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Evaluation of the anaerobic biodegradability of household organic waste in the Kinshasa City

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ABSTRACT

Introduction

Anaerobic digestion (AD) can be used to produce a more environmentally friendly form of energy from municipal solid waste (MSW) and to clean up urban environments. The application of this technology to MSW produced in Kinshasa city (KC) is highly desirable to alleviate the waste management problem it currently faces and to combat indoor air pollution caused using biomass energy, which causes over 1.1 million deaths in Africa every year. However, the successful implementation of AD technology in Kinshasa City (KC) will depend on the level of anaerobic biodegradability (AB) of its MSW.

Purpose

This work aimed to assess the AB of household organic waste (HOW) produced in large quantities in the KC, namely: leaves and stems of *Manihot utilissima* (MU), leaves of *Mangifera indica* (MI), leaves of *Persea americana* (PA), stems of *Amaranthus viridis* (AV), stems of *Ipomoea batatas* (IP) and peels of *Musa paradisiaca* (MP).

Methods

AB tests were carried out under mesophilic conditions in 750 mL bottles. These bottles were filled with a mixture consisting of 6.7 g, 13.3 g, or 50.0 g DW/L of these waste products (substrates); inoculum, at a substrate/inoculum ratio of 4:1 and water, at a substrate/water ratio of 1:4. After 70 days of AD, the methane yield (BMP) of each substrate was then calculated.

Results

The highest BMP (0.09534 ± 0.00024 m3 CH4/kg.VS) was recorded for stems of AV at a concentration of 13.3 g DW/L, while leaves of PA at a concentration of 50.0 g DW/L gave the lowest BMP (0.00860 ± 0.00023 m3 CH4/kg.VS). Stems of AV stems and leaves of MU, and peels of MP showed good AB, compared with stems of IB and leaves of MI and PA. Co-digestion of these wastes is desirable to further improve their AB and enable the implementation of household-scale AD technology in KC.

Conclusion

The results obtained confirm that this waste could be recycled by AD to produce a clean form of energy for cooking and to clean up the KC.

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INTRODUCTION

Rapid population growth, economic expansion, and rising living standards have accelerated the rate of municipal solid waste (MSW) generation, making waste management a major challenge worldwide (Yang et al., 2023; Adedara et al., 2023; Kang et al., 2023; Anuardo et al., 2022; Jagun et al., 2022). In Africa, over 90 % of MSW produced is disposed of in open dumps and uncontrolled landfills (Kang et al., 2023; UNEP, 2018). The resulting landfill sites are ideal breeding grounds for diseases such as malaria, typhoid, cholera, dengue, yellow fever, etc., and cause major environmental pollution of the air, soil, and water bodies (Kang et al., 2023; Adedara et al., 2023; Fernando and Zutshi, 2023).

In KC, the capital of the Democratic Republic of the Congo (DR Congo) and Africa's third largest city, MSW management is a much-neglected sector (Kang et al., 2023; ACCP, 2019). It is estimated that only 14 % of the solid waste produced in the KC is handled by waste management structures, out of an annual total estimated at over 4 million tons (Kang et al., 2023; ACCP, 2019; INS, 2017). The rest of the garbage piles up in mounds along the city's main arteries, creating illegal dumps all over the city, making the KC one of the dirtiest cities in Africa (Kang et al., 2023; ACCP, 2019). The lack of effective waste management policies, structures, and financial or technical resources is at the root of this deplorable situation in the KC (Kang et al., 2023; Simbu et al., 2022; Mambanzulua, 2015a). However, the MSW produced in the VK, as in the large urban cities of developing countries, come mainly from residential areas (households) (Kang et al., 2023; Phuong et al., 2021; Simbu et al., 2022; Hiligsmann et al., 2006). In addition, more than 65 % of household waste produced in the KC is of organic origin, of which more than 94 % is vegetable waste (Kang et al., 2023; ACCP, 2019; INS, 2017; Mambanzulu et al., 2015b).

In addition to these environmental and health problems linked to the lack of MSW management, the KC also faces a serious energy access problem. It is estimated that only 15 % of the population has access to electricity or some form of clean energy regularly (INS, 2017). This situation is due not only to the non-existence or obsolescence and/or vulnerability of its electricity grids but also to the precarious standard of living of the KC population in terms of regular access to electricity (INS, 2017; Bogaert et al., 2018; Shuku, 2011). As a result, biomass, especially charcoal, accounts for an average of 70 % of the energy consumed by households in the KC (INS, 2017; Shuku, 2011). The use of this form of energy has many harmful consequences, notably on the environment: deforestation and loss of biodiversity, and on public health: indoor (domestic) air pollution. The latter causes respiratory illnesses and over 1.1 million deaths in Africa every year (WHO, 2023; HEI, 2022; Chandrasekaran et al., 2023; Oke et al., 2022).

The large quantities of mainly vegetable organic waste produced in VK households could provide a free source of raw materials for AD technology to produce a more environmentally friendly form of energy (biogas) for household cooking and sanitation (Salamattalab et al. 2024; Zhang et al., 2022; Balasundaram et al., 2022; Fan et al., 2021; Wassie and Adaramola, 2019). The application of this AD technology to the HOW produced in the KC will provide an effective response to the waste management problems it has been facing in recent decades, and to the air pollution in its households caused using biomass energy. However, the successful implementation of this AD technology in KC will depend on the level of AB of its waste. For this reason, the present work has set itself the objective of evaluating, under mesophilic conditions and without pre-treatment (except for waste size reduction), the AB of HOW produced in the KC. This study focused on seven organic wastes produced daily in large quantities in KC households: leaves and stems of MU, leaves of MI, leaves of PA, stems of AV, stems of IP, and peels of MP (Makumbelo et al., 2002; Diasolua et al., 2002; INS, 2017; Mambanzulua et al., 2015b). During the study, the optimum conditions for achieving good methane yields and for processing large quantities of waste were identified, separately for each type of waste. This study will pave the way for optimal biogas production in KC households, from the AD of its organic waste.

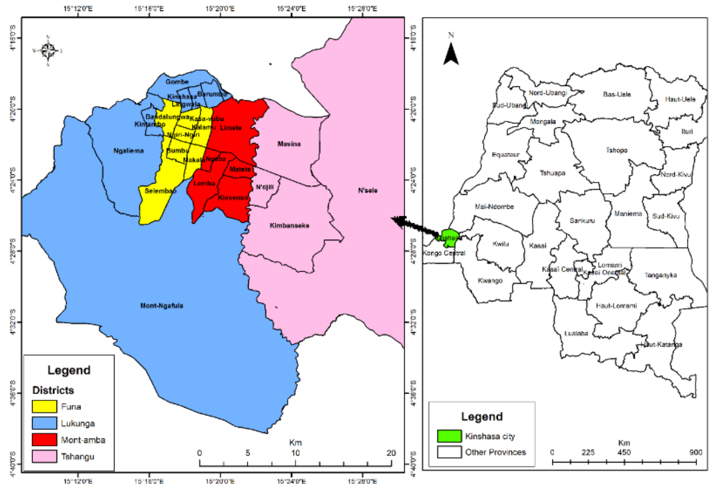
METHODS

Substrates and inoculum sampling

The substrates, namely stems of MU, IP, and AV, leaves of MU, MI, and PA, and peels of MP **(Figure 1)**, were sampled in 40 households taken at random from the four KC districts, with 10 households per district **(Figure 2)**. Samples of this waste were then identified by the Herbarium of the Biology Department of the University of Kinshasa. Cow dung (CD), used as inoculum in this work, was collected from the abattoir of Masina township in the Tshangu district. It was then stored at room temperature in a hermetically sealed plastic container for analysis and subsequent use in the tests that followed.

Figure 1:
Samples of leaves of PA (1), MI (2), and MU (3); stems of IB (4), AV (5), and MU (6); peels MP (7) and CD (8)

Figure 2: Cartography of DR. Congo, location of KC and substrates and inoculum collection sites



Substrates and inoculum characterization

Physico-chemical analysis of substrates and inoculum

Substrates and inoculum were characterized by determining the following parameters: pH, moisture content (%M), dry matter or total solids (%DW or %TS), organic matter or volatile solids (%OM or %VS), and ash (%ash); total Kjeldahl nitrogen (TKN), total organic carbon (TOC), protein, carbohydrates, and lipids.

The pH of substrate and inoculum samples was determined by mixing them in distilled water in the equivalent ratio of 1:5 (30 g of sample in 150 mL distilled water). The mixture was stirred and left to stand for 1 hour, after which the pH was measured directly in the liquid phase (Sundberg et al., 2011).

The DW represents the dry solid matter obtained after evaporation of the water contained in fresh samples (substrates or inoculum). The DW percentage (% DW) of our substrates and the inoculum were determined by the weight loss in the desiccator operated in the Memmert oven, heated at a constant temperature of 105 °C until a constant weight is obtained (Oke et al., 2022; Hakimi et al., 2021; APHA, 2005; Nikiema et al., 2020). The proportion of water contained in the substrate or fresh inoculum, which characterizes their moisture content, was obtained by subtracting the percentage of dry matter from 100 % of the fresh matter. The VS of substrates or the inoculum which constitutes their organic matter was obtained by calcining the sample at a temperature of 550 °C until constant weight was observed (Hakimi et al., 2021; APHA, 2005; Nikiema et al., 2020). The ash content was calculated by the difference between the 100 % of the DW and the %VS. TOC determination was performed using the modified Walkley and Black method (De Vos et al., 2007; CEAEQ, 2014; Black and Evan, 1965). The method involved the oxidation of organic matter by excess potassium dichromate in an acid medium (96 % concentrated sulfuric acid), at high temperature and in the presence of the catalyst (Ag_2SO_4) from a 1 g sample of substrate or inoculum. The Kjeldahl method was used for the determination of nitrogen in substrates and inoculum (Afilal et al., 2014; Mariotti et al., 2016; Nikiema et al., 2020; Sáez-Plaza et al., 2013). Total protein content was then calculated by multiplying TKN by a factor of 6.25 (Mariotti et al., 2016; Nikiema et al., 2020; Sáez-Plaza et al., 2013). The carbohydrate content was determined following the Indole reaction method of Ashwell (Ashwell, 1957; Tamuli et al., 2013; Afilal et al., 2014) in which reducing and non-reducing sugars were estimated taking glucose as standard. Lipid content was determined according to the Soxhlet extraction method using hexane as solvent (AFNOR, 1981; Nikiema et al., 2020).

Analysis of secondary metabolites in substrates

Analysis of secondary metabolites involved qualitative analysis of polyphenols, flavonoids, anthocyanins, tannins, quinones, alkaloids, and saponins; and quantitative analysis of total polyphenols, total flavonoids, and total tannins. To identify the presence or absence of these secondary metabolites, specific phytochemical tests based on staining, precipitation, or turbidity reactions were carried out, using the methods of Wagner *et al.* 1984 and Brunton 1995 (Wagner et al., 1984; Brunton, 1995; Rathee et al., 2012, Ipona et al., 2023).

The total polyphenol content of the various substrates was determined by spectrophotometric assay, using the Folin-ciocalteu method (Ipona et al., 2023; Paz et al., 2020; Zengin and Aktumsek,2014; Dall'Acqua et al., 2021). Total polyphenol content was expressed as mg gallic acid equivalent per gram of DW (mg GAE/g DW). The standard equation of the curve used was Y= 0.006X + 0.002; (R^2= 0.997). While the aluminum trichloride ($AlCl_3$) method was used for the determination of total flavonoids (Chang et al, 2002; Dall'Acqua et al., 2021). Total flavonoid content was expressed as mg Quecertine equivalents (QE) per gram of DW. The standard equation of the curve was: Y= 0.009X + 0.006; (R^2= 0.999). The tannin assay benefited from the reduction of the phosphotungstic reagent by the tannins (Gueye et al.,2022; Ipona et al., 2023; Joslyn, 1970). The blue coloration obtained was evaluated colorimetrically against a control (tannic acid). Total tannin content, expressed in mg tannic acid equivalent (TAE) per gram of DW, was calculated using the relationship: Y= 0.443X + 0.264; (R^2= 0.720).

Evaluation of anaerobic biodegradability

Tests to assess the AB of different substrates were carried out according to the procedure described by Rodriguez *et al.* (Rodriguez et al., 2005) and Wang et al. (Wang et al., 1994). These tests were carried out in triplicate in 750 mL bottles, filled with a mixture consisting of :

i. 6.7 g or 13.3 g or 50.0 g DW/L of each substrate, reduced to a size of around 2 cm in diameter.
ii. inoculum, at a substrate/inoculum ratio of 4:1
iii. water, at a substrate/water ratio of 1:4.

Thus filled, the 63 assembled digesters were hermetically sealed and kept at room temperature, which was 36.13 ± 1.60°C during the experiment. No pre-treatment was applied to the waste and no chemicals (NaOH, $CaCO_3$, buffer solution, etc.) were added to the digesters.

Monitoring of the volume and composition of the biogas produced was carried out periodically for 70 days, using plastic syringes with capacities ranging from 5 to 50 mL to measure the volume of biogas (Fujishima et al., 2000; Owen et al., 1979; Mambanzulua et al., 2015a; Hiligsmann et al., 2011), and using the method of CO_2 absorption by NaOH (KOH) to obtain the amount of methane produced (Induchoodan et al., 2022; Mambanzulua et al., 2015a; Hiligsmann et al., 2011). The BMP of each substrate was calculated by dividing the volume of methane produced (m^3) by the weight (kg) of VS of the substrate put into the digester.

RESULTS AND DISCUSSION

Physical and chemical characterization of substrates and inoculum

The physico-chemical characteristics of the substrates and inoculum are given in **Table 1**.

Table 1:
Physico-chemical characteristics of substrates and inoculum

Parameters analyzed	Stems of MU	Leaves of MU	Leaves of MI	Leaves of PA	Stems of AV	Stems of IB	Peels of MP	Inoculum
M (%)	54,50 ± 0,71	30,00 ± 7,07	38,57 ± 0,04	32,00 ± 2,83	59,50 ± 2,12	55,50 ± 9,19	47,50 ± 0,71	78,40 ± 0,20
DW (%)	45,50 ± 0,71	70,00 ± 7,07	61,43 ± 0,04	68,00 ± 2,83	40,50 ± 2,12	44,50 ± 9,19	52,50 ± 0,71	21,60 ± 0,20
VS (% DW)	85,72 ± 1,33	85,74 ± 0,58	71,02 ± 4,10	77,21 ± 0,09	80,31 ± 2,46	88,18 ± 5,62	87,63 ± 1,18	16,10 ± 0,40
Ash (% DW)	14,28 ± 1,33	14,26 ± 0,58	28,98 ± 4,10	22,79 ± 0,09	19,69 ± 2,46	11,82 ± 5,62	12,37 ± 1,18	83,90 ± 0,40
TOC (mg/g MS)	530,50 ± 15,40	310,10 ± 16,12	400,70 ± 11.5	884,70 ± 20,37	552,90 ± 17,82	574,67 ± 18,83	352,35 ± 15,20	486,40 ± 13,05
TKN (mg/g MS)	23,80 ± 0,30	37,44 ± 2,00	8,55 ± 1,20	22,25 ± 1,77	20,50 ± 1,41	23,36 ± 1,13	10,52 ± 0,52	26,10 ± 1,40
C/N	22,29	8,28	46,87	39,76	26,97	24,60	33,49	18,64
pH	6,22 ± 0,05	6,37 ± 0,01	5,51 ± 0,31	5,93 ± 0,02	5,71 ± 0,06	4,78 ± 0,02	6,59 ± 0,02	8,70 ± 0,40
Lipid content (mg/g DW)	33,25 ± 1,23	47, 50 ± 2,70	22,77 ± 8,20	73,95 ± 8,74	37,26 ± 7,21	48,00 ± 2,83	70,05 ± 4,17	70,00 ± 5,40
Carbohydrate content (mg/g DW)	489,58 ± 8,90	410, 00 ± 16,70	225,59 ± 10,50	696,58 ± 26,47	277,86 ± 10,10	197,97 ± 9,59	465,25 ± 20,55	142,00 ± 8,10
Protein content (mg/g DW)	148,76 ± 2,65	234,00 ± 12,00	47,19 ± 7,50	139,07 ± 11,05	128,13 ± 8,81	146,00 ± 7,07	65,73 ± 3,22	163,13 ± 8,75

Table 2:
Secondary metabolites and total polyphenol, tannin, and flavonoid content in substrates

Bioactive secondary metabolites	Substrates						
	Stems of MU	Leaves of MU	Leaves of MI	Leaves of PA	Stems of AV	Stems IB	Peels of MP
Polyphenols	+	+	+	+	+	+	+
Flavonoids	-	+	+	+	-	-	-
Alkaloids	+	+	+	+	+	-	+
Tannins	+	+	+	+	+	+	+
Anthocyanins	-	+	-	-	-	-	-
Leuco anthocyanins	-	-	-	-	-	-	-
Saponins	-	-	+	-	-	-	-
Coumarins	-	+	+	+	-	-	-
Free quinones	-	-	-	-	-	-	-
Linked quinones (Anthraquinones)	-	-	-	+	-	-	-
Reducing sugars	-	-	-	-	-	-	-
Steroids and Terpenoids	-	-	-	-	-	-	-
Catechic tannins	+	+	+	+	-	-	-
Gallic tannins	-	-	-	-	-	+	-
Total polyphenols (mg GAE/g DW)	44,89 ± 0,10	132,06 ± 0,10	191,11 ± 0,26	82,28 ± 0,10	27,11 ± 0,10	46,17 ± 0,17	44,44 ± 0,10
Total tannins (mg TAE/g DW)	0	0,25 ± 0,001	0,64 ± 0,001	0,11 ± 0,001	0	0	0
Total flavonoids (mg QE/g DW)	11,19 ± 0,07	15,67 ± 0,11	32,82 ± 0,06	15,85 ± 0,13	9,19 ± 0,06	9,07 ± 0,06	9,41 ± 0,06

+: Presence of metabolite
-: Absence of metabolite

%M of HOW produced in large quantities in KC ranged from 30.00 to 59.50 %. The %VS of this waste ranged from 71.02 to 88.18 %. The values of these two parameters (%M ~ 45% and %VS ~ 80%) are indicative of the suitability of these waste for AD, to valorize their rich organic matter content into biogas (Wikandari et al., 2013; Garcia-Peña et al., 2011).

However, their % DW or %TS ranged from 40.50 to 70.00%. These high dry matter contents (%DW ~ 54.63%) (Cao et al., 2020; Monnet, 2003), could increase the retention time of these wastes in the digesters and consequently affect their performance. To avoid this problem, the CD was used as an inoculum in the AD of these wastes. The CD is known for its high methanogenic activity and shorter retention time for biogas production (Hakimi et al., 2023; Hussien et al., 2021). In addition, reducing the size of these wastes to around 2 cm in diameter and using a substrate/water ratio of 1:4; instead of 1:3 which is widely used (Hakimi et al., 2023; Putri et al., 2012; Hakimi et al., 2021), were also beneficial in minimizing the adverse effects of their high dry matter content in the digesters during AD. This approach also enabled us to further increase the moisture content of our substrates in the digesters by supplying water, a very important material for hydrolysis and acetogenesis, two crucial stages in AD (Hakimi et al., 2023; Fan et al., 2021; Putri et al., 2012; Zhang et al., 2005).

Furthermore, for good AD and methane yield, the recommended C/N ratio range is between 20 and 35 (Zhang et al., 2022; Ma et al., 2020; Li et al., 2019; Cerón-Vivas et al., 2019; Li et al., 2017a). Only the C/N ratios of stems of MU (C/N:22.29), of AV (C/N:26.97), and IB (C/N:24.60) and peels of MP (C/N:33.49) were within this range. The C/N ratio of the leaves of MU was 8.28, while those of the leaves of MI and PA were 46.87 and 39.76 respectively. This low C/N ratio for the leaves of MU would cause an accumulation of ammonia in the digester during AD, thereby inhibiting the activity of methanogenic archaea (Zhang et al., 2022; Cerón-Vivas et al., 2019; Khalid et al., 2011). The high C/N ratios of the leaves of MI and PA would lead to a decrease in the concentrations and buffering capacity of the buffer substances in the reaction medium. This would result in acidification of the reaction medium and, consequently, reduced methane production by methanogenic archaea, which are highly sensitive to pH variations (Zhang et al., 2022; Zhang et al., 2021; Cerón-Vivas et al., 2019; Paul and Dutta, 2018).

The pH range for good AD is around neutral between 6.8 and 7.5, with an optimum pH of 7 (Fan et al., 2021; Cerón-Vivas et al., 2019; Zhou et al., 2016). However, these organic wastes from VK households had pH values ranging from 4.78 to 6.59. These pH values, below 7, would therefore be unfavorable to the hydrolysis and acidogenesis stages during the AD of this waste, and would also be detrimental to the activity of the methanogenic archaea (Cerón-Vivas et al., 2019; Zhang et al., 2005). Indeed, because of the accumulation of volatile fatty acids produced during the acidogenesis stage and also during acetogenesis, the already acidic pH values in the digesters containing these wastes could fall further into overly acidic zones, thereby inhibiting the biological activity of the methanogenic archaea. Stems of IB with a pH value of 4.78 proved to be the most acidic of all the wastes studied. Thus, the addition of basic pH inoculum (pH ~ 8.70) to seed the digesters, in a substrate/inoculum ratio of 4:1, adjusted the initial pH values in all digesters containing these substrates to the range between 6.81 and 7.57. These values were thus within the range indicated for good AD and, above all, above 5.3, the minimum pH required to maintain the activity of methanogenic archaea (Fan et al., 2021; Cerón-Vivas et al., 2019; Zhou et al., 2016; Wu et al., 2010; Wang et al., 2014).

The lipid dosage revealed that the leaves of PA and the peels of MP had high lipid contents of 73.95 and 70.5 mg/g DW respectively. At first sight, these lipid-rich organic wastes would give a high yield of methane production compared with other wastes with low lipid contents (Li et al., 2017a; Angelidaki and Sanders, 2004). On the other hand, this source par excellence of volatile fatty acids, which supply acetic acid, the basic substrate to produce over 70% of methane by acetoclastic methanogens, could also, in the event of accumulation of the acids formed, lead to a drop in pH and thus limit the AD (Fan et al., 2021; Li et al., 2017a; Sun et al., 2014; Li et al., 2017b; Liu et al., 2015).

Leaves PA with a very high carbohydrate content (696.58 mg/g DW), could cause the pH to drop in very acidic areas, thus inhibiting the activity of methanogenic archaea. Indeed, the rapid and easy decomposition of these carbohydrates, producing large quantities of volatile fatty acids in the digester, would be faster than the conversion of these volatile fatty acids into biogas by methanogenesis (Li et al., 2017a; Paritosh et al., 2017). Leaves of MU with a high protein content of 234.00 mg/g DW, would present risks associated with high ammonia concentration in the digester, and inhibit methanogenic activity. This high protein content in the leaves of MU was reflected in their low C/N ratio (8.28), which is unfavorable for good AD (Zhang et al., 2022; Cerón-Vivas et al., 2019; Khalid et al., 2011; Li et al., 2017a; Chen et al., 2008). Although the C/N ratio is often considered to be the parameter of choice for assessing the biodegradability of organic matter (Li et al., 2017a; Schnurer and Jarvis, 2010, U Kun Kiran et al., 2014), however, none of these physico-chemical parameters characteristic of these HOW produced in the KC, alone, could be determinant of the kinetics and especially the yield of their AD process. The influences of these parameters could act synergistically or even antagonistically during their AD in digesters. In addition, AD efficiency also depends on other parameters such as the temperature at which this process takes place, the substrate loading rate in the digester, intermediate products formed in the digesters, the characteristics of the inoculum used, the existence or absence of inhibitors in the substrates, etc. (Fan *et al.*, 2021; Cao *et al.*, 2020).

Secondary metabolites in substrates

Table 2 reports the results of qualitative identification of secondary metabolites and quantitative analysis of total polyphenols, total tannins, and total flavonoids in aqueous substrate extracts.

The results of secondary metabolite analyses revealed the presence of polyphenols and tannins in all substrates. Flavonoids and coumarins were present in the leaves of MU, MI, and PA, while the stems of IB, MU, and AV and the peels of MP did not contain them. Alkaloids were present in all substrates except stems of IB. Total polyphenol concentrations in these HOW ranged from 27.11 to 191.11 mg GAE/g DW. Total tannin concentrations were very low in all substrates, ranging from 0.11 to 0.64 mg TAE/g DW. With a total flavonoid concentration of 32.82 mg QE/g DW, the leaves of MI contained a high concentration of these secondary metabolites, while the other substrates showed an average total flavonoid concentration of around 10 mg QE/g DW. The leaf substrates (leaves of MU, MI, and PA) had high contents of polyphenols, tannins, and total flavonoids, compared with peels of MP and stems of AV, IB, and MU.

The various secondary metabolites identified in these different substrates (leaves, stems, and peels) are produced by plants to protect themselves against microbial and insect attacks (Wikandari et al., 2013; Mambanzulua et al., 2015a). Naturally present in these organic plant wastes, these secondary metabolites with biological activities against micro-organisms, could inhibit the AB process of these wastes (Balasundaram et al., 2022; Li et al., 2023; Wikandari et al., 2013; Levén et al., 2012; Akassou et al., 2010). However, the methanogenic toxicity of secondary metabolites depends on their concentration, their molecular structure (presence or absence of aromatic rings, presence or absence of many hydroxyl groups, length of the hydrocarbon chain, etc.), and also the temperature and nature of the microbial consortium in the reaction medium (inoculum used) (Balasundaram et al., 2022; Levén et al., 2012; Akassou et al., 2010; Wikandari et al., 2013). In addition, several studies have shown that certain secondary metabolites, notably polyphenols, and other aromatic compounds, can undergo bioconversion during AD, leading to their biodegradation without affecting the activity of methanogenic archaea (Levén et al., 2012; Levén and Schnürer, 2005; Chen et al., 2008). Efficient degradation of phenolic secondary metabolites during AD was reported in the mesophilic temperature range than in other temperature ranges (thermophilic or psychrophilic) (Levén et al., 2012; Chen et al., 2008; Levén and Schnürer, 2005).

Tannins, with their numerous hydroxyl groups (-OH), hardly represent an inhibitory effect on methanogenesis (Akassou et al., 2010). Therefore, with the low concentrations found in the waste under examination, their inhibitory effects on the AD of these substrates would not be of concern. On the other hand, the presence of flavonoids with two aromatic rings and various biological activities, such as antibacterial, antiviral and anti-inflammatory effects, is a major advantage (Wang et al., 2021; Xiong et al., 2023; Lu et al., 2005), and coumarins, reputed to be highly toxic to methanogenic archaea (Akassou et al., 2010; Borja et al., 1997), could be detrimental to the AD of the leaves of MU, PA, and MI, compared with peels of MP and stems of AV, IB, and MU, which do not contain them.

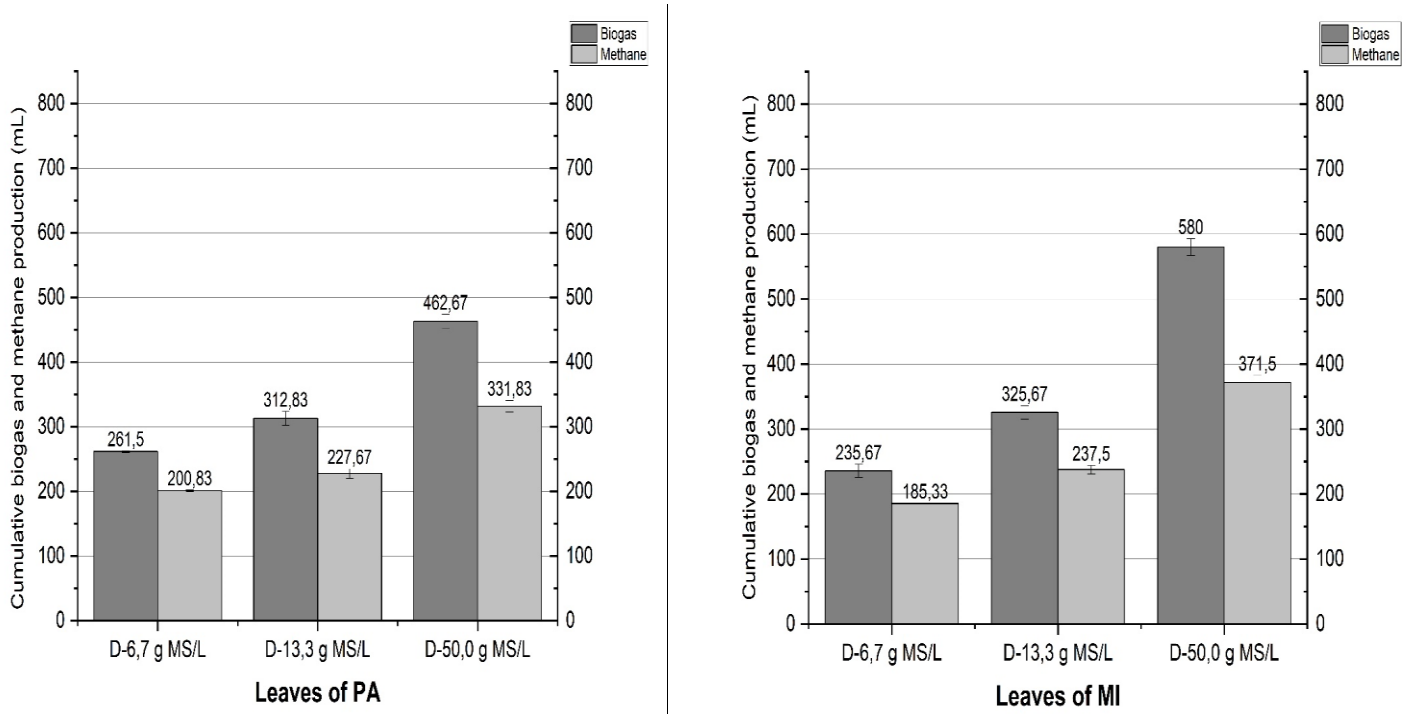
Leaves of MI showed higher levels of total flavonoids and total polyphenols than the leaves of MU and PA. Therefore, in the event of AD inhibition by these secondary metabolites, the biodegradation of the leaves of MI would be more detrimental than that of the leaves of MU or PA.

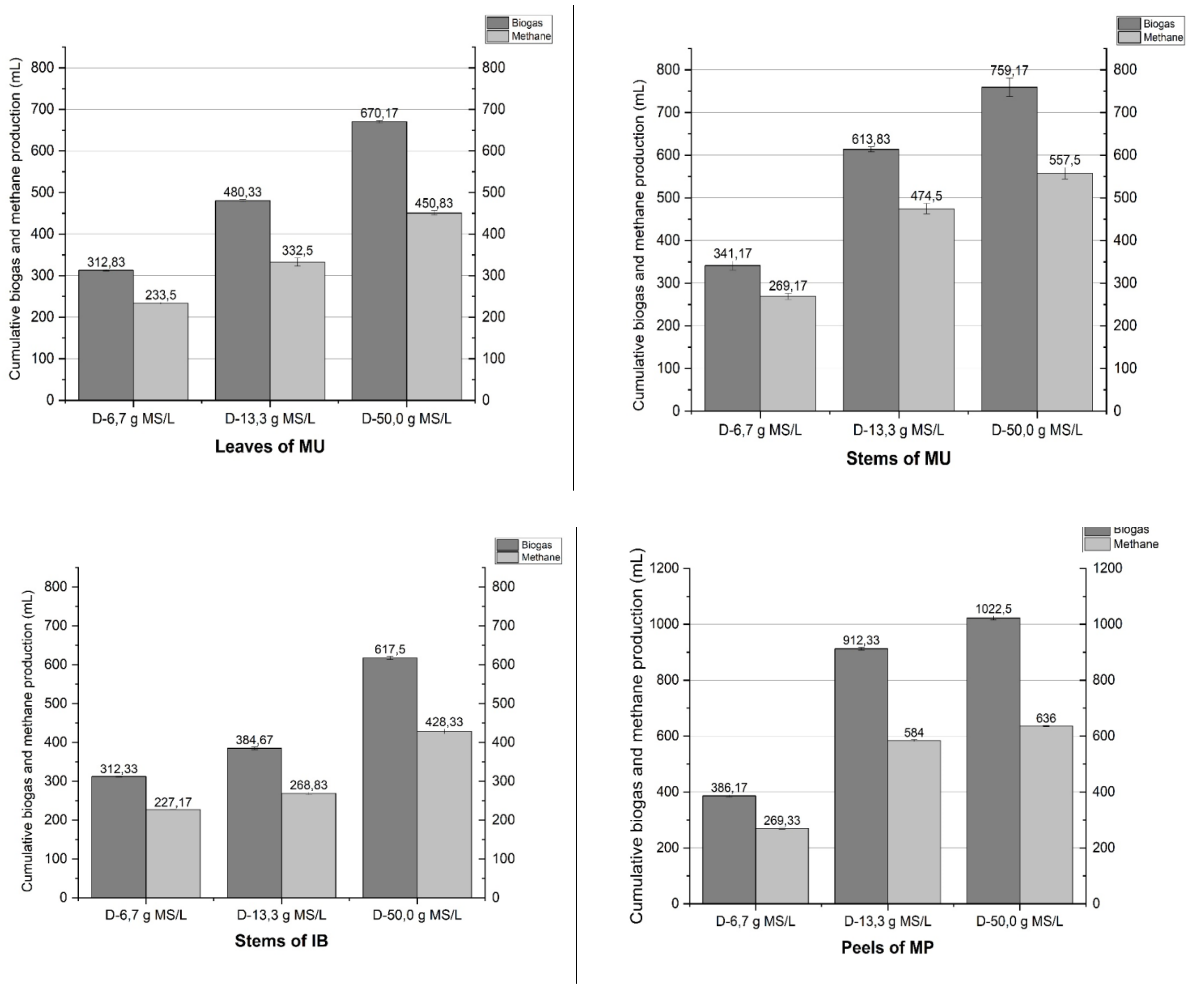
Peels of MP and stems of MU, IB, and AV showed high volatile solids contents (80.31 - 88.18%), low dry matter contents (40.50 - 52.50%), and C/N ratios in the range indicated for good AD (22.29 - 33.49), compared with the leaves of MU, MI, and PA. In addition, these substrates also had low concentrations of total polyphenols, total flavonoids, and total tannins, compared with the leaves of MU, MI, and PA. As a result, these substrates (peel of MP and leaves of MU, IB, and AV) could have a better AB and consequently a better methane yield than the leaves of MU, MI, and PA. Nevertheless, the rigid walls of the stems of MU, IB, and AV and the peels of MP, rich in lignocellulosic compounds, could compromise their biodegradability, by limiting their solubility in the reaction medium during the hydrolysis step (Balasundaram et al., 2022; Zhang et al., 2022; Karki et al., 2021).

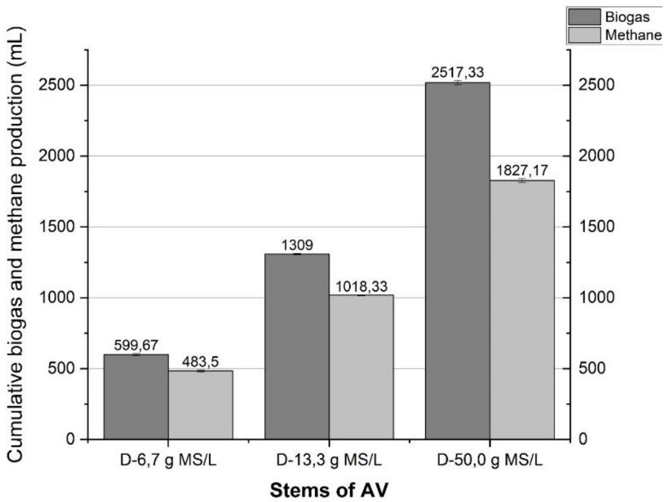
Evaluation of anaerobic biodegradability of substrates

Biogas production was monitored for 70 days in the different digesters containing each substrate at concentrations of 6.7, 13.3, and 50.0 g DW/L, to assess their AB. These assays were carried out under mesophilic conditions, suitable for tropical regions such as KC. **Figure 3** shows the cumulative biogas and methane production obtained for each substrate.

Figure 3:
Cumulative biogas and methane production recorded after 70 days of AD of the leaves of PA, MI, and MU, stems of MU, AV, and IB, and peels of MP




Biogas
Methane
Cumulative biogas and methane production (mL)
312,83
233,5
480,33
332,5
670,17
450,83
D-6,7 g MS/L
D-13,3 g MS/L
D-50,0 g MS/L
Leaves of MU
341,17
269,17
613,83
474,5
759,17
557,5
Stems of MU
312,33
227,17
384,67
268,83
617,5
428,33
Stems of IB
386,17
269,33
912,33
584
1022,5
636
Peels of MP



After 6 days of AD, biogas, and methane production were detected in all digesters, highlighting the suitability of all these organic wastes for anaerobic biological treatment. The relatively short delay before biogas production was due to the use of CD as inoculum and a substrate/water ratio of 1:4. Indeed, CD was necessary to supply the reaction medium with microorganisms with high methanogenic activity and shorter retention time. The addition of water to this ratio was necessary to facilitate hydrolysis of the organic waste and solubilization of the released molecules.

The highest total cumulative biogas volume (2517.33 ± 15.54 mL) was recorded in digesters containing the stems of AV at a concentration of 50.0 g DW/L, while the lowest volume (462.67 ± 11.02) at this concentration of 50.0 g DW/L was recorded in digesters containing the leaves of PA. These discrepancies in biogas production by these wastes would appear to be due to certain factors determining AD, linked to the nature of the substrates (C/N, pHi, and so on) but also to the inhibitory effects of certain intermediates formed in the digesters during the AB process of these wastes.

The peels of MP and the stems of AV and MU, with C/N values in the optimum range for a good AD (C/N: 20-35), gave high biogas production, compared with leaves of MU, MI, and PA, which did not have this ratio in the recommended range. On the other hand, the stems of IB with a C/N ratio of 24.60, within the margin required for a good AD, had lower biogas production than the leaves of MU (C/N: 8.28), which had this C/N ratio far below the range indicated for a good AD. This observation allowed us to confirm that, although the substrate's C/N ratio had a strong influence on its AB, biogas production did not depend solely on this substrate characteristic, but also on other factors that set the total initial and temporal composition of the digester.

The percentages of methane in the biogas produced by these organic wastes decreased with increasing concentration in the digester. This could be explained by the increase in dry matter/total solids (DW or TS) content in the reaction medium. Indeed, increasing the DW content in the digester, while on the one hand beneficial for the availability of the raw material to the micro-organisms, on the other hand also increases the content of toxic compounds and contributes to rapid acidification of the reaction medium, if the buffer capacity (alkalinity) prevailing there is not sufficient to compensate for the excess of volatile fatty acids that will be generated (Cerón-Vivas et al., 2019; Chen et al., 2015; Fan et al., 2021). This excess acidity, together with the high levels of certain active secondary metabolites, would affect the activity of the methanogens in the digesters and consequently methane production. The highest methane percentage (80.66 ± 2.71%) was recorded in digesters containing the stems of AV at a concentration of 6.7 g DM/L, while the lowest methane percentage (62.20 ± 0.24%) was recorded for the peels of MP at a concentration of 50.0 g DW/L.

Methane yields (BMP) after 70 days of AD for the different substrates are reported in **Table 3**. This **Table** also reports the initial pH values (pH_i) in the digesters, measured at the start of AD, the pH values measured after 47 days of AD (pH_{47}), and the final pH values (pH_f), measured at the end of AD (on the 70th day of AD).

Table 3:
BMP of digested substrates, pHi, pH47, and pHf in the different digesters

Substrates	Concentration (g DW/L)	BMP (m^3 CH_4/kg.VS)	pH_i	pH_{47}	pH_f
Stems of MU	6,7	0,04686 ± 0,00173	7,25 ± 0,05	4,89 ± 0,01	4,01 ± 0,01
	13,3	0,04162 ± 0,00107	7,18 ± 0,03	4,82 ± 0,01	3,86 ± 0,01
	50,0	0,01301 ± 0,00031	7,15 ± 0,02	4,72 ± 0,01	3,72 ± 0,01
Leaves of MU	6,7	0,04065 ± 0,00046	7,30 ± 0,01	5,39 ± 0,02	5,23 ± 0,01
	13,3	0,02916 ± 0,00088	7,26 ± 0,02	5,24 ± 0,01	5,01 ± 0,01
	50,0	0,01052 ± 0,00013	7,24 ± 0,01	4,10 ± 0,01	4,03 ± 0,03
Leaves of MI	6,7	0,03895 ± 0,00016	6,90 ± 0,01	5,65 ± 0,01	5,34 ± 0,01
	13,3	0,02515 ± 0,00070	6,87 ± 0,06	5,17 ± 0,01	4,90 ± 0,01
	50,0	0,01046 ± 0,00031	6,85 ± 0,14	4,78 ± 0,02	4,60 ± 0,01
Leaves of PA	6,7	0,03882 ± 0,00037	7,30 ± 0,01	5,66 ± 0,01	5,50 ± 0,01
	13,3	0,02217 ± 0,00070	7,28 ± 0,03	5,45 ± 0,01	5,35 ± 0,01
	50,0	0,00860 ± 0,00023	7,25 ± 0,04	4,55 ± 0,01	4,01 ± 0,01
Stems of AV	6,7	0,08837 ± 0,00163	7,41 ± 0,02	5,52 ± 0,03	5,44 ± 0,01
	13,3	0,09534 ± 0,00024	7,40 ± 0,03	5,53 ± 0,02	5,46 ± 0,01
	50,0	0,04550 ± 0,00033	7,38 ± 0,04	5,43 ± 0,01	5,38 ± 0,01
Stems of IB	6,7	0,03845 ± 0,00176	6,83 ± 0,04	5,11 ± 0,01	5,07 ± 0,01
	13,3	0,02292 ± 0,00029	6,82 ± 0,02	5,00 ± 0,01	4,89 ± 0,01
	50,0	0,00971 ± 0,00014	6,81 ± 0,02	4,66 ± 0,05	4,49 ± 0,02
Peels of MP	6,7	0,04588 ± 0,00081	7,57 ± 0,02	5,65 ± 0,01	4,73 ± 0,02
	13,3	0,05011 ± 0,00027	7,54 ± 0,02	5,63 ± 0,01	4,72 ± 0,01
	50,0	0,01452 ± 0,00005	7,52 ± 0,05	4,73 ± 0,04	4,60 ± 0,01

The BMP of HOW from the KC under investigation ranged from 0.00860 to 0.09534 m^3 CH_4/kg.VS. These BMP are lower than the values found in the literature, which range from 0.190 to 0.591 m^3 CH_4/kg.VS (Li et al., 2017a; Gunaseelan, 2004). This could be explained by the lack of use of buffer solutions during the AD of these substrates, to avoid the influence of excess acidity that occurred in the digesters during the hydrolysis and acidogenesis stages. Indeed, after 47 days of AD, a significant drop in biogas production was observed in almost all digesters at all concentrations, except in digesters containing the leaves of MU at a concentration of 6.7 g DW/L, the stems of AV at concentrations of 6.7, 13.3 and 50.0 g DW/L, and the peels of MP at concentrations of 6.7 and 13.3 g DW/L. The pH values measured on the 47th day of AD in the various digesters were below 5.3 **(Table 3)**, the minimum value required to maintain methane production by methanogenic archaea (Fan et al., 2021; Wu et al., 2010). However, digesters containing the leaves of MU (6.7 g DW/L), the stems of AV (6.7, 13.3, and 50.0 g DW/L), and the peels of MP (6.7 and 13.3 g DW/L) gave pH values above this minimum value, which had justified continued biogas production in these digesters. The maintenance of pH values above 5.3 in these digesters could be explained by the alkalinity developed in the reaction medium, which was sufficient to compensate for the excess acidity produced in the medium to avoid drastic pH drops (Cerón-Vivas et al., 2019; Chen et al., 2015; Fan et al., 2021).

On the 47th day of AD, digesters containing the leaves of PA at concentrations of 6.7 and 13.3 g DW/L and the leaves of MI at 6.7 g DW/L also had pH values above this minimum value of 5.3. However, an inhibition of biogas production was recorded in these digesters. This could be explained by the release into the reaction medium of certain AD inhibitors contained in these two substrates. Indeed, several studies have shown that leaves of AP possess many medicinal properties, notably antiviral, antifungal, anticonvulsant, anti-inflammatory, antitussive, antianalgesic, antihypertensive, antidiabetic, and cure urinary tract infections, bronchitis, and rheumatism. This justifies their widespread use in traditional African medicine, as well as in many tropical and subtropical countries around the world (Park et al., 2019; Ojewole and Amabeoku, 2006). Other studies carried out on the leaves of PA have identified various monoglycoside flavonoids and other compounds such as persin and pinenes in this substrate (Park et al., 2019; Peter et al., 1995). Persin, for example, is a bioactive compound that is already showing antibacterial effects at concentrations of 60 to 100 ppm (Peter et al., 1995).

These secondary metabolites, which are toxic to the microbial flora, were able to inhibit the methanogenic activity of methanogenic archaea in digesters containing the leaves of PA. In the case of the leaves of MI, the complete inhibition of biogas production observed could be justified by the presence in these substrates of bioactive secondary metabolites, notably mangiferin, which has strong antimicrobial activity. Several pharmacological studies have also shown that aqueous extracts of the leaves of MI possess numerous virtues, including antioxidant, antimicrobial, antidiabetic, antiallergic, antihelminthic, anticancer, cholesterol-lowering and immunomodulating properties (Mambanzulua et al., 2015a; Imran et al., 2017; Zhang et al., 2014). These biological activities, carried by the secondary metabolites contained in the leaves of MI, are thought to underlie the inhibition, over time, of their AD. However, for the stems of IB and MU, the inhibition of biogas production observed on 47th day of AD for all concentrations (6.7, 13.3 and 50.0 g DW/L), is thought to be due to the drop in pH to values below 5.3 **(Table 3)**.

On the 70th day of AD, no significant biogas production was recorded in all digesters except in digesters containing the stems of AV, where a continuation of biogas production was observed. This would be justified by pH values above 5.3 in all digesters containing this substrate.

The BMP obtained after 70 days of AD for the leaves of MU (0.04065 m^3 CH_4/kg.VS) and the leaves of MI (0.03895 m^3 CH_4/kg.VS) at the concentration of 6.7 g DW/L, were higher than those obtained by Mambanzulua *et al.* (Mambanzulua et al., 2015a), after 100 days of AD of the same substrates, at the same concentration and temperature of 30 °C. The yields found by Mambanzulua *et al*. were 0.036 and 0.023 m^3 CH_4/kg.VS for the leaves of MU and MI respectively. This difference in BMP for the same substrates and at the same concentration would be due, among other things, to the difference in temperature at which the AD of these substrates was carried out, but also to the nature of the inoculum used. The CD used as inoculum in this work has a shorter retention time for biogas production and, above all, a high methanogenic activity, compared with the anaerobic sludge from activated sludge used as inoculum in the work by Mambanzulua et al. (Hakimi et al., 2023; Hussien et al., 2021; Ashekuzzaman and Poulsen, 2011).

The results obtained in this study also show that the BMP values found for the same substrate decreased with increasing concentration in the digester (loading rate). This can be explained by the increase in total solids in the reaction medium, which affects digester performance by lowering the pH following the release and accumulation of large quantities of volatile fatty acids in the medium. However, for digesters containing the leaves of PA and MI, this finding could also be justified by the increased concentration of certain AD-inhibiting secondary metabolites in the reaction medium. However, the exception was for digesters containing the stems of AV at the concentration of 13.3 g DW/L, where the BMP was 0.09534 m^3 CH_4/kg.VS while for the concentration of 6.7 g DW/L, it was 0.08837 m^3 CH_4/kg.VS. The same applies to peels of MP for concentrations of 6.7 g DW/L (BMP: 0.04588 m^3 CH_4/kg.VS) and 13.3 g DW/L (BMP: 0.05011 m^3 CH_4/kg.VS).

After 70 days of AD, the stems of AV at the concentration of 13.3 g DW/L gave the highest methane yield of all digested substrates (0.09534 m^3 CH_4/kg.VS), while the leaves of PA at the concentration of 50.0 g DW/L gave the lowest methane yield (0.00860 m^3 CH_4/kg.VS).

CONCLUSION

This study is a contribution to the knowledge of the level of AB of HOW produced daily in large quantities in the KC to detect their suitability for biological treatment, especially by AD. The results obtained on the assessment of the AB of these HOW in the KC showed that this waste can well be valorized by AD to produce a form of clean energy for cooking at the household level in the KC and to ensure the sanitation of their environment. In addition, this study revealed that the stems of AV and MU, the peels of MP, and the leaves of MU had a good AB, compared with the stems of IB and the leaves of MI and PA. However, co-digestion of these KC household organic wastes should improve their methane yield and thus ensure the successful implementation of AD technology in the KC.

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