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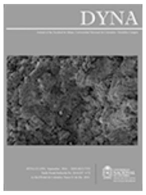

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Influence of the mixing ratio on the anaerobic co-digestion of municipal biowaste with domestic wastewater sludge on methane production

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Abstract

Anaerobic co-digestion (A-Co) is a viable option to overcome the disadvantages of mono-digestion. This research presents the results of biochemical methane potential assays (BMPs) assessing the A-Co of municipal biowastes (MBW) from one city which perform source separation and selective collection with domestic wastewater sludge (DWS) from a municipal wastewater treatment plant (WWTP) in different DWS: MBW mixing ratios. Besides methane production, it was evaluated the hydrolysis (through a first-order kinetics model and the modified Gompertz model), since this is the limiting step of the A-Co of solid wastes. The results showed that A-Co of DWS with MBW is feasible and that DWS: MBW mixing ratio generated a synergistic effect in the process. The mixing ratio DWS:MBW that produced the largest quantities of methane (105.6 $mLCH_4 \cdot gVS^{-1}$), optimal hydrolysis constants (K_h) and shortest lag phase (under 3.3 days) was 20:80.

Keywords: Anaerobic co-digestion; Hydrolysis; Municipal biowaste; Primary sludge; Renewable energy.

Influencia de la proporción de mezcla en la codigestión anaerobia de biorresiduos municipales con lodos de aguas residuales domésticas sobre la producción de metano

Resumen

La codigestión anaerobia (CA) es una de las opciones viables para superar las desventajas de la mono-digestión. Esta investigación presenta los resultados del Potencial Bioquímico de Metano (PBM) donde se evaluó la CA de biorresiduos municipales (BM) de una localidad que realiza separación en la fuente y recolección selectiva con lodos de aguas residuales domésticas (LARD) provenientes de una planta de tratamiento de aguas residuales en diferentes proporciones de mezcla. Se evaluó la producción de metano mediante el modelo modificado de Gompertz y la hidrólisis a través del modelo cinético de primer orden, ya que esta es la etapa limitante en la CA de residuos sólidos. La proporción de mezcla LARD:BM donde se lograron las mayores producciones de metano (105.6 $mLCH_4 \cdot gSV^{-1}$), mejores constantes de hidrólisis (K_h) y fases de latencia cortas (menores a 3.3 días) fue 20:80.

Palabras clave: Biorresiduos municipales; Codigestión anaerobia; Energía renovable; Hidrólisis; Lodo primario

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1. Introduction

Growing urbanization generates a continuous and progressive amount of many types of wastes (wastewater and solid waste). Therefore, it is important to find alternatives for the treatment and final disposal of such wastes [1]. In Latin America, municipal biowaste (MBW) accounts for 50-70% of the municipal solid waste (MSW), whereas in Colombia, it accounts for 65% [2,3]. Treatment approaches have

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changed in recent years, and rather than disposing these type of wastes, they can be used as an alternative source of energy, being the biological processes the most appropriate process since it contribute to mitigate the effects of associated greenhouse gases and environmental impacts, such as the proliferation of vectors, production of leachates and loss of land value for landfills [2].

The anaerobic digestion (AD) of single substrates (mono-digestion) presents some drawbacks linked to substrate properties as: i. Sewage Sludge (SS), characterized by low organic loads; ii. the organic fraction of municipal solid waste (MBW) could have contained improper materials as well as a high concentration of heavy metals, among others. The anaerobic co-digestion (A-Co) is the simultaneous AD of two or more substrates, is a feasible option to overcome the drawbacks of mono-digestion and to improve the economic viability of AD facilities due to higher methane production which is an important source of renewable energy [4,5].

In addition, A-Co focused on mixing substrates favors positive interactions, i.e. macro- and micronutrient equilibrium, moisture balance and/or dilute inhibitory or toxic compounds, increased digestion rate, improved substrate stabilization, improved buffer capacity of the process, optimized rheological properties of the waste and improves economic conditions due to the potential of combining different flows and large amounts of waste in the same treatment system [6,7].

According to Hartmann et al. [7], A-Co can be used for different types of waste. Currently, transport cost of the co-substrate from the generation point to the AD plant is the first selection criteria. Despite this fact, it is still important to choose the best co-substrate and to select the best mixing ratio with the aim of promoting synergies, diluting harmful compounds, optimize the methane production and not disrupt digestate quality [4].

Mata-Alvarez, et al. [5] reported that the MBW has typically been used as co-substrate for domestic wastewater sludge (DWS) because, in many towns, WWTP were already equipped with anaerobic digesters. Indeed, A-Co of MBW and DWS is reflected as a way to reduce significantly the treatment costs of both wastes. For this reason, the A-Co of MBW and DWS has the potential to provide significant production of renewable energy, enhances the economic viability of the wastewater treatment plants (WWTP) and generates some economic and environmental benefits on municipal biowaste (MBW) disposal in sanitary landfills.

The proportion of substrates required by A-Co in order to produce renewable energy has been widely studied. Although it has been reported that these ratios have a different influence on A-Co depending on the nature of the substrates, the optimal values depend on their characteristics and should be assessed for each case and inoculum used. Lesteur et al. [8] stated that the optimal substrate ratio depends on aspects such as the potential production of volatile fatty acids (VFAs), buffer capacity and contribution of nutrients. Ponsá et al. [9] evaluated the 83:17 ratio of MBW using vegetable oil, animal fat, cellulose and protein as co-substrates and confirmed that the production of biogas by A-Co decreased notably when the waste contained high amounts of fat and protein.

On the other hand, Callaghan et al. [10] assessed A-Co from pig manure and MBW at four mixing ratios (80:20, 70:30, 60:40 and 50:50) and found that the mix with the highest MBW content produced the best results regarding biogas production. Tandukar et al. [11] evaluated the effect of the organic load on the A-Co of primary sludge with restaurant grease traps, waste activated sludge and gum waste. They achieved greater methane production and better quality of digestate, especially in the content of nitrogen.

The hydrolysis is the limiting step in the AD, has been identified, as a major difficulty that occurs during this stage is the solubility of the substrate. This restricts the rate and extent of degradation of the particulate organic matter present in solid waste, which causes interference in the overall efficiency of the process in terms of methane production [12].

Studies of the biochemical and physicochemical processes that occur during A-Co of MBW with DWS showed that hydrolysis is the main limiting stage of the process and indicated that the biological process is affected by several factors that should be investigated experimentally and required the use of kinetics models [13-15].

Most kinetics models correspond to first-order kinetics. Krishna et al. [16] recommend the use of these models to enhance the study of hydrolysis and the modified Gompertz model is the most frequently used for such studies. These models can infer A-Co performance during important stages, such as acidogenesis and acetogenesis, and provide an understanding of biomass dynamics within the bioreactors.

In the literature there are few studies on DWS from WWTP with chemically assisted primary treatment (CAPT) technology, despite the fact that this technology predominates in developing countries as a solution to improve treatment efficiency of wastewater to solve their problems regarding sanitation, only around 6% of these achieved an acceptable treatment[12]. The use of CAPT significantly improves BOD removal (>55%) although the sludge amount increases [13,14] due to the use of coagulants, which may affect the physicochemical properties of the sludge and therefore can affect the A-Co thereof.

This research shows the A-Co as an alternative to improve treatment of DWS, using the MBW as a co-substrate. In addition, were evaluated different mixing ratios in order to identify both the synergistic or antagonistic effect such as the methane production.

2. Methodology

2.1. Experimental location

The assays were performed at an altitude of 970 m above the sea level. The room temperature was 23.6ºC on average, and the controlled temperature of the experiment was 30 ± 0.5ºC.

2.2. Experimental stage

2.2.1. Characterization of substrates (MBW and DWS) and inoculum

The MBW were collected from a solid waste treatment plant (SWTP) in a Colombian city where integrated MSW management was held, including source separation and selective collection of waste. On average, 10200 $kg \cdot week^{-1}$ of MSW are generated, and MBW accounted for 66% of the total waste, which is equivalent to 6732 kg·week^{-1}[3]. The sampling and characterization of MBW were performed following the recommendations of Sakurai [17]. The DWS was obtained from a municipal WWTP that employs ferric chloride in the chemically enhanced primary treatment.

Prior to physicochemical characterization and BMP testing, all inert material (stones, metal, charcoal, bone) and slowly degrading material (plastic, rubber, and leather) were removed from the MBW samples [18]. Afterwards, the materials were subjected to grinding as recommended by Sharma et al. [19] using a Waring Commercial CB15 blender at a speed of 15800 rpm for 1 minute (standard equipment speed).

The physicochemical characterization of the substrates was performed according to ICONTEC [20] and APHA [21]. The variables used for the characterization were pH (Units), humidity (%), total alkalinity TA, total bicarbonate alkalinity TBA (mgCaCO$_3$·L^{-1}), VFAs (mg·L^{-1}), acetic acid (mg·L^{-1}), propionic acid (mg·L^{-1}), butyric acid (mg·L^{-1}), palmitic acid (mg·L^{-1}), oleic acid (mg·L^{-1}), total and oxidizable carbon (%), UV$_{254}$ (cm^{-1}), total and filtered chemical oxygen demand (COD, mg·L^{-1}), biological oxygen demand (BOD, mg·L^{-1}), total nitrogen (%), total ammonia nitrogen (mg·L^{-1}), cellulose (%), starch (%), lignin (%), ether extract (mg kg^{-1}), proteins (%), carbohydrates, (%), raw fiber (%), total solids TS and volatiles VS (mg·L^{-1}).

The biodegradable fraction (BF) of the MBW and TPS was determined according to the methodology suggested by Espinosa et al. [22] (Eq. 1):

$$BF = 0.83 - 0.028\,LC \tag{1}$$

Where BF is the biodegradable fraction in relation to the volatile matter (VM); 0.83 and 0.028 are empirical constants; and LC is the lignin content in the VM, expressed as a dry weight percentage.

Additionally, the non-ionized ammoniacal nitrogen and ammonium ion present in the substrates were quantified using Eqs. (2) and (3) as suggested by Sterling et al. [23]:

$$NH_3 = 1.13\,x10^{-9}.\frac{NH_3T}{H^+} \tag{2}$$

$$NH_3T = NH_3 + NH_4^+ \tag{3}$$

Where NH$_3$ is the non-ionized ammoniacal nitrogen (mg·L^{-1}); NH$_3$T is the total ammoniacal nitrogen (mg·L^{-1}); H$^+$ is the concentration of hydrogen ions (mol·L^{-1}); and NH$_4^+$ is the ammonium ion (mg·L^{-1}).

In order to ensure favorable conditions for A-Co, an inoculum concentration of 1.5 gVS·L^{-1} was used in the BMP assays [24]. The inoculum was obtained from an anaerobic digester of the municipal WWTP of DWS, and was characterized in terms of physicochemical variables determined from the substrates. Additionally, Specific Methanogenic Activity-SMA (gCOD·gVS^{-1}·d^{-1}) test were carried out [25]. The MBW, DWS and inoculum were preserved at temperatures below 4°C for periods shorter than seven days until the assays were performed.

The results for physicochemical characteristics of the substrates and inoculums were processed by descriptive statistical methods.

2.2.2. Influence of DWS:MBW mixing ratios on methane production

The biogas was quantified according to the manometric method using an OxiTop® instrument to monitor the pressure. This equipment consists of a 250 mL reactor with a measuring head that is inserted in the reactor mouthpiece as well as a control that uses an infrared interface to transfer the data. The assays were performed in a WTW TS 606-G/2-i incubator with manual intermittent mixing during 20 days. The sample volume was 200 mL, and 50 mL were retained in the reactor for storage of the produced biogas as recommended by Aquino et al. [25].

The techniques to determine methane production are standardized methods used to provide improved conditions for the AD of the substrates. A solution of macronutrients and micronutrients was used to maintain stable conditions during the BMP assays [26], with a pH adjusted to 7.0 using NaHCO$_3$ solution at 4%.To capture CO$_2$ of biogas, NaOH pearls were used and composition of biogas was determined by gas chromatography (Chromatograph GC2014).

The volume of methane for standard conditions (SC) was determined according to the methodology proposed by Giménez et al. [27]. Thus, the fraction of dissolved methane in the experimental units was considered. The substrate-inoculum (S/I) ratio was 0.25 gVS$_{substrate}$·gVS$_{inoculum}^{-1}$ for all the experimental units. Moreover, the concentration of inoculums was maintained at a constant value, following the recommendations of Raposo et al. [28] for BMP assays without continuous stirring.

Only the mixing ratios were modified while maintaining the same organic load. The DWS:MBW mixing ratios assessed were 100:00, 80:20, 60:40, 40:60, 20:80 and 00:100, respectively. Each mixing ratios were replicated (n=2), and an additional unit that only contained inoculum with distilled water was used as the control for the determination of endogenous methane production.

The synergistic effect of co-digestion could be estimated as an additional methane yield for co-substrates over the weighted average of the individual substrate's BMP. If the differential (BMP–Weighted BMP) was positive and greater than the standard deviation (SD) of BMP, the synergistic effect could be confirmed. The weighted BMP of co-substrates were calculated with Eq. (4) [29]:

$$Weighted\,BMP = BMP_{DWS} * P_{DWS} + BMP_{MBW} * P_{MBW} \tag{4}$$

Where weighted BMP is the weighted average of BMP for co-substrates (mLCH$_4$·gVS^{-1}); BMP$_{DWS}$ and BMP$_{MBW}$ are the experimental methane yields for DWS and MBW, respectively (mLCH$_4$·gVS^{-1}); P$_{DWS}$ and P$_{MBW}$ are the percentage of DWS and MBW, respectively on a VS basis.

The C/N ratio was determined in each reactor according to the recommendations of Chiumenti [30] using Eq. (5):

$$\frac{C}{N_M} * M_{total} = \frac{C}{N_{MBW}} * M_{MBW} + \frac{C}{N_{DWS}} * M_{DWS} \tag{5}$$

Where C/N$_M$ is the C/N ratio in the substrate mixture for each reactor; M$_{total}$ is the mass of the substrate mixture in each reactor (gVS); C/N$_{MBW}$ is the C/N ratio in the MBW; M$_{MBW}$ is the MBW mass in each reactor (gVS); C/N$_{DWS}$ is the C/N ratio in the DWS; and M_{DWS} is the DWS mass in each reactor (gVS).

2.2.3. Kinetic Models

In order to determine the effect of the different mixing ratios on the BMP assay, an analysis of variance (ANOVA) and Tukey's test at $p<0.05$ were applied by using the software R (i386 3.0.2). The response variable was the BMP. A statistical mixed model (Eq. 6) was used to determine the effect of the different ratios using the software Polymath 5.0:

$$y = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3 + \beta_4 x^4 + \varepsilon \tag{6}$$

Where y is the response variable (BMP), ε is the random error, β_0 is the coefficient associated with ratio 1 of the quantitative predictor variable, β_1 is the coefficient associated with ratio 2, β_2 is the coefficient associated with ratio 3 and so on. Then, to validate this model, a variance analysis was performed.

In order to analyze hydrolysis as a limiting stage, a first-order kinetics model [31] and the modified Gompertz model were applied. In the first-order kinetics model, Eqs. (7) and (8) were applied in order to obtain the concentration (mol· L^{-1}) from the pressure and the hydrolysis constant, respectively.

$$M(t) = \frac{P(t)}{R * T} \tag{7}$$

Where M(t) is the methane concentration at time t (mol·L^{-1}); P(t) is the pressure registered by the OxiTop® instrument (atm) at time t; R is the ideal gas constant (atm· L·mol^{-1}·K^{-1}); and T is the experiment temperature (K).

$$LN\left(\frac{M_U}{M}\right) = K_h t \tag{8}$$

Where LN is the natural logarithm; t is the time (d); Mu is the methane production at the end of the experiment (mol· L^{-1}); M is the remaining gas production over time (M=Mu-M(t)); and K_h is the hydrolysis constant (d^{-1}).

The modified Gompertz model can identify significant parameters related to the hydrolysis stage of anaerobic digestion, such as the maximum production rate, maximum production and lag phase, which emphasizes the time when the substrate is transformed and its relation to the stage of the methane production. So as to apply this model, the volume data obtained for each mixing ratio assessed during the experiment were used. The model corresponds to a sigmoid function expressing methane production in the reactor as a function of time (Eq. 9)[32]:

$$V_{CH4}(t) = P_{max} * \exp\left[-exp\left(\frac{R_{max} * \exp(1)}{P_{max}} * (\lambda - t) + 1\right)\right] \tag{9}$$

Where $V_{CH4(t)}$ is the cumulative methane production (mL·h^{-1}); P_{max} is the maximum cumulative production at the end of the experiment (mL); R_{max} is the maximum rate of methane production (mL·h^{-1}), λ is the lag phase (hours); and t is the methane generation time (hours).The determination coefficient (R^2) was used as criterion to assess the fitted models through the software Polymath 5.0 and Microsoft Excel 2007.

3. Results and discussion

Table 1 shows the results of the physicochemical analysis for the substrates (MBW and DWS) and inoculum.

Table 1.
Physicochemical characterization of MBW, DWS and inoculums.

Parameter	**Units**	**MBW**^{n}**	**DWS**^{n}**	**Inoculum**^{n}**
pH	-	5.5±0.1	5.0±0.1	7.2±0.1
Humidity	%	77±3.1	95.±5.8	94±6.4
TA	mg CaCO$_3$·L^{-1}	4447±1249	2015±856	6270±357
TBA	mg CaCO$_3$·L^{-1}	-	-	3390±442
VFAs	mg·L^{-1}	10595±1086	2969±120	1657±105
Acetic acid	mg·L^{-1}	<1.0	<1.0	<1.0
Propionic acid	mg·L^{-1}	<1.0	<1.0	<1.0
Butyric acid	mg·L^{-1}	<1.0	<1.0	<1.0
Palmitic acid	mg·L^{-1}	1391±50	3202±180	.N.D
Oleic cid	mg·L^{-1}	1118±100	2181±200	N.D
TOC*	%	38±2.3	30±5.2	10±1.1
Oxidizable carbon	%	5.7±1.7	3±0.5	1.56±0.4
UV$_{254}$	cm^{-1}	>3.30	>3.30	>3.30
COD$_{total}$	mg·L^{-1}	137839±72267	87321±5622	54853±4562
COD$_{filtered}$	mg·L^{-1}	35604±3600	70060±1201	4049±123
BOD	mg·L^{-1}	45333±1800	2717.±223	1274±103
Total nitrogen*	%	1.70±1.0	0.9±0.5	0.5±0.3
Ammoniacal nitrogen	mg·L^{-1}	324±55	122±13	393±5
Ammoniacal nitrogen ion ammonium - NH^+_4	mg·L^{-1}	324±48	122±46	N.D
Non-ionized ammoniacal nitrogen - NH_3	mg·L^{-1}	0.13±0.05	0.02±0.01	N.D
Cellulose	%	1.5±3.6	0.05±0.01	0.01±0.00
Starch	%	<0.10	<0.10	<0.10
Lignin	%	1.2±2.2	0.05±0.001	0.01±0.00
Ether extract	mg·kg^{-1}	9600±4600	12000±200	5000±2100
Proteins	%	2.7±5	0.6±0.1	0.7±0.1
Carbohydrates	%	9.6±0.6	1.4±0.1	0.03±0.0
Raw fiber	%	2.7±0.4	1.6±0.02	0.02±0.00
Total solids	mg·L^{-1}	113037±15241	59700.±8563	74090±5120
Volatile solids	mg·L^{-1}	930167±28528	33950±7899	28259±6542
BF	-	0.80	0.83	N.D
SMA	gCOD·gVS^{-1}·d^{-1}	N.D	N.D	0.008

*Dry material; **Average values; n; number of samples: 5; N.D .not determinated.

Source: Presentation of authors

Generally, the values for pH, humidity, TA, TBA and VFAs of the MBW listed in Table 1 correspond to the normal values for acidified waste and are similar to those obtained by other authors, such as Pesta [33] and Zupančič et al. [34]. The low pH values are related to the high contents of humidity (because of the high amounts of raw food waste), which favors the production of VFAs and low alkalinity, indicating that an alkaline solution with enough buffer capacity should be used to neutralize the acidity during A-Co of MBW[35]. Additionally, a chromatography analysis of the VFAs shows that long-chain and branched-chain fatty acids are more abundant (palmitic acid and oleic acid), and this result could extend the time required for hydrolysis and microbial acclimatization[36].

All the organic matter indicators determined for the MBW show high values due to the physical composition of the MBW according to Oviedo et al. [3]. Additionally, the ratio of $COD_{filtered}$ to COD_{total} (0.26) showed high quantities of particulate material that can affect the stage of hydrolysis of the organic matter.

pH determines the dominant form of nitrogen and it is an important factor in biological processes because of the probable inhibition of microbial activity[33]. In MBW, the dominant compounds of nitrogen corresponded to ammoniacal nitrogen in two forms: NH_4^+ (324.32 $mg \cdot L^{-1}$) and NH_3 (0.13 $mg \cdot L^{-1}$), which is the most toxic form. Based on the buffer capacity, Parawira et al. [37] and other authors recommend nitrogen concentrations of 1100 $mg \cdot L^{-1}$ so as to maintain an adequate A-Co of this type of waste.

The MBW presented a C/N ratio ranged from 20 to 30, which has positive effects on the A-Co and may be related to the high contents of protein [38]. The values of the VS/TS ratio (0.82) and BF ratio (0.80) also indicated high contents of organic matter and low contents of material of vegetable origin that is difficult to degrade, such as lignin [39]. These results are similar to those obtained by Chen et al. [40].

Generally, cellulose and lignin contents of MBW goes from 40 to 60% and from 10 to 15%, respectively[41]. However, the values obtained in this study were lower because of the source separation of waste, thus reducing the paper, diaper and pruning waste. The starch content was also low, which was possibly caused by the storage time in housings (between 3 and 4 days), which favors fermentation and sucrose formation. Other compounds, such as ether extract (lipids), proteins, raw fiber and carbohydrates, were present in quantities similar to those reported by Chen, et al. [40] for this type of waste.

The DWS presented normal values of pH, humidity, TA, TBA and VFAs[42]. Compared to the MBW, the DWS presented different characteristics in terms of VFA composition (dominance of long-chain VFAs[43]) and high contents of organic matter. However, other variables, such as humidity, $COD_{filtered}$, COD_{total}, and VS/TS and C/N ratios, were different due to the substrate composition (e.g., low protein content, higher amount of water, etc.). The higher contents of ether extract can affect the hydrolysis process and methane production, which was reported by Cirne, et al. [43] for waste with high lipid contents.

The DWS:MBW mixing ratios may facilitate the A-Co due to the synergy among nutrients, which favors the enzymatic processes that occur during the hydrolytic stage and transformations in the subsequent stages [7].

The inoculum presented values typical of anaerobic sludge from municipal WWTP, which uses chemically assisted primary treatment with values of pH, AT, and BA that are indicative of a good buffer capacity that favors A-Co[33]. The value of the VS/TS ratio is low from the point of view of the activity of the biomass present in the sludge, although it is typical of anaerobic reactors at municipal WWTP, which uses chemically assisted primary treatment. Nevertheless, it is important to understand that the VS/TS ratio alone is not a good indicator of the inoculum quality, due to the microbial diversity present in the sludge[44].

3.1. Influence of the DWS: MBW ratios on methane production

Fig.1 illustrates the BMP during A-Co of the DWS and MBW, which lasted 20 days (480 hours). The CH_4 produced for the different mixing ratios ranged from 61.28 to 105.6$mLCH_4 \cdot gVS^{-1}$. The CH_4 produced at 5 days (120 hours) for the 00:100 and 20:80 mixing ratios accounted for more than 70% of the total; in contrast, the mixing ratios 40:60, 60:40, 80:20 and 100:00 only represented 48, 15, 15 and 12% respectively of the methane production. This behavior can be attributed to the high content of long chain fatty acids and lipids in DWS (palmitic and oleic acid), which according to Hidalgo et al. [24] and Esposito et al. [45], destabilizes the metabolic process performed by methanogenic microbial consortium and reduces methane production.

The results of the ANOVA ($p<0.05$) showed significant differences between the BMP for the mixing ratios assessed. This variable has an important influence on the activity of the microorganisms involved along the different stages, especially during methane production. The effect of mixing ratios can also be observed in the lag phase, which were 2.6 and 3 days, respectively for 00:100 and 20:80 ratios, whereas for the remaining mixing ratios, the duration ranged from 4 to 7 days.

Moreover, Tukey's test ($p>0.05$) evidenced no significant differences between the 00:100 and 80:20 or between the 40:60, 60:40, 80:20 and 100:00 mixing ratios. However, significant

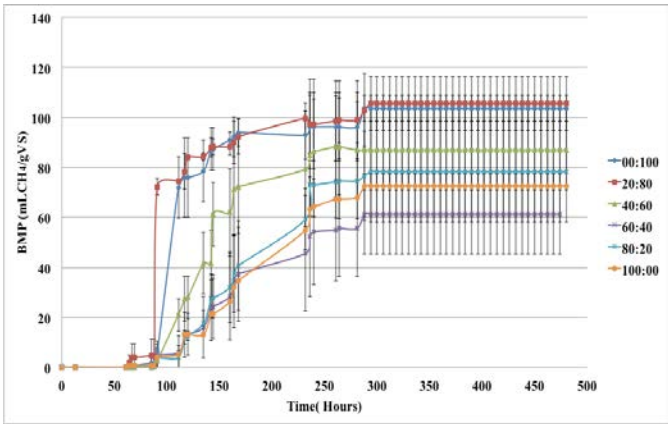

Figure 1. BMP for the different mixing ratios assessed.
Source: Presentation of the authors

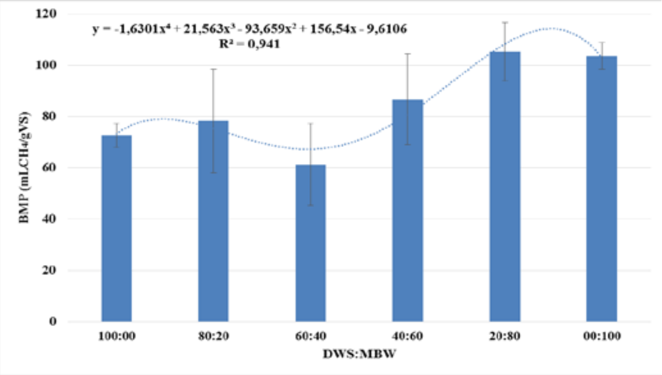

Figure 2. Prediction model for effect synergistic or antagonistic for different DWS:MBW mixing ratios.
Source: Presentation of authors

Table 2.
Synergistic or Antagonistic effect evaluation of A-Co DWS: MBW mixing ratios.

DWS: MBW mixing ratios	C/N	BMP (mL·gVS^{-1})	Weighted BMP	Differential (BMP-Weighted BMP)	Increasing or Decreasing rate of methane yield (%)	S/A
100:00	33.5	72.6	-	-	-	-
80:20	25.8	78.3	78.8	-0.5	0.7(decreasing)	A
60:40	23.9	61.3	85.0	-23.7	38.7(decreasing)	A
40:60	23.1	86.9	91.2	-4.2	4.9 (decreasing)	A
20:80	22.6	105.6	97.4	8.2	7.8(increasing)	S
00:100	22.3	103.6	-	-	-	-

A: Antagonistic effect; S: Synergistic effect.
Source: Presentation of the authors

differences were observed for the remaining cases, which indicates that MBW concentrations over 80% produce similar positive impacts on methane production during A-Co of DWS, thereby favoring digestion of the DWS, which has a high content of long-chain VFA´s and are characterized by reduced methane production[46].

Regarding synergistic or antagonistic effect showed in Fig. 2 (R^2=0.94) it was observed strong synergistic effects in the process for different DWS:MBW mixing ratios. The BMP is affected when higher quantities of DWS are used, and the lowest methane production is obtained for mixing ratio 60:40 (Table 2). These results are similar to those obtained by Esposito et al. [45], who stated that higher MBW contents in the A-Co process produce a greater amount of methane, which is attributed to the high biodegradability of the substrate.

Table 2 shows the BMP and C/N ratios when different DWS and MBW mixing ratios are used. The range of optimal values for A-Co is indicated and corresponds to ratios between 20 and 30.

According to Table 2, the C/N ratios in each experimental unit ranged from 22.6 to 25.8, indicating that the contribution of C and N by the different substrates was within the required range for co-digestion processes. Therefore, these substrate ratios presented improved pH, alkalinity, VFA and the necessary nutrients conditions to allow growth of the microbes that perform the biological processes[38]. However, the DWS:MBW proportion 100:00 presented C/N ratios that were outside the range of AD, which indicates lower or higher contents of carbon than required. Therefore, it is evidenced the synergistic effect which is generated by incorporating MWB in A-Co of DWS, thus improving methane production and also ensuring a balance of nutrients necessary for the process.

3.2. Influence of the different proportions on hydrolysis during the A-Co of DWS and MBW

Table 3 presents the results for the hydrolysis constants and lag phase for the different DWS:MBW mixing ratios.

With regard to the first-order kinetics model, the hydrolysis constants were within the range of values corresponding to the digestion of substrates containing high amounts of carbohydrates, which are found in sludges from municipal WWTP [15]. However, the hydrolysis constant values were dependent of DWS:MBW mixing ratio. Therefore, the highest values of the hydrolysis constant were obtained for the 00:100 and 20:80 mixing ratio.

The K_h value was 0.37, which represents a higher conversion rate of substrate into methane, whereas the other proportions presented K_h values ranging between 0.25 and 0.28 d^{-1}. The decreasing trend in K_h values can be explained by the increase in the fat concentration related to the higher amounts of DWS in each reactor. This result was suggested by Iacovidou et al. [47], who stated that the presence of lipids contributes to the formation of long-chain VFAs characterized by their toxicity to AD processes. Furthemore, Kim et al. [48] indicated that the addition of MBW to the A-Co process with DWS causes an increase in the values of K_h, which was found in the present study.

Concerning the adjustment to the nonlinear regression Gompertz model, an inversely proportional relationship was observed between the length of the lag phase and methane production (similar to what was observed in the case of K_h). The shortest lag phase was observed when substrate ratios of 00:100 and 20:80 were used, which produced corresponding values of 80 h (3.3 d) and 67 h (2.8 d). This result can be explained due to MBW contain organic matter with high quantities of carbohydrates that are easily assimilated by the microorganisms during this stage and promote rapid acclimatization to the substrate, which can be observed in the methane production.

Table 3.
Hydrolysis constants and lag phase.

DWS: MBW mixing Ratio	First-order Kinetics Model		Modified Gompertz Model			
	K_h (d^{-1})	R_1^2	λ^a	R_{max}^b	P_{max}^c	R_2^2
100:00	0.25±0.04	0.80	106±6	0.04±0.01	5.5±0.2	0.99
80:20	0.28±0.05	0.85	98±9	0.04±0.02	4.9±0.1	0.99
60:40	0.26±0.03	0.74	94±7	0.04±0.02	4.6±0.1	0.99
40:60	0.27±0.05	0.77	90±5	0.05±0.01	5.9±0.2	0.99
20:80	0.37±0.06	0.93	67±8	0.06±0.03	7.8±0.2	0.98
00:100	0.37±0.05	0.84	80±6	0.09±0.02	7.4±0.2	0.99

a (hours); b (mL·h^{-1}); c (mL) R_1^2: determination coefficient for first-order kinetics model; R_2^2: determination coefficient Gompertz model.
Source: Presentation of authors

The values for the lag phase process obtained for 40:60, 60:40, 80:20 and 100:00 mixing ratios were over 90 h. These results show that if the proportion of DWS is high, a longer residence time in the reactor is required in order to ensure that hydrolysis occurs; in addition, larger reactor volumes and higher costs during implementation and operation are also required.

The results of R_{max} and P_{max} obtained for the ratios with 80% MBW were 0.064 $mL \cdot h^{-1}$ and 7.8 mL, respectively. These values are expected for the different stages of methane production, which can be observed in the BMP results. In the reactors where the amount of MBW accounted for less than 80%, the results obtained for R_{max} and P_{max} ranged from 0.04 to 0.050 $mL \cdot h^{-1}$ and 4.6 to 5.9 mL, respectively, which is related to the low methane production.

4. Conclusions

The A-Co of DWS and MBW is based on a synergic effect related to the supplied nutrients, especially C and N. For the mixing ratios with 20% of MBW the ratios C/N were between 22.6 and 25.8, which makes this process suitable for its use with this type of waste produced by the communities of the country.

The DWS:MBW mixing ratio affects the A-Co. When the amount of MBW accounted for more than 80% of the mixture, the co-digestion performance was optimal. In contrast, the use of lower quantities of MBW destabilizes the process, most likely due to limitations in the hydrolysis of the organic matter and inhibition processes related to VFA accumulation (especially long-chain), which may be toxic for the microbial consortia involved in methane production. When the amount of DWS used accounts for more than 20% of the mixture, the co-digestion of DWS and MBW entails higher implementation costs (larger reactor volumes) and operational complexity.

Although the values of the hydrolysis constant of DWS and MBW (K_h) were within the usual range of values obtained in the literature, the K_h was affected by the substrate ratio. In the present study, the optimal conditions corresponded to 20:80 mixing ratio; under these circumstances, the length of the lag phase was shorter (less than 3.3 days), and faster substrate degradation as well as higher methane production were also obtained.

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References

[1] Jingura, R. and Matengaifa, R., Optimization of biogas production by anaerobic digestion for sustainable energy development in Zimbabwe. Renewable and Sustainable Energy Reviews, 13(5), pp. 1116-1120, 2009. DOI:10.1016/j.rser.2007.06.015

[2] Wang, H. and Wang, C., Municipal solid waste management in Beijing: characteristics and challenges. Waste Management & Research, 31(1), pp. 67-72, 2013. DOI: 10.1177/0734242X12468199

[3] Oviedo-Ocaña, R., Marmolejo-Rebellón, L. and Torres-Lozada, P. Evaluation of the adittion of wood ashes to control the pH of substrates in municipal biowaste composting. Ingeniería, Investigación y Tecnología, 15(3), pp. 469-478, 2014. DOI:10.1016/S1405-7743(14)70355-5

[4] Martín-González, L., Colturato, L., Font, X. and Vicent, T. Anaerobic co-digestion of the organic fraction of municipal solid waste with FOG waste from a sewage treatment plant: Recovering a wasted methane potential and enhancing the biogas yield. Waste Management, 30(10), pp. 1854-1859, 2010. DOI:10.1016/j.wasman.2010.03.029

[5] Mata-Alvarez, J., Dosta, J., Romero-Güiza, M., Fonoll, X., Peces, M. and Astals, S., A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renewable and Sustainable Energy Reviews, 36, pp. 412-427, 2014. DOI:10.1016/j.rser.2014.04.039

[6] Balussou, D., Kleyböcker, A., McKenna, R., Möst, D. and Fichtner, W., An economic analysis of three operational co-digestion biogas plants in Germany. Waste and Biomass Valorization, 3(1), pp, 23-41, 2012. DOI 10.1007/s12649-011-9094-2

[7] Hartmann, H. and Ahring, B., Anaerobic digestion of the organic fraction of municipal solid waste: Influence of co-digestion with manure. Water Research, 39(8), pp. 1543-1552, 2005. DOI:10.1016/j.watres.2005.02.001

[8] Lesteur, M., Bellon-Maurel, V., Gonzalez, C., Latrille, E., Roger, J., Junqua, G. and Steyer, J., Alternative methods for determining anaerobic biodegradability: a review. Process Biochemistry, 45(4), pp. 431-440, 2010. DOI:10.1016/j.procbio.2009.11.018

[9] Ponsá, S., Gea, T. and Sánchez, A., Anaerobic co-digestion of theorganic fraction of municipal solid waste with several pure organic co-substrates. Biosystems Engineering, 108(4), pp. 352-360, 2011. DOI:10.1016/j.biosystemseng.2011.01.007

[10] Callaghan, F., Wase, D., Thayanity, K. and Forster, C., Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure, Biomass Bioenergy, 22(1), pp. 71-77, 2002. DOI:10.1016/S0961-9534(01)00057-5

[11] Tandukar, M. and Pavlostathis, S., Co-digestion of municipal sludge and external organic wastes for enhanced biogas production under realistic plant constraints. Water Research, 87, pp. 432-445, 2015. DOI:10.1016/j.watres.2015.04.031

[12] Zhang, P., Estudio y propuesta de mejora del proceso de depuración de la EDAR de Zhongyuan (China). Tesisi de Grado en Ciencias Ambientales, Escuela Politecnica Superior de Gandia, Universidad Politecnica de Valencia, Valencia, España, 2013.

[13] Xu, G., Yan, Z., Wang, Y. and Wang, N., Recycle of Alum recovered from water treatment sludge in chemically enhanced primary treatment. Journal of Hazardous Materials, 161(2-3), pp. 663-669, 2009. DOI:10.1016/j.jhazmat.2008.04.008

[14] Poon, C. and, Chu, C., The use of ferric chloride and anionic polymer in the chemically assisted primary sedimentation process. Chemosphere, 39(10), pp. 1573-1582, 1999. DOI:10.1016/S0045-6535(99)00055-7

[15] Trzcinski, A. and Stuckey, D., Determination of the hydrolysis constant in the biochemical methane potential test of municipal solid waste. Environmental Engineering Science, 29(9), pp. 848-854, 2012. DOI:10.1089/ees.2011.0105

[16] Kafle, G., Kim, S. and Sung, K., Ensiling of fish industry waste for biogas production: A lab scale evaluation of biochemical methane potential (BMP) and kinetics. Bioresoruce Technology, 127, pp. 326-336, 2013. DOI:10.1016/j.biortech.2012.09.032

[17] Sakurai, K., Método sencillo del análisis de residuos sólidos, Lima-Peru, CEPIS/OPS, Peru, 2000.

[18] Mukherjee, S., Kumar, S. and Devotta, S., Influence of Nitrogen of Anaerobic Digestion of Municipal Solid Waste in a Laboratory Scale. Journal of the IPHE [Online]. 9(4), 2008. [date of reference March 25th of 2016]. Available at: http://indiaenvironmentportal.org.in/files/Influence%20of%20nitrogen.pdf

[19] Sharma, K., Mishra, I., Sharma, M. and Saini, J., Effect of Particle Size on Biogas Generation from Biomass Residues. Biomass, 17(4), pp. 251-263, 1988. DOI:10.1016/0144-4565(88)90107-2

[20] ICONTEC., Norma Técnica Colombiana 5167. Productos para la Industria Agrícola, Productos Orgánicos Usados como Abonos o Fertilizantes y Enmiendas de Suelo, Colombia, 2004, 32 P.

[21] APHA. Standard methods for examination of water and wastewater, Washington D.C., A.W.W.A.a.W.E. Federation, Estados Unidos, 2005.

[22] Espinosa, M., López, M., Pellón, A., Mayarí, R. y Fernández, A., La fracción orgánica de los residuos sólidos urbanos como fuente potencial de producción de biogás. Revista CENIC Ciencias Biológicas [Online]. 38(1), 2007. [date of reference March 25th of 2016]. Available at: http://www.redalyc.org/articulo.oa?id=181221557003

[23] Sterling, M., Lacey, R., Engler, C. and Ricke, S., Effects of Ammonia Nitrogen on H2 and CH4 production during anaerobic digestion of dairy cattle manure. Bioresource Technology, 77(1), pp. 9-18, 2001. DOI:10.1016/S0960-8524(00)00138-3

[24] Hidalgo, D. and Martín-Marroquína, J., Effects of inoculum source and co-digestion strategies on anaerobic digestion of residues generated in the treatment of waste vegetable oils. Journal of Environmental Management, 142, pp. 17-22, 2014. DOI:10.1016/j.jenvman.2014.04.004

[25] Aquino, S., Chernicharo, C., Foresti, E. Florencio, M. e Monteggia, L., Metodologias para determinação da atividade metanogênica específica (Ame) em lodos anaeróbios. Engenharia Sanitaria e Ambiental, 12(2), pp. 192-201, 2007. DOI: 10.1590/S1413-41522007000200010

[26] Owen, W., Stuckey, D., Healy, J., Young, L. and McCarty, P., Bioassay for Monitoring Biochemical Methane Potential and Anaerobic Toxicity. Water Research, 13(6), pp. 485-492, 1979. DOI:10.1016/0043-1354(79)90043-5

[27] Giménez, J., Martí, N., Ferrer, J. and Seco, A., Methane recovery efficiency in a submerged anaerobic membrane bioreactor (SANMBR) treating sulphate-rich urban wastewater: Evaluation of methane losses with the effluent. Bioresoruce Technology, 118, pp. 67-72, 2012. DOI: 10.1016/j.biortech.2012.05.019

[28] Raposo, F., Banks, C., Siegert, I., Heaven, S. and Borja, R., Influence of inoculum to substrate ratio on the biochemical methane potential of maize in batch tests. Process Biochemistry, 41(6), pp. 1444-1450, 2006. DOI:10.1016/j.procbio.2006.01.012

[29] Chen, X., Yan, W., Sheng, K. and Sanati, M., Comparison of high-solids to liquid anaerobic co-digestion of food waste and green waste. Bioresource Technology, 154, pp. 215-221, 2014. DOI:10.1016/j.biortech.2013.12.054

[30] Chiumenti, A., Modern composting technologies. USA: Emmaus, PA: JG Press., 2005.

[31] Liew, L., Shi, J. and Li, Y., Methane production from solid-state anaerobic digestion of lignocellulosic biomass, Biomass and Bioenergy, 46, pp. 125-132, 2012. DOI:10.1016/j.biombioe.2012.09.014

[32] Lay, J., Li, Y., Noike, T., Endo, J. and Ishimoto, S., Analysis of enviomental factors affecting methane production from high-solids organic. Water Science and Technology, 36(6-7), pp. 493-500, 1997. DOI:10.1016/S0273-1223(97)00560-X

[33] Pesta, G., Anaerobic digestion of organic residues and waste, USA: Springer New York, 2007.

[34] Zupančič, G. and Roš, M., Determination of chemical oxygen demand in substrates from anaerobic treatment of solid organic waste. Waste and Biomass Valorization, 3(1), pp. 89-98, 2012. DOI: 10.1007/s12649-011-9087-1

[35] Abdulkarim, B.I. and Abdullahi, M.E., Effect of buffer ($NaHCO_3$) and waste type in high solid thermophilic anaerobic digestion, International Journal of ChemTech Research, 2, pp. 980-984, 2010

[36] Sundberg, C., Franke-Whittle, I., Kauppi, S., Yu, D., Romantschuk, M., Insam, H. and Jönsson, H., Characterisation of source-separated household waste intended for composting. Bioresource Technology, 102(3), pp. 2859-2867, 2011. DOI: 10.1016/j.biortech.2010.10.075

[37] Parawira, W., Murto, M., Zvauya, R. and Mattiasson, B., Anaerobic batch digestion of solid potato waste alone and in combination with sugar beet leaves. Renewable Energy, 29(11), pp. 1811-1823, 2004. DOI:10.1016/j.renene.2004.02.005

[38] Mata-Alvarez, J., Macé, S. and Llabares, P., Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresoruce Technology, 74(1), pp. 3-16, 2000. DOI:10.1016/S0960-8524(00)00023-7

[39] Takáčová, A., Mackuľak, T., Smolinská, M., Hutňan, M. and Olejníková, P., Influence of selected biowaste materials pre-treatment on their anaerobic digestion. Chemical Paper, 66(2), pp. 129-137, 2012. DOI: 10.2478/s11696-011-0107-1

[40] Chen, X., Romano, R. and Zhang, R., Anaerobic digestion of food wastes for biogas production. International Journal of Agricultural and Biological Engineering, 3(4), pp. 61-71, 2010. DOI: 10.3965/j.issn.1934-6344.2010.04.0-0

[41] Juanga, J., Optimizing dry anaerobic digestion of organic fraction of municipal solid waste, MSc. Thesis, School of Environment, Resources and Development, Asian Institute of Technology, Bangkok, Thailand, 2005.

[42] Torres-Lozada, P., Díaz-Granados, J. and Parra-Orobio, B.A., Effects of the incorporation of drinking water sludge on the anaerobic digestion of domestic wastewater sludge for methane production. Water Science and Technology, 72(6), pp. 1016-1021, 2015. DOI: 10.2166/wst.2015.291

[43] Cirne, D., Paloumet, X., Björnsson, L., Alves, M. and Mattiasson, B., Anaerobic Digestion of Lipid-Rich Waste—Effects of Lipid Concentration. Renewable Energy, 32(6), pp. 965-975, 2007. DOI: 10.1016/j.renene.2006.04.003

[44] Demirel, B. and Scherer, P., The Roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: A review. Reviews in Environmental Science and Bio/Technology, 7(2), pp. 173-190, 2008. DOI: 10.1007/s11157-008-9131-1

[45] Esposito, G., Frunzo, L., Giordano, A., Liotta, F., Panico, A. and Pirozzi, F., Anaerobic co-digestion of organic wastes. Reviews in Environmental Science and Bio/Technology, 11(4), pp. 325-341, 2012. DOI: 10.1007/s11157-012-9277-8

[46] Davidsson, Å., Lövstedt, C., la Cour Jansen, J., Gruvberger, C. and Aspegren, H., Co-digestion of grease trap sludge and sewage sludge. Waste Management, 28(6), pp. 986-992, 2008. DOI: 10.1016/j.wasman.2007.03.024

[47] Iacovidou, E., Ohandja, D. and Voulvoulis, N., Food waste co-digestion with sewage sludge - Realising its potential in the UK. Journal of Environmental Management, 112, pp. 267-274, 2012. DOI: 10.1016/j.jenvman.2012.07.029

[48] Kim, H., Han, S. and Shin, H., Anaerobic co-digestion of sewage sludge and food waste using temperature-phased anaerobic digestion process. Water Science and Technology [Online]. 50(9), 2004. [date of reference March 25th of 2016]. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15581001

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