

Characterization of Wet Mesophilic Biomethanization of Three Types of Materials: Chicken Dung, Rabbit Poop and Pig Slurry

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Abstract: Biomethanization is a process leading to the production of biogas. Characteristics effects of some materials on biomethanization results are not well known by now. That is the reason of studying the effect of the chemical composition of chosen substrates that are chicken dung, pig slurry and rabbit poop on biomethanization characteristics. These substrates of 1 mm particles size and 13.33% water content were first subjected to chemical analysis. Experimentation consisted of mixing 1.3 kg of each substrate with 6.2 L of water for a 15% dry matter content in the final mixture. Biomethanized cow dung (0.81 L) was added as inoculum to each mixture to give a ratio of inoculum volume to mixture volume of 10%. The biomethanization temperature was maintained at 38°C during all the process. The evolution of the composition and the biogas yield of each substrate was monitored using respectively an infrared biogas analyser and a digital manometer installed on each experimental unit. The main results were as follows: the C/N ratio was highest in rabbit poop (28.57), followed by pig slurry (14) and finally chicken dung (11). The organic matter content was also highest in rabbit poop (80%), but followed by chicken dung (65%) and pig slurry (50%). The final methane content was highest in rabbit poop (58.61%), followed by chicken dung (51.59%) and pig slurry (50.83%). The final percentage of carbon dioxide was highest in the pig slurry (12.62%), followed by the rabbit poop (11.31%) and finally the chicken dung (9.98%). In terms of biogas yield and hydraulic retention time, rabbit poop gave the highest yield of 0.109 m³.kg⁻¹ of dry matter in 37 days. This were followed by chicken dung with 0.067 m³.kg⁻¹ of dry matter in 27 days and pig slurry with 0.037 m³.kg⁻¹ of dry matter in 20 days. In the light of these results, the main conclusion is that, more the organic matter content is high and C/N ratio is in the optimal range of 25 to 30, higher are biogas yield and methane content, and longer is the hydraulic retention time.

Keywords: Substrate Characteristics, Biogas Composition Evolution, Yield, Hydraulic Retention Time

1. Introduction

Energy is indispensable for all activities of living beings,

and humans in particular. The global energy deficit is still very high to date in Africa, with an estimate that by the year 2030 if no efforts are made, access to electricity and clean cooking technologies will be low at 42% and 56%

respectively [7]. One of the most interesting forms of energy is biogas, as it has multiple advantages that could help solving some environmental problems. These include: waste treatment; capture of a highly toxic flammable greenhouse gas CH_4 which can be used as energy source for electricity generation [20]; as fuel in gas-powered vehicle, and as cooking gas. Other advantages are that, digestate obtained after biomethanization process can be used as fertilizer in agriculture [19], in the production of biochar [12] and phenol [23] through pyrolysis, and for waste pre-treatment before another biomethanization [5]. Biogas is obtained after the process known as biomethanization or also named as anaerobic fermentation treatment of organic matter (OM) for biogas production mainly consisting of CH_4 (50 - 75%) and CO_2 (25 - 50%) by volume [1]. This process is carried out in four main steps seen from the angle of the evolution of the biogas composition in the biodigester [3]. The present research works focuses little on the evolution of the composition of the biogas during the process. Some authors did it, but in natural methanization over a period of more than 10 years [3]. So by now, little research works have been done on controlled biomethanization processes over reasonable periods of less than 60 days which is often the maximum time observed. Also, some studies showed the links that might exist between the evolution of this biogas composition and the initial substrate characteristics. However, it is said that the yield, hydraulic retention time (HRT) and final composition of the biogas are closely related to the nature and composition of the substrate [2], but also to the biomethanization conditions [4]. This study therefore aimed to highlight the influence that the chemical composition of a substrate could have on the characteristics of the different biomethanization phases. This work is important because nowadays, the challenge in this domain is to optimise yield of biogas and reduce the hydraulic retention time. For this purpose, some practices are commonly applied in the field, like: making the reactor becoming anaerobic by replacing O_2 with inert gases like CO_2 or N_2 ; evacuating first produced biogas after a moment from the beginning because containing too much CO_2 ; or injecting a quantity of H_2 for biogas enrichment [24]. All these practices are applied not at the same period, but not at the right time to get biogas maximum yield and the minimal HRT. And this is because the biogas composition evolution depending of chemical characteristics of substrate is not well known. So, knowing the biogas composition evolution depending on substrate characteristics can help knowing with is the perfect timing to apply any method for optimising biogas yield and HRT.

2. Materials and Methods

2.1. Choice, Treatment and Analysis of Substrates

The three types of substrates used were pig slurry (PS), chicken dung (CD) and rabbit poop (RP), as shown in figure

1. They were selected based on their availability in the context of the study. They were collected in Dschang, Cameroon, whose geographical coordinates are $5^{\circ}25'$ and $5^{\circ}30'$ North Latitude and between 10° and $10^{\circ}5'$ East Longitude.

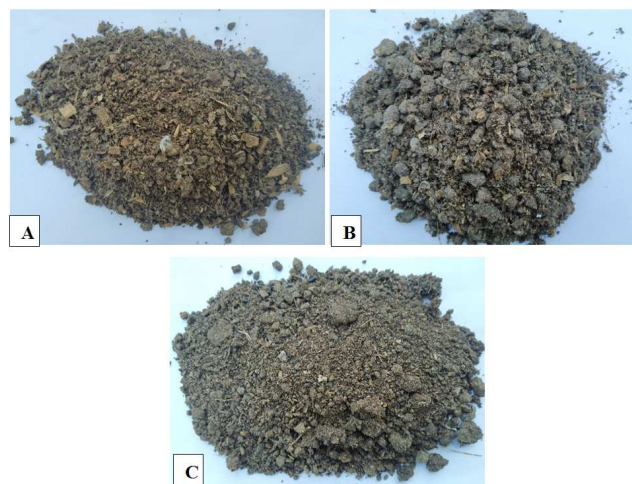


Figure 1. (A) chicken dung, (B) rabbit poop, (C) pig slurry.

The above substrates were dried at 25°C in an electric dryer for 5 days until a common water content of 13.33% was reached. They were then crushed with a pestle and sieved with a 1 mm mesh sieve. Each sieved samples was directly subjected to bromatological analysis using standard methods [17] at the soil analysis and environmental chemistry laboratory of University of Dschang. The investigated parameters in this experiment are those that would affect the quantitative and qualitative yield of biogas [14, 2]. They are among others: carbohydrate (%Gl), lipid (%Li) and protein (%Pr) contents, dry matter content (%DM), organic matter content (%OM), mineral content (N, P, K, Na, Ca, Mg), ash content, energy, C/N ratio, water content, pH.

2.2. Experimental Units and Experimentation

The experimental reactor for each unit is a 14 L capacity metal cylinder. A mass of 1.3 kg of each substrate sample with 13.33% moisture content was weighed with an electric scale and mixed with 6.2 L of water to obtain a mixture with 15% dry matter content [13] for wet digestion. To inoculate the units, previously methanized cow dung was anaerobically fermented at 38°C for 7 days to remove any remaining biogas potential [25]. A volume of 0.81 L of this inoculum was added to the mixture of each unit, representing 10% of the total volume of the final mixture of each unit which was 8.17 L [21]. Once the mixture was made for each substrate, it was introduced into a reactor. In order to ensure constant mesophilic anaerobic fermentation at 38°C [25], the reactors were half-submerged in a marine bath containing a 2000 W heating resistor, water as a heat transfer fluid, and an automatic temperature control system installed. The system is illustrated in the block diagram in Figure 2.

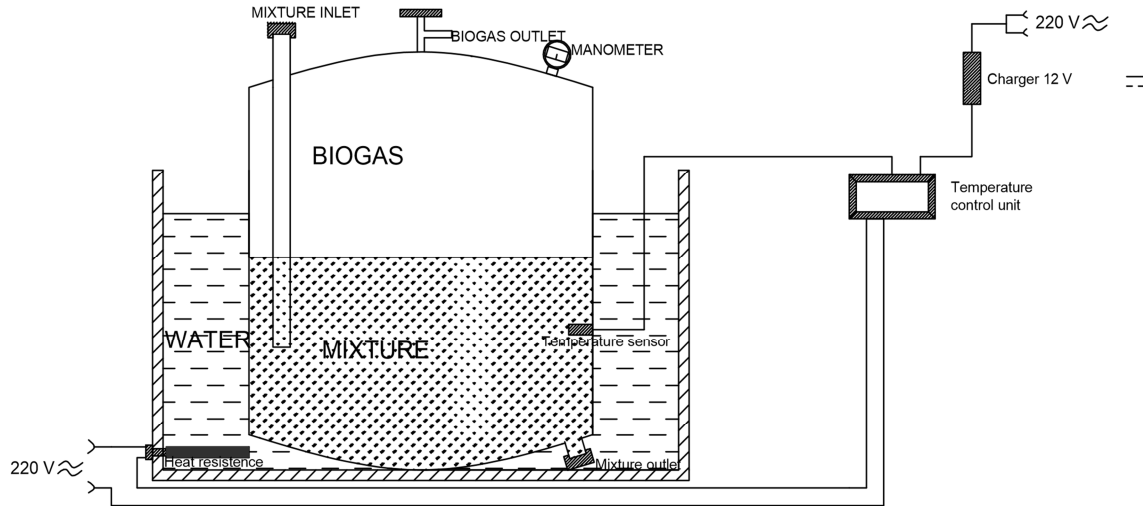


Figure 2. Schematic diagram of the biomethanization units.

The number of repetitions per biomethanization unit of each substrate were three.

2.3. Monitoring the Evolution of the Biogas Composition and Delimiting the Phases

a) Monitoring the evolution of the biogas composition for each substrate

The evolution of biogas composition was monitored every 24 hrs for the duration of the hydraulic retention time (HRT) using a Shenzhen Yiyuntian Electronics Co, Ltd. analyser model MS400. The analysed gas types with measurement range were as follows: CH₄ (0-100% volume); CO₂ (0-100% volume); H₂ (0-40000 ppm); O₂ (0-30% volume).

The absolute pressure in the gasometer of each unit was measured using a digital manometer with an accuracy of 0.01 bar. Two measurements were made per day, before and after the biogas analysis, in order to estimate the pressure drops after each sampling for analysis and also the quantities of biogas lost. The remaining quantities and the additionally produced quantities at the time of the next sampling. The cumulative quantities of each gas species over the duration of the process were estimated, and the evolution curves of each gas species for each of the substrates were made using EXCEL2013 software.

b) Delimitation of the different average phases of biomethanization

The delimitation of phases was based on the classification according to the evolution of the biogas composition. For this, reference was taken to the general behaviour of the gas types at each of the biomethanization phases [3].

2.4. Establishment of the Evolution of Biogas Yield

a) Evolution of biogas volume of each unit

The estimation of biogas volume in each unit was based on data obtained after measuring absolute gas pressures and calculating cumulative quantity in moles of biogas every 24 hrs after biogas analysis. These data were obtained earlier when monitoring the evolution of the chemical composition

of biogas in each unit. Equation 1 was applied to each of these periods in order to obtain an evolution of the cumulative volume in normal temperature and pressure conditions (NTPC) of biogas produced by each unit.

$$V_{bci} = n_{bci} \times V_{mol \text{ NTPC}} \quad (1)$$

Where: V_{bci} is the cumulative volume of biogas produced (m³)

n_{bci} is the cumulative quantity of biogas produced (mol)

$V_{mol \text{ NTPC}}$ is the molar volume in NTPC (22,4 L.mol⁻¹)

b) Evolution of biogas yield per unit mass of dry matter

Biogas yield is a way of estimating the volume of biogas based on a certain dry mass of substrate used for biomethanization. Therefore, in addition to the evolution of cumulative biogas volume for each unit, the cumulative yield was estimated using equation 2.

$$\eta_{bci} = \frac{V_{bci}}{\%MS \times m_i} \quad (2)$$

Where: η_{bci} is the cumulative biogas yield per unit substrate dry matter (m³/kg of DM)

V_{bci} is the cumulative biogas volume produced (m³)

$\%MS$ is the percentage of substrate dry matter (%)

m_i is the initial substrate weight (kg).

2.5. Estimated Hydraulic Retention Time

This HRT for each unit refers to the time in days, from the setting up of the unit, until the moment when no more variation of biogas pressure is observed on the manometer. After delimiting phases of biogas composition evolution for each substrate, the duration or HRT of each of these phases was also observed.

2.6. Statistical Analysis of the Data

All data were subjected to the analysis of variance (ANOVA) test, and results were compared using Fisher test. The means separation and classification was performed by Duncan's test. The software used for all these analyses was

SPSS 23, at a probability level of 5%.

3. Results and Discussion

3.1. Characteristics of the Substrates

The obtained chemical composition of substrates used is presented in Table 1 below.

Table 1. Chemical composition of the substrates.

Parameters	Pig slurry	Rabbit poop	Chicken dung
pH	7.1	7.0	7.9
Lipids (%)	15.66	2	15
Carbohydrates (%)	3.43	26.66	5.78
Proteins (%)	11.11	8.75	18.11
Water content (%)	13.33	13.33	13.33
Dry matter (%)	85	85	85
Organic matter (%)	50	80	65
Ash (%)	50	20	35
C/N	14	28.57	11
Energy (Cal)	199.11	124.25	230.57
Na (mg/kg)	1506.8	315.5	3106.1
K (mg/kg)	11929.84	2366.325	28767.7
Ca (mg/kg)	11960	2880	25560
Mg (mg/kg)	1090.35	826.2	1502.26
N (%)	1.78	1.40	2.9
P (P ₂ O ₅) %	1.41	0.20	1.45
K (K ₂ O) %	1.43	0.23	3.45

The first observation made on results reveals that substrates are different in terms of characteristic values. Pig slurry and chicken dung have a higher lipid, protein and energy content than rabbit poop. On the other hand, rabbit poop has a higher C/N (28.57), followed by pig slurry (14) and chicken dung (11). These results are in perfect agreement with some researches [10] who obtained a C/N ratio of slurry averaging 14 and poultry droppings around 11. Rabbit poop also has the highest organic matter content (80%), followed by chicken dung (65%) and pig slurry (50%). These differences may be explained by the fact that pigs, hens and rabbits have different digestive systems in terms of feed degradation and nutrient removal. They could also be explained by the fact that the dietary compositions of these three types of animals are fundamentally different, as ingredients are not necessarily the same, and in different proportions [22, 8, 15]. Hence, the importance of having carried out these analyses because in another context, different results could have been obtained.

3.2. Evolution of the Chemical Composition of Biogas of the 3 Types of Substrates

The graphs showing the evolution curves of the different types of gases are figure 3 for CH₄, figure 4 for CO₂, figure 5 for O₂ and figure 6 for H₂.

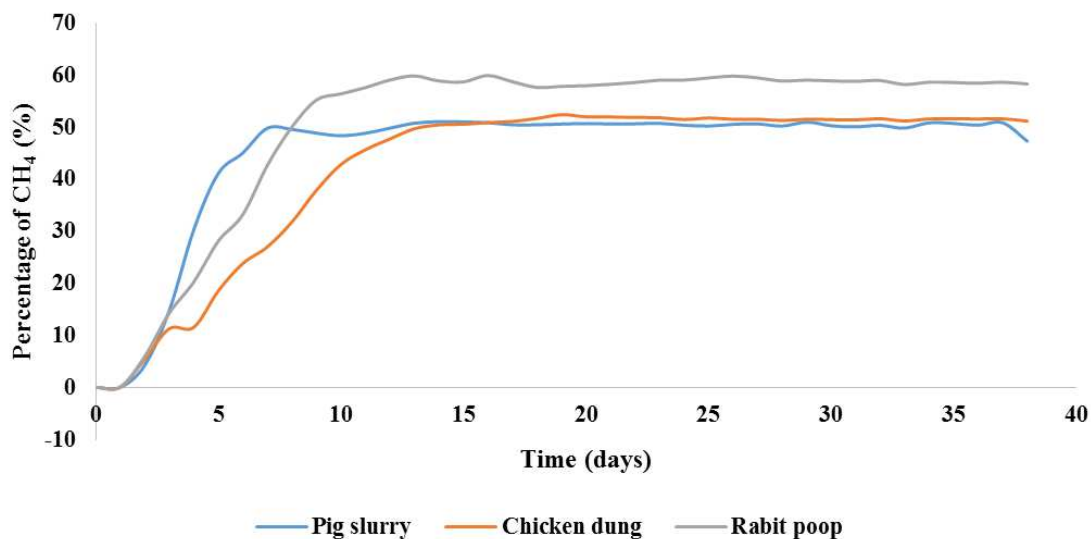


Figure 3. CH₄ evolution curve of the studied substrates.

3.2.1. Evolution of Methane During Biomethanization of Substrates

The observation of Figure 3 shows an almost identical growth of the CH₄ percentage during the first three days until reaching a percentage of 14.73% for pig slurry, 14.26% for rabbit poop and 11.28% for chicken dung. Thereafter, pig slurry stood out with a faster growth until it reached its CH₄ percentage of 49.78% on day 7, followed by rabbit poop with 43.62% and chicken dung with 26.98%. After this seventh day, CH₄ percentage of pig slurry became almost constant around 49 and 50% until the end with a percentage of

50.83%. The CH₄ content of rabbit poop continued to increase and exceed that of pig slurry on day eight, reaching 59.83% on day thirteen, and then fluctuated between 58 and 59% to reach a final value of 58.61% CH₄. The CH₄ content of chicken dung also continued to increase and exceed that of pig slurry on day 17 with a value of 51.06%, then remained constant around 51% until reaching a value of 51.59% at the end. Statistical analysis reveals that final CH₄ percentage of rabbit poop (58.61%) is significantly higher than those of pig slurry (50.83%) and chicken dung (51.59%). These CH₄ percentages are nevertheless within the range of 50-75% [1], despite the fact that biogas was not dehydrated before

analysis, so part of the volume is H_2O . Proteins generally give the highest CH_4 content, followed by lipids and finally carbohydrates [2]. But here we find that rabbit poop, although it is lower in protein (8.75%) and lipids (2%) than chicken dung (18.11% and 15% respectively) and pig slurry (11.11% and 15.66% respectively), it still has the highest CH_4 content. This is firstly due to the fact that it also has a significantly higher carbohydrate content (26.66%) than

chicken dung (5.78%) and pig slurry (3.43%). Secondly, rabbit poop has a C/N ratio (28.57) within the optimal biomethanization range of 25-30 [5, 10], and is significantly higher than pig slurry (14) and chicken dung (11). Finally, rabbit poop has a significantly higher organic matter content (80%) than chicken dung (65%) and pig slurry (50%), making it more suitable for degradation and methane production.

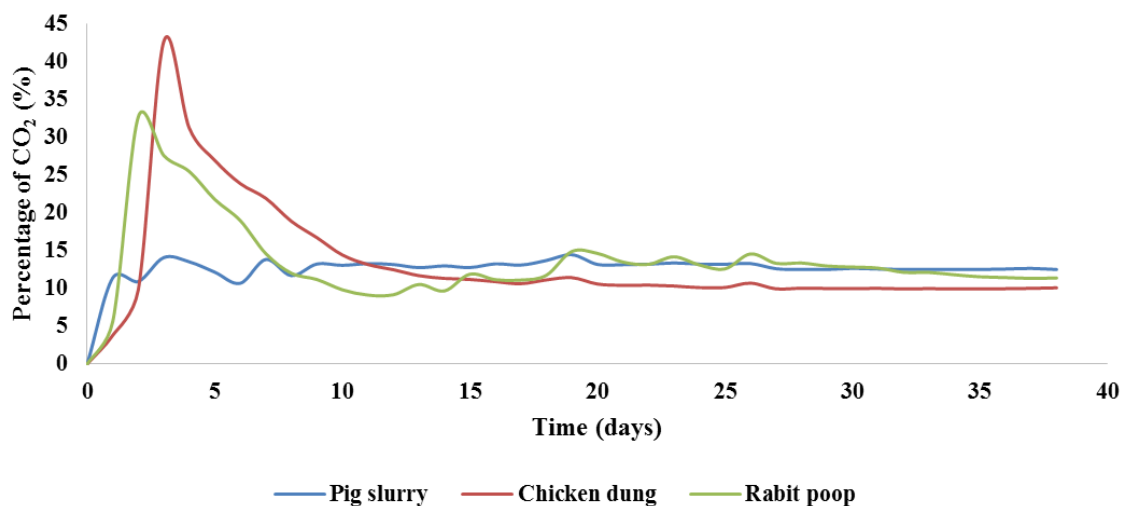


Figure 4. CO_2 evolution curve of the studied substrates.

3.2.2. Evolution of Carbon Dioxide During Biomethanization of Substrates

Figure 4 shows an increasing CO_2 production from day one for all three substrates. But very soon, pig slurry stood out and reached 11.38% after the first day, showing a rapid degradation (hydrolysis and acidogenesis) producing CO_2 , while rabbit poop was at 5.61% and chicken dung at 3.79%. This could be due to the lower organic matter content (50%) in pig slurry, meaning a lower organic load for the same microbial seed size than rabbit poop and chicken dung which have significantly higher organic loads (80% and 65% respectively). CO_2 level of rabbit poop then increased faster to maximum value on day 2 at 32.66%, followed by pig slurry dropping to 10.89% and chicken dung increasing to 9.79%. Thereafter, chicken dung CO_2 percentage continued to grow until it reached its maximum value at 42.64% on the third day, while rabbit poop percentage dropped to 27.55%, and pig slurry percentage fluctuated between 12 and 13% to reach 12.62% at the end. After the third day, CO_2 levels of chicken dung and pig slurry continued to fall, reaching values of 11.16% and 11.86% respectively on day 15. Their levels then fluctuated around these values until at the end they reached 9.98% for chicken dung significantly lower than 11.31% for rabbit poop and 12.62% for pig slurry. The observation made here is that these CO_2 levels are much lower than the usual standards often located between 25 and 50% CO_2 [1]. This can be explained by the fact that in this study, biogas samples were not dehydrated before analysis, which means that in relation to the overall volume of biogas, a non-negligible part is

occupied by the water vapour present. However, despite this, it could still be said that maintaining the experimental units under optimal biomethanization conditions allowed good CO_2 consumption for the production of CH_4 by hydrogenotrophic methanogens. This is not the case in biodigesters located in natural conditions, with all temperature variations that do not favour microbial activity.

3.2.3. Evolution of Oxygen During Biomethanization of Substrates

The graph in figure 5 shows the general behaviour of O_2 during biomethanization of the three studied substrates. It can be seen that O_2 percentage dropped rapidly from 20.78% to 0.62% for pig slurry and 1.12% for rabbit poop after the first day, compared to 7.58% for chicken dung, which is significantly higher. This can be explained by the fact that rabbit poop and pig slurry have more neutral pH values (7.0 and 7.1 respectively) than chicken dung (7.9) at the beginning of the process before various variations due to reactions. Aerobic microorganisms in this phase grow best at neutral pH, so O_2 consumption was faster in pig slurry and rabbit poop than in chicken dung. Chicken dung continued to drop in O_2 to 0.28% on day 6, while pig slurry dropped to 0.78% and rabbit poop rose sharply to 8.23% before dropping back to 0.04% the next day. The rest of the process saw O_2 levels of the three substrates fluctuate around values ranging from 0.04% to 2% until day twenty-seven. After this period, O_2 level continued to fluctuate, reaching at the end 0.03% for rabbit poop significantly lower than 1.97% for pig slurry and 2.41% for chicken dung. This corroborates the results [9] of O_2 levels between 0 and 2% of the final biogas.

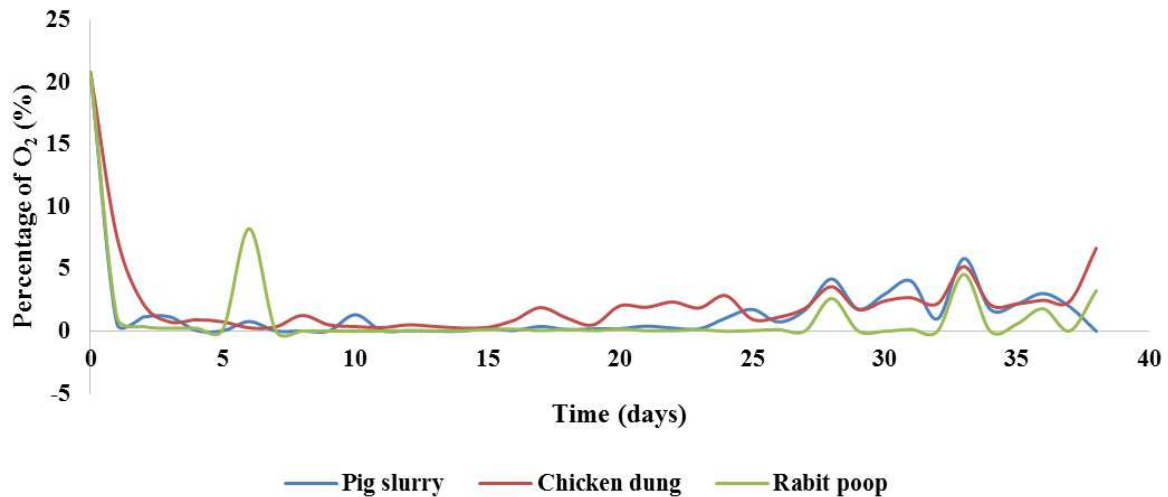


Figure 5. O_2 evolution curve of the studied substrates.

3.2.4. Evolution of Dihydrogen During Biomethanization of Substrates

The general observation is that H_2 is present throughout the process in very low quantities, not exceeding 0.40%. Absent from the medium at the beginning, a first rapid increase of H_2 level takes place on the second day to reach the maximum value in rabbit poop at 0.397% and in pig slurry at 0.025%, followed by chicken dung which reached its maximum value 0.123% on the fourth day. The most responsible parameter for this behaviour would be the

organic matter content. This is because it is higher in rabbit poop (80%), followed by chicken dung (65%) and pig slurry (50%), hence the different performances observed. After day 5, there followed a long period until day 33 with this H_2 value varying from 0.000 to 0.001% for all substrates. During the rest of the time, this value rose slightly to oscillate between 0.003% and 0.060% until it finally reached 0.017% for pig slurry, 0.014% for rabbit poop and 0.021% for chicken dung. This is usually observed with H_2 levels not exceeding 1% in the final biogas composition [9].

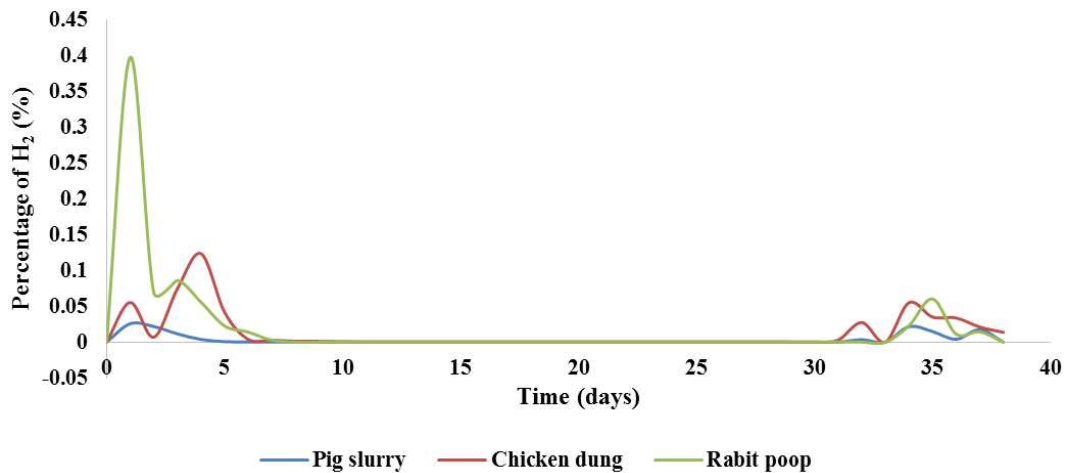


Figure 6. H_2 evolution curve of the studied substrates.

3.2.5. Overall Evolution of Biogas Composition During Biomethanization of Substrates

Figure 7 shows the graph representing the overall evolution of the average composition of the biogas produced by the three substrates over the HRT period which was 37 days.

The graph in figure 7 shows the average evolution over time of the four gases types in the biogas, namely CH_4 , CO_2 , O_2 and H_2 , produced by the three substrates studied. It can be

seen from this graph that there are four phases of biogas composition evolution [3] that can be clearly defined over a period of 37 days, but with the difference that it extends over 10 years in natural biomethanization.

Firstly, there is aerobic fermentation phase, which lasted for the first two days of the process. During this phase, O_2 level dropped from 20.78% to 1.24%, but CO_2 and CH_4 levels appeared and grew rapidly, reaching 17.78% and 5.19% respectively. Hydrogen is in trace form at 0.032%. This composition is almost similar to the universal graph [3]

which shows at this stage about 20% of CO_2 , traces of O_2 , H_2 and CH_4 . This could be explained by the fact that O_2 was consumed by the facultative aerotolerant microorganisms to hydrolyse the substrate into monomers which will then be used as an energy source for other metabolisms [16]. Next, anaerobic microorganisms begin to produce CO_2 and CH_4 , which increase more as O_2 level in the environment decreases.

Then comes the second phase, which is that of acid anaerobic fermentation lasting one day. During this phase, O_2 level remained low at 0.71%. CO_2 reached its maximum value of 28.08% at the end of this phase, and CH_4 continued to rise to 13.42%. H_2 is still present as a

trace at 0.057%. This behaviour is similar but with different values from the universal graph [3] which shows the maximum value of CO_2 and H_2 at 80% and 20% respectively, O_2 and CH_4 in traces). This is due to the fact that the medium being anaerobic, it produces monomers from hydrolysis, volatile fatty acids (propionate, butyrate, valerate, pyruvate and lactate) making the medium acidic, and containing alcohols (ethanol and methanol) [11]. In the course of these reactions, CO_2 also appears as a product, hence its increase in the medium. At the same time, part of this CO_2 is combined with H_2 produced to give CH_4 , that is why CH_4 also increases in the medium and H_2 remains as a trace.

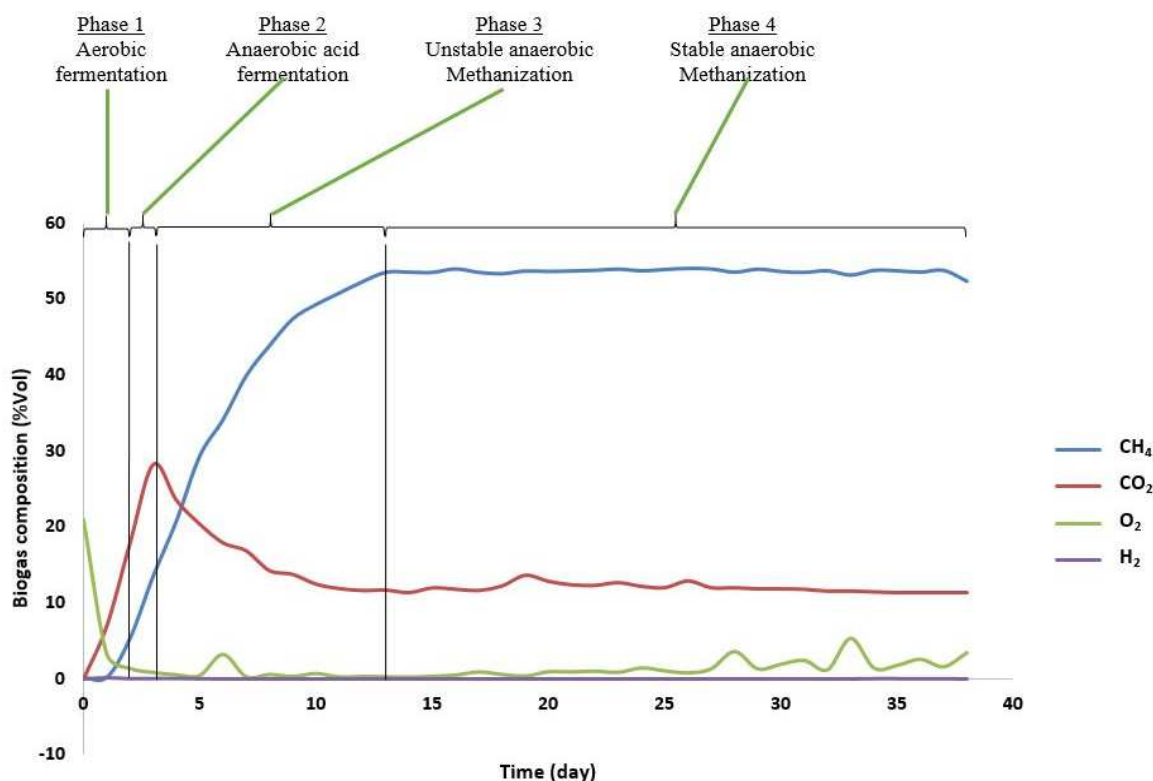


Figure 7. Average curve of the overall evolution of the biogas composition during biomethanization.

The third phase was that of unstable anaerobic methanization, which lasted for the next 10 days. It was marked by a trace presence of O_2 (between 0.14% and 3.09%) and H_2 with its maximum value at 0.06%. CO_2 level continued to decrease until it reached 11.63% on the thirteenth day. CH_4 level continued to increase, exceeding CO_2 after day 4, reaching 53.42% on day 13. This behaviour is similar but with some deviations in percentage values from that of the universal graph [3] of which about 55% CH_4 , 45% CO_2 , O_2 and H_2 in traces are present. Explanation given for this behaviour is that as the environment was already anaerobic, population of methanogens increased exponentially, leading to an increase in CH_4 . For the production of CH_4 , acetoclast methanogens used acetate produced from the fatty acids and alcohols of the previous phase. And hydrogenotrophic methanogens used H_2 that had reached its maximum value, in combination with a large part

of CO_2 present in the medium, to produce CH_4 , hence the drop in the levels of CO_2 and H_2 and the increase in CH_4 level during this phase.

The fourth and final phase was stable anaerobic methanization, which lasted for the last 25 days. It was characterised by the maintenance of relatively constant margins of CH_4 (53.24 to 53.94%) and CO_2 (11.12 to 13.57%). O_2 and H_2 still in trace with respective percentage margins of 0.10 to 5.20% and 0.00 and 0.03%. This behaviour is similar but with some deviations in percentage values from that of the universal graph [3], in approximately maintaining the composition of 55% CH_4 , 45% CO_2 , O_2 and H_2 in traces. This behaviour would be due to the fact that during this last phase, there was continuity of CH_4 production by acetoclast methanogens, but a scarcity of H_2 production, thus causing a stabilisation of CO_2 level. This made it possible to maintain a constant difference between CH_4 and

CO₂ percentages, which averaged 41.24% until the end of the process.

The results expressed by the graph of figure 7 can now serve as a basic graph for every one wishing to apply any method to optimize biogas production. Because it illustrates phases periods, the behaviour of the different gases types within each phase, and this in a reasonable period of time of 37 days usually observed for controlled biomethanization.

3.3. Evolution of Biogas Yield and HRT of Biomethanization of the 3 Substrate Types

Evolution of biogas yield for each substrate is related to its HRT, because when it becomes constant, it means that there is no more additional biogas production, thus marking the end of biomethanization process. Figure 6 below shows the evolution of biogas yield during the process for each of the 3 substrates.

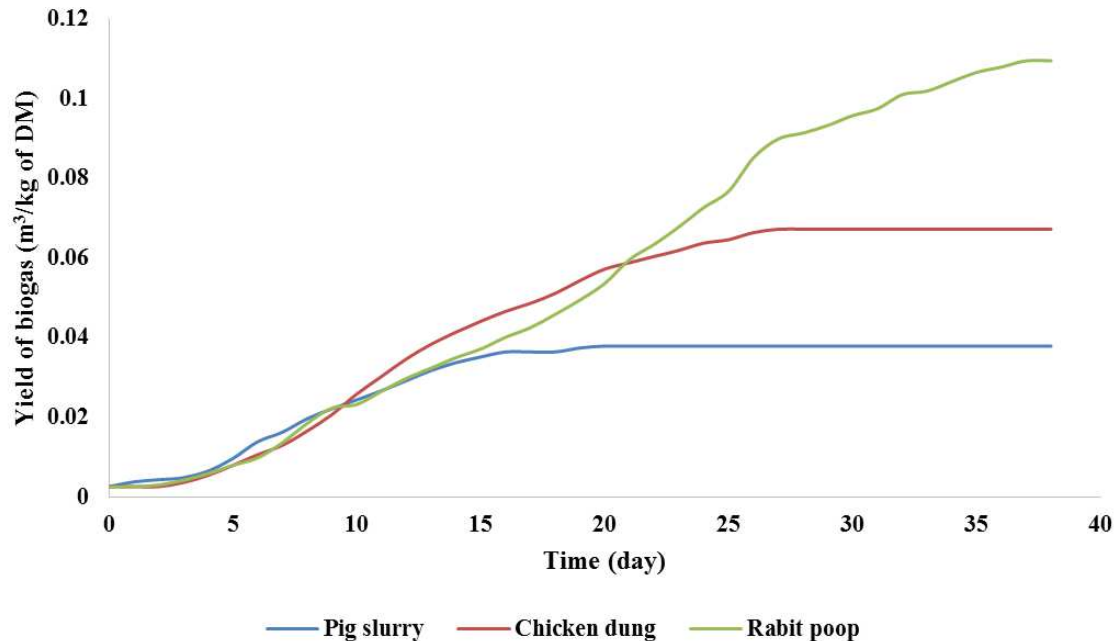


Figure 8. Evolution curve of the biogas yield of the studied substrates.

Figure 8 illustrates that the three substrates showed the trend of almost similar evolution during the first ten days, reaching on average a biogas yield of 0.024 m³.kg⁻¹ of dry matter. After the tenth day, chicken dung stood out from rabbit poop and followed by pig slurry by having the highest yield until the twenty-first day. From day twenty-two until the end of the process on day thirty-seven (37 days), rabbit poop dominated, achieving a final yield of 0.109 m³.kg⁻¹ of dry matter. It was significantly higher than chicken dung, which grew to its maximum yield of 0.067 m³.kg⁻¹ of dry matter by day 27 and remained constant for the rest of the time. Pig slurry were significantly the lowest with a maximum yield of 0.037 m³.kg⁻¹ of dry matter on day 20 and remained constant for the rest of the time. These results are in line with results of a yield of 0.036 m³.kg⁻¹ of dry matter for the pig slurry [6], but with a lower yield than that of this study of 0.044 m³.kg⁻¹ of dry matter for chicken dung. The HRT required for biomethanization of these substrates were 37 days for rabbit poop, 27 days for chicken dung and 20 days for pig slurry, in line with 15 days for slurry in wet mesophilic digestion [6]. The most convincing parameter with the greatest influence on biogas yield and HRT is OM content [18]. Rabbit poop has the highest yield (0.109 m³.kg⁻¹ of dry matter) and HRT (37 days) with the highest organic matter content (80%). It is followed by chicken dung with a

yield of 0.067 m³.kg⁻¹ of dry matter) and HRT of 27 days with the organic matter content of 65%. And finally pig slurry with a yield of 0.037 m³.kg⁻¹ of dry matter and 20 days HRT with the lowest organic matter content of 50%.

4. Conclusions

The three types of substrates studied, that are pig slurry, chicken dung and rabbit poop, have different characteristics in terms of chemical composition, according to laboratory analyses. Rabbit poop is richer in organic matter content (80%), carbohydrate (26.66%) and C/N ratio (28.57) which is optimal for biomethanization. Pig slurry and chicken dung on the other hand have higher lipid (15.66% and 15.00% respectively), protein (11.11% and 18.11% respectively) contents. Following biomethanization process, it was found that the initial characteristics of the substrate have influence on the evolution of the process, with the C/N ratio influencing more the evolution of the chemical composition of the biogas, and the organic matter content influencing more the yield and the HRT. This is due to the fact that the results at the end of the process give a CH₄ percentage of 58.61% and a CO₂ percentage of 11.31% for rabbit poop, followed by chicken dung with a CH₄ percentage of 51.59% and a CO₂ percentage of 9.98%, and finally pig slurry with a

CH₄ percentage of 50.83% and a CO₂ percentage of 12.62%. The yield and HRT evolved in the same direction with the substrate composition, with rabbit poop giving the highest yield of 0.109 m³.kg⁻¹ of dry matter and the longest HRT of 37 days, followed by chicken dung with a yield of 0.067 m³.kg⁻¹ of dry matter and an HRT of 27 days, and finally pig slurry with the lowest yield of 0.037 m³.kg⁻¹ of dry matter and a lowest HRT of 20 days.

5. Recommendations for Follow up

Future studies could focus on: establishing a model to predict the behaviour of the biogas compositional evolution as a function of the chemical characteristics of any substrate; applying various biogas purification methods in each phase of the biogas composition evolution, to find out which phase is optimal in terms of yield and energy efficiency as many of these methods are also energy consuming.

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