

# Optimization of biomethane production by anaerobic co-digestion of mango waste, legume residues, pig manure and bovine manure

Désiré Traoré<sup>1\*</sup>, Dayéri Dianou<sup>2</sup> and Alfred S. Traoré<sup>1</sup>

<sup>1</sup> Centre de Recherche en Sciences Biologiques Alimentaires et Nutritionnelles (CRSBAN), Université Ouaga I, Pr Joseph KI-ZERBO, 03 BP 7131 Ouagadougou 03, Burkina Faso

<sup>2</sup> Centre National de la Recherche Scientifique et Technologique (CNRST), 03 BP 7192 Ouagadougou 03, Burkina Faso.

\* Corresponding author: Désiré Traoré; e-mail: [desiretraore@gmail.com](mailto:desiretraore@gmail.com)

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## ABSTRACT

Numerous technologies have been developed in recent years to improve anaerobic digestion conditions for methane production. Among these technologies, the co-digestion of substrates was shown to be the most efficient. The objective of this study was to determine the combinations of mango waste (MW), vegetable residues (VR), bovine manure (BM) and pig manure (PM), providing the most efficient substrate for biogas and methane productions from anaerobic digestion process. The study was carried out in presence of pig manure sludge (PMS) and bovine manure sludge (BMS) as inoculums. The physicochemical parameters of the different substrates were determined by standard methods of biochemistry, while biogas and methane produced were analyzed by gas chromatography. Dry matter of substrates was  $22.61 \pm 0.3\%$ ,  $15.3 \pm 0.3$ ,  $11.78 \pm 0.6$  and  $8.04 \pm 0.02\%$  for pig manure, bovine manure, vegetable residues and mango waste, respectively. The best productions of biogas (43880  $\mu$ l, 41170 and 410220  $\mu$ l) and methane (26108  $\mu$ l, 25513 and 25030  $\mu$ l) were obtained with the combined substrates PM/VR (1/3), PM/MW (1/1) and PM/VR (1/1), respectively.

**Keywords:** Co-digestion, biogas, biomethane, mango waste, vegetable residues, bovine manure, pig manure.

## 1. INTRODUCTION

Anaerobic digestion is a complex biochemical process carried out in absence of oxygen and involving different microbial communities in the conversion of organic matter into biogas [1, 2]. This technology is treating agricultural residues, animal waste, household waste, waste from urban and industrial centers [3, 4]. However, the substrate nature may limit its bioconversion into methane [5]. So, Petit [6] showed that whatever the type, an organic waste incubated alone, is not able to meet all the criteria of anaerobic digestion.

Thus, thermal treatment [7, 8], acid treatment [9], alkaline treatment technologies [10] were developed to improve anaerobic digestion of biomass. Other biogas yield enhancement processes have been achieved by adding micronutrient solutions [11, 12]. However, co-

digestion has been shown to improve efficiently the conditions of anaerobic digestion and consequently, to increase the yield of biomethane [6, 13].

The objective of this study was to determine the proportions of mango waste (MW), vegetable residues (VR), bovine manure (BM) and pig manure (PM), in a mixture leading to the best biomethane production in an anaerobic co-digestion process.

## 2. MATERIALS AND METHODS

### 2.1. Collection and processing of substrates and inoculums

Samples of mango waste (MW), vegetable residues (VR), bovine manure (BM) and pig manure (PM) were used as substrates in this study. Mango waste was

collected at two mango processing plants (GEBANA in Bobo Dioulasso and DAFANI in Orodara) and in two fruit markets in Ouagadougou (*Sankara yaar* and *Kaatre yaar*). Vegetable residues were collected from the restaurants of Ouagadougou University at Zogona and Patte d'Oie (two districts of Ouagadougou city) and from two vegetable markets located in the same districts. The vegetable residues consisted of a mixture of courgette peel, potato, eggplant and lettuce leaves, cabbage leaves, and onion. Bovine manure and pig manure were freshly taken from *Pagalayiri* cattle market and from a farm at *Nioko II*, respectively. Bovine manure sludge (BMS) and pig manure sludge (PMS) served as inoculums in this study. The different treatments carried out on substrates and inoculums are detailed in our previous studies [14, 15].

## 2. 2. Anaerobic digestion methodology

We used 120 ml and 250 ml bottle as digesters. For each test, 250 ml digesters were loaded with 75 ml of inoculum to which various co-suspensions of substrates were then added (Table 1). These digesters were used to monitor and measure the amount of biogas produced. At the same time, 28 bottles of 120 ml were prepared under the same conditions in order to monitor biogas and methane production. Pig manure sludge (PMS) and bovine manure sludge (BMS) were used as inoculums in the co-digestion of vegetable residues, mango waste and pig manure, and in the one of vegetable residues, mango waste and bovine manure, respectively.



**Figure 1:** Digesters for biomethanization used in the study. A: 250 ml septum vials; B: septum vials of 120 ml.

**Table 1:** Organic load of co-substrates in relation to digester type.

Co-substrate	Substrate amount/digester (g VM)	
	250 ml digester	120 ml digester
PM/MW (1/1)	4.8 g + 4 g	2.6 g + 2.2 g
PM/MW (3/1)	7.4 g + 2 g	4 g + 1.1 g
PM/MW (1/3)	2.4 g + 6.1 g	1.32 g + 3.3 g
PM/VR (1/1)	4.8 g + 5 g	2.6 g + 2.75 g
PM/VR (3/1)	7.4 g + 2.5 g	4 g + 1.37 g
PM/VR (1/3)	2.4 g + 4 g	1.32 g + 4 g
BM/MW (1/1)	4.2 g + 4.1 g	2.3 g + 2.2 g
BM/MW (3/1)	6.5 g + 2 g	3.5 g + 1.1 g
BM/MW (1/3)	2.2 g + 6.1 g	1.2 g + 3.3 g
BM/VR (1/1)	4.2 g + 5 g	2.3 g + 2.75 g
BM/VR (3/1)	6.5 g + 2.5 g	3.5 g + 1.37 g
BM/VR (1/3)	2.2 g + 4 g	1.2 g + 4 g

PM= pig manure, MW= mango waste, VR= vegetable residues, BM= bovine manure, VM= volatile matter

## 2.3. Analytical methods

Mango waste, vegetable residues, Pig and bovine manure samples were analyzed for total solids, volatile solids, ash mater, pH and total organic carbon, using standard methods [16,17]. The pH was measured using a digital pH meter (WTWpH340).

The methane content in biogas was determined as described in our previous studies [14, 15]. The reactor headspace gas (500µl) was analyzed at three (3) days

interval using a thermal conductivity gas chromatograph Girdel series 30 catharometer, equipped with porapak Q 80/100 and Q 100/120 columns assembled in parallel and connected to a thermal conductivity detector (TCD) and a potentiometric recorder (SERVOTRACE type sefram Paris 1 mV). The temperature was set at 90°C for the injector, 60°C for the column and 100°C for the detector; N<sub>2</sub> was used as carrier gas. Methane standard (90% purity) supplied by Burkina Industrial Gas,

allowed to establish the following regression equation from that the production of CH<sub>4</sub> during the experiments was deduced: Volume CH<sub>4</sub> (μL) = 0.1094 X - 5.0911 (r<sup>2</sup>= 0,9958), with X the area of methane peak.

### 2.4. Statistical analysis

The data collected were subjected to analysis of variance (ANOVA) using XLSTAT-Pro 7.5.2 software. Means were compared through Fisher test to determine significant differences among variables at α= 0.05.

## 3. RESULTS AND DISCUSSION

### 3.1. Evolution of pH during the digestion process

Overall, pH is one among the most important parameters for methanation; the control of its variation, is fundamental for correct functioning of reactors. A stable pH indicates a system in equilibrium

and an efficient methanization. Table 2 shows the pH values of various substrate combinations measured at the beginning and at the end of digestion.

Initial pH indicates pH value measured immediately after the preparation of substrate and final pH indicates pH value measured at the end of anaerobic digestion. From the results obtained, it appears that the initial pH values vary between 7.17 and 7.48 and the final pH between 6.99 and 7.6, both for the co-digestion of mango waste and vegetable residues with pig manure (PM/MW, PM/VR), and the one of mango waste and vegetable residues with bovine manure (BM/MW, BM/VR). These results corroborate those of Igoni et al. [16] who demonstrated that bacteria involved in anaerobic digestion have an optimal activity around pH 7.

**Table 2:** pH at the beginning and at the end of the anaerobic digestion of combined substrates from bovine manure, pig manure, mango waste and vegetable residues.

Co-digestion	Substrates combination	Initial pH	Final pH
PM/MW/VR	PM/MW (1/1)	7.35 ± 0.079	7.3 ± 0.066
	PM/MW (3/1)	7.48 ± 0.11	7.37 ± 0.02
	PM/MW (1/3)	7.23 ± 0.037	7.31 ± 0.02
	PM/VR (1/1)	7.44 ± 0.049	6.99 ± 0.27
	PM/VR (3/1)	7.42 ± 0.037	7.47 ± 0.1
	PM/VR (1/3)	7.27 ± 0.015	7.66 ± 0.03
BM/MW/VR	BM/MW (1/1)	7.17 ± 0.011	7.18 ± 0.005
	BM/MW (3/1)	7.34 ± 0.09	7.17 ± 0.02
	BM/MW (1/3)	7.17 ± 0.011	7.2 ± 0.011
	BM/VR (1/1)	7.37 ± 0.06	7.23 ± 0.083
	BM/VR (3/1)	7.43 ± 0.02	7.19 ± 0.036
	BM/VR (1/3)	7.32 ± 0.02	7.25 ± 0.017

PM= pig manure, MW= mango waste; VR= vegetable residues; BM= bovine manure

Globally, the results presented in Table 2 clearly reveal a normal anaerobic digestion course of the different combinations of substrates. Indeed, pH decreasing is a sign of problems arising in the anaerobic digestion process [19], while the increase of pH inhibits the metabolic pathways of methanogenic bacteria [20, 21].

Moreover, the pH range recorded in this study confirms a normal evolution of our different substrates co-digestion, compared to the results of Rizk and Bergamasco [22] who reported that co-digestion

stabilizes pH, volatile fatty acids production in the digester and consequently, increases biogas yields.

### 3.2. Determination of substrate combinations for optimum biomethane production

The anaerobic digestion of the substrates taken separately (MW, VR, PM, BM) served as controls in this specific case. Co-digestion was carried out on pig manure (PM) and bovine manure (BM) combined in different proportions with mango waste (MW) and vegetable residues (VR), respectively. The results obtained are presented in Tables 3 and 4.

**Table 3:** Productions of biogas and methane (CH<sub>4</sub>) by co-digestion of mango waste, vegetable residues and bovine manure, with bovine manure sludge as inoculum (means of 3 replicates).

Substrate/co-substrate	Biogaz (μl)	CH <sub>4</sub> (μl)	CH <sub>4</sub> (%)
MW	13968a	7072.42a	50.63a
VR	15882a	9392.61a	59.14c
BM	21108b	12454.01b	53.42ab
BM/VR (1/1)	23333b	12464.72b	59.05c
BM/MW (3/1)	23280b	13504.96b	58.01bc
BM/VR (3/1)	25978c	14888.01c	57.93bc
BM/MW (1/3)	28559d	18454.72d	64.64d
BM/VR (1/3)	31796e	19242.90e	60.52cd
BM/MW (1/1)	32290e	20636.53e	63.91d

In a column, values sharing a same letter are not significantly different according to the Fisher test at 5% threshold. BM= bovine manure; MW= mango waste; VR= vegetable residues;

**Table 4:** Productions of biogas and methane (CH<sub>4</sub>) by co-digestion of mango waste, vegetable residues and pig manure, with pig manure sludge as inoculum (means of 3 replicates).

Substrate/Co-substrate	Biogas (µl)	CH <sub>4</sub> (µl)	CH <sub>4</sub> (%)
MW	21970a	12030a	57.63a
VR	24280ab	14350ab	59.15a
PM	28450bc	17550bc	61.69bc
PM/MW (3/1)	22132a	12030a	54.38a
PM/VR (1/3)	43880d	26108d	59.53a
PM/VR (3/1)	30960c	18690c	60.40b
PM/VR (1/1)	41022d	25030d	61.69bc
PM/MW (1/1)	41170d	25513d	61.97bc
PM/MW (1/3)	32670c	20570c	62.97c

In a column, values sharing a same letter are not significantly different according to the Fisher test at 5% threshold. **PM**= pig manure; **MW**= mango waste; **VR**= vegetable residues.

In the co-digestion test using bovine manure sludge as inoculum, we recorded small volumes of biogas and methane with the substrates incubated separately: 13968 µl and 15882 µl of biogas, 7072.42 µl and 9392.61 µl of methane, for mango waste (MW) and vegetable residues (VR), respectively, compared to the amounts obtained from combined substrates: 28559 µl, 31796 and 32290 µl of biogas, 18454.72 µl, 19242.9 and 20636.53 µl of methane, for the co-substrates BM/MW (1/3), BM/VR (1/3) and BM/MW (1/1), respectively (Table 3).

The best productions of biogas and methane in this test were obtained with the co-substrates BM/VR (1/3) and BM/MW (1/1) (Table 3). However, no significant difference was observed between the 2 co-substrates for biogas and methane productions, respectively (Table 3).

With pig manure sludge as inoculum, low biogas and methane productions were recorded with mango waste (MW) alone: 21970 µl and 12030 µl, respectively (Table 4). The best productions of biogas (43880 µl, 41170 and 410220 µl) and methane (26108 µl, 25513 and 25030 µl) were obtained with PM/VR (1/3), PM/MW (1/1) and PM/VR (1/1), respectively (Table 4). These productions are not significantly different between them for biogas and methane productions, respectively, although significantly higher than those obtained from the substrates incubated separately and from the other co-substrates (Table 4).

In some cases, the productions of biogas or methane doubled when moving from mono-digestion to co-digestion, although these productions were substantially equal in both cases for some substrates, using either bovine manure sludge or pig manure sludge as inoculum, i.e. PM and PM/MW (1/1), BM and BM/VR (1/1) or BM and BM/MW (3/1) (Tables 3 and 4).

The low volumes of biogas and methane observed in anaerobic digestion of the substrates incubated alone, compared to the volumes recorded in the co-digestion, could be explained by the fact that: (i) co-digestion usually contributes to dilute toxic elements, and to additional nutrients and adequate moisture supply [23, 24]; (ii) the mixing of substrates creates a

compensatory effect which could prevent the inhibition of methanation due to excessive production of ammonia or volatile fatty acids which are limiting factors in digestion of substrates taken separately [25-27]; (iii) co-digestion stabilizes the process and increases biogas production [28-32].

In agreement with the results of Anhuradha and Mullai [33], we found that biogas or methane production from the mixture of two substrates is better than the production of each substrate incubated separately. Our results are also confirmed by those of other authors who showed a considerable increase in the volume of biogas and the methane level in co-digestion [2, 30]. Pouech and Coudure [2], in a co-digestion study of agro-industrial waste with pig slurry found that the production of biogas is tripled with the introduction of a mixture consisting of 95% slurry and only 5% agro-industrial waste. In particular, they obtained a daily production of 270 Nm<sup>3</sup>/d of biogas in the co-digestion phase, compared with 90 Nm<sup>3</sup>/d in the slurry phase alone. Callaghan and Wase [30] demonstrated that the co-digestion of vegetable residues with cow dung and chicken droppings increases the biogas yield from 20% to 50% and the methane content from 0.23 to 0.45 m<sup>3</sup> CH<sub>4</sub> / kg of volatile matter.

These fluctuations in gas production may be explained by the variation of some factors, in the sense that some proportions of substrates in the co-digestion may reduce or increase the organic load of the digester leading to : (i) a considerable slowing of the digester and a decrease of microbial activity may prolonged biogas generation time [34] with consequently, an accumulation of volatile fatty acids leading to the production of a poor biogas with low methane level; (ii) an increase in the microbial activity and, consequently, an increase in the methane yield if the mixture of the substrates leads to a reduction in the organic load of the digester [35, 36].

However, at qualitative level, if the biogas produced is less rich in methane, this reduction can be largely offset by the increase in production and vice versa [2]. Table 5 shows the productions of biogas and methane during the digestion of various co-substrates with bovine manure sludge or pig manure sludge as inoculum.

**Table 5:** Productions of biogas and methane (CH<sub>4</sub>) in relation to substrates combination in presence of bovine manure sludge or pig manure sludge as inoculum (means of 3 replicates).

Substrates combination	Biogas (µl)	CH <sub>4</sub> (µl)	CH <sub>4</sub> (%)
PM/VR (1/3)	43880a	26108a	59.50efg
PM/MW (1/1)	41170a	25513a	61.97bcd
PM/VR (1/1)	41022a	25030a	61.02cdef
PM/MW (1/3)	32670bc	20570bc	62.96ab
BM/MW (1/1)	32290bc	20636.53bc	63.91ab
BM/VR (1/3)	31796c	19242.9c	60.52def
PM/VR (3/1)	30960cd	18690cd	60.37def
BM/MW (1/3)	28559de	18454.72de	64.62a
BM/VR (3/1)	25978ef	14888.01f	57.31f
BM/MW (3/1)	23280f	13504.96f	58.01ef
BM/VR (1/1)	23333f	12464.72g	53.42gh
PM/MW (3/1)	22132fg	12030gh	54.36g

In a column, values sharing a same letter are not significantly different according to the Fisher test at 5% threshold. **PM**= pig manure; **BM**= bovine manure; **MW**= mango waste; **VR**= vegetable residues.

From these results obtained, it appears clearly that the combinations of substrates with pig manure in the presence of pig manure sludge as inoculum presented the best yields of biogas and methane. The best productions of biogas (43,880 µl) and methane (26108 µl) were obtained with the co-substrates PM/VR (1/3) (43880 µl biogas and 26108 µl methane) followed by PM/MW (1/1) (41170 µl biogas and 25513 µl methane) and PM/VR (1/1) (41022 µl biogas and 25030 µl methane) (Table 5). These biogas and methane productions, although not significantly different from one to another, showed significant differences with those of the other substrate combinations.

#### 4. CONCLUSION

The study aimed to find out the most efficient co-substrates for biomethane production during anaerobic digestion process. The results obtained showed clearly that biogas and methane productions from anaerobic digestion of substrates tested separately, were significantly lower than the productions from co-substrates digestion (p <0.0001). Productions can double when going from mono-digestion to co-digestion. Based on a comparative analysis of substrates and co-substrates digestion results, we can assume that co-digestion really improves the parameters of anaerobic digestion, along with biomethane production. Especially, the study revealed that the production can double from mono- to co-digestion. Substrates combinations with pig manure in presence of pig manure sludge as inoculum presented the best yields of biogas and methane. Pig manure supplemented to vegetable residues in 1/3 ratio (PM/VR-1/3) appeared the most efficient co-substrate for biogas and methane productions. Overall, this study findings will be helpful to the National Program of Biogas for providing more renewable energy, along with biomethane to populations for a sustainable development.

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