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## **Biogas Production from Fish Waste and Filao Litter**

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**Abstract** The growing exploitation of fossil fuel reserves to meet the high demand of energy is becoming the main source of environmental pollution. So it urges to find safe, renewable and sustainable energy resources. This copes with Senegal new governmental energy policies which aim to decrease the dependence to fuel importations and wood consumption, as well as to develop renewable energies. In this context, valorization of waste into energy for productive uses has been identified, for Senegal, as an alternate for environmentally sustainable economic growth and progression towards meeting environmental mitigation targets. Moreover such valorization allows to address another challenging problem for the developing countries: the disposal of municipal and industrial solid waste which is a major contributor to soil pollution. This study focuses on the investigation of co-digestion of two wastes: filao litter and fishery waste that cause many environmental problems in Senegal. These two organic matters present different contents in terms of carbon and Nitrogen. The first presents a high carbon to nitrogen ratio (76.609), while the latter has a low ratio (6.751). Thus, taken separately, these substrates won't have interesting biomethane potential, but their combination may produce a great co-substrate for biomethanization process. For methanization, we obtained biogas production with a maximum percentage of 51.3% methane under a variable temperature ranging from 23.6 °C to 27.8 °C.

**Keywords** biogas; biomethanization; co-digestion; TOC; N; C/N; methane

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### **Introduction**

In Senegal, renewable energies have not been widely used. They were rather confined to the experimental state in the field of photovoltaic energy. In 2015, Senegal launched an economic development plan (Emergent Senegal Plan), in which increasing energy production from renewable sources is considered a top priority for the development of the economy. In addition, new government energy policies aim to reduce dependence on imported fossil fuels and the consumption of wood [1].

In this context, the valorization of waste into energy for productive use has been identified as a potential lever for economic growth and progress towards environmental protection objectives. These wastes come mainly from the agro-food industries, fishing and urban wastewater discharges [2, 3]. With a growing and increasingly diversified consumption throughout the country, and particularly in coastal areas, the production of waste is constantly increasing in quantity. It generates enormous nuisances on the environment and, consequently, public health. We can see that fish waste causes enormous problems on markets and fishing docks because of the odors it gives off. At the same way, Senegal has an important deposit of filao litter in the Niayes area. This area has become more and more inhabited, the filao litter can be the cause of bush fires and insalubrity of this strip. The valorization of this fish waste and filao litter in methanization would be an alternative for the reduction of the impact of this waste on the environment.



Methanization or anaerobic digestion is a process of degradation of organic matter by micro-organisms without oxygen inside hermetic tanks called digesters in wet or dry environment. [4, 5, 6, 7].

Filao litter has a high carbon content and low nitrogen value. Fish waste has a low carbon to nitrogen ratio based on studies that have been conducted on the characterization of organic waste [8]. Hence the interest to combine it with the filao litter to increase its C/N ratio in order to obtain a methanizable co-substrate.

This transformation of matter can answer a double objective of energy valorization of waste, namely the recovery of biogas and the stabilization of organic waste. The biogas produced is used as fuel and the digestate produced solid of the anaerobic digestion is usable as fertilizer in the agricultural lands.

The present work aimed to study the production of biogas by co-digestion of fish waste and filao litter. In this perspective, we will make a physico-chemical characterization in the laboratory to evaluate the methanogenic potential of the co-substrate before performing a biogas production test. In the latter, we monitored the pH evolution and the biogas composition ( $\text{CH}_4$  ;  $\text{CO}_2$  and  $\text{H}_2\text{S}$ ).

## Materials and Methods

### Feedstock Collection and Preparation

The raw materials used were fish waste and filao litter. They were collected, sorted and crushed because anaerobic digestion is more suitable for fine particle substrates. The fish waste (Figure 1) was collected at the central market of Thies, while the filao litter (Figure 2), a plant found mostly on the seashore of sand dunes, was collected on the beach of Mboro. After collection, these two wastes were sorted, crushed and well preserved to avoid alteration. The filao litter was kept dry, while a quantity of one kg of fish waste (sardinella waste) was kept cold ( $0 - 4^\circ\text{C}$ ). Physico-chemical analyses are subsequently performed on the substrates. Figures 1 and 2 show the processes of fish waste and filao litter preparation. A mixed substrate consisting of 50% fish waste and 50% filao litter was also prepared.



Figure 1: Fish waste preparation. A: collection. B: sorting. C: grinding. D: final product.





Figure 2: Filao litter presentation. A: Collection. B: Sorting, C: Ground litter

### Physico-chemical characterization of substrates

The determination of the physico-chemical parameters of the substrates, namely organic matter (OM), mineral matter (MM), chemical oxygen demand (COD), total organic carbon and total nitrogen, was carried out using standardized methods often used in the literature. In order to have conclusive results, two different determination methods were used in this study.

### Dry Matter

The dry matter content is deduced from the water content measured by loss of mass of the sample after passage in the oven at 105 °C for 24 hours. It is determined in triplicate in crucibles previously dried on masses of 1 to 10 g of material [8, 9]. The percentage of dry matter of the organic waste is determined in relation to the weight of the sample before and after drying until the stabilization of the mass, as well as the humidity, by the following formulas:

$$\% \text{ DM} = \frac{M_1}{M_0} \times 100 \quad (1)$$

$$\% \text{ H} = 100 - \% \text{ MS} \quad (2)$$

% DM: percentage of dry matter

$M_1$ : Mass dried at 105 °C

$M_0$ : Mass of the initial sample

% H: percentage of humidity

### Organic Matter

Any mass of the sample after drying,  $M_1$  is introduced into a capsule previously cleaned and dried for the determination of organic matter (OM). The whole is placed in a muffle furnace for calcination at a temperature of 600 °C for 6 hours.

After cooling the capsule containing the mineral matter (ash) is weighed again and a mass  $M_2$  (ash mass) is noted. The percentage of organic matter is obtained by the difference between the dry matter and the mineral matter.

The percentage of organic matter (OM) is determined by the following equation.

$$\% \text{ OM} = \frac{(M_1 - M_2) \times 100}{M_1} \quad (3)$$



$M_2$  : Ash mass after calcination at 600 °C

### Mineral Matter

Mineral matter (MM) is the mass of ash obtained after incineration at 600 °C for 6 hours. It has the expression of total ash. Accordingly, the measurement of the weight of the residual ash fraction by loss on ignition allowed the determination of the percentage of the mineral fraction in the given waste by the following formula

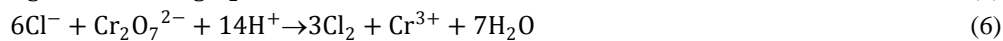
$$\% \text{ MM} = \frac{M_2 \times 100}{M_1} \quad (4)$$

% MM: percentage of mineral matter.

### Chemical Oxygen Demand

The Chemical Oxygen Demand is a measure of organic matter contained in natural waste; whether or not it is biodegradable.

It is done under the action of a strong oxidant (potassium dichromate  $K_2Cr_2O_7$ ), in the presence of a strong acid ( $H_2SO_4$ ) and under energetic conditions (2h reflux). Most of the organic matter is oxidized to  $CO_2$  and  $H_2O$ . Silver sulfate is used as a catalyst ( $Ag_2SO_4$ ). The presence of halogens (chlorides, bromides and iodides) causes interferences. They consume part of the catalyst and the dichromate.



The sample is refluxed in an acidic medium and in the presence of a known quantity of potassium dichromate in excess via a COD neutralizer consisting of a temperature controller and a hot plate heated to 155 °C. Once the reaction is completed, the residual dichromate is dosed by the addition of ammonium sulfate (ASF).

The indicator used is the ferroine which at the equivalence changes from blue-green to red. The amount of dichromate consumed is measured by difference. The initial quantity of dichromate is determined by the control. This procedure allows to take into account the inevitable losses of the oxidant. The COD expressed in mg/L is determined as follows:

$$\text{COD} = \frac{(A-B) \times N_{ASF}}{V_{\text{sample}}} \times 8000 \quad (7)$$

A: volume in ml of ASF used to determine the control

B: volume of ASF used to determine the sample

$N_{ASF}$  : ASF normality

V: volume of the sample

### Total Organic Carbon

The total organic carbon was also determined by the volumetric titration method. Indeed a solution of potassium dichromate is added to the sample in the presence of sulfuric acid. After the reaction, the quantity of dichromate that did not react with the sample is determined to establish the concentration of total organic carbon. Thus the results are expressed in % C. Considering the concentrations of ferrous sulfate and potassium dichromate, each ml of dichromate that reacts corresponds to 4 mg of carbon [10].

The percentage of carbon in the sample expressed in % C is determined as follows:

$$\% \text{ C} = \frac{(A-B) \times 20 \times 0,004 \times 100}{P \times A} \quad (8)$$

C: concentration of total organic carbon in the material

A: volume of ferrous sulfate used for the control (ml)

B: volume of ferrous sulfate used for the sample (ml)

20: volume of dichromate added initially

0.004: number of grams of C per ml of dichromate

P: weight of the titrated sample expressed on dry basis

100: factor to obtain a percentage



### Total Organic Nitrogen

The method used is a method for the determination of nitrogen (N) content in agricultural land (in ammonium, nitrate, nitrite and organic form). It is based on Kjeldahl mineralization, but the catalyst used is titanium dioxide (TiO<sub>2</sub>), which is much less harmful to the environment than selenium. The principle is based on three steps: a mineralization phase, a distillation phase and a titration phase.

### Digestion or Mineralization

Protein nitrogen from organic waste is converted to ammonia nitrogen by oxidation of the organic material in concentrated sulfuric acid at high temperature in the presence of a catalyst and a salt.

### Distillation

Before steam distillation of ammonia, ammonia must be released as the salt (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> by the addition of a concentrated solution of excess NaOH: the ammonia is then steam distilled and trapped in a boric acid solution. The ammonia reacts with the boric acid to form ammonium borate salts.

### Titration of the Distillate

Ammonia in the form of ammonium borates is titrated directly with a standardized acid solution (HCl) and a colored indicator. A blank is made by putting all the reagents except the sample, to subtract the ammonia contained in the reagents [11, 8].

The nitrogen content is expressed in milligrams per gram of dry matter. It is obtained from the following equation:

$$N = \frac{V_1 - V_0 \times c(H^+) \times 14}{m} \times (100 + wH_2O) / 100 \quad (9)$$

N: Nitrogen content (mg /g) ;

V<sub>1</sub>: Volume in milliliter of chloridric acid used for the determination of the sample;

V<sub>0</sub>: Volume in milliliters of chloridric acid used for the blank;

c(H<sup>+</sup>): The concentration in H<sup>+</sup> of the chloridric acid used is equal to 0.02M;

14 : Molar mass of nitrogen;

wH<sub>2</sub>O : The water content, expressed as a percentage by mass of the sample;

M: the mass in grams of the air-dried sample.

### Phosphorus

Phosphorus (P) analysis was done by the photometric method using an AL800 spectrophotometer and AQUALYTIC tank tests. The organic matter undergoes mineralization in an acidic medium by transforming the organic phosphoric compounds into orthophosphate. The solution thus obtained is put in a volumetric flask of one liter and supplemented with distilled water. The method for determining total phosphate LR with a spectrophotometer cuvette test in the range (0.07 – 3) mg/l P is used.

### Design of the experimental setup

The digester designed for this study is a home-made bioreactor operating in batch mode. The digester is filled with the organic matter to be treated and left until its exhaustion, called residence time. If all the material is degraded, the digester is emptied and a new cycle starts.

Our device was realized at the Laboratory of Sciences and Techniques of Water and Environment (LaSTEE) of the Polytechnic School of Thiès. It consists of an assembly of a bottle of mineral water of 1.25 liter and a bucket of 5 kg. The bottle is pierced all around its lateral surface with an opening coefficient to ensure a fluidity of the substrate from the bottle to the bucket. The base of the bottle is attached to the bottom of the bucket. It is designed to withstand environmental conditions.

After assembly, the cap is drilled with a hole and connected to a 9 mm diameter, 1 m long pipe for biogas recovery. The other end of the pipe is equipped with a valve.



A tightness test has been carried out. This consisted of putting soap foam around the cap and pumping air into the bottle. If bubbles escape, the digester is not watertight. The water tightness was reinforced with Teflon until there are no more bubbles.

### **Biogas production test**

Methanization is an anaerobic phenomenon so the degradation takes place inside the bottle. The biodigester is placed in a thermally insulated building where the temperature variation (23.6 and 27.8) is not too important for a period of 82 days. During all this time, regular measurements (every 2 days) of the biogas produced are made with a biogas analyzer and the monitoring of the extrinsic parameters (pH and temperature).



*Figure 3: Experimental setup*

### **Monitoring of Biogas Composition**

The biogas analyzer used in this study is a GEM 5 000 (Geotech [www.geotechuk.com](http://www.geotechuk.com), United Kingdom). This apparatus allows to measure among others the percentages in  $\text{CH}_4$ ,  $\text{CO}_2$ ,  $\text{O}_2$ . For  $\text{CH}_4$  and  $\text{CO}_2$ , the analyzer can measure ranges from 0 to 100% with an increased accuracy of  $\pm 0.5\%$  after calibration, while for  $\text{O}_2$  the measurement range is 0 to 25%. It also determines the rate of hydrogen sulfide expressed in parts per million (ppm). It comes with the GAM (Gas Analyser Manager Software) with USB port for data retrieval and analysis. The biogas analyzer measures the static and differential pressure of the gas produced, but it does not have a biogas storage system.



*Figure 4: Biogas analyzer*



### **Monitoring of the temperature and pH of the substrates**

Temperature and pH are physico-chemical parameters that also influence the yield of biogas production. They were measured using a portable pH meter type HANNA 83 141 (HANNA instruments, [www.hannainst.com](http://www.hannainst.com), Romania). The calibration was done with a buffer solution of pH 4 and 7. It is composed of a temperature and pH probe.

## **Results and Discussion**

### **Physicochemical Characteristics of the Substrates**

Given the importance of the composition of the substrate in all bioprocesses, especially methanization, the test of biogas production is always preceded by a physicochemical characterization study of the substrate in question. The content of certain parameters is not negligible for the methanogenic potential of the organic matter. This work, carried out on a laboratory scale, reveals the main results of the physico-chemical characterization of fish waste and filao litter and of the biogas produced by the co-substrate.

### **Dry Matter**

The results of the dry matter content of the fish waste, filao litter and the mixture are recorded in the table below.

The fish waste has a dry matter content of 27.913%. This value is slightly higher than the frequently quoted percentage for methanatable organic matter [8]. This is a good indication for the valorization of the waste into methane. This result is in perfect corroboration with those of M. E. Afilal *et al.* who used fish waste and other types of waste such as poultry viscera waste, pet food waste and broiler droppings [8].

On the other hand, filao litter has a dry matter content of 97.843% and a moisture content of 2.156%. These results are similar to those of Afilal *et al.* in the study of methane fermentation of different organic substrates such as aromatic and medicinal plants (MAP), microbial leavening and the mixture of MAP with cow dung. These substrates had moisture contents of 7%, 11% and 6%, respectively, and produced acceptable biogas yields [12].

Indeed, plants with low moisture content are organic waste rich in cellulose which is a source of carbon for bacteria. Therefore, the filao litter alone does not have a methanogenic potential, but its richness in carbon constitutes an asset for co-digestion.

For a substrate to be suitable for biogas production, the feedstock must contain at least 10% to 15% dry matter [2]. In this study, the fish waste and filao litter mixture has a dry matter content of 64.52%, which is well above the minimum value for a substrate to be methanizable [6]. This is a favorable asset for the substrate. This content is comparable to the values found in the work of Afilal *et al.* [12].

### **Organic Matter**

In this study, fish waste and filao litter had organic matter contents of 83.60% and 90.50%, respectively. This richness in organic compounds is a considerable advantage for methanization [13], except that this parameter does not inform on the biodegradability of the substrate, and is far from being sufficient to determine the methanogenic potential of a substrate. Moreover, it has been found that despite its high organic matter content, fish waste has a low biogas production yield [8].

And, the work of Boutouta *et al.* in the Study of biomethanization in batch reactor applied to solid waste of the city of Blida present an organic matter content of 90.60% [14]. Moreover, the biodegradability of solid waste is verified by its content of COD and BOD<sub>5</sub> unlike fish waste and filao litter. So we can say that the organic matter content is not enough to validate the biodegradability of a substrate

### **Mineral Matter**

It represents the part of the non-degradable matter. Ash represents 16.40% of the fish waste. This value is higher than the value found for filao litter 9.51%, and even for the mixture 11.11%.

It can be said that the degradable part of the fish waste is less than that of the two substrates and almost all of the filao litter is total volatile solid.



### The C/N ratio

Fish waste reveals a carbon content of 49.18% and a nitrogen content of 7.28% which gives it a C/N ratio of 6.75. Then the frequently quoted range is between 20 and 30 for good biomethanization with good biogas yield [15, 16, 17].

The low C/N ratio of fish waste is due to the excessive presence of nitrogen on the fish waste and its high protein content which can promote the formation of hydrogen sulfide. These results can be compared with other organic wastes such as poultry viscera waste, broiler droppings and pet waste whose C/N ratios are 2.42; 3.68 and 8.21 respectively [8].

Fish waste as well as these wastes mentioned above have low C/N ratios. Hence the need to co-digest with other carbon-rich waste with low nitrogen content to optimize biogas production.

And, filao litter has a carbon content of 53.23% higher than fish waste and a nitrogen percentage of 0.69% lower than fish waste. It has a C/N ratio of 76, 61% which is higher than the optimum for methanogenesis. This high C/N value was found mostly in substrates that are rich in cellulose [18].

Indeed, filao litter, very rich in organic matter and carbon, with a low nitrogen content, could be considered as a good co-substrate for the valorization and optimization of the biogas yield of fish waste, which has a low C/N ratio, unlike filao litter.

### Physico-chemical Characteristics of the Co-substrate

The co-substrate is a mixture of 50% fish waste and 50% filao litter. This co-digestion was done in order to find another way to valorize the filao litter, and to improve the biogas yield from fish waste with a low C/N ratio. The following table presents the physico-chemical characteristics of the two substrates and the co-substrate.

### Organic Matter

The co-substrate, a mixture of fish waste and filao litter in equal proportions gives an organic matter content of 88.89%. Like the fish waste and the filao litter, the percentages of organic matter found in this experiment are largely superior to the characteristic value for a substrate to be biodegradable. The co-substrate is therefore rich in organic matter necessary for anaerobic digestion.

### C/N Ratio

The co-substrate has a total organic carbon content of 52.29% and a total nitrogen percentage of 3.65%, for a carbon to nitrogen ratio of 41.67. This ratio is not in the range frequently cited for good anaerobic digestion of a substrate. This is due to the fact that the proportions chosen for the mixture do not give a very good methanogenic potential. Therefore, it would be necessary to change the mixture proportions while using 25% filao litter and 75% fish waste to optimize the C/N ratio.

Apart from biogas production, the digestate from the digestion of the co-substrate would make a good quality compost due to its nitrogen and phosphorus content in the substrate, according to M'Sadak et al. [19].

**Table 1:** Physico-chemical characteristics of the substrates

Parameters	Fish waste	Litter of filao	Co-substrate
DM (%)	27.91	97.84	64.52
H (%)	72.09	2.16	35.48
OM (%)	83.60	90.49	88.89
MM (%)	16.40	9.51	11.11
COD mg O <sub>2</sub> /L	664.76	215	446.99
C (%)	49.18	53.23	52.29
N (%)	7.28	0.69	3.99
P (%)	0.02	0.37	0.21
C/N	6.75	76.60	41.67





## Methanogenic Potential of Substrates

### Evolution of pH

The pH is relatively easy to measure and is often the only parameter of the liquid phase that can be measured in real time. Its change is an indicator of the stability of the anaerobic digestion process, since microorganisms can only develop in a specific pH range.

Figure 6 shows the evolution of the pH during the residence time. During this study, the sampling was done by twodays

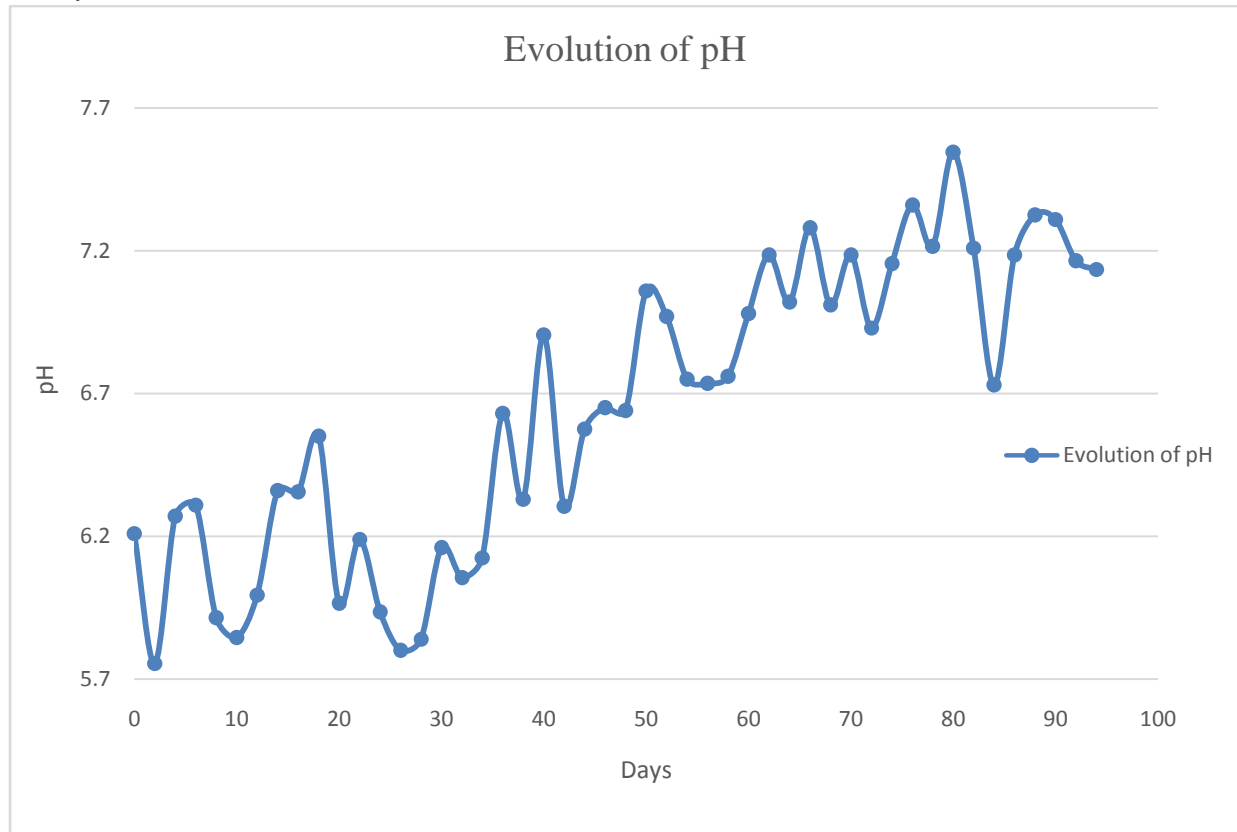


Figure 6: pH evolution curve as a function of time

It reflects a pH value of 6.2 at equilibrium from day 2 to day 28 the pH fluctuates between 5.75 and 5.84 before rising to 6.16 on day 30. The drop in pH can be explained by a degradation of organic matter and the formation of organic acids and fatty acids. Therefore, this period corresponds to the hydrolysis and acidification phase. Similar results were reported in several works on methanization [20, 21, 22].

From the 32nd day of production, the pH varies between 6.12 and 6.98 until the 60th day. This period marks the beginning of the methanogenesis phase with an ascending production of methane and a decrease in carbon dioxide. It corresponds to the alkalization phase with slower pH changes. This phase agrees well with the work of Douag-Tirishin, and al. [23]. From the 62nd to the 82nd day, the pH turns around 7, a value that favors the development of methanogenic bacteria responsible for methane formation. And on day 84, there is a slight drop in pH to a value of 6.73. The latter marks the end of methanization.

### Evolution of Methane Production

Figure 7 below shows the evolution of the production of methane, carbon dioxide and hydrogen sulfide as a function of time in an anaerobic environment. The biogas obtained is composed mainly of methane and carbon dioxide.



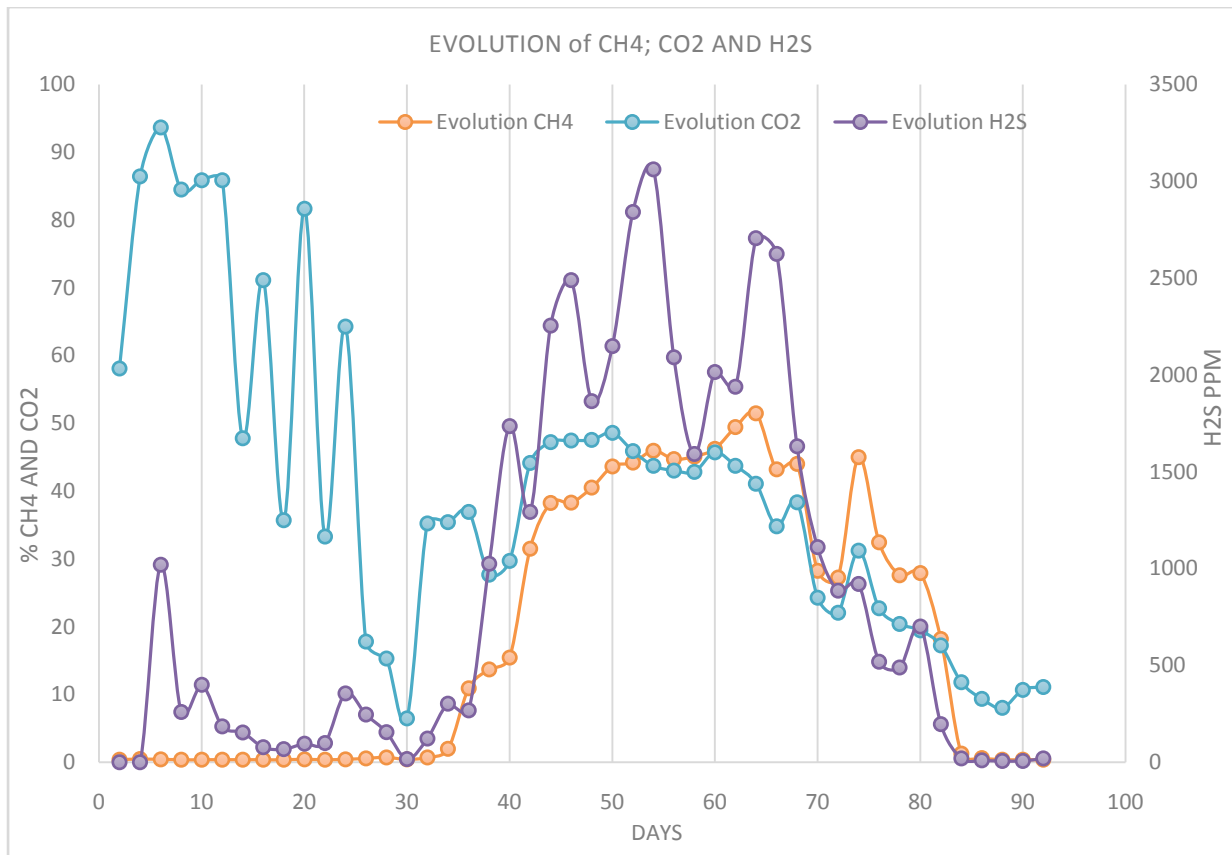


Figure 7: Evolution of the percentage of methane on the production of biogas

There is a low biogas production with a percentage of methane that is almost constant at 0.4% and a low production of  $H_2S$  expressed in parts per million ranging from 0 to 354.5 from day 1 to day 24. During this time, the percentage of carbon dioxide ( $CO_2$ ) is very high with a maximum of 93.65%. This phase corresponding to hydrolysis, acidogenesis and acetogenesis, is very long.

The duration of this phase is explained by the fact that the organic matter brought by the co-substrate is difficult to degrade. Indeed, the filao litter is largely made up of lignocellulosic matter, which is very difficult to biodegrade [24]. Few bacteria are equipped with enzymatic equipment allowing them to use the lignite.

From the 26th day, the production of biogas increases progressively until a maximum value with a percentage of methane of 51.5 % at the 64th day accompanied by a decrease of the percentage of carbon of 41.1 % and a content in  $H_2S$  of 2706 PPM. The production kinetics of this phase correlates well with the work of Boutouta, et al. [14].

This increase is followed by a consequent decrease of the biogas production with a percentage of methane ranging from 43.45% to 0.6% before stabilizing from day 86 to 92 on a value of 0.35% methane. During this time, the percentage of carbon dioxide decreased from 38, 35% to 11.1% and the hydrogen sulfide content flushed from 2625 to 19.5 PPM. It can be said that the production of carbon dioxide during the phase of acetogenesis would lead to the formation of methane during methanogenesis. And the presence of hydrogen sulfide shows that this gas should be purified for its use in engines. So these results are in good agreement with all the studies that have been done on methanization saying that carbon dioxide is transformed into methane. The consequent production of biogas supposedly inflammable reflects the good functioning of our device. The evolution of methane production was similar to that observed in the work of Tahri, et al. over a residence time of 70 days [4]. The difference is that in this study the best performance in terms of volume of biogas produced is recorded in the mixture of 75% poultry waste and 25% slaughterhouse waste and the low values are recorded in the mixture of 50% slaughterhouse waste and 50% poultry waste. Our study also correlates well with that of Kalloum et al. in the case of unadjusted pH [20].

In this study, the methanogenic phase was triggered from the 60th day with a low volume of biogas production contrary to the case of the adjusted pH. This evolution can be always compared with the one reported by Igoud et al. in their studies on a first approach to the characterization of biogas produced from bovine manure [21].

Our results are also in perfect agreement with those of Xiao, Wu, et al. [25] who combined pig manure with three agricultural residues namely corn stalks, pear straw and wheat straw. Their results showed that all agricultural residues have biogas production with significant methane percentage.

### Conclusion

This experimental work of anaerobic co-digestion was carried out in two stages: a phase of physico-chemical characterization which reveals that the Co-substrate presents an important methanogenic potential with an organic matter content of 88.89% and a carbon/nitrogen ratio of 41.67; and a biomethanization phase of fish waste and filao litter at equal proportions. This biogas production test evolving over a residence time of 94 days presents a gas with a maximum percentage of 51.3% methane under a variable temperature ranging from 23.6°C to 27.8°C.

To optimize the methanogenic potential of the substrate, it would be important to change the mixture proportions by applying a ratio of 25% filao litter and 75% fish waste. This proportion would lead to the best C/N ratio and therefore a good methanogenic potential. It should be noted that methanation is very sensitive to temperature variation.

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