Comparative biochemical analysis during the anaerobic digestion of lignocellulosic biomass from six morphological parts of Williams Cavendish banana (Triploid *Musa* AAA group) plants

Short title: Banana lignocellulosic biomass and anaerobic digestion

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Significance statement: In this original paper, we present the biochemical composition and potential methane production from the anaerobic digestion of each type of lignocellulosic waste from a banana cultivar (Williams Cavendish: triploid *Musa* AAA group). These wastes are usually abandoned in the plantation after the fruits have been harvested. There is great interest in obtaining energy from this generally neglected biomaterial, particularly in the contexts of global warming and sustainable development.

Abstract: We studied banana lignocellulosic biomass (BALICEBIOM) that is abandoned after fruit harvesting, and assessed its biochemical methane potential, because of its potential as an energy source. We monitored biogas production from six morphological parts (MPs) of the "Williams Cavendish" banana cultivar using a modified operating procedure (KOP) using KOH. Volatile fatty acid (VFA) production was measured using high performance liquid chromatography. The bulbs, leaf sheaths, petioles–midribs, leaf blades, rachis stems, and floral stalks gave total biogas production of 256, 205, 198, 126, 253, and 221 mL g^{-1} dry matter, respectively, and total biomethane production of 150, 141, 127, 98, 162, and 144 mL g^{-1}, respectively. The biogas production rates and yields depended on the biochemical composition of the BALICEBIOM and the ability of anaerobic microbes to access fermentable substrates. There were no significant differences between the biogas analysis results produced using KOP and gas chromatography. Acetate was the major VFA in all the MP sample culture media. The bioconversion yields for each MP were below 50%, showing that these substrates were not fully biodegraded after 188 d. The estimated electricity that could be produced from biogas combustion after fermenting all of the BALICEBIOM produced annually by the Cameroon Development Corporation–Del Monte plantations for 188 d is approximately 10.5×10^6 kW h (which would be worth 0.80–1.58 million euros in the current market). This bioenergy could serve the requirements of about 42000 people in the region, although CH_4 productivity could be improved.

Keywords: anaerobic digestion, banana lignocellulosic biomass, biochemical analysis, bioenergy, biogas production, sustainable development

Introduction

After decades of rapid industrialization, global warming (mostly caused by greenhouse gases, such as CO_2) is causing imbalances with unfortunate consequences in the biosphere. This threat requires appropriate solutions that will allow the preservation and promotion of new industrial development. Interest in using biomass as an alternative source of energy is growing worldwide (Hall et al. 1998; Chandra et al. 2012), and it is prominent among the solutions currently being considered because biomass is a renewable primary energy source. However, the use of "first generation" biofuels is being increasingly questioned because human foodstuffs are used to produce fuel. The "second generation" of biofuels are mainly produced from renewable non-food biomass resources, particularly lignocellulosic materials, because these are the most abundant (and cheap) types of non-food materials available from plants. The production of a number of biofuels from lignocellulosic biomass has been described, including bioethanol (Ogier et al. 1999; Galbo et al. 2002; Didderen et al. 2008; Balat 2011), biohydrogen (Guo et al. 2010; Cheng et al. 2011), biomass pellets (Gil et al. 2010; Ståhl et al. 2011), and biomethane (Ward et al. 2008; Chandra et al. 2012; Barakat et al. 2012).

The biomethanation of carbohydrates in waste biomass generally comes from the reactions

$$C_nH_{2n}O_n + nH_2O \rightarrow nCO_2 + 2nH_2 \tag{1}$$

and

$$C_nH_{2n}O_n \rightarrow n/2CH_4 + n/2CO_2. \tag{2}$$

Methane production using anaerobic digestion technology is becoming more common around the world because of its economic and environmental benefits. All types of biomass can generally be used as substrates, as long as they contain fermentable carbohydrates, proteins, and fats as major components. However, for technical and economic reasons, some materials are preferred over others, and biogas yields and compositions are greatly affected by the chemical composition of the anaerobic digestion feed materials (Mital 1996). Many agricultural and municipal waste biomass resources are currently used to produce biomethane, but very few reports of biomethane production from banana lignocellulosic biomass (BALICEBIOM) have been published. Biomethane production from banana pseudo-stems under thermophilic and mesophilic conditions has been achieved (Kalia et al. 2000), and methane production from banana leaves has been studied in a plug flow digester (Chanakya et al. 2012). However, to the best of our knowledge, there has been no study of the entire range of BALICEBIOM materials in which each of the six main morphological parts (MPs) of the banana plant, after harvesting the fruit, have been separately and comparatively studied for their suitability for biogas production. The six main MPs that can be used for biogas production represent about two thirds of the banana plant, the other third being the fruits, in terms of fresh matter (Kamdem et al. 2011). The worldwide annual production of bananas and plantains is approximately 125 million tonnes (FAO 2010), which generates about 250 million tonnes of fresh lignocellulosic biomass waste (Kamdem et al. 2011).

As a leading African producer of bananas and plantains, Cameroon produces about 7.2 million tonnes of fresh BALICEBIOM waste, containing about 11.2% dry matter (DM) (Lassoudière 2007; FAO 2010; Kamdem et al. 2011). The

BALICEBIOM that is generated in Cameroon (from the intensive production of bananas for export) is mainly from the "Dwarf Cavendish" and "Williams Cavendish" cultivars. Because it is taller and more robust, the "Williams Cavendish" cultivar produces more waste. These wastes are, unfortunately, discarded or burned after the fruit has been harvested. The possibility of the biomethanation of the six MPs that are available from the waste biomass from "Williams Cavendish" banana production was studied to assess the impact of using it on attempts to protect the environment, avoid energy shortages, and promote sustainable development in banana-producing countries. A comparative biomethanation and biochemical analysis study was performed to attempt to understand the anaerobic digestion of these materials and to assess their biogas production potentials. The biochemical methane potential (BMP) assay was used in our experiments (Owen et al. 1979).

Materials and methods

Sample preparation

The lignocellulosic biomass of mature "Williams Cavendish" banana (*Musa* AAA group) plants was collected randomly from an industrial plantation at the Cameroon Development Corporation (CDC)-Del Monte Cameroon base in Moussaka, southwest Cameroon. After the mature fruits had been harvested, the entire lignocellulosic biomass of the plant was cut down by cutlass and knife. This biomass was carefully separated into six MPs, which were bulbs, leaf sheaths, petioles–midribs, leaf blades, rachis stems, and floral stalks, and these MPs were cut into pieces with diameters of approximately 50 mm, then washed and rinsed four times to remove pesticide residues. The six MPs were then sun- and air-dried for 30 d then ground in a laboratory blender to give particles of approximately 1 mm diameter. The bulbs, leaf sheaths, petioles–midribs, leaf blades, rachis stems, and floral stalks had DM contents of 90.2, 94.6, 92.3, 83.9, 90.1, and 88.7%, respectively.

Analysis of total organic carbon and nitrogen

The total organic carbon (TOC) content was determined using a method based on that described by Allen et al. (1974), but modified by others (Lechien et al. 2006; CEAEQ, 2011). A total of 0.025 g DM of each sample was analyzed. The TOC percentage in the sample, expressed as a percentage of DM, was calculated using the equation

$$C = \frac{(Va-Vb) \times Vd \times n \times 100}{Va \times m}, \tag{3}$$

where C is the organic carbon concentration (% DM), Va is the volume of ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) solution (0.504 M) used for the blank titration (mL), Vb is the volume of $FeSO_4.7H_2O$ solution (0.504 M) used to titrate the BALICEBIOM sample after oxidation (mL), Vd is the initial volume of potassium dichromate ($K_2Cr_2O_7$) solution (0.167 M) used for the oxidation (mL) (Vd = 10 mL), n is a factor representing the quantity of carbon (g) oxidized per mL of $K_2Cr_2O_7$ solution (n = 0.004 for the oxidation of complex organic matter, such as BALICEBIOM), and m is the oxidized sample DM (g). The glucose equivalent (GE) was then estimated from the TOC content according to the relationship

$$GE = C/40, \tag{4}$$

where GE is the TOC content (C, as a percentage of the DM) divided by the TOC content of a glucose molecule (40%). The GE value gives the energetic potential of a lignocellulosic material during fermentation, and also takes into account the energetic potential of indirectly fermentable molecules and biopolymers, such as lignin. The maximum GE values were below 2.5, because no biopolymer or biomaterial is currently known that is made of pure carbon.

The total nitrogen content was determined using the Kjeldahl method, using a Kjeldahl Selecta Alcodest still apparatus (Conklin-Brittain et al. 1999).

Preparation of the media

A BMP assay medium (Wang et al. 1994) was used. The anaerobic sludge that was used as an inoculum and blank sample was taken from a 20 L stirred anaerobic digester in the Walloon Centre of Industrial Biology, which is used for BMP assays of different agro-food organic wastes. This lab-scale digester was inoculated 2 years before the study reported here, with sludge collected from a full-scale anaerobic digester that is used to treat activated sludge from a municipal wastewater treatment plant. The sludge was stored at 4 °C under an anaerobic atmosphere (nitrogen), and it had a DM content of 20% (w/v). Glucose monohydrate was used as a positive control sample. Before fermentation, the concentration of each sample in the culture medium was 20 g L^{-1} (w/v).

Culture conditions and experimental procedures

The BMP assay was performed according to the procedure published by Wang et al. (1994), and all experiments were carried out in triplicate in 100 mL sterile glass serum bottles. DM (1 g) of each banana biomass sample was introduced into a 100 mL bottle containing 45 mL of BMP medium, and 5 mL of the anaerobic sludge inoculum was added. Each positive control sample consisted of 1 g of glucose monohydrate and 5 mL of the inoculum in a 100 mL sterile glass serum bottle containing 45 mL of BMP medium. Each blank sample was 5 mL of the anaerobic sludge inoculum and 45 mL of BMP medium. No energetic substrate was added to the blank samples. The final culture volume of each sample before incubation at 30 °C was 50 mL. The pH was adjusted with a 0.5 N KOH solution to achieve an initial pH of 7.3 in each sample, and a maximum variation during the culture period of pH 0.15 was maintained. The sample bottles were capped tightly with rubber septa and sealed with aluminum seals, and nitrogen was passed into the bottles to flush out air and other gases before the incubation (Hiligsmann et al. 2011). The bottles were then incubated at 30 °C and monitored for 188 d.

Monitoring and analytical methods

KOH operating procedure

Water containing 9 N KOH in 100 mL gas replacement equipment was used to monitor the biogas production and composition, and the absorption potential of the KOH solution was regularly measured using gas mixtures containing 0, 20, 35,

80, and 100% CO_2 (Hiligsmann et al. 2011). The H_2 (for the first 8 d of fermentation), CH_4, and CO_2 contents were determined for each gas sample using the procedure published by Hiligsmann et al. (2011) but adapted for the biomethanation of BALICEBIOM.

Gas chromatography and high performance liquid chromatography analysis

H_2, CH_4, and CO_2 were determined using a method described by Hamilton et al. (2010), and separation was achieved using a Hewlett Packard 5890 Series II gas chromatograph (GC; Agilent Technologies, Santa Clara, CA, USA) equipped with a 30 m long, 0.32 mm id Alltech GAS PRO GSC column (Grace, Deerfield, IL, USA) in series with a 20 m long, 0.25 mm id Chrompack CARBOPLOT P7 column (Agilent Technologies) and a thermal conductivity detector. The carrier and reference gas was He, and a mixture of N_2 (15%), CO_2 (35%), and CH_4 (50%) was used to calibrate the instrument for determining the proportions of CH_4 and CO_2 in the biogas. A mixture of H_2 (80%) and CO_2 (20%) was used to allow the fraction of H_2 in the biogas produced to be determined. The GC injection port, the thermal conductivity detector chamber, and the oven were maintained at 90, 110, and 55 °C, respectively.

Volatile fatty acid (VFA) concentrations in the culture medium were determined using an Agilent 1110 series high performance liquid chromatograph (HPLC; Agilent Technologies) equipped with a Supelcogel C-610H column (Sigma-Aldrich, St Louis, MO, USA) preceded by a Supelguard H precolumn (Sigma-Aldrich). The columns were kept at a temperature of 40 °C, and the isocratic mobile phase was 0.1% H_3PO_4 (in ultrapure, "milliQ", water), at a flow rate of 0.5 mL min^{-1}. A differential refraction index detector, kept at 35 °C, was used. This analysis took 35 min at a maximum pressure of 60 bar (Masset et al. 2010). The instrument was calibrated using 0.125, 0.25, 0.5, 1, 2, and 4 g L^{-1} solutions of glucose, acetate, ethanol, propionate, and butyrate.

Results and discussion

Carbon and nitrogen contents

To calculate the C/N ratios, the C and N contents of the samples were determined using the TOC procedure (using potassium dichromate solution) and the Kjeldahl method, respectively. The GE and organic matter contents were also calculated. The GEs and the C, organic matter, and N contents of each MP are presented in **Table 1**.

The floral stalks had the lowest carbon contents (30.40% of the DM), and the data confirmed that the floral stalks play the role of mineral conduit, resulting in it having a relatively high mineral content (26.1% of the DM) (Lassoudière 2007; Oliveira et al. 2007). The bulbs and leaf blades have nutritional roles, and had the highest C contents, 41.60 and 40.20% of their DMs (Lassoudière 2007). The leaf blades had the highest N contents and the leaf sheaths and petioles–midribs had the lowest, as was found by Oliveira et al. (2007). The C/N ratio influences the anaerobic digestion process, as described by Mital (1996), and the rachis stems and floral stalks may be more suitable for the biomethanation process than the other MPs.

Cumulative production of biogas

Taking into account the initial amount of N_2 in the gas and its dilution by the biogas produced, it was possible to analyze the gas composition and, therefore, the volumetric biogas (CH_4 and CO_2) production for each gas sample taken. The cumulative biogas production volumes are presented in **Fig. 1a–h**, each point representing the mean of three measurements. The vertical lines around the points represent the standard deviations (SDs). These results allow the biogas production performances of the six different MPs obtained from the BALICEBIOM to be compared. High biogas production rates in the early stages of glucose fermentation meant that gas samples had to be collected with short time intervals at the beginning of the fermentation (for the first 8 d), but after this stage gas samples were collected at longer time intervals (38, 58, 73, 114, 142, and 188 d). The gas was collected when the pressure in the collection bottles was high enough to deliver enough gas for analysis. The glucose samples showed relatively good total biogas production (TBP; 331 mL g^{-1} of DM, corrected for biogas production by the inoculum) after 188 d of fermentation, without any pretreatment, as presented in **Fig. 1b,** compared with the six MPs (**Fig. 1c–h**). The overall TBP from bulbs, leaf sheaths, petioles–midribs, leaf blades, rachis stems, and floral stalks after 188 d was 256, 205, 198, 126, 253, and 221 mL g^{-1} of DM, respectively, which represents productivities of 1.13, 0.91, 0.88, 0.56, 1.12, and 0.98 mL L^{-1} h^{-1}, respectively. The total methane production (TMP; corrected for inoculum and hydrogen production) from glucose, bulbs, leaf sheaths, petioles–midribs, leaf blades, rachis stems, and floral stalks was 50, 150, 141, 127, 98, 162, and 144 mL g^{-1} of DM, respectively. However, Kalia et al. (2000) found banana stem TMP of 196 and 171 mL g^{-1} of DM after 57 d of digestion time (DT) in mesophilic conditions and 24 d in thermophilic conditions, respectively.

The relatively low TMP performance of glucose after 188 d of DT indicated that protein, minerals, and vitamins present in the raw banana waste biomass had a great influence on methane production. These results confirmed that banana bulbs, leaf sheaths, rachis stems, and floral stalks have chemical compositions that make them suitable for anaerobic digestion and biogas production (Oliveira et al. 2007; Kamdem et al. 2011). The high lignin contents of the leaf blades and petioles–midribs (24.3 and 18% of DM, respectively) strongly affect their digestibility and, consequently, affect their TBP performances (Oliveira et al. 2007; Kamdem et al. 2011). The N content of each MP is presented in **Table 1**, and this may also explain their TBP performances because a C/N ratio of 20–30 has been suggested to be the optimum for the anaerobic digestion process (Mital 1996). Gerardi (2003) suggested that a C/N ratio of at least 25 is required for optimal biogas production. The results shown in **Fig. 1a–h** and **Table 1** demonstrate that the biogas production from the six MPs was affected by the C/N ratio. For example, the TBP and TMP from rachis stems could be explained by the C/N ratio (28.12), because, as has been described by Shandra et al. (2012), a very high C/N ratio causes methanogens to rapidly consume the N to meet their protein requirements and then not be able to react with the remaining C, leading to low volumes of gas being produced. A C/N ratio that is too high indicates a lack of N, leading to restrictions on protein formation and, therefore, on energy production and structural material metabolism by the microorganisms. This was probably the case for leaf sheaths, which had the highest C/N ratio (57.23) of the MPs, and was confirmed by the TBP and TMP being lower for the leaf sheaths than the rachis stems. On the other hand, if the C/N ratio

is very low, N will be liberated and accumulated as NH_4^+, and the presence of excess NH_4^+ will increase the pH of the biodigestate in the digester. Toxic effects become apparent in the methanogen population when the pH becomes higher than 8.5. This should be the case for the leaf blades, which had the lowest C/N ratio (18.23), and the lowest TBP and TMP, of the MPs.

One volume of inoculum and nine volumes of the substrate were incubated together to assess the ability of the microbial mixture to adapt during the metabolism of the substrate (hydrolytic, acidogenic, acetogenic, and methanogenic microorganisms were present in the inoculum). The relatively small quantity of inoculum (1 volume) used may explain the relatively long retention times and low productivities found in our study compared with other studies (Kalia et al. 2000; Chanakya et al. 2012). For example, Kalia et al. (2000) performed anaerobic digestions of banana stems in mesophilic conditions using twice the inoculum volumes that we used, and using banana biomass concentrations of 2% (w/v) in the fermentation medium. These authors achieved a TBP of 271 mL g^{-1} after 57 d of DT, the productivity being 4 mL L^{-1} h^{-1}, and, under thermophilic conditions, they achieved a TBP of 217 mL g^{-1} after 24 d of DT, the productivity being 7.5 mL L^{-1} h^{-1}. Chanakya et al. (2012) used 10 times the inoculum volumes that we used to digest bananas leaves, and achieved a TBP of 350 mL g^{-1} after 30 d of DT, the productivity being 9.7 mL L^{-1} h^{-1}.

The overall TBP from the sludge (blank) after 188 d of DT was 31 mL g^{-1} of DM, and the CH_4 and CO_2 production rates were 11 and 20 mL g^{-1} of DM, respectively (**Fig. 1a**). These performances were actually achieved after 8 d, and did not change through the remaining DT. The sludge had low concentrations of VFAs and other biodegradable substrates (such as sugars, other fatty acids, and amino acids) that were accessible for the microorganisms to metabolize, which explained why the biogas production from blank samples appeared to end quickly (after 8 d of biomethanation) with relatively little biogas produced (31 mL g^{-1} of DM). Monomers within the samples or formed during the hydrolytic phase were accessible to the microbes. According to Gerardi (2003), these monomers were taken up by anaerobic bacteria with different metabolic abilities and degraded to form short-chain (C_1–C_5) organic acids (butyric, propionic, and acetic acids), alcohols, H_2, and CO_2.

The glucose and BALICEBIOM samples gave exponentially increasing biogas production for the first 8 d of incubation, probably because accessible soluble monomers were rapidly transformed by acidogenic bacteria in the culture media. This was in agreement with a study by Masset et al. (2010), in which the biogases produced during the first 8 d of anaerobic digestion of sugar (such as glucose) were mainly H_2 and CO_2. The gas generation rates from all of the MPs were initially high and then gradually declined in our study. The slow production of CH_4 could be explained by the regeneration time for hydrolytic and acid forming bacteria being significantly shorter than the regeneration times of methanogenic microorganisms, and the slow growth of methanogens causes CH_4 production to have a relatively long start-up phase (Deublein et al. 2008; Chandra et al. 2012). As in other studies, the results could also be explained by the accessible substrates (sugars, proteins, and lipids) being almost completely metabolized after 8 d of DT (Mital 1996; Yadvika Santosh Sreekrishnan et al. 2004). The slow rate of

methanogenesis observed after 8 d showed that the monomers were completely used and the microorganisms probably adapted their metabolisms to the long and slow process of hydrolyzing the lignocellulosic substrates in the MPs during this phase.

The banana biomass samples were ground to form particles with different diameters (approximately 1 mm) before they were exposed to anaerobic digestion, so the values and variations in the SDs shown in **Fig. 1a–h** could be explained by the absence of mixing conditions during the fermentation, which would allow random heterogeneity to develop within the medium. Mixing ensures that a sample is, and remains, homogeneous and that organic material is transferred efficiently to the active microbial biomass, releases gas bubbles trapped in the medium, and prevents the sedimentation of relatively dense material. Mixing, therefore, stops the SD being high. Heterogeneity in a system has been linked to the formation of anaerobic granules that have been shown to greatly affect anaerobic digestion (Ward et al. 2008). These granules have been found to be made of extracellular polymeric substances, a combination of proteins and carbohydrates that acted as biofilm generation supports within the bioreactor (Liu et al. 2004). For these reasons, biogas production rates from all of the samples also depended on direct interactions between the organic matter and the microbial biomass community. It has been postulated that propionate oxidizing bacteria and methanogenic archaea live in close proximity in granules and use hydrogen and formate as electron carriers (De Bok et al. 2004), the association enhancing biogas production.

CH$_4$ and CO$_2$ volumetric kinetics

The biogas produced in the serum bottles was collected after 2, 8, 38, 58, 73, 114, 142, and 188 d of incubation, and transferred to the replacement equipment that was filled with KOH, to allow the CO$_2$ to be absorbed. The CH$_4$ and CO$_2$ volumes in the biogas were determined at each sampling time and their volumetric proportions were calculated using the equations

$$\% \, CO_2 = \frac{Vol \, CO2}{Vol \, CO2 + Vol \, CH4} \times 100 \qquad (5)$$

and

$$\% \, CH_4 = \frac{Vol \, CH4}{Vol \, CO2 + Vol \, CH4} \times 100. \qquad (6)$$

The results are presented in **Fig. 2a–g**, in which each point represents the mean volumetric proportion of three measurements at each DT. These results were confirmed using GC measurements. The production of H$_2$ and CO$_2$ from glucose and the six banana MPs increased exponentially during the first 2 d of DT; then decreased between 2 and 8 d, except in the glucose and leaf blade samples, in which the gas production rates continued almost unchanged.

As mentioned above, the biogas produced during the first 8 d of biomethanation was essentially composed of CO$_2$ and H$_2$. The exponentially increasing biogas production phase was related to the metabolism of directly accessible polymers and monomers by hydrolytic and acidogenic bacteria, forming H$_2$, CO$_2$, ethanol, and acetic, propionic, butyric, and lactic acids. The glucose and proteins were rapidly used up by the microorganisms, so the exponentially increasing phase continued only up to 8 d of DT in the glucose samples (i.e., the samples in which glucose was the energy-supplying substrate) and the leaf blade

samples (which were rich in proteins) (Gerardi et al. 2003). The decrease in gas production by the bulb, petiole–midrib, rachis stem, and floral stalk samples between 2 and 8 d of DT was probably linked to the acetogenesis phase, in which 4 mol of H_2 are combined with 2 mol of CO_2 to produce 1 mol of acetic acid, following the reaction

$$4 H_2 + 2 CO_2 \rightarrow CH_3COOH + 2 H_2O. \tag{7}$$

This decreases the volume of H_2 produced during the exponentially increasing phase, as described above. GC analysis of the biogases produced was performed after 14 d of DT to verify that H_2 was being consumed within the culture vessel. The results showed that, after 14 d of DT, the biogas from all of the banana MP samples we studied contained less than 1% H_2. In contrast, the gas produced by the glucose samples contained about 20% H_2, indicating that acidogenic activities in the glucose samples continued after 14 d of DT. There were no signs of significant acidogenic activity in the other samples after the same DT. It appears, therefore, that biogas produced from banana MPs between 8 and 188 d of DT was essentially a mixture of CH_4 and CO_2.

Without taking the hydrogen production during the first 8 d into account, the mean overall CH_4 content in the biogases produced from glucose, bulbs, leaf sheaths, petioles–midribs, leaf blades, rachis stems, and floral stalks over 188 d of DT was 15, 59, 69, 64, 78, 64, and 65%, respectively. These results are comparable to the results of a study by Kalia et al. (2000), in which the mesophilic and thermophilic anaerobic digestion of banana leaf sheaths gave 72 and 79% methane in the biogas, respectively. We found that leaf blades produced less biogas (126 mL g^{-1}) than the other MPs, but that the biogas produced from the anaerobic digestion of leaf blades was the richest in methane (78%). The leaf blades had the lowest C/N ratio (18.23), and this confirms that the N content of the substrate greatly influences the metabolism of the methanogens.

Production of volatile fatty acids

To assess VFA metabolism, each culture medium was analyzed by HPLC after 14 and 188 d of DT. Three major VFAs (butyric, propionic, and acetic acids) and one alcohol (ethanol) were found, as shown in **Fig. 3a**. Butyrate was the dominant VFA compound in the glucose samples after 14 d, with a concentration of 3.29 g L^{-1}, whereas acetate was dominant in all six MP culture media, reaching 3.72 g L^{-1} in the rachis stem samples.

The glucose samples had the highest concentrations of monomer substrates that were directly accessible to the anaerobic microorganisms, but the acetate concentration in these samples were surprisingly low compared with the concentrations in the banana waste samples. Consequently, the production of methane from the glucose samples was low, with an overall average of 15% of the TBP, as described above. As was also described earlier, the H_2 concentration after 14 d of culture was higher in the glucose samples (at about 20% of the biogas), confirming that methane production also depends on the rapid conversion of H_2 to acetate, and this production occurred when the H_2 partial pressure was low. As described by Chernicharo (2007), the low level of acetate production could be explained by H_2 retro-inhibiting the hydrogen-producing acetogenic bacteria that are required. The metabolism of these bacteria is only possible if H_2 is eliminated progressively.

Homoacetogenic bacteria, which produce acetate by reducing H_2 and CO_2 or from other VFAs and alcohols, were present at lower concentrations than the hydrogen-producing acetogenic bacteria that are required, so the acetate concentration is lower, leading to a low rate of methane production. It was also apparent that hydrogenophilic methanogenic bacteria, such as *Methanobrevibacter* and *Methanobacterium*, which reduce CO_2 with H_2 to produce methane, may be more active in the banana waste substrate samples than in the glucose samples. The association between the hydrogen-producing bacteria and methanogenic bacteria appeared to be the key step in the biomethanation reaction. Our results indicate that H_2 retro-inhibition of the hydrogen-producing acetogenic bacteria required may not have been significant, or may not have occurred at all, in the banana waste biomass samples, but did occur in the glucose samples.

The traces of ethanol found in the glucose (**Fig. 3a** and **b**), leaf sheath (**Fig. 3a**), and leaf blade (**Fig. 3b**) samples showed that the metabolic pathway leading to the production of ethanol was also exploited by anaerobic microorganisms in these samples. As is shown in **Fig. 3b**, the high acetate concentration (5.49 g L^{-1}) in the glucose samples at the end of the fermentation confirmed that the progressive accumulation of acetate produced by homoacetogenic bacteria was linked to the slow metabolism rate of methanogenic bacteria. As discussed earlier, this was probably caused by the low N content in the culture medium, because N in the serum bottle was principally derived from biotin and cysteine, which were used to prepare the BMP culture medium. The low concentrations (<1 g L^{-1}) of VFAs found in all of the banana biomass samples after 188 d of DT indicate that the fermentable and accessible organic matter had been completely anaerobically digested.

Glucose equivalents and conversion yields

The overall biogas (H_2+CH_4+CO_2) production and VFA residues (g g^{-1} of the sample DM) in the bottles were calculated after 188 d of DT. The GE value is linked to the C content of a sample, so samples richer in C, such as bulbs, leaf sheaths, and rachis stems, gave high GE values.

The variability in C contents and GE values in the samples, shown in **Table 1** and **Fig. 4**, could be explained by the difference between the C content (as a percentage of the substrate) present in major polysaccharides, such as cellulose and starch (44.4%), and present in major monosaccharides, such as glucose (40%), according to the hydrolytic reaction

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow (C_6H_{12}O_6)_n . \tag{8}$$

The lignin content also explained our results, because it has a higher C content than the other major types of organic matter, such as cellulose, hemicelluloses, starches, proteins, and pectin, present in the banana biomass samples (Oliveira et al. 2007). It can be seen from **Fig. 4** that only the glucose samples (at 69%) had conversion yields above 50%. The conversion yields for all of the MPs tested was below 50%, the actual yields being 32.69, 34.41, 28.42, 20.39, 33.00, and 38.16% for bulbs, leaf sheaths, petioles–midribs, leaf blades, rachis stems, and floral stalks, respectively. The relatively high conversion yield (38.16%) found using floral stalks was probably cause by its low lignin and high starch content, because starch is more easily accessible and biodegradable than cellulose and hemicelluloses (Kamdem et al. 2011). The lowest conversion yield was found

for the leaf blade samples (20.39%), which could be explained by the high lignin content of leaf blades (Oliveira et al. 2007; Kamdem et al. 2011). Lignin protects cellulose and hemicelluloses from hydrolysis by cellulase and hemicellulase, leading to the incomplete degradation of the lignocellulosic biomass material. It appears, therefore, that pre-treatment to remove the lignin would increase the digestion yield from BALICEBIOM. Barakat et al. (2012) demonstrated that methane production from sugar and lignin-derived molecules is possible after lignocellulosic biomass thermal pre-treatment, and they measured methane potentials of 105, 430, 450, and 453 mL g^{-1} from the biodegradation of vanillin, furfural, 5-hydroxymethylfurfural, and syringaldehyde, respectively. Our results show that the BMP test used in this study did not lead to the total fermentation of the biodegradable organic matter without sample pre-treatment.

Annual energy estimations for biogas production from wastes produced by an agro industrial company in Cameroon

The average proportions of bulbs, leaf sheaths, petioles–midribs, leaf blades, rachis stems, and floral stalks in BALICEBIOM are 11, 12, 17, 41, 7, and 13%, respectively (Kamdem et al. 2011). So, in this study, the overall mean biogas and CH_4 production were 183.27 and 125.25 mL g^{-1} of BALICEBIOM, respectively, after 188 d of DT. As is shown in **Table 2**, an estimated 10.5×10^6 kWh of electricity could be derived from these biogases. Cameroon has a population of more than 20 million and an annual national electrical consumption of about 5×10^9 kWh, so its per capita electrical consumption is about 250 kWh per annum (CIA 2013).

Using these values, we estimated that the BALICEBIOM produced by CDC–Del Monte, after 188 d of anaerobic digestion, could produce 0.2% of the annual electricity requirement of Cameroon. This could serve about 42000 people (which is more than the population of the Njombe-Penja agricultural district). The market value (MV) of the electricity produced would be between 0.52×10^9 and 1.04×10^9 FCFA (0.80–1.58 million euros), and this energy could generate an important income for the company and the population of the local area, and could contribute to the sustainable development of the country. Fertilizer produced from the digestate could generate another important income from the biomethanation process.

Conclusions

We have demonstrated, by monitoring the biodegradation process, that it is possible to transform banana waste biomass into a clean energy vector, biomethane. This could offer advantages in terms of expanding industrialization and reducing the amount of waste banana products by using the lignocellulosic biomass produced, and could be beneficial in the context of global warming. The BMP assay used in this study led to TBP of 256, 205, 198, 126, 253, and 221 mL g^{-1} of DM and TMP of 150, 141, 127, 98, 162, and 144 mL g^{-1} of DM from the anaerobic digestion of bulbs, leaf sheaths, petioles–midribs, leaf blades, rachis stems, and floral stalks, respectively. Overall CH_4 concentrations of more than 50% were found in the biogas produced after 188 d of DT using each of the MPs tested. We verified that the biogas yield depends on the C/N ratio and on the accessibility of the anaerobic microbes to the fermentable banana substrates. We also showed that, using optimal

conditions, the yield of biogas in general, and CH_4 in particular, strongly depends on the biochemical composition of each banana MP. It appears that the anaerobic digestion of BALICEBIOM involved an ethanol metabolic pathway and that some major VFAs (butyric, propionic, and acetic acids) were produced during the digestion. This study proved that the quantity of anaerobic microbes present in the inoculum affected the anaerobic degradation and, therefore, biogas productivity from banana waste biomass. Using low-cost and easy-to-use KOP equipment to analyze the amount of biogas produced and its composition seemed to be a good alternative to using more costly and complicated GC equipment. The KOP could, therefore, be suitable for cheap and reliable biogas analysis during anaerobic digestion. Banana-producing countries could become less dependent on fossil fuels and less prone to energy shortages by producing biomethane from banana lignocellulosic waste biomass, which would also lead to benefits in terms of environmental protection and sustainable development. This study shows that this could be feasible. An estimated 4.63×10^6 m^3 of biogas, containing 68.34% of methane (v/v), could be produced from 188 d of anaerobic digestion of the BALICEBIOM produced by CDC–Del Monte Cameroon, and this biogas could be used to produce 0.2% of the electricity consumed in Cameroon. This energy could serve about 42000 people (more than the population of the Njombe-Penja agricultural district in Cameroon). The MV of this electricity would be between 0.52×10^9 and 1.04×10^9 FCFA (0.80–1.58 million euros), so the energy could generate an important income for the company and the population of the agricultural district and contribute to the sustainable development of Cameroon. Fertilizer produced from the digestate could also be a significant source of revenue from the biomethanation process. However, further studies need to be performed to improve the biogas productivity, and pre-treatments and the co-biomethanation of all six MPs studied in this work need to be investigated to reduce the DT and to optimize the production of CH_4.

Abbreviations

BMP: Biochemical methane potential

BALICEBIOM: Banana lignocellulosic biomass

C/N: Carbon/ nitrogen ratio

CDC: Cameroon Development Corporation

DM: Dry matter

DT: Digestion time

FCFA: *Franc des Colonies Françaises d'Afrique*

GE: Glucose equivalent

GC: Gas chromatography

HPLC: High performance liquid chromatography

KOP: operating procedure using KOH

MP: Morphological parts

MV: Market value

SD: Standard deviation

TOC: Total organic carbon

TBP: Total biogas production

TMP: Total methane production

VFA: Volatile fatty acid

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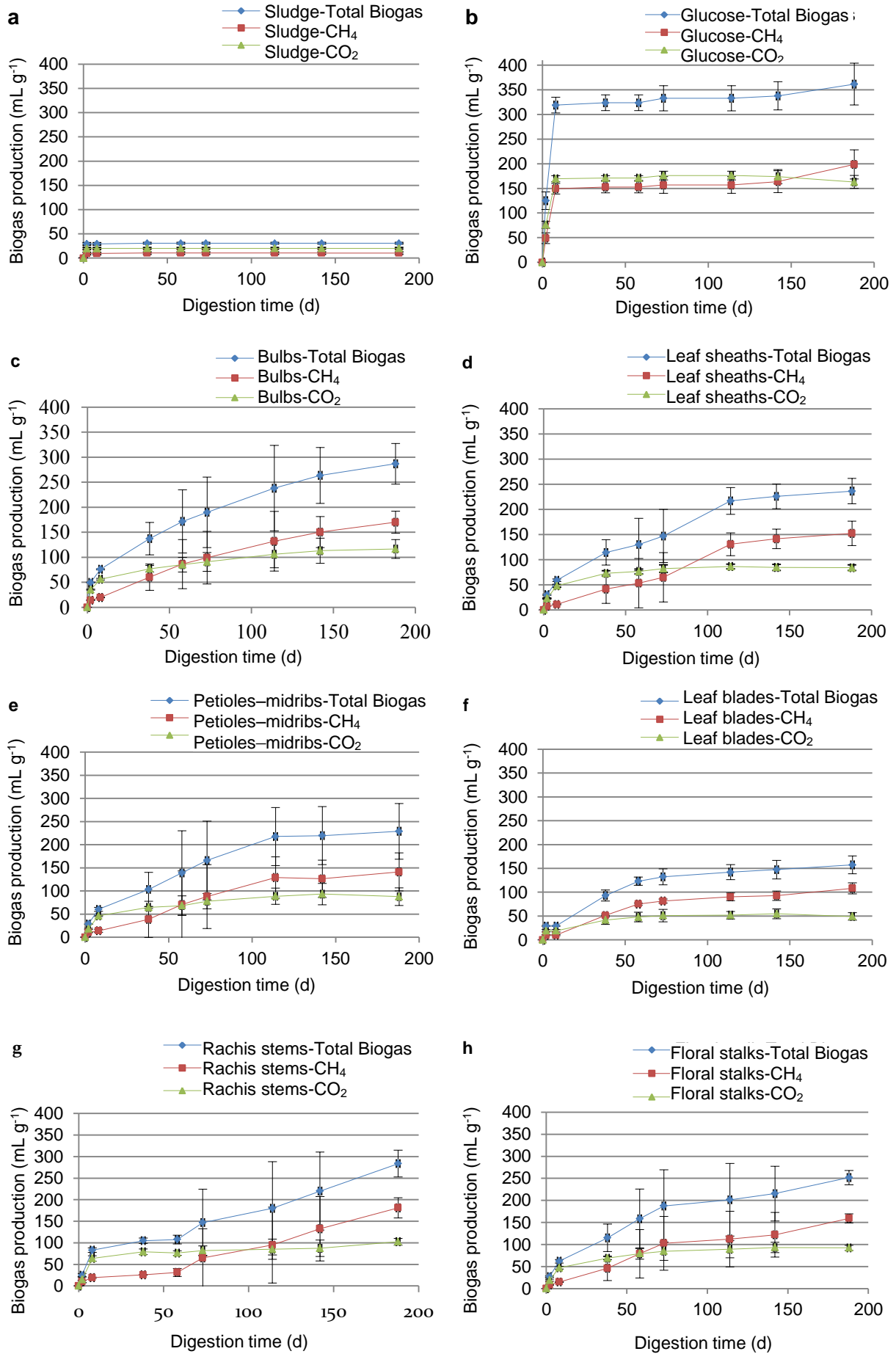
Fig.1 Cumulative biogas production (mL g^{-1} ± standard deviation) from the anaerobic digestion of samples. (a) Sludge, (b) Glucose, (c) Bulbs, (d) Leaf sheaths, (e) Petioles–midribs, (f) Leaf blades, (g) Rachis stems, (h) Floral stalks
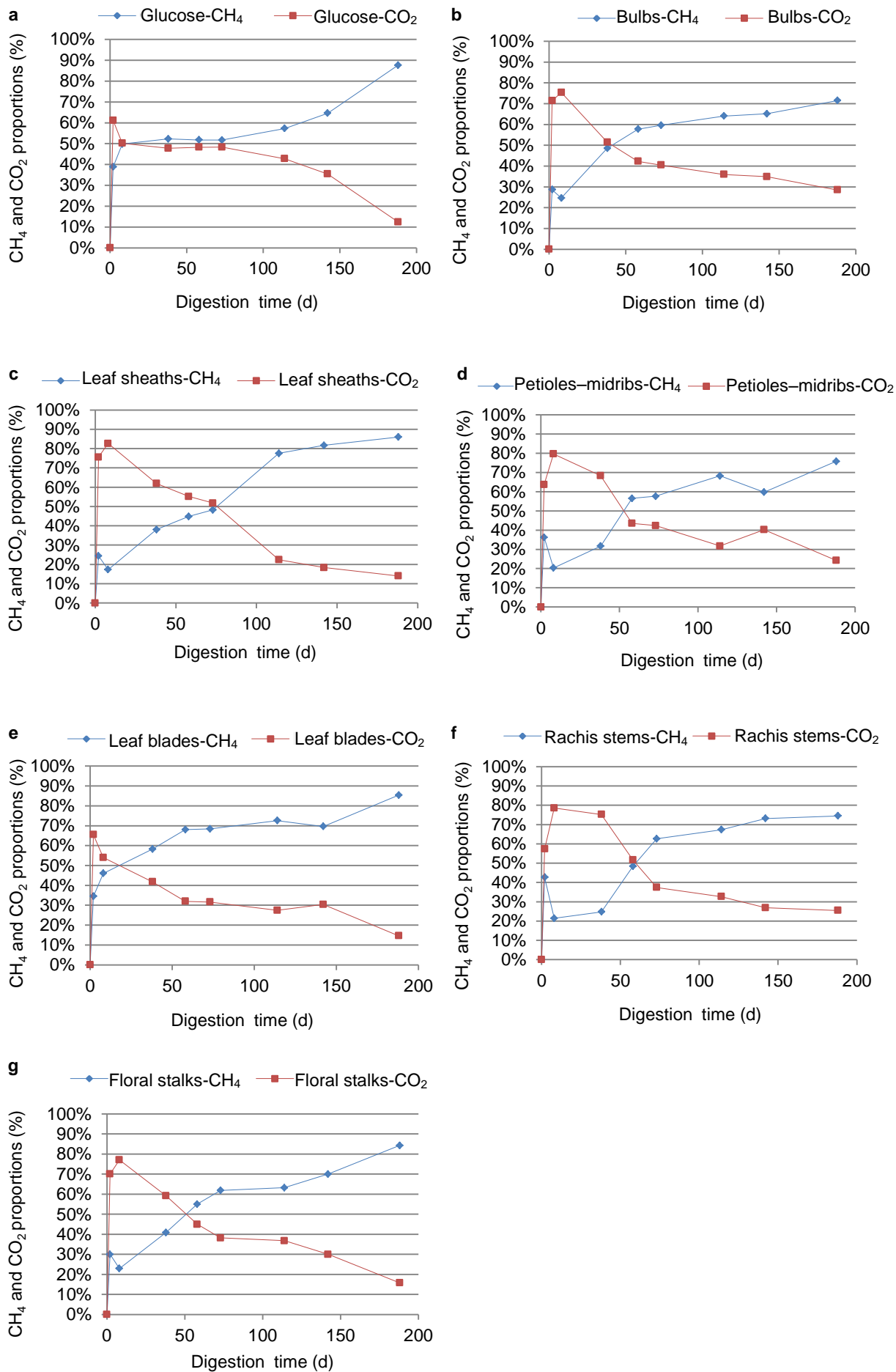
16

Fig. 2 Volumetric kinetics of CH_4 and CO_2, expressed as percentages. (a) Glucose, (b) Bulbs, (c) Leaf sheaths, (d) Petioles–midribs, (e) Leaf blades, (f) Rachis stems, (g) Floral stalks
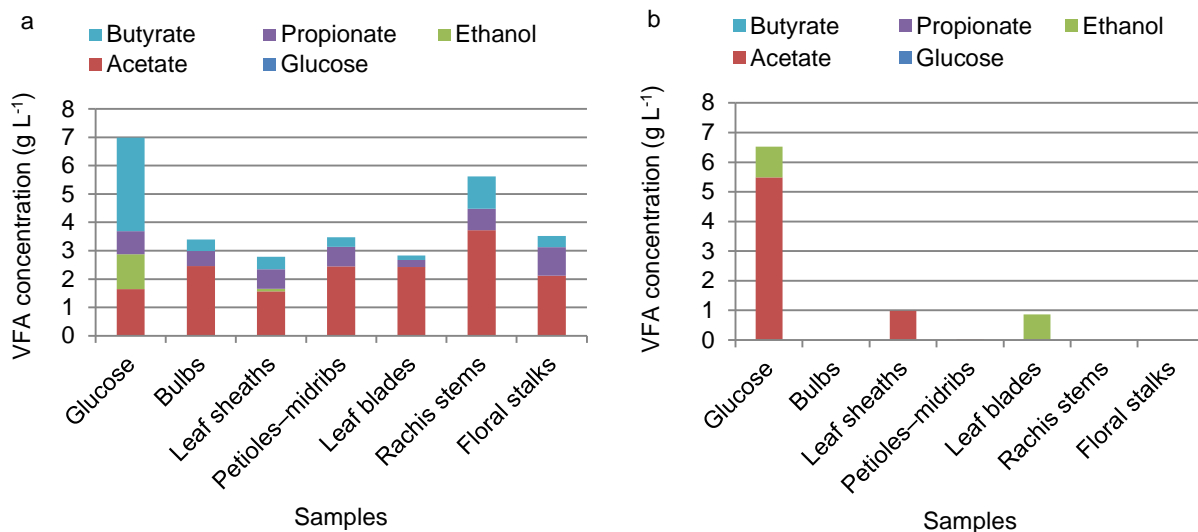
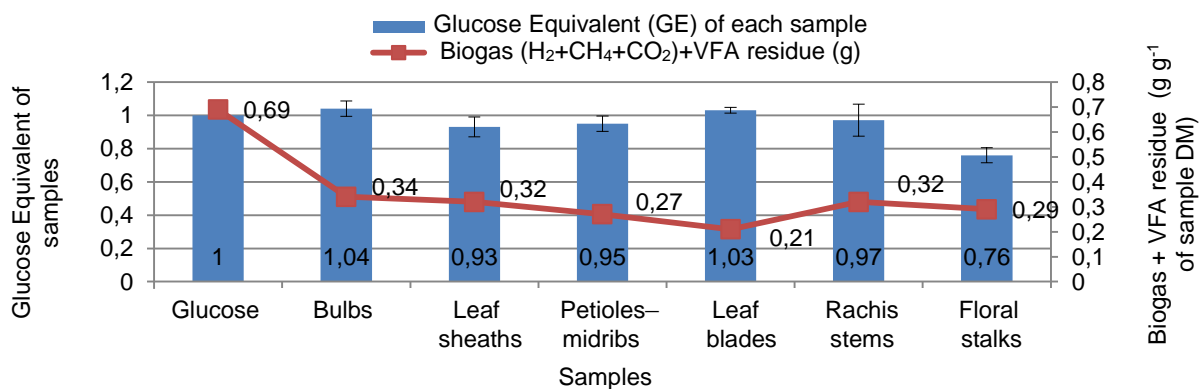**Fig.3** Volatile fatty acid concentrations during the anaerobic digestion. (a) after 14 d, (b) after 188 d

Fig. 4 Glucose equivalents (GEs) for each sample type and the overall production of biogas ($H_2+CH_4+CO_2$) and volatile fatty acid (VFA) residues after 188 d of anaerobic digestion

Table 1 Chemical composition of each of the morphological part of the banana plant

Components	Morphological parts					
	Bulb	Leaf sheaths	Petioles midrib	Leaf blades	Rachis stem	Floral stalk
Carbon content (% of DM)	41.60 ± 1.84	37.20 ± 2.40	38.00 ± 1.84	41.20 ± 0.70	38.80 ± 3.85	30.40 ± 1.84
Glucose Equivalent (per g of DM)	1.04 ± 0.05	0.93 ± 0.06	0.95 ± 0.05	1.03 ± 0.02	0.97 ± 0.10	0.76 ± 0.05
Organic matter** (% of DM)	83.62 ± 3.69	74.77 ± 4.82	76.39 ± 3.69	82.80 ± 1.40	77.98 ± 7.74	61.10 ± 3.69
Nitrogen content (% of DM)	0.92 ± 0.01	0.65 ± 0.02	0.69 ± 0.00	2.26 ± 0.00	1.38 ± 0.07	1.42 ± 0.01
C/N ratio	45.22	57.23	55.07	18.23	28.12	21.41

**Calculated by multiplying the carbon content by 2.01, as described by Giroux and Audesse (2008)

Table 2 Estimation of the energy that could be generated from the biogas that could be produced annually by CDC–Del Monte Cameroon from the anaerobic digestion of Banana lignocellulosic biomass for 188 d

Compounds	Components	Value
Annual biomass production[a] (metric tons)	Fruits	123×10^3
	Fresh BALICEBIOM	226×10^3
	Dry BALICEBIOM	25.24×10^3
Biogas from anaerobic digestion	Biogas (m^3)	4.63×10^6
	CH_4 (m^3)	3.16×10^6
	CH_4 (%)	68.34
Energy production from biogas combustion[b] (kWh)	Total LHV	31.43×10^6
	Electricity	10.50×10^6
Desserved population[c]		42000
MV[d] (FCFA and €)		0.52–1.04×10^9 FCFA (0.80–1.58 million euros)

[a]: Calculated from the annual production of dessert bananas in Cameroon (Kamdem et al. 2011)
[b]: Calculated from the lower heating value (LHV) of 1 m^3 of CH_4 (9.94 kWh m^{-3}) and electricity production by co-generation (Kamdem et al. 2011)
[c]: Estimated from the annual electricity consumption per inhabitant (CIA 2013)
[d]: MV (market value), calculated using current prices, excluding tax, in Cameroon. The current (2013) price is between 50 and 99 FCFA kWh^{-1} (AES-SONEL, 2013)