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Recovery of fibers and biomethane from banana peduncles biomass through anaerobic digestion

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ABSTRACT

Banana crop produces large quantities of post-harvest biomass wastes. Some of them are a potential resource of raw materials such as natural fibers, which can be used as reinforcement for composite materials. The recovery of fibers, after bioconversion of the more digestible soft tissues to biogas was assessed for peduncles of three banana varieties (Grande Naine (GN), Pelipita (PPT) and CRBP969). Fibers were sieved out from the digestate. Biogas was monitored manometrically and with gas chromatography. PPT peduncle produced both the highest fibers recovery (0.2 g_DM_fiber/g_DM_initial_substrate) and methane production (260 ml_CH_4/g_COD_initial_substrate) after 74 days of anaerobic digestion. This variety was the most suitable candidate to combine both fiber recovery and biomethane production through anaerobic digestion. GN peduncle fibers degraded in less than 20 days. This variety was more convenient for biomethane production (around 210 ml_CH_4/g_$COD_{initial_substrate}$). The amount and the quality of recovered fibers strongly depended both on the duration of anaerobic digestion and the banana variety. This work showed that anaerobic digestion was an effective bioprocess alternative to mechanical decortication and biological retting processes for fiber extraction from banana peduncles biomass.

Introduction

Banana is now widely cultivated in Asia (continent of origin), Latin America, Caribbean countries, and Africa, where its fruits contribute to food security and socio-economical life. Edible varieties arose from hybridization of *Musa acuminata* (AA) and *Musa balbisiana* (BB) (Stover and Simmonds, 1987). With a world production of more than 138 million ton in 2010, bananas and plantains are the seventh most important food crop after maize, rice, wheat, potato, soybean and cassava (FAOSTAT, 2012). But, fruit represents only about one third of the total fresh banana plant weight (Kamdem et al. 2011). For the year 2012, production reached 1.4 million ton in Cameroon, the African leading producer of bananas and plantains, resulting in about 90,000 ton as dry matter of agro-industrial residues (FAOSTAT, 2012).

Banana intensive cropping produces large quantities of post-harvest organic residues such as pseudo-stems, peduncles, bulbs, leaf sheath and rachis, representing about 70% of the total fresh plant weight. These residues are very often gathered as big roadside piles within which non-controlled fermentation leads to emission of volatile organic compounds, greenhouse gases, and contributes to spread mosquitoes and pathogens, with the corresponding environmental and health burdens (Awedem et al., 2016). Some of these residues are mainly constituted of cellulose and lignin, which are difficult to degrade under normal windrow composting conditions (Chanakya and Sreesha, 2012; Tiappi et al., 2015). They are a potential resource of natural fibers, which can be used in papers and textile, and as reinforcement for composite materials for aeronautics and cars (Baiardo et al., 2002; Gañán et al., 2008; Saikia et al., 1997).

In spite of potential applications of this type of residues and even their environmental impact, very few works related to fiber extraction and isolation have been reported. Some results have been published on the fiber isolation from banana pseudo-stem, leaf sheath and rachis through mechanical decortication and biological retting processes.

Abbreviations: BMP, Biochemical methane potential; CARBAP, African Research Centre on Bananas and Plantains; CRBP, Previous name of CARBAP; COD, Chemical oxygen demand; DM, Dry matter; FM, Fresh matter; GC, Gas chromatography; GN, Grande Naine; PHP, Plantations Haut Penja; PPTA, Pelipita; VS, Volatile solids.

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Table 1
Botanical accession of the studied varieties.

Variety	Analysis code	Specie/genetic group	Subgroup	Origin	Fruit type
Grande Naine	GN	AAA	Cavendish	Asia (China/Vietnam)	Dessert
Pelipita	PPT	ABB	Pelipita	Philippines	Cooking banana
CRBP969	CRBP969	AAAB	(hybrid plantain)	CARBAP (Cameroon)	Plantain

Mechanical decortication leads to deformation or breakages of β-glucosidic linkages present in cellulose and hemicellulose, while biological retting process leads to chemical changes of non-cellulosic components of the cell wall, such as pectins (Chanakya and Sreesha, 2012; Gañán et al., 2008). The recalcitrant properties of this biomass limit the recovery of fibers by both extraction methods (Chanakya and Sreesha, 2012; Gañán et al., 2004, 2008; Saikia et al., 1997). Other results have been reported on the bioconversion of banana residues to biogas (Bardiya et al., 1996; Chanakya et al., 2009; Chananchida et al. 2014; Gunaseelan, 2004; Kalia et al. 2000; Kamdem et al. 2013; Awedem et al., 2016). However, the bioconversion yields for biogas production found in the literature are currently below 50% *w*/w (e.g. 48% *w*/w for banana stem (Kalia et al. 2000); 46, 42, 40 and 28% *w*/w for banana peduncles, bulbs, leaf sheaths and leaf blades, respectively (Kamdem et al. 2013)), showing that these substrates are not fully biodegraded, even after many days of degradation time and the use of physical, chemical and mechanical pretreatments.

Nowadays, chemical, physicochemical and mechanical methods of pretreatment are investigated to improve degradation of the less digestible fibers. However, the additional costs required for this stage (such as high temperature, pressure or enzymes) can make the process more expensive and lower its industrial attractiveness (Dionisi et al., 2015). In the opposite way, the present work investigates anaerobic digestion as a bioprocess for combining both fiber recovery and biogas production. To the best of our knowledge, there has been no study with such an approach with banana peduncles biomass. We compared the degradation of soft tissues from peduncles of three banana varieties with contrasted properties (Grande Naine (export dessert banana), Pelipita (locally used plantain), CRBP969 (phytopathogen resistant hybrid-plantain)), to release fibers. We assessed the recovery yield of both biogas and fibers. The influence of the duration of anaerobic digestion on fiber recovery as well as fibers stiffness during the bioprocess was also investigated.

Materials and methods

Sample preparation

Banana peduncles of the varieties Grande Naine (GN, dessert banana), CRBP969 (previous name of CARBAP, hybrid plantain), and Pelipita (PPT, cooking banana) were obtained from the *African Research Centre on Bananas and Plantains* (CARBAP) and *Plantations Haut Penja* (PHP, only for the variety Grande Naine), in Cameroon. The varieties selected for this study are described in Table 1 (Awedem et al., 2015; Tiappi et al., 2015; Awedem et al., 2016). After the mature fruits had been harvested, the peduncles were collected. The harvested peduncles were cut into pieces with size of approximately 80 cm and stored at −20 °C. The samples were thawed just before use and cut with a knife into sticks of 4.5 cm length and maximum 2.5 cm diameter.

Chemical analysis

The dry matter (DM) content of the samples was determined after drying at 105 °C for at least 24 h. The dry residue was subsequently burned in a furnace at 550 °C for 24 h. The mass loss was defined as the volatile solids (VS). The chemical oxygen demand (COD) and ash content were determined according to Standard Methods (Clesceri et al., 1999). Soluble COD (CODs) was measured with the COD Cell Test method (Spectroquant® kits 1.14541.0001 and 1.14555.0001, Spectroquant® ThermoReactor 620, Photometer SQ200, Merck Germany) according to the provider's instructions. Cellulose, hemicelluloses and lignin contents were analyzed following the Van Soest method as extensively described in another study (Escarnot et al., 2010). Their contents were measured gravimetrically as residues of sequential non-enzymatic extraction procedures leaving the neutral detergent fibers (NDF, includes hemicelluloses, cellulose and lignin), the acid detergent fibers (ADF, includes cellulose and lignin) and acid detergent lignin (ADL). Then, the neutral detergent fiber (NDF) method provided data on the cellulose and hemicelluloses content by difference.

Anaerobic digestion

The anaerobic digestion assay was performed according to the method described by Awedem et al. (2016). The inoculum was prepared by incubating for 10 days at 35 °C under anaerobic conditions, a methanogenic primary inoculum maintained in the laboratory, with freshly collected activated sludge, as a substrate in a ratio of 0.3 $g_{COD\_activated\_sludge}/g_{COD\_methanogenic\_primary\_inoculum}$. The activated sludge was collected at the Chastre municipal wastewater treatment plant (Mont-Saint-Guibert, Belgium). Upon arrival in the laboratory, the sludge was left to settle in the dark at 4 °C for 24 h. The clear

Fig. 1. Fiber recovery (left: digestate on a sieve; right: fibers cleaning into a beaker).

Table 2
Banana peduncle composition.

Parameter	GN PHP	GN CARBAP	PPT	CRBP969
DM (%FM)	4.795 ± 0.005	4.215 ± 0.003	8.175 ± 0.005	6.349 ± 0.003
VS (%DM)	70.99 ± 0.02	74.17 ± 0.01	82.22 ± 0.02	88.12 ± 0.08
COD (g_COD/g_DM)	1.59 ± 0.02	1.44 ± 0.01	1.10 ± 0.1	1.14 ± 0.01
Cellulose (%DM)	36.4 ± 0.2	36.4 ± 0.3	37.1 ± 0.9	35.3 ± 0.1
Hemicellulose (%DM)	14.0 ± 0.3	16.4 ± 0.6	19.3 ± 0.9	20.5 ± 0.9
Lignin (%DM)	4.7 ± 0.0	5.2 ± 0.3	7.9 ± 0.0	5.8 ± 0.0
H/L ratio(%DM)[a]	10.8 ± 0.2	10.2 ± 0.7	7.2 ± 0.28	9.6 ± 0.3
Ash (%DM)	29.01 ± 0.02	25.83 ± 0.01	17.78 ± 0.02	11.88 ± 0.08

DM: dry matter; FM: fresh matter; VS: volatile solids; COD: chemical oxygen demand; PHP: plantations Haut Penja; CARBAP: African Research Centre on Bananas and Plantains. Average values and standard deviations of three determinations.
[a] Holocelluloses (cellulose + hemicelluloses)/lignin.

supernatant was removed to concentrate the sludge to 15–20 $g_{\_COD}$/L prior to use.

Bioreactors consisted of 1 L Schott Duran GL 45 glass bottles, with a two-way Luer polycarbonate valve (Fisher Scientific) connected at the top side. The bioreactor bottle was capped with a PBT screw-cap, containing a PTFE-coated silicone seal. Each bioreactor was checked to be airtight and resistant to internal pressure before each use. Two similar experiments were performed, one with 100 g of peduncles and the other with 200 g of peduncles. Each bioreactor was filled with the inoculum (10 $g_{\_COD}$), and incubated at 35 °C for at least 2 h in order to allow the rebalancing of CO_2 between the liquid phase and the gas phase. A known mass of banana peduncle substrate (100 g or 200 g depending on experiment) at 35 °C was added in order to reach a COD ratio ranging from 0.2 to 0.4 $g_{COD\_substrate}/g_{COD\_inoculum}$, depending on the experiment. Demineralized H_2O was added to complete the volume to 600 mL. Each bioreactor headspace was flushed for 2 min with a constant flow of nitrogen gas in order to ensure the absence of oxygen in the bioreactors prior to hermetic closure. The bioreactors were incubated at 35 °C in the dark under batch conditions for a maximum of 100 days, with intermediate sacrifices of some bioreactors. Each experiment was performed in triplicate, with negative control consisting of water in the place of the sample, in order to determine the biogas produced by the inoculum alone. The end of anaerobic digestion was determined when the production of biogas of the reactors with substrate plus inoculum did not exceed the biogas production of the reactors with the inoculum alone.

The biogas production was monitored using a UNIK type manometer (5000 PTX5072-TA-A3-CA-H0-PA, GE Measurement & Control Solutions) connected to the bioreactor through a 2 way valve. The manometer was equipped with a display (DMS-40LCD-4/20S , Datel Inc., Mansfield, MA, USA) calibrated for absolute pressure ranging from 900 to 1300 mbar with an accuracy of 0.1 mbar. The pressure was converted to gas volume using the ideal gas law with the headspace volume of each bioreactor determined independently. The gas pressures were monitored at regular intervals to ensure that the absolute pressure was maintained below 1150 mbar. Gas samples were collected using a polypropylene syringe closed with a two-way Luer polycarbonate valve (Fisher Scientific) every day during the first two weeks, every two days during the next three weeks and when the pressure in the collection bottles was high enough to deliver enough gas for analysis.

Gas composition was determined using a two channel Gas chromatography (Compact GC, GLOBAL ANALYSER SOLUTIONS™, Interscience, Belgium) equipped with a thermal conductivity detector on each channel: the first channel equipped with a RI-QBond column (10 m × 0.32 mm) allowed to separate and analyze CO_2. The elution was performed under isotherm conditions at 60 °C with helium as the carrier gas at 20 mL/min. The second channel had a RI-QBond column (2 m × 0.32 mm) followed by a Molsieve 5A column (7 m × 0.32 mm). The elution was performed under isotherm conditions at 70 °C with argon as the carrier gas at 10 mL/min. The columns placed in series permitted successively to separate CO_2 from the other gases as in the first channel (first column); then the H_2, O_2, N_2 and CH_4 gases were separated in the second column while the CO_2 was back-flushed through the first column. The detectors were heated at 90 °C and the filaments at 170 °C. Argon and helium, and calibrated mixtures of H_2, N_2, CO_2, CH_4, and air were used to calibrate the instrument for determining the proportions of CH_4, H_2 and CO_2 in the biogas. The biogas and methane productions of the inoculum were subtracted from the productions of each bioreactor to determine the net production attributed to the substrate.

Fiber recovery

At different times during digestion, some reactors were sacrificed. The fibers were recovered from the digestate using sieves with a mesh size of 0.5 mm. After shaking the bioreactors, the mixed liquor was poured onto the sieve. The fibers retained on the sieves were washed with a fresh water jet until the filtrate became clear. After washing, fibers were transferred into a beaker for a second cleaning by agitating the fibers suspended in fresh water to eliminate non-fibrous residues; the operation was repeated at least 5 times to get clean fibers (Fig. 1). Once extracted, fibers were taken for qualitative and quantitative analyses or stored at −20 °C, when the analyses were not done on the same day.

Data treatment

For the statistical treatment of the results, the mean value and the standard deviation were used when three values were available and the mean value and the deviation from the mean when two values were available.

Results and discussion

Banana peduncles composition

The proximate composition of banana peduncles samples is summarized in Table 2. PPT peduncle had the highest dry matter and lignin

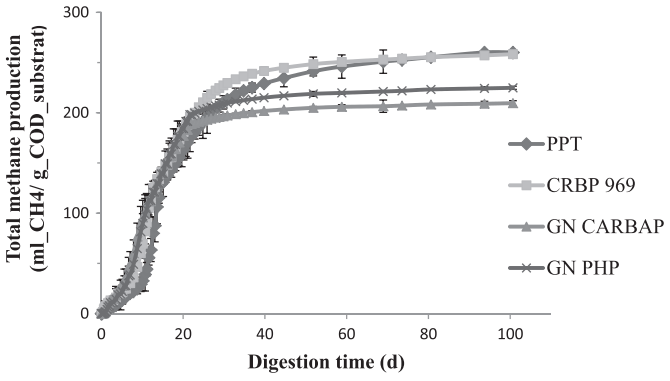

Fig. 2. Cumulated methane production (biogas with 50–55% CH_4). Vertical lines correspond to the standard deviations.

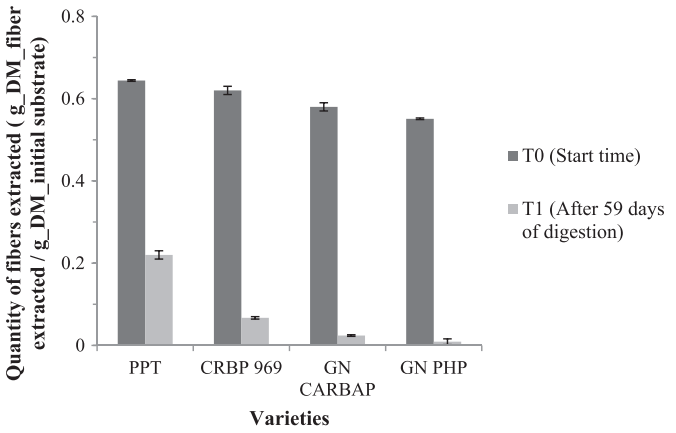

Fig. 3. Recovery yield of fibers in initial banana peduncles and after 59 days of anaerobic digestion.

contents while CRBP969 peduncle had the highest volatile solid content. GN peduncle had the highest ash content among the three varieties. The COD of dry matter from GN peduncle was higher than that observed with the two other varieties, and higher than expected for polysaccharides, suggesting the presence of more reduced organic molecules. PPT peduncle had also the relatively lower H/L ratio as compared to the two other varieties. These results are in general similar with the literature concerning the global chemical composition of banana peduncles (Cordeiro et al., 2009; Oliveira, et al., 2007; Tiappi et al., 2015; Uma et al., 2005).

Anaerobic digestion (BMP)

Fig. 2 shows the methane production kinetics of peduncles from the three banana varieties. Digestion of PPT peduncle was slower at the beginning but finally reached the higher total production among all varieties. The late start of the digestion with PPT peduncles would probably be due to its lignin content (Table 2). The amount of methane produced from the three varieties was almost similar until day 22 of digestion (Fig. 2). The COD biodigestibility ranged from 60 (GN CARBAP) to 75%$_{COD\_CH4/COD\_substrate}$ (PPT). The methane content (%CH_4) in the biogas ranged from 50% to 55% *v*/v for all the varieties, and GN peduncle had the highest methane percentage in the biogas (55% *v*/v).Values obtained for methane content were generally higher than observations made by Kalia et al. (2000), Chanakya and Sreesha, (2012) and Kamdem et al. (2013) with pseudo-stem, leaves and peduncles of various varieties of banana, respectively.

Fiber extraction

The initial fiber fraction and the fiber fraction extracted after 59 days of digestion time are shown in Fig. 3. The amount of fibers recovered after digestion strongly decreased with time, especially for GN variety. While the initial fibers contents were similar for all varieties (Fig. 3), the amount of fibers recovered after 59 days of anaerobic digestion was higher for PPT peduncle than for other varieties (0.2 versus 0.1 g_DM_fiber recovered/g_DM_initial substrate). Fibers from PPT peduncle resisted better to the digestion by anaerobic microorganisms, as compared to the others varieties. That difference might be related to the higher lignin content of PPT peduncles (Table 2). These results are in accordance with Tiappi et al. (2015) who noticed that the enzymatic hydrolysis of fibers from GN peduncle was very fast, as compared to the PPT peduncle, possibly linked with the difference of H/L ratio.

Changes in fiber composition during digestion

Fig. 4 presents the evolution of fiber composition during anaerobic digestion for PPT and GN PHP peduncles. The total ash which is initially present in both peduncles at day 0 (stage 1), disappeared at stages 2 and 3 (days 59 and 74 for PPT; days 22 and 43 for GN PHP, respectively) of digestion. This ash has probably been dissolved and contributed to the increase of the soluble fraction (Fig. 4). For PPT, the relative contents of cellulose and especially lignin in residual fibers increased with the digestion time while the hemicellulose fraction remained almost unchanged, as no sharp difference was observed (Fig. 4, left). The cellulose fraction from GN PHP peduncle strongly decreased with anaerobic digestion time while the soluble fraction and to some extent the hemicellulose fraction increased during digestion (Fig. 4, right).

Balance of fiber recovery

The amount of fibers recovered after anaerobic digestion is shown in Fig. 5. The amount of cellulose, hemicellulose and lignin recovered from PPT peduncle after 59 days of digestion was reduced by 73, 77 and 42%, respectively, as compared to the amount present in the initial substrate. The total amount of fibers recovered decreased with digestion time much faster for GN PHP than for PPT. For PPT peduncle, the fiber composition remained almost unchanged (Fig. 4). After 74 days of digestion time, an amount of 0.16 g_DM_fiber/g_DM_initial_substrate was still recovered from the PPT peduncle (Fig. 5).

For the GN PHP peduncles, the fibers almost disappeared after only 43 days of digestion, most probably due to degradation or fragmentation (Fig. 5). The amount of cellulose, hemicellulose and lignin recovered after 22 days of digestion was reduced by 98, 97 and 91%, respectively, as compared to the amount present in the initial substrate. The fiber composition (Fig. 4) reveals a degradation of the cellulose fraction. This degradation or fragmentation of cellulose fraction has significantly contributed to its high and quick methane production kinetic during the first twenty-two days of anaerobic digestion (Fig. 2). However, this degradation has also decreased the final amount of recovered fibers during the bioprocess (Fig. 5). Therefore, it seems

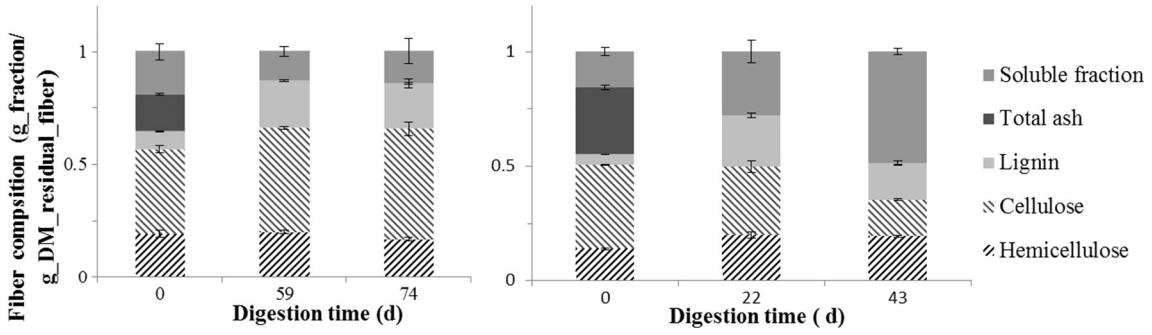

Fig. 4. Composition of the fibers recovered after increasing digestion time for PPT (left) and GN PHP (right).

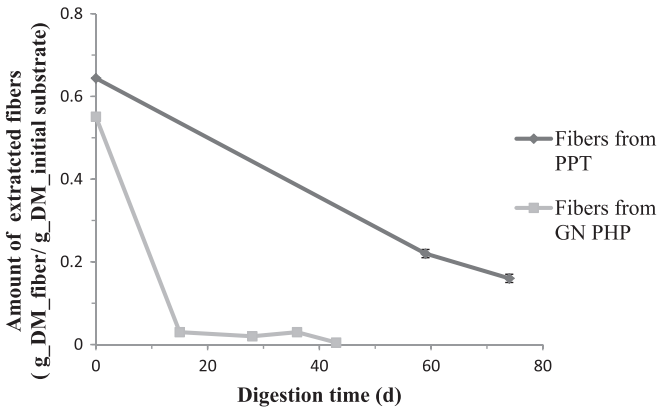

Fig. 5. Amount of fiber recovered during digestion.

that substrates with fiber components easily degradable like those from GN PHP are most suitable for biomethane production and not for fiber recovery through anaerobic digestion. Otherwise, the digestion time should not probably exceed 15 days.

Indeed, Tiappi et al. (2015) had reported that cellulose from GN peduncles was more easily hydrolyzed by cellulase, as compared to PPT. The lower content in guaiacyl units of the lignin fraction from GN peduncles as compared to PPT (Tiappi et al., 2015) suggests the presence of tissues with less lignocellulosic fibers, but more metabolically active cells, with cell walls that contain less crystalline cellulose and are better swollen by water, that can explain a better access of hydrolytic enzyme to digest the GN tissues and the higher degradation of fibers or the lower final amount of fibers recovered from GN variety.

Fig. 6 shows that fibers extracted from PPT digestate peduncle ranged from 2 to 5 cm in length and about 0.5 mm in diameter, while those extracted from GN PHP peduncle were shorter and thinner (0.5 to 1 cm in length and 0.02 mm in diameter) (Fig. 6). The relatively high content of lignin (Table 2) which is known to give rigidity to cellular wall could explain the higher resistance of PPT fibers. For GN PHP peduncles, a digestion time of 25 days would probably be suitable enough for the fiber recovery. The fiber fraction observed around that duration was high and the quality, separability and recovery by sieving were good as well. This observation is consistent with Chanakya and Sreesha (2012) who reported that a digestion time of 27 days was the optimum for fiber recovery from banana leaf. The digestion time and the variety both influenced the amount of fiber recovery and the fiber stiffness.

Influence of variety on optimal fiber/biomethane recovery

Anaerobic digestion process allowed the conversion of soft peduncles tissues to biomethane and the recovery of less digestible fibers. Due to the fast GN fiber degradation, it appears that it is not worth to recover fibers from peduncles of this variety. The best way to valorize GN peduncles seems to be their conversion to biogas only. The biogas produced could then be converted by several pathways according to the specific needs. Each hectare of banana crop generates 220 Mg of wet residues and banana peduncles represent 7% (15.4 Mg_peduncles/(ha·year)) of these wet residues (Kamdem et al., 2011; Reddy et al., 2003). If we take these data into account, the yield of biomethane produced annually with the dessert banana peduncles used in this study will be in the range of 200–204 m^3-/(ha·year) (Fig. 2).

For PPT peduncles, as compared to GN peduncles, the fibers were more resistant to degradation. In addition to soft tissue, some parts of the fibers were degraded before 40 days of anaerobic digestion (Fig. 5) and probably contributed to the total methane production (Fig. 2). PPT peduncles seem to be more suitable for the recovery of both fibers and biomethane production than GN variety. The best digestion time to recover an important amount of fibers and biomethane ranged from 30 to 40 days of anaerobic digestion. With 15.4 Mg_peduncles/(ha·year), a yield of 0.2 Mg_dry_fiber/(ha·year) and 260 m^3_CH_4/(ha·year) would be supplied from the PPT variety after 74 days of digestion time. If these fibers are recovered around 40 days of anaerobic digestion, the methane production would be 235 m^3_CH_4/(ha·year) and the amount of fiber recovered annually would be doubled (0.4 Mg_dry_fiber/(ha·year).

Such natural fibers have attractive technical, economic and environmental advantages. They are used in the manufacture of several materials for aeronautics, cars, papers and textile (Gañán et al., 2008; Saikia et al., 1997). Anaerobic digestion can then be considered as an alternative bioprocess to mechanical decortication and biological retting processes used for fiber extraction, as reported by others investigations (Chanakya and Sreesha, 2012; Gañán et al., 2008). However, it would be necessary to identify the suitability of the different banana varieties for fiber extraction and the most adequate digestion time for each one, in order to get the best performance in terms of fibers recovery and biomethane production.

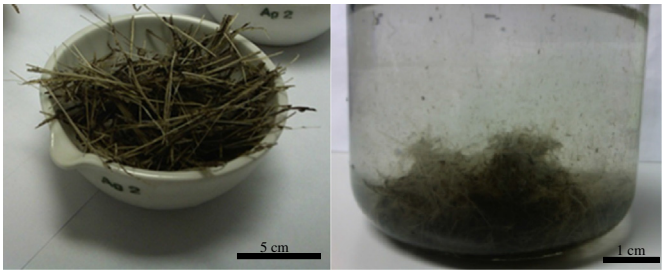

Fig. 6. Cleaned fibers recovered after 59 and 36 days of anaerobic digestion for PPT (left) and GN PHP (right) peduncles respectively.

Conclusion

Peduncles of three banana varieties (GN, PPT and CRBP969) were anaerobically digested in order to convert soft tissues to biomethane and recover the less digestible fibers. The bioprocess was found to be effective as a method for fiber extraction from banana peduncles. The amount and the quality of fibers strongly depended on both the duration of anaerobic digestion and the banana variety. GN and CRBP969 varieties were found to be more convenient for methane production than for fiber recovery, due to their lower initial fiber content and more digestible fibers, as compared to PPT variety. The total methane produced annually from GN banana peduncles ranged from 200 to 204 m^3/(ha·year). PPT peduncle was more suitable for the recovery of fiber together with some biomethane production. The optimum digestion time was around 40 days and would allow the recovery of 0.4 Mg_fiber/ha/year and 235 m^3_CH_4/ha/year, respectively. Anaerobic digestion would be a good bioprocess to isolate and recover natural lignocellulosic fibers. Nevertheless, fiber properties and solubility to various applications remain to be determined.

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