustainably blend with gasoline or replace gasoline as a transportation fuel. Global production of bioethanol stands at 29.1 billion gallons in

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2019 [17]. In 2013, the USA produced 51 billion liters of ethanol and consumed 50 billion liters whereas Brazil produced 23.5 billion liters and consumed 20.9 billion liters [34]. There is an argument that diverting food crops into producing bioethanol will give rise to food-fuel competition [30]. Balat [6] reported that using corn for bioethanol production increased the price of beef, chicken, pork, eggs, bread, cereals, and milk from 10 % to 30 % in the USA. Thus, the production of bioethanol from food sources only is not sustainable, but a more sustainable way will be utilizing agricultural wastes such as corn stover (CS) and fast-growing grass.

CS is the non-grain part of the maize plant and comprises stalks, cobs, leaves, and husks. The interest in CS as feedstock for bioethanol production stems from the fact that it is substantially available due to its close link with maize production and also is a rich source of carbohydrate. In estimating the quantity of CS available, Kim and Dale [23] determined the ratio of CS to corn to be 1, which implied that similar quantities of CS exist per every production of maize. World maize production stands at 1.1 billion tonnes in 2018 [16]. CS generally contains about 40.67 % cellulose, 31.10 % hemicelluloses, and 11.70 % lignin [11]. Van Dongen *et al.* [12] reported CS to consist of 57 % total sugar and 30.9 % lignin. Comparatively, Li *et al.* [25] also reported CS to contain 39.4 % cellulose (corn stalk rind) and 20.1 % lignin. The cellulose and the hemicellulose portions of CS are hydrolyzed to release fermentable sugars mainly glucose and xylose for fermentation to ethanol. This sustainable approach helps to minimize the use of food as feedstock for ethanol fuel production. Ethanol from such lignocellulosic biomass is usually referred to as cellulosic ethanol. Ethanol yield per tonne of dry CS is reported by Weiss *et al.* [45] to be 374 l. However, it is important to note that the actual yield of ethanol is usually found to be about 60–90 % of the theoretical and that it depends solely on the nature of the CS and the process employed [47].

Maize production in Ghana for the past fifteen years (2000–2018) has been recorded to be in the million tonnes and, therefore, suggests that similar quantities of CS may be available for bioethanol production [16]. Ghana depends heavily on petroleum fuels for transportation and generation of electricity and, therefore, necessitates the need to explore renewable sources of fuels such as cellulosic bioethanol. Gasoline accounts for 24–42 % of the share of the petroleum products produced from the crude oil import and also consists of about 29–37 % of the total petroleum product consumption [14]. The major consumption of petroleum products is in the transportation sector. The transportation sector accounts for about 78 % of the consumption of petroleum products [13]. Thus, the National Bioenergy Policy (NBP) for Ghana targets the replacement of 10 % of petroleum fuels with biofuels by 2020 and 20 % by 2030 [14]. In the quest of achieving the NBP, this work was to estimate the quantities of CS available in the country and their suitability for bioethanol production.

Materials and Methods

Chemicals

Ethanol (≥ 99.97 %), sulphuric acid (≥ 95 % w/w), nitric acid (≥ 68 %), acetic acid (≥ 99 %), sodium hydroxide (≥ 98 %), potassium bromide (≥ 99 %), disodium hydrogen phosphate (≥ 99 %), potassium phosphate (≥ 99 %), peptone, potassium hydroxide (≥ 85 %), 3, 5-dinitrosalicylic acid (≥ 98 %), phenol (≥ 99.5 %), sodium sulphite (≥ 98 %), glucose (≥ 99.5 %) and potassium dichromate (≥ 99 %) were used as received without further purification. Cellulase, xylanase, and beta-glucosidase enzymes were kindly donated by Novozymes Denmark.

Test material

Maize planted on 0.07 hectare (0.67 acres) field of the Maize Department, Crops Research Institute (CRI), Council for Science and Industrial Research (CSIR), Station Variety Trial 2 farm in Kumasi was used as the test material in this study. The soil was well-drained loam soil and the application of fertilizer, herbicide, and pesticide were done following standard practices of the Maize Department. The field had a dimension of 27×25 m² and was divided into 4 major columns separated by 1 m alley. Each column was 27×5 m² and was divided into 18 plots. Each plot had dimension 0.75×5 m² and was separated from another by 0.75 m alley. The field consisted of maize plants of nine (9) different high yielding local varieties of maize. The maize varieties included *Fu 20–90 DWDP*, *Fu 20–90 DYFP*, *GH 90 DWD Pop*, *GH 90 DYF Pop*, *Omankwa*, *Pioneer 30 B 80*, *Pioneer 30 W 40*, *Pioneer 30 D 55*, and *Kwadaso local*. The plants were cultivated for 111 days and the plant population was 40,474 ha⁻¹.

Biomass assessment

Border plants were not selected for the biomass assessment because of the greater exposure to sunlight and wind. Biomass was collected after 111 days of maturity of the plants. The harvesting of the maize plants was put into two categories; first of all, the harvesting of the maize heads and secondly the collection of the remaining CS which consisted of the maize plant stalks, leaves, and husks. The harvesting of the plants was done according to plots. A plot was to contain 48–49 maize plants, however, due to the windy nature of the area, some plots recorded as low as 20 plants. In all, 2,732 plants were harvested from the 72 plots. Typically, the heads of corn were harvested from all the maize plants in a plot, dehusked and the field weight (FW) measured. The weight of the corn cobs was determined by subtracting the weight of the grain from the FW of the dehusked head of corn corrected for its moisture content (MC). The remaining CS including the stalks, leaves, and husks were collected and the FW determined. This was repeated for all the plots to determine the

yield of biomass. It must be noted that a collection of maize plant stalks, leaves, and husks was done using systematically selected 530 standing plants. The maize plants were manually harvested by cutting the plants about 5 cm above the ground. The MC of the grain was determined using a moisture analyzer machine whilst that of the CS was obtained by oven drying of samples of the biomass to constant weight.

Analysis of the physicochemical composition of corn stover

The CS from *Pioneer 30 W 40* maize plants was collected, dried, milled (using mechanical hammer mill), and sieved by passing through a 16-mesh sieve to obtain a powder. The total solids, ash, extractives, and lignin contents of the CS were determined following methods reported by the National Renewable Energy Laboratory (NREL, U. S. A.) [18–20,37–39]. The cellulose and hemicellulose contents were determined using a method published by the Technical Association of Pulp and Paper (TAPPI), U.S. A. [1].

Total solid (TS)

Approximately 1.0 g of CS was weighed into a pre-dried porcelain crucible and dried in a convection oven at 105 ± 3 °C to constant weight. The drying took 6 h. The difference in the weight of the CS before and after drying was used to calculate the % MC. The % TS is calculated by subtracting the % MC from 100 %.

Ash content (AC)

Approximately 2.0 g of CS was weighed into a pre-heated porcelain crucible and ignited at 575 ± 25 °C in a furnace for 24 ± 6 h to constant weight and then cooled in a desiccator. The weight of the ash was calculated by the difference between the empty pre-heated crucible and the crucible containing the ash. The % AC was calculated by dividing the weight of the ash by the oven-dry weight (at 105 ± 3 °C) of the initial CS times 100 %.

Extractives content (EC)

The extractives portion of the CS was obtained using a Soxhlet apparatus and ethanol as solvent. The receiving round bottom flask and thimble were cleaned and dried for 12 h at 105 ± 3 °C to obtain their initial weights before the extraction. Approximately, 10 g of CS was extracted with 200 ml of ethanol overnight and the extracted solid was washed several times into a flask. The ethanol extractives were combined and the ethanol removed leaving the extractives which were dried at 40 ± 2 °C overnight and weighed. The % EC was calculated by dividing the weight of extractives by the oven-dry weight of the initial CS times 100 %.

Total lignin (TL)

The TL of biomass is the sum of the acid-insoluble lignin (AIL) and acid-soluble lignin (ASL). The AIL is the residue remaining after extensive acid hydrolysis of the biomass corrected for the AC. ASL refers to a small portion of the lignin that is solubilized during the hydrolysis process. AIL was determined gravimetrically whilst ASL was determined using a Shimadzu UVmini-1240 UV-Vis spectrophotometer. The ethanol-extracted CS was used for the experiments. Approximately, 0.3 ± 0.01 g of the extracted CS (w_1) was mixed with 3 ml of 72 % w/w H_2SO_4 in a test tube and placed in a water bath at 30 °C and hydrolyzed for 2 h. The sample was stirred every 15 min to ensure complete mixing and wetting. The content of the test tube was transferred into an autoclavable bottle and diluted to 4 % acid concentration by adding 84.00 ml of water. The sample was then autoclaved for 1 hour at 121 ± 3 °C and allowed to cool to room temperature. The hydrolyzed sample solution was filtered through a previously oven-dried glass gooch filtering crucible using a vacuum pump. Approximately 30 ml of the filtrate was taken to determine the ASL. The lignin residue remaining (constitutes AIL and acid-insoluble ash (AIA)) in the crucible was dried at 105 ± 3 °C for 3 h to constant weight (w_2). The lignin residue was then transferred into a pre-ignited porcelain crucible and ignited at 575 ± 25 °C for 3 h to obtain AIA (w_3). The AIL content (%) of the CS was calculated using equation 1:

$$\%AIL = \frac{W_2 - W_3}{W_1 \times T} \times 100 \quad (1)$$

Where T is the total solid (%) of the ethanol extracted CS

The ASL was determined using the filtrate after acid hydrolysis of the ethanol extractives-free CS. The absorbance of the filtrate was measured at 205 nm and a 4 % sulfuric acid solution was used as a reference blank. The % ASL was calculated using equation 2:

$$\%ASL = \frac{\frac{A}{b \times a} \times df \times V \times \frac{L}{1000 \text{ mL}}}{\frac{W \times T_{fmat}}{100}} \times 100 \quad (2)$$

where A the absorbance at 205 nm, df the dilution factor, b the cell path length, 1 cm, a the absorptivity, equal to 110 l/g-cm unless experimentally determined for a given biomass material, V the volume of filtrate equal to 87 ml, W the initial CS sample weight in grams, T is % TS of the biomass sample.

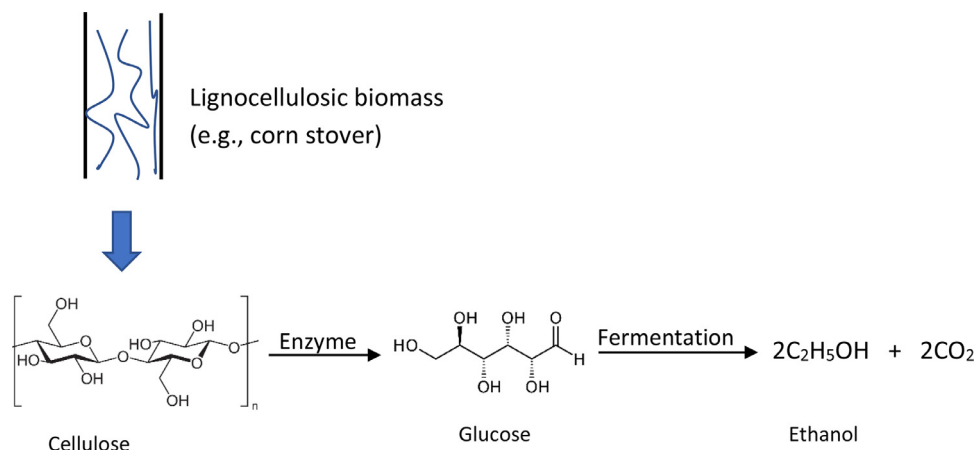


Fig. 1. Schematic diagram for conversion of lignocellulosic biomass into ethanol.

Carbohydrate content (CC)

The cellulose was determined gravimetrically using the nitric acid – acetic acid method whilst the hemicellulose was determined by subtracting the sum (cellulose content, lignin, extractive, and ash) % from 100 %. Nitric acid – acetic acid solution was prepared by mixing 90 ml of nitric acid and 732 ml acetic acid in a volumetric flask (1000 ml) and making up to the mark with distilled water. The solution was allowed to stabilize for more than 8 hours to ensure homogeneity. Approximately 0.1 g of CS was weighed into a 250 ml beaker and 25 ml of the nitric acid – acetic acid solution was added and refluxed for 30 min. After cooling to room temperature, the delignified solid was filtered in a pre-weighed gooch filtering (or sintering) crucible and the residues (i.e. cellulose) dried at 105 °C for 3 h and weighed. The % CC was calculated by dividing the weight of the cellulose by the oven-dry weight of the initial CS times 100 %.

Sodium hydroxide pretreatment

The sodium hydroxide pretreatment of the CS was done using an autoclave at a constant temperature of 121 °C. The catalyst load or alkalinity of 0.5 %, 1 %, 2 %, 3 %, and 4 % of NaOH were varied at residence times of 30, 60, 90, 120 min of autoclaving. Typically, 2.5 g of CS was weighed into the autoclavable bottle and 47.5 ml of the respective NaOH solution was added. The bottle and its content were then loaded into a preconditioned autoclave and autoclaved at 121 °C for the target residence time. The bottle was removed and cooled in a water bath to room temperature. The solids remaining were washed with water to neutrality, filtered, and dried in an oven at 105 °C for 3 h to constant weight, and the weight was recorded as solid yield. The extent of the pretreatment was assessed by measuring the (i) solid yield (i.e. the % weight of solid remaining after pretreatment), (ii) cellulose content of the pretreated sample, and (iii) extent of delignification (specifically the AIL). The FT-IR spectra for the untreated and pretreated CS were obtained by using FT-IR spectrometer and KBr (a pellet of CS: KBr ratio of 1:149 pellet was made for the measurement).

Enzymatic hydrolysis and simultaneous saccharification and fermentation (SSF)

Fig. 1 shows a schematic diagram for the conversion of lignocellulosic biomass into ethanol. The hydrolysis system consisted of 5 % of pretreated CS, 0.4 % disodium hydrogen phosphate, 0.8 % potassium phosphate, 1 % peptone, 5 % cellulase, 0.25 % xylanase, 0.6 % beta-glucosidase and 86.95 % distilled water. Before the addition of the enzymes, the pH of the hydrolysis system was adjusted to 5 using dilute sulphuric acid and KOH solutions. The system was sterilized, stirred and the hydrolysis temperature was set at 38 °C and kept for 168 hours. The extent of the hydrolysis was monitored by measuring the reducing sugars using 3, 5-dinitrosalicylic acid (DNS) colorimetric method. The DNS reagent was prepared by dissolving 10 g of 3, 5-dinitrosalicylic acid, 10 g of NaOH, 2 g of phenol, and 0.5 g sodium sulphite in distilled water and making the total volume to 1000 ml. The reagent was kept at 4 °C in a freezer. A calibration curve was developed from a 10 mg/ml glucose stock solution (using 0 (blank), 0.1, 0.3, 0.5, 0.7 and 0.9 ml of stock). The aliquot hydroxylate withdrawn from the hydrolysis system was heated to denature the enzymes and then centrifuged to obtain the supernatant. To the supernatant, 2 ml of the DNS reagent was added and then heated in boiling water for 10 min to develop a red dark colour. Approximately, 1 ml of the resulting solutions were diluted to 50 ml and the absorbance read at 510 nm using a Shimadzu UVmini-1240 UV-Vis spectrophotometer.

Similarly, the SSF system was prepared to keep the yeast (*Saccharomyces cerevisiae*, common bakers' yeast) and yeast extract concentration at 1 % and 0.5 % of the hydrolysis system. The ethanol content in the SSF samples was analyzed using the potassium dichromate (PDC) colorimetric method [3, 22, 26]. The PDC reagents prepared were 40 mg/ml potassium dichromate solution, 4.3 (pH) acetate buffer, and sulphuric acid solution. To 1 ml sample aliquot, 2.5 ml of the dichromate

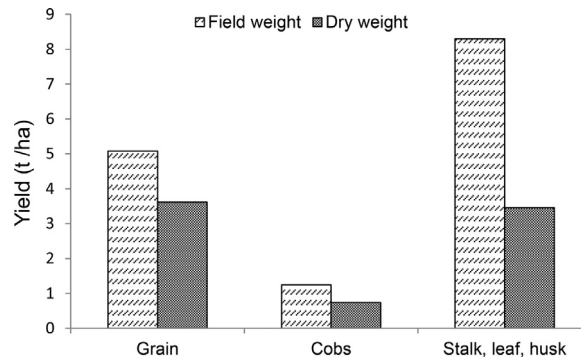


Fig. 2. Yield of fractions of maize plant in tonnes per hectare (t/ha).

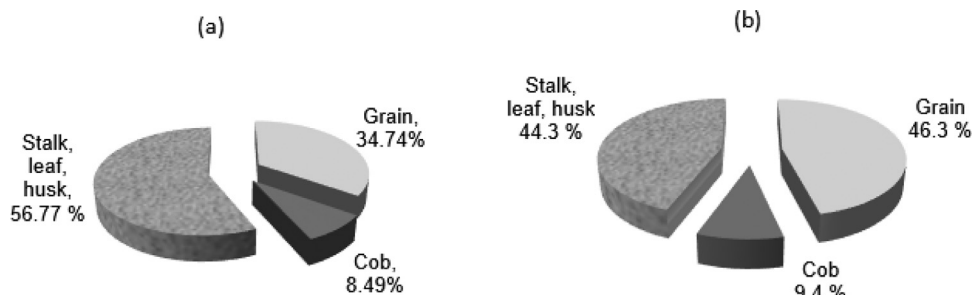


Fig. 3. Weight distribution of maize plant (a) field weight basis (b) dry weight basis.

reagent, 2.5 ml of buffer, and 12.5 ml of sulphuric acid solution was added and heated in boiling water for 10 minutes to develop a dark green colour. The resultant solution was diluted to 50 ml and the absorbance was measured at 578 nm. A calibration curve was developed using 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of 1.574 mg/ml ethanol stock solution.

Results and Discussion

Corn stover yield

Fractions of the maize plant

Fig. 2 shows the yield in tonnes per hectare (t/ha) of the different fractions of the maize plant. The fractions were put together as (a) grain, (b) cob, and (c) stalk, leaf, and husk (SLH). The stalk, leaf, and husk were grouped for simplicity in estimating the CS quantities. The weight of the biomass taken on the field was termed as field weight whilst the actual weight of the biomass corrected for its MC was termed as the dry matter weight. The average yield of grain, cob, and SLH were found to be 5.08 (2.05), 1.24 (0.5), and 8.30 (3.36) t/ha (t/ac) respectively on-field dried weight basis. Thus, the distribution of the aboveground field dried matter was 34.74 % for grain, 8.49 % for cob, and 56.77 % for SLH (Fig. 3(a)). Similarly, Shinnars and Binversie [36] obtained the field weight distribution after grain harvest to be 15 % for cob and 85 % for stalks, leaves, and husks (i.e. 56 %, 21 %, and 8 % respectively). Sokhansanj *et al.* [40] also obtained the field weight distribution to be 15 % for cob and 86 % for stalks, leaves, and husks (i.e., 50 %, 22 %, and 14 % respectively). The variation in the values may be as a result of the different MC of the biomass considered.

The average MC of the cobs and SLH were 40.77 % and 58.35 % respectively, which compared closely to 50 % MC of CS reported by Pordesimo *et al.* [32]. The average MC of the grain was 28.01 % which compares closely to 30 % reported by Shinnars and Binversie [36]. The dry matter yields of grain, cob, and SLH were obtained to be 3.61 (1.46), 0.73 (0.30), and 3.46 (1.40) t/ha (t/ac) respectively (Fig. 2). Thus, the percentage weight distribution of the corn plant fractions was 46.3 % for grain, 9.4 % for cob and 44.3 % for SLH on a dry weight basis (Fig. 3(b)) and the total yield of dry CS was 4.19 t/ha (1.70 t/ac). Similarly, Pordesimo *et al.* [31] investigated the dry weight distribution of corn plant fractions and found corn cobs to be 8.2 %, and SLH to be 45.9 % (i.e. stalk 27.5 %, leaf 11.4 %, and husk 7.0 %). In summary, the CS portion of the maize plant was 54 % against 46 % for grain, implying that huge quantities of CS are available annually.

Ratio of grain to corn stover

Fig. 4 illustrates the ratio of the yield of grain to CS. This relation is necessary for predicting the available quantities of CS given the production figures for corn. The R^2 value was found to be 0.97 which implied that there was a linear correlation between the production quantities of corn and CS. Thus, the ratio of grain to CS (or *product to residue*) is found to be

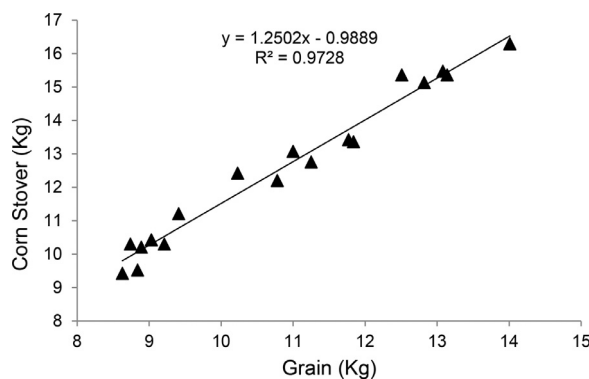


Fig. 4. Relationship between the yield of grain and corn stover.

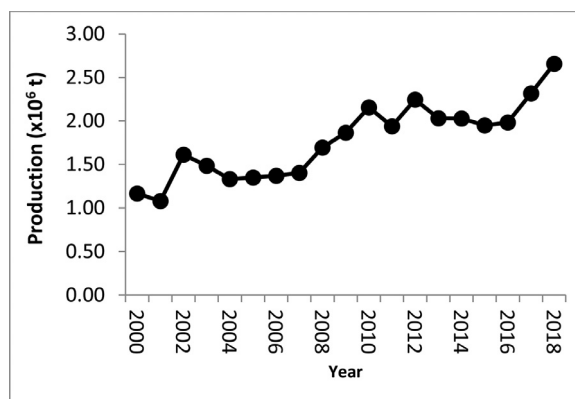


Fig. 5. Estimated quantities of corn stover (in million tonnes) available annually in Ghana.

approximately 0.865. Comparatively, Atakora [4] obtained the ratio of grain to cob to be 1.0 whereas Duku *et al.* [13] obtained the grain to stalk ratio to be 1.50. Kim and Dale [23] and Pordesimo *et al.* [31] also reported grain to CS ratio to be 1.0 and 0.66–0.86 respectively. The ratio of grain to CS obtained in this work closely agrees with that of Kim and Dale [23] and Pordesimo *et al.* [31]. The plant fraction ratios of grain to total dry matter, cobs to total dry matter, and SLH to total dry matter are 0.46, 0.09, and 0.44, respectively. The 0.46 ratio for grain to total dry matter agrees with 0.45 to 0.55 found by Sokhansanj *et al.* [40]. Also, CS to total dry matter ratio was found to be 0.54 which falls within the range 0.43–0.59 reported by Pordesimo *et al.* [32].

Consequently, the available quantities of CS produced in Ghana annually are shown in Fig. 5 taking into consideration production figures of maize from FAOSTAT. Maize production increased between the year 2000 and 2018 by 149 % which suggests potential rise in quantities of CS in the future. The mean annual production of maize in Ghana from the year 2000 to 2018 stands at 1.538×10^6 t and the corresponding possible available quantities of CS is calculated to be 1.77×10^6 t. However, considering a sustainable approach to the collection of lignocellulosic biomass, only 40 % of the available quantities of CS, 0.708×10^6 t, could be realized according to Kadam and Mcmillan [21]. This approach ensures that part of the CS is left on the field to improve soil nutrients and prevent soil erosion.

Yield of dried matter from different maize variety

Fig. 6 shows the yield of dry matter from different varieties of maize grown in Ghana. From Fig. 6, Pioneer 30 W 40 recorded the highest yield of dry matter followed by GH 90 DWD Pop and Pioneer 30 D 55. The highest yield of grain recorded was 3.9 t/ha for Pioneer 30 W 40 which was comparable to 3.6 and 3.8 t/ha reported by Morris *et al.* [27] for Kawanzie and Dorke SR maize varieties grown in Ghana respectively. Omankwa, GH 90 DYF Pop, and Fu 20–90 DWDP recorded relatively lower yield of dry matter. All the maize varieties showed higher yield for CS compared to the grain. According to this study, Pioneer 30 W 40 seemed to be the best candidate among the 9 maize varieties for promoting food security and bioethanol production because it recorded the highest yield of grain and CS. Also, the varieties such as GH 90 DWD Pop, Pioneer 30 D 55, Fu 20–90 DYFP, and Pioneer 30 B 80 which yielded relatively higher yield of grain and CS are also likely candidates.

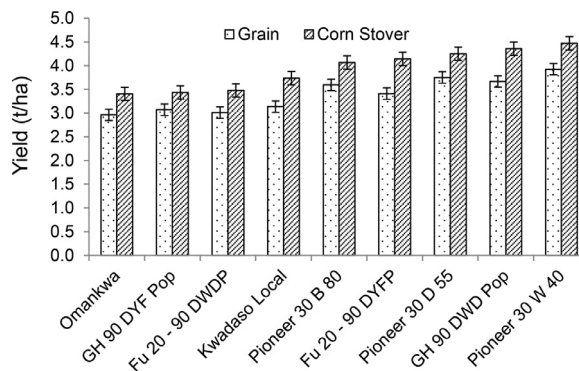


Fig. 6. Dry matter yield of local maize varieties in Ghana.

Table 1

Characteristics of Corn Stover from *Pioneer 30 W 40* maize variety.

Corn stover fraction	Total Solid (%)	Moisture Content (%)	Ash (%)	Extractives (%)	Lignin (ALL) (%)	Cellulose (%)	Hemicellulose (%)
Cobs	95.65	4.36	2.23	3.67	29.61	35.58	28.91
Stalk	93.39	6.61	7.03	8.16	30.62	42.41	11.78
Husk	93.89	6.11	2.96	3.76	30.54	31.17	31.57
Leaves	90.31	9.69	7.93	9.65	30.96	41.26	10.20
Average	93.31	6.69	5.03	6.31	30.50	37.72	20.62

Standard deviation: 0.06–3.66.

Physicochemical characteristics of corn stover

Table 1 shows the results of the physicochemical characteristics of the CS obtained from the *Pioneer 30 W 40* maize variety. The TS of the cobs, husks, stalks, and leaves, on a dry weight basis, were determined to be 95.64 %, 93.89 %, 93.39 %, and 90.32 %, respectively. The mineral or inorganic contents (i.e., % AC) constituted 2.23 %, 7.03 %, 2.96 %, and 7.93 % of the cobs, stalks, husks, and leaves, respectively. Comparatively, Li *et al.* [25] reported % AC for corn stalk rind, stalk pith, and leaf to be 9.9 %, 10 %, and 11.3 %, respectively. Aden *et al.* [1] and Capunitan and Capareda [9] reported AC to be 5.2 % and 6.62 % of corn stover, respectively. The stalks and leaves contained the highest ethanol extractives of 8.15 % and 9.64 % compared to the low values of 3.68 % and 3.76 % for cobs and husks, respectively. Capunitan and Capareda [9] reported corn stover to contain 18.1 % extractives which are higher compared to this work. The high ash and extractives contents of the leaves and husks maybe because they contain more pigment and are exposed to the environment than the cobs and husks.

Lignin is a complex phenolic polymer that is mostly insoluble in mineral acids. The percentage of acid-insoluble portions of lignin (ALL) in the cobs, stalks, husks, and leaves was 29.61 %, 30.62 %, 30.54 %, and 30.96 %, respectively. The percentage of acid-soluble portions of lignin (ASL) in the cobs, stalks, husks, and leaves was 4.89 %, 2.01 %, 5.68 %, and 2.40 %, respectively. Thus, the total lignin content was 34.25 % of the whole corn stover. However, the ASL is the small portion of lignin that solubilizes into solution during acid hydrolysis and its analysis is quite challenging since there is always spectroscopic interference from other soluble substances in the hydrolysis solvent. The lignin content reported in this work is mainly the ALL, in which the average was found to be 30.50 % for the whole stover. This value compares closely to 30.9 % and 29.0 % reported by Van Dongen *et al.* [12] and Eylen *et al.* [15].

The portions of corn stover that are carbohydrates are the cellulose and hemicellulose biopolymers. The cobs, stalks, husks, and leaves contained 35.58 %, 42.41 %, 31.17 %, and 41.26 % of cellulose, respectively. The stalks had the highest cellulose followed by the leaves and the least were the husks. The whole stover had cellulose content of 37.72 %. Chen *et al.* [11] and Torre *et al.* [44] reported cellulose content of 40.67 % and 31 %, respectively, which compares to this work. Li *et al.* [25] also, reported cellulose contents of 39.4 % and 31.3 % for the stalks and leaves. The hemicellulose contents were calculated to be 28.91, 11.78, 31.57, and 10.20 % for the cobs, stalks, husks, and leaves, respectively, which compares with the range 18.5–31.10 % reported by Chen *et al.* [11], Torre *et al.* [44] and Weiss *et al.* [45]. The total carbohydrate content of the whole stover was obtained to be 58.34 % which represents a valuable source of sugars for ethanol production.

Pretreatment of corn stover

Pretreatment processes are usually employed to extract the carbohydrate component of lignocellulosic biomass in a form suitable for enzymatic hydrolysis. Alkaline pretreatment appears to increase lignin solubilization, cellulose swelling, and the porosity of the biomass [28]. The alkaline catalyst cleaves the ester bonds connecting the lignin and hemicellulose, thereby removing the lignin and some part of the hemicellulose into the solution. Sodium hydroxide is one of the alkaline catalysts most studied for pretreatment of various biomasses. The main factors considered in sodium hydroxide pretreatment are

Table 2
Evaluation of sodium hydroxide pretreatment of corn cobs

Pretreatment conditions			Results			
NaOH load (%)	Solid load (%)	Autoclaving (121 °C) period	Solid Yield (%)	Cellulose (%)	Lignin (%)	Delignification (%)
0.50%	5.00%	30 min	64.97	52.19	22.14	29.73
1.00%	5.00%	30 min	59.1	60.19	17.48	44.5
2.00%	5.00%	30 min	54.66	66.09	17.48	44.52
3.00%	5.00%	30 min	47.99	67.81	18.75	40.47
4.00%	5.00%	30 min	45.65	72.76	14.69	53.38
0.50%	5.00%	60 min	61.55	52.7	21.11	32.98
1.00%	5.00%	60 min	58.08	59.52	18.42	41.53
2.00%	5.00%	60 min	53.42	63.62	17.37	44.86
3.00%	5.00%	60 min	48.84	69.25	15.99	49.35
4.00%	5.00%	60 min	45.29	73.29	12.76	59.5
0.50%	5.00%	90 min	64.32	56.08	21.46	31.89
1.00%	5.00%	90 min	57.89	59.18	13.83	56.1
2.00%	5.00%	90 min	52.95	64.78	11.67	62.96
3.00%	5.00%	90 min	49.48	68.14	11.5	63.49
4.00%	5.00%	90 min	45.53	74.3	9.8	68.9
0.50%	5.00%	120 min	63.34	56.48	14.61	53.61
1.00%	5.00%	120 min	59.15	62.57	20.61	34.57
2.00%	5.00%	120 min	54.81	67.78	18.11	42.5
3.00%	5.00%	120 min	51.74	72.24	14.9	52.63
4.00%	5.00%	120 min	46.38	78.85	14.07	55.33

Standard deviations: solid yield = 0.28–1.21, cellulose = 0.41–3.06, lignin (or delignification) = 0.05–5.76.

Table 3
Effect of two NaOH pretreatment processes on different parts of corn stover

Corn stover fraction	(i) 4 % NaOH, 90 min autoclaving at 121 °C				
	Solid Yield (%)	Cellulose (%)	Lignin (%)	Delignification (%)	
Cobs	45.53	74.3	9.8	68.9	
Husks	36.74	73.44	8.23	73.88	
Leaves	34.77	66.7	8.32	73.58	
Stalks	44.42	79.9	7.04	77.66	
Overall stover	40.37	73.58	8.35	73.50	
(ii) 3 % NaOH, 120 min autoclaving at 121 °C	Cobs	51.74	72.24	14.9	52.63
	Husks	38.51	70.56	6.36	79.82
	Leaves	35.79	67.4	6.54	79.25
	Stalks	44.59	81.27	4.83	84.67
	Overall stover	42.66	72.87	8.16	74.09

the sodium hydroxide load or concentration, biomass load, time, and temperature. In the present study, the biomass load and temperature were kept constant at 5.0 % and 121 °C, respectively. Table 2 shows an evaluation of various pretreatment conditions applied to corn cobs.

The sodium hydroxide load was observed to be the most sensitive pretreatment parameter compared to time. Increasing the NaOH % load led to a decrease in the solid yield and lignin content, and an increase in the cellulose contents of the corn cobs (Fig. 7(a), (b), (c)). Specifically, when the NaOH % loads were 0.5 % and 4.0 % at constant pretreatment time of 30 minutes, the solid yields, lignin and cellulose content were found to be 64.97 %, 22.14 %, 52.19 % and 45.46 %, 14.69 %, 72.76 % respectively. Thus, increasing the NaOH % by 87.5 % reduced the solid yield and lignin content by 30 % and 34 %, respectively, and increased the cellulose content by 39 %. This observation agrees with Sun *et al.* [41], that the kinetics of delignification of CS at high temperatures (>100 °C) follows a first-order reaction since the progress of the reaction depend mainly on the concentration of the alkali. The pretreatment time had a minor effect on the cellulose content (Fig. 7(d)). However, it must be noted that there is the possibility of precipitation of the solubilized lignin onto the surfaces of the pretreated biomass [48].

Table 3 shows the effect of two NaOH pretreatment processes on different parts of CS. The two NaOH pretreatment processes or conditions are (i) 4.0 % NaOH, 90 min, 5.0 % solid loading, and (ii) 3.0 % NaOH, 120 min, 5.0 % solid loading. Both pretreatment processes yielded similar results. The highest solid yield of 51.74 % was obtained for the corn cobs and the lowest of 35.79 % for the corn leaves. This observation may be associated with the percentage of extractives contained in each biomass fraction which was removed alongside the lignin during the pretreatment process. Corn cobs had the least extractives of 3.67 % and recorded the highest solid yield whilst corn leaves had the highest extractives of 9.65 % and recorded the lowest solid yield. Cornstalk recorded the highest cellulose content of 81.27 % and the lowest was 67.40 % for corn leaves. Cornstalk recorded the highest percentage of delignification of 84.67 % and the lowest of 52.63 % was recorded

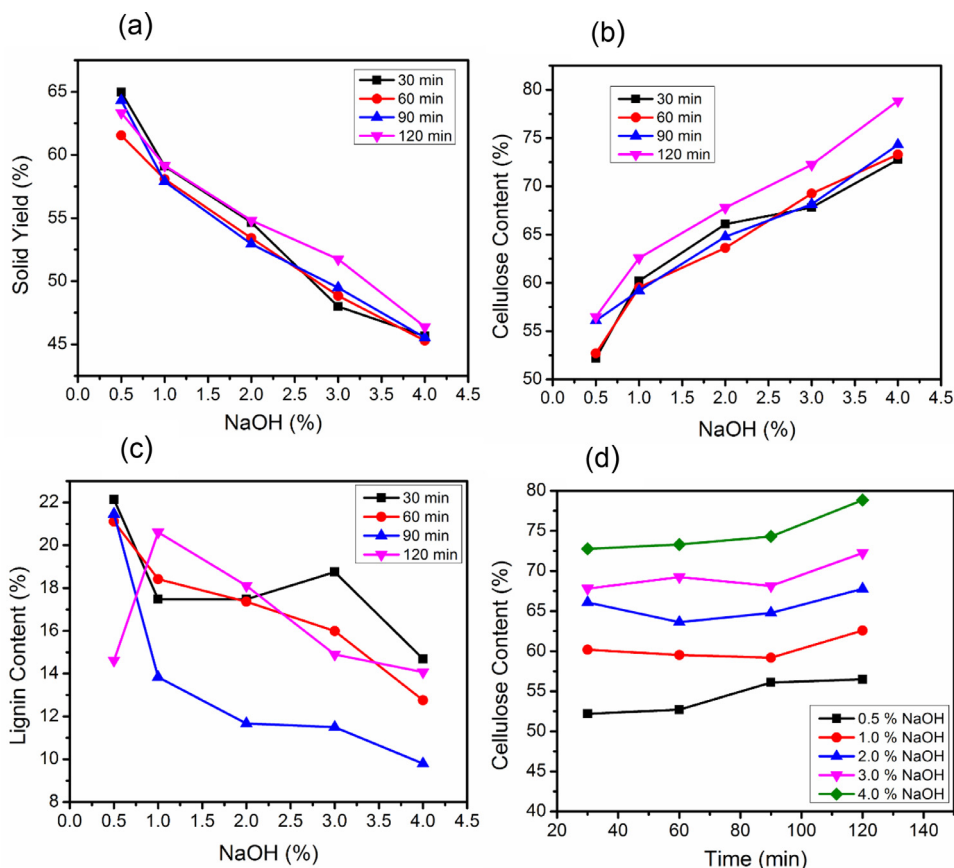


Fig. 7. Effect of pretreatment parameters: NaOH concentration on (a) solid, (b) cellulose and (c) lignin contents of corn cobs. (d) Effect of pretreatment time on cellulose content of corn cobs.

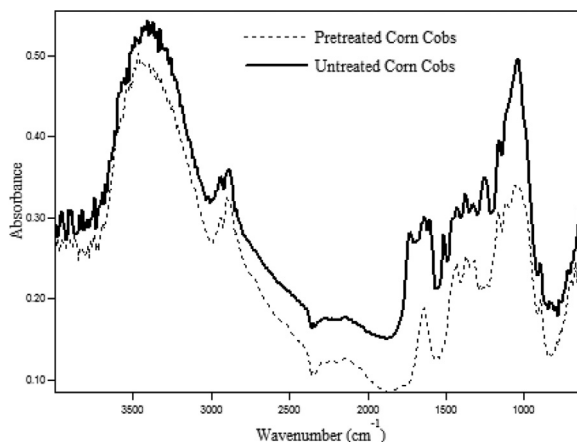


Fig. 8. FT-IR spectra for untreated and sodium hydroxide pretreated corn cobs.

for corn cobs. The corn stalks were the most affected by pretreatment methods, followed by corn husks, corn leaves, and then corn cobs. This might be due to the rigidity of the corn cobs compared to the other fractions.

The effect of the sodium hydroxide pretreatment on CS is also shown qualitatively in the FT-IR spectra (Fig. 8). The untreated (consisted of 35.58 % cellulose, 28.91 % hemicellulose, and 29.61 % lignin) and pretreated (consisted of 72.24 % cellulose, 12.86 % hemicellulose, and 14.9 % lignin) corn cobs showed similar FT-IR spectra. Both spectra had significant peaks at 3500–3000 cm⁻¹ representing OH stretching, peaks at 3000–2500 cm⁻¹ representing C-H stretching, and peaks at 2000–1000 cm⁻¹ which are characteristic of the cellulose, hemicellulose, and lignin since they all consist of C=O (1510–

Table 4
Theoretical ethanol yield of corn stover

Ethanol Yield	
<i>Ethanol from Cellulose</i>	
Dry stover	1 tonne (1000 kg)
Cellulose content	× 0.3772
Cellulose conversion and recovery efficiency	× 0.76
Ethanol stoichiometric yield	× 0.51
Glucose fermentation efficiency	× 0.75
Ethanol yield from glucose	109.652 kg (138.976 l)
<i>Ethanol from Hemicellulose</i>	
Dry stover	1 tonne (1000 kg)
Hemicellulose content	× 0.2062
Hemicellulose conversion and recovery efficiency	× 0.9
Ethanol stoichiometric yield	× 0.51
Xylose fermentation efficiency	× 0.5
Ethanol yield from xylose	47.3229 kg (59.978 l)
<i>Ethanol yield per 1 tonne (1000 kg) of corn stover</i>	
Total ethanol yield in kilograms	156.9749 kg
Density of ethanol	0.789 kg/l
Total ethanol yield in liters	198.95 l

1560 cm^{-1}), C-O-C (1232 cm^{-1}) and C-O-(H) (~1050 cm^{-1}) [46]. However, the spectrum for the untreated corn cobs is of higher peak intensities than the spectrum for the pretreated corn cobs. The reduction in peaks intensities was significant in the region of 2000–1000 cm^{-1} . This might be as a result of the reduced lignin content in the pretreated corn cobs.

Lignin is reported to be rich in O-CH₃ (methoxy), C-O-C (aryl-alkyl ether), and C=C (benzene stretching) groups which absorb at 1470, 1232, and 1632 cm^{-1} respectively [46]. The reduction of these absorbing groups in the lignin during pretreatment consequently led to a reduction in the intensities of the peaks in the spectrum for the pretreated corn cobs. Kim *et al.* [24] also determined the FT-IR spectra for untreated corn stover and ammonia recycle percolation pretreated CS and observed lignin characteristic peaks at 1218, 1268 (C-O of guaiacyl ring), 1315 (C-O of syringyl ring), and 1502–1600 cm^{-1} (aromatic skeletal vibration). Hence, the difference in the spectra of the raw and pretreated corn cobs indicated the reduction in the lignin content of the corn cobs when treated with sodium hydroxide.

Enzymatic hydrolysis and simultaneous saccharification and fermentation (SSF)

The highest conversion of corn cobs, corn stalks, and corn leaves to sugars was observed within 24, 48, and 24 h of reaction respectively. The highest concentration of reducing sugars of 157.96 mg/ml was obtained within 24 h of enzymatic hydrolysis of corn cobs representing 60.29 % of conversion. Corn stalks hydrolysis also yielded 128.74 mg/ml of reducing sugars within 48 h of reaction representing 56.30 % conversion. Corn leaves yielded a relatively low concentration of reducing sugars of 28.05 mg/ml within 24 h representing 38.25 % of conversion. Sarkar *et al.* [35] obtained a similar sugar concentration of 86 mg/ml when they performed enzymatic hydrolysis of 2 % NaOH pretreated corn straw (at 80 °C, 1 h) with cellulase of *Trichoderma reesei* ZU-02 and cellobiose of *Aspergillus niger* ZU-07. It must be noted that the conventional method used to estimate the sugars does not target non-reducing sugars.

The highest ethanol yield obtained for corn cobs, stalks, and leaves was 0.045, 0.024, and 0.009 l/kg respectively. The ethanol yields obtained were low compared to the 0.29 l/kg reported by Kim and Dale [23]. The ethanol concentration dropped drastically after 48 h of fermentation. No amount of ethanol was determined in the SSF system of the corn stalks and corn leaves after 120 and 48 h of fermentation, respectively, whereas, the ethanol concentration of 0.015 l/kg was determined in the fermentation of corn cobs even at 168 h. The inhibition of the fermentative microorganism by a high concentration of sugars and the consequent conversion of ethanol to byproducts such as acetic acid may explain why the concentration of ethanol dropped after 48 h of fermentation.

Theoretical ethanol yield of corn stover

The theoretical conversion factor for converting cellulose to ethanol by *S. cerevisiae* is reported to be 0.568 by Zhang *et al.* [47]. Thus, 1 g of cellulose yields theoretically 0.568 g ethanol [47]. Also, according to Badger [5], 100 g of glucose theoretically yields 51.4 g ethanol and 48.8 g CO₂. In the calculation of theoretical ethanol yield of CS, Badger [5] assumed cellulose conversion and recovery ratio of 0.76, stoichiometric ethanol yield from glucose ratio of 0.51, glucose fermentation efficiency of 0.75, hemicellulose conversion and recovery ratio of 0.90, stoichiometric ethanol yield from xylose of 0.51 and xylose fermentation efficiency of 0.50. Thus, the ethanol yield that could be realized from a tonne of CS was calculated to be 198.95 l of ethanol (Table 4). The actual yield of ethanol obtained was 45 l per tonne of CS, representing 22.6 % of the theoretical. The theoretical ethanol yield compares with 227 l and 290 l ethanol per tonne of C reported by Badger [5] and Kim and Dale [23], respectively. The 0.708 × 10⁶ t of CS generated (i.e. 40 % of the annual CS production) in Ghana has an ethanol potential of 140.855 × 10⁶ l.

Conclusion

The assessment of quantities, alkali pretreatment, enzymatic hydrolysis, and fermentation of CS obtained from local maize varieties in Ghana has been assessed. The dry weight distribution of the corn plant was found to be 46.3 %, 9.4 %, and 44.3 % of grain, cob, and stalk-leaves-husk respectively. The ratio of grain to CS (*product to residue*) was determined to be 0.865. Thus, the available average quantity of dry CS was calculated to be 1.77×10^6 t per year in Ghana. Among the 9 maize varieties considered in this study, *Pioneer 30 W 40* was found to yield the highest quantity of CS. The physicochemical characteristics of the CS obtained from *Pioneer 30 W 40* were 93.31 % TS, 6.69 % MC, 5.03 % AC, 6.31 % EC, 30.50 % lignin, 37.72 % cellulose and 20.62 % hemicellulose. Sodium hydroxide pretreatment of the CS yielded a cellulose-rich substrate of 81.27 % and lignin removal of up to 84.67 %. Enzymatic hydrolysis of the CS substrate yielded a reducing sugar concentration of 157.96 mg/ml within 24 h. The corn cobs fraction appeared to yield more sugar compared to the other fraction. However, the ethanol yield obtained (0.045 l/kg) was low and is associated with factors related to enzyme inhibition and byproduct formation. The theoretical ethanol yield per tonne of CS was calculated to be 198.95 l. Thus, about 708,000 t of dry CS (i.e., 40 % of the mean annual CS production – sustainable CS collection) could theoretically yield about 140.855×10^6 l of ethanol. This quantity of ethanol could be channeled into blending with gasoline in Ghana for transportation to reduce petroleum imports and emissions of greenhouse gases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors wish to thank Novozymes Denmark for donating enzymes for this work. The Maize Department, [Crops Research Institute](#) (CRI), [Council for Science and Industrial Research](#) (CSIR), Kumasi, Ghana is also appreciated for providing their field and maize crops for the study. The Energy Commission, Ghana is acknowledged for providing the funds for this project.

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