

Response of the Bacterial Consortium under Supersaturation Conditions in an Anaerobic Co-Digestion Environment between *Typha domingensis* and Bovine Manure

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Abstract

Oversaturation in an anaerobic co-digestion environment occurs when two substrates with different bulk densities are used. In this case, the co-substrate with the higher bulk density has its ratio gradually increased without changing the water supply initially, while the ratio of the second co-substrate is decreased. In our case study, the ratio of *Typha domingensis*, which has a higher density than cattle manure, will gradually increase from 10% to 50%, the point at which the anaerobic environment becomes supersaturated. Since the optimum pH is between 6.1 and 7.1, the system's temperature is dependent on the ambient temperature with a temporary variation between 28 and 36 degrees Celsius. This necessitates examining other parameters of the reaction medium that could explain this phenomenon. The relevant parameters are salinity, conductivity, and oxidation-reduction potential. The limit values obtained for these parameters are [2.4; 4.5 mS and 35.2 mV], respectively. This led us to question the performance of the overall process in terms of biogas composition, achieving a maximum CH₄ concentration of 55.59%.

Keywords

Typha domingensis, Oxidation-Reduction Potential, Salinity, Conductivity, Supersaturated, Density

1. Introduction

The functioning of anaerobic fermentation processes depends on the bacterial consortium and its adaptation to and response to the used substrate. Several families of microorganisms are involved in methanogenesis. These families include, among others: Coprococcus, Anaerolineae, Prophyromonadaceae, Bacteroidales, Ethanoli-*genens*, Ruminococcaceae, Pseudomonas, Enterobacteriaceae, Clostridiaceae types I and II, Oxobacter, Caloramator, Syntrophobacter, and Syntrophomonas. Microbiological analysis of the substrate in the reaction medium shows that the phenotype changes from one medium to another. This explains the appearance of wild phenotypes. These wild phenotypes encompass large families of microorganisms that can adapt or disappear during the anaerobic digestion process [1] [2]. This has allowed us to conclude that most of the bacterial population belongs to the large family of non-fermentative bacteria considered endogenous. When we focus specifically on methane production, we see that certain microorganisms are involved. These include: Methanobacteriaceae, Methanospirillaceae, Methanosaetaceae, and Methanosarcinaceae. Depending on the temperature and other environmental conditions, different types of microorganisms may be present, making anaerobic digestion processes very complex from a microbiological point of view [3] [4]. Anaerobic digestion is essentially subdivided into four phases, which depend on the alkalinity of the environment. Thus, the microorganisms found in an anaerobic environment depend on the phase or stage. For example, Bacteroidetes and Firmicutes are responsible for hydrolysis; Bacteroidetes, Chloroflexi, Firmicutes, and Proteobacteria are responsible for acidogenesis; Pelotomaculum, Smithella, and Syntrophobacter are responsible for acetogenesis; and Methanobacterium, Methanobrevibacter, Methanoculleus, Methanospirillum, and Methanothermobacter are responsible for methanogenesis [5]-[8]. These families of bacteria work symbiotically to ensure proper methanogenesis. In the case of anaerobic co-digestion, another factor comes into play: the bulk density of the co-substrates. This occurs when the co-substrate with the highest bulk density is gradually increased until it reaches supersaturation. In fact, in the case of our study, supersaturation can be observed in the bulk density as soon as the proportion of *Typha domingensis* exceeds 30%, without increasing the water content, which is three-quarters of the mixture. This results in the digestate leaving the pilot plant becoming extremely thick, which tends to block the digester. In our study, the two co-substrates used were cattle manure and *Typha domingensis*. The *Typha domingensis* ratio, which has the highest volumetric density, was increased in the mixture from 10% to 50%, while the manure ratio was decreased from 90% to 50% in inverse proportion. The test was carried out in HOMEBIOGAS 2.0. This technique provides insight into the role of microorganisms in biogas production under extreme conditions. However, pre-treatment is essential when using *Typha*.

2. Materials and Methods

Long present in the Senegal River valley since the construction of the Diama Dam,

Typha domingensis is a plant that has often been used by riverside populations as fencing for houses and orchards, but also for craftwork. Today, the plant is the subject of several studies for its use as a building material or as fodder for livestock, and one of the studies that caught our attention is its use for food packaging [9]. However, the real limitation to its use as livestock feed and packaging remains the presence of certain heavy metals such as Cd, Ni and Pb [9]. This is why one of the most efficient ways of using it seems to be for biogas production. The use of *Typha domingensis* as a co-substrate in anaerobic digestion requires pre-treatment. This pre-treatment starts with cutting on-site and mixing with the co-substrate, which is cattle manure. The pre-treatment of cow manure starts with collection and mixing. These steps are shown in Figure 1.

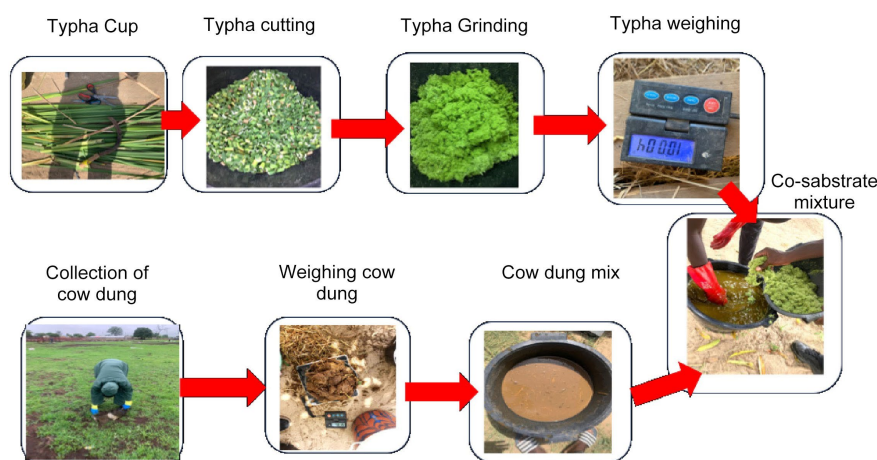


Figure 1. The process of preparing co-substrates (*Typha domingensis* and cow manure) prior to treatment.

Indeed, while the pre-treatment of cow dung involves only three steps—collection with shovels, weighing and mixing with water—*Typha domingensis* undergoes four steps: on-site cutting, cutting into small pieces, grinding to reduce size, weighing, and finally mixing with the co-substrate. The tools used for pre-treatment are:

- A sickle for cutting Typha on site.
- Pairs of scissors for cutting.
- A spice grinder for grinding.
- An SF-890 scale for weighing.
- Bowls for mixing.

After mixing the co-substrates, the next step is to load the biodigester. This was done in a way that took into account the condition of progressive supersaturation, which is linked to the higher density of *Typha domingensis* compared to cow manure. The following ratios were used: 10% Typha/90% cow dung (CD), 20% Typha/80% CD, 30% Typha/70% CD and 40% Typha/60% CD, with the final ratio being 50% Typha/50% CD. The water supply ratio is 1 kg of co-substrate to 3 kg of water. This equates to 15 kg of water for every 5 kg of co-substrate. The daily

load is 60 kg of mixture, with two loads per week. The amount of water was chosen to prevent clogging of the digestate outlet and to facilitate movement of the load carriers in the reaction medium. Co-digestion of the substrates was carried out in an Israeli-made HOMEBIOGAS 2.0 digester. Throughout the test, the digester was loaded twice a week and each batch was used for two weeks. The HOMEBIOGAS 2.0 is a very interesting model because, as well as being mobile, it makes it easy to measure functional parameters in the reaction environment. And each parameter was measured three times a day, except for the biogas composition, which was measured once a day.

To achieve this, we use a dedicated measuring device for each parameter to be monitored. In our study, we monitor ambient temperature, biodigester temperature, pH and the elemental composition of the biogas produced. However, in addition to these traditional parameters, we were interested in others such as redox potential, salinity and conductivity. These additional parameters provided a more comprehensive insight into the activity of microorganisms during the anaerobic fermentation process. All measurement points are shown in **Figure 2**. Temperatures, pH and redox potential were recorded using a pH meter, while salinity and conductivity were measured using a conductivity meter. The elemental composition of the biogas was analyzed using an Optima 7 (MRU) gas-phase analyzer. Additionally, the ambient temperature was recorded at a water basin located 2 meters from the biodigester. This was done to account for temperature variation in the material. The data collected while monitoring these parameters provided us with a comprehensive database. This enabled us to plot the characteristic curves of the studied parameters.



Figure 2. Measuring devices arranged on the HOMEBIOGAS 2.0.

3. Results and Discussion

3.1. Changes in Room Temperature

Temperature is an essential parameter in mechanization. Whether ambient or system temperature, it determines how our system operates. These include psychrophilic, mesophilic and thermophilic modes. The ambient temperature defines the

bioreactor's temperature range. In our study, as mentioned above, the ambient temperature was measured in the water basin situated just two meters from the HOMEBIOGAS unit. This was done to obtain the most stable temperatures possible and to respect the state of the matter in both environments. Taking a closer look at the ambient temperature evolution, we can see that the lowest temperatures were recorded in the morning, with a minimum value of 28°C (**Figure 3**).

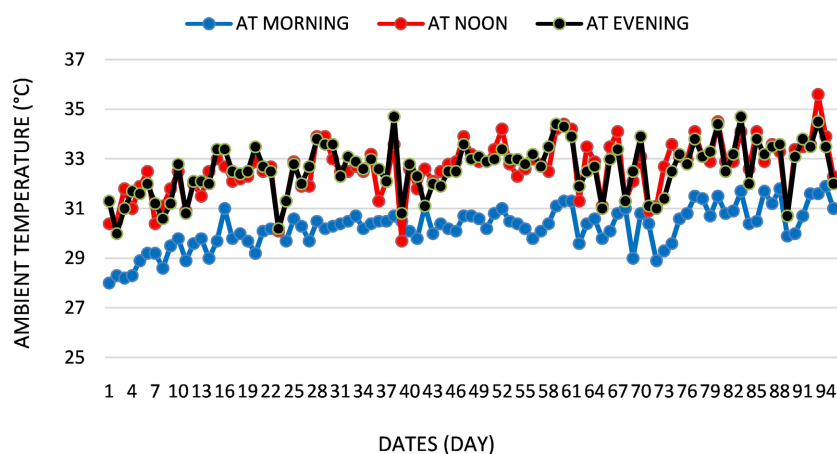


Figure 3. Variation in ambient temperature over time. AT MORNING: Ambient temperature morning (°C); AT NOON: Ambient temperature noon (°C); AT EVENING: Ambient temperature evening (°C).

This is because morning temperatures are recorded between 7 am and 9 am. In this northern region of Senegal, morning temperatures are the mildest from May to July. The same pattern is followed by midday and evening temperatures, with the highest temperature recorded at midday, reaching 35.8°C (**Figure 3**). With ambient temperatures ranging from 28°C to 35.8°C, and given the heat concentration effect of the black HOMEBIOGAS material, the reaction medium temperature should reach the optimum mesophilic level of around 37°C. Let us now observe the temperature curve of the bioreactor to see if this is the case.

3.2. System Temperature Evolution

The temperature of anaerobic digestion reactors depends on the ambient environment in which they are installed. In fact, in regions with a Sahelian climate, such as this northern part of Senegal, the daily temperatures can reach 40°C during certain periods of the year [10], meaning that anaerobic digestion processes often do not require heat input to operate in mesophilic mode.

The temperature variation in the reaction environment depends strictly on the evolution of the outside temperature and the absorption capacity of the material used to construct the pilot digester. The double wall of the HBG2, which is made of plastic with an outer black tarpaulin coating, behaves like a black body by absorbing all the radiation it receives from the sun. This characteristic of the device

favors midday temperatures over evening temperatures due to the concentration of heat received. This increases the activity of the bacterial population. Let us observe the effect of these two phenomena on the system's temperature throughout the experimental period using the curves in **Figure 4**. The system temperature fluctuates throughout the experimental period. Low temperatures are recorded in the morning, ranging from 25°C to 33.6°C; median temperatures are recorded in the evening, ranging from 29.8°C to 40.9°C; and maximum temperatures are recorded at midday. The minimum temperature recorded at midday was 28.9°C, while the maximum temperature was 41.9°C (**Figure 4**). Examining the climate types, we can conclude that our experiment was conducted in a mesophilic climate, with temperatures ranging from 25°C to 41.6°C [10]. This is a defining feature of countries south of the Sahara, meaning that additional heat is not required for mesophilic regimes compared to countries with a temperate climate. Temperature is an important parameter for the performance of anaerobic fermentation systems. It provides a clear indication of reaction kinetics, characterized by the movement of charge carriers, *i.e.*, oxidation-reduction potential, or redox.

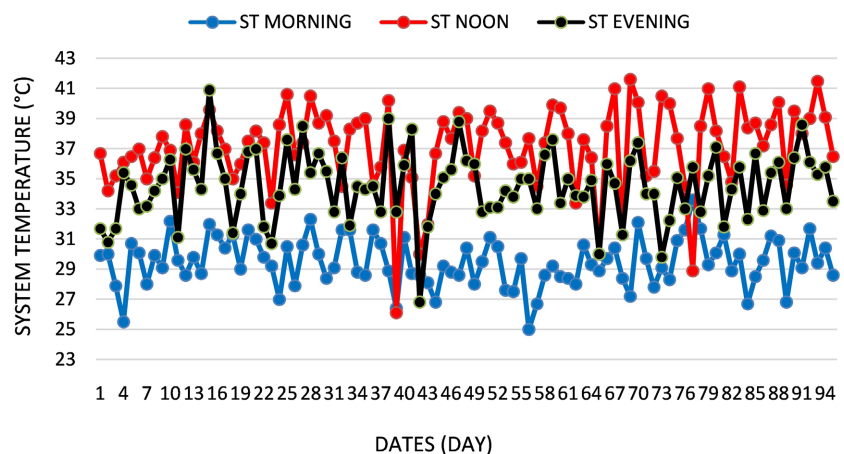


Figure 4. Variation in pilot temperature over time. ST MORNING: System temperature morning (°C); ST NOON: System temperature noon (°C); ST EVENING: System temperature evening (°C).

3.3. The Evolution of the Redox Potential in the System

Redox potential is considered a key indicator of reaction kinetics in an anaerobic environment. It depends on the pairs present in the mixture. However, the transfer of ions between these acid/base pairs is strictly temperature-dependent. Compared to the potential obtained in the pilot activation process, the redox potential of co-digestion between *Typha domingensis* and cow manure is specific due to the contribution of the co-substrate. Thus, the shape of the three curves can be likened to radio waves (**Figure 5**).

This wave-like appearance is due to the environment becoming richer in acid/base pairs.

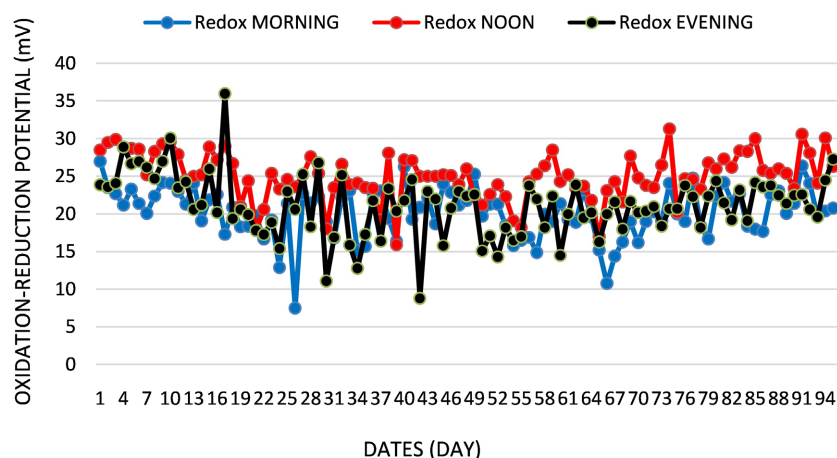


Figure 5. Variation in the pilot's redox potential over time. Redox MORNING: Oxidation-reduction potential morning (mV); Redox NOON: Oxidation-reduction potential noon (mV); Redox Evening: Oxidation-reduction potential evening (mV).

As the redox potential depends on the temperature of the system, the midday values are generally the highest, followed by the evening and morning values, which appear to evolve similarly over time. However, the maximum redox potential value of 36 mV was recorded in the evening. The minimum value was around 7.5 mV and was recorded in the morning. The accepted values for redox couples in this study generally range from 7.5 to 36 mV (Figure 5). Looking closely at the limits of these intervals reveals a difference compared to the digestion of simple cow dung, where the values ranged from 12.5 mV to 37.3 mV. But if the redox potential is imposed by temperature variation, what would be the case for pH? In the literature, redox potential values are often found to be a function of the phase of mechanization. Thus, we observe that the redox potential of acidogenesis is higher than that of methanogenesis, with respective values of 32.71 mV and 28.97 mV [11]. These differences can be explained by the state of the organic matter present in the reaction medium, the medium's pH level, and the types of microorganisms involved in each stage of anaerobic digestion. All of these parameters can impact biogas production.

3.4. Changes in pH Levels in the Pilot Plant

Like temperature, pH provides information about the various processes involved in anaerobic digestion. One aspect we can observe through the evolution of pH is the consistency of the substrate that has already been digested at the biodigester outlet. This digested substrate is called digestate. Since the data is measured at the outlet of the system, the digestate discharged during a load is always different from that discharged during data collection. This is because the digestate discharged from the digester by pressure during the data collection hours contains fine particles of Typha fibers, which form a more compact substance at the outlet. This enables us to observe precisely how the concentration of the reaction medium evolves with the gradual increase in the proportion of Typha in the mixture over time (Figure 6).

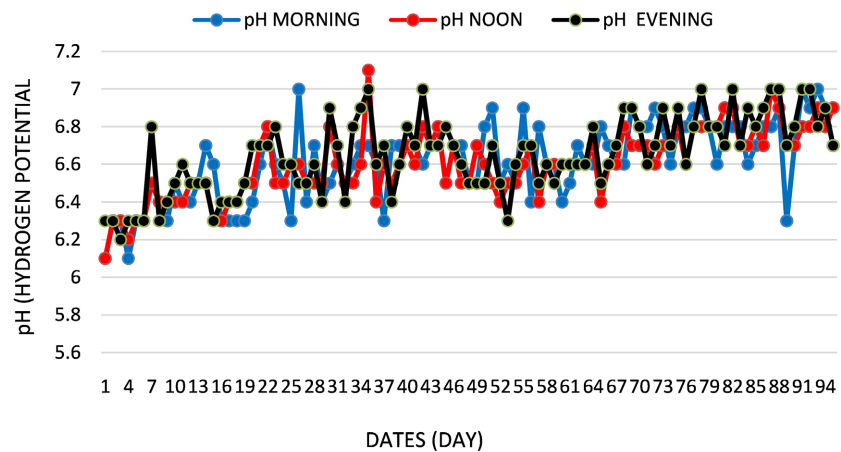


Figure 6. Variation in the system's pH over time. pH MORNING: Hydrogen potential morning; pH Noon: Hydrogen potential noon; pH Evening: Hydrogen potential evening.

In fact, the evolution of the characteristic pH curves throughout our study appears consistent, with only minor differences. Since the start of the study, the pH curves have been rising from 10% Typha and 90% cow manure to 30% Typha and 70% cow manure. However, from 30% to 40% Typha, there is a slight decrease in pH values. Tests have shown us that the pH value increases as the substrate becomes more concentrated. So, what could explain this drop in pH values? Projecting these values over time shows that they coincide with the period from 21/07/2023 to 06/09/2023. This period corresponds to the rainy season across the country. Therefore, the rain could have diluted the digestate at the outlet, reducing its concentration. However, another possible explanation could lie in the nature of the cow dung during this winter period. The manure is often very liquid, because the cows, which had originally been fed dry straw and maize silage, had their diet changed to forage crops and fresh grass.

If the manure used is liquid, this can reduce the medium's concentration despite the increase in Typha. This could explain the slight drop in pH values during this period. However, this decrease in concentration will be corrected by the sequential increase in the amount of Typha in the mixtures, causing a slight rise in pH values. Thus, between 40% and 50% Typha, we will see an upward trend in the characteristic pH curves until the end of the study (see **Figure 6**). This confirms our observations. However, despite the oversaturation observed in terms of bulk density, the pH remains neutral, which allows for good growth of the bacterial consortium and the maturation of the process. But what about the other parameters that depend on the concentration of the reaction medium?

3.5. Changes in Conductivity and Salinity

Conductivity and salinity are two parameters that are new to our study and are rarely found in anaerobic digestion tests. In fact, despite extensive research, we have never found a paper that mentions them in the literature. However, studying

these two parameters together has enabled us to gain a better understanding of reaction kinetics and/or the movement of charge carriers in anaerobic environments. We decided to study them together because their characteristic curves have the same shape, despite the values not being of the same order of magnitude (**Figure 7** and **Figure 8**). As can be seen from **Figure 7** and **Figure 8**, the conductivity and salinity values increase as the amount of Typha in the initial mixture increases, until the critical or limit values are reached, beyond which the solution becomes saturated. This decreases the movement of charge carriers, resulting in a drop in conductivity and salinity. In our case, the limit concentration is approximately 30% Typha. Above this, the mixture becomes increasingly concentrated, affecting the concentration of the reaction medium (**Figure 7** and **Figure 8**).

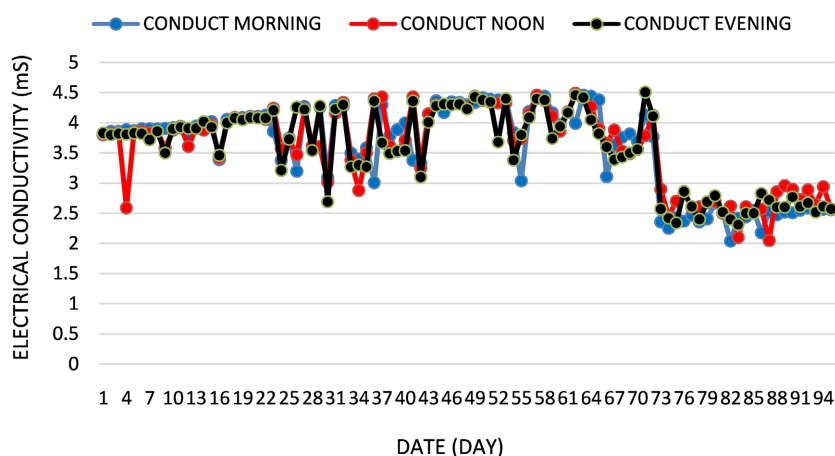


Figure 7. Variation in the system's electrical conductivity over time. CONDUCT MORNING: Electrical conductivity morning (mS); CONDUCT NOON: Electrical conductivity noon (mS); CONDUCT EVENING: Electrical conductivity evening (mS).

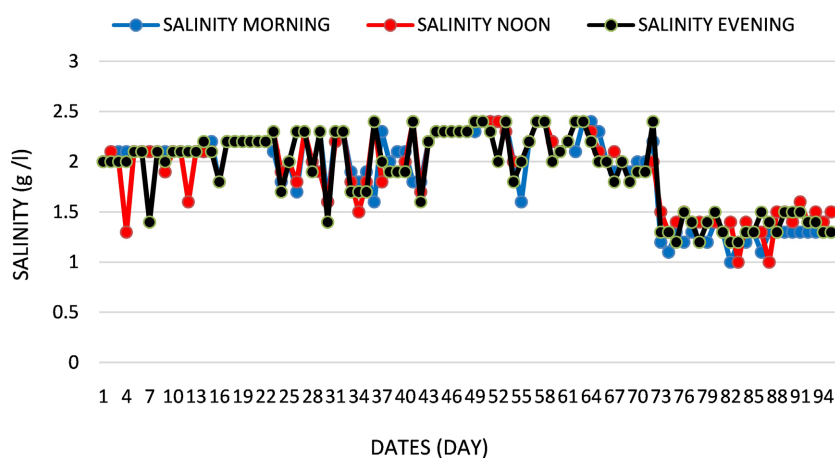


Figure 8. Changes in the salinity of the pilot over time. SALINITY MORNING: Concentration of NaCl ion in solution at the morning; SALINITY NOON: Concentration of NaCl ion in solution at the noon; SALINITY EVENING: Concentration of NaCl ion in solution at the evening.

Looking more closely at the maximum and minimum values, we have the following ranges: [0.9 to 2.5] for salinity and [2.2 mS and 4.6 mS] for conductivity. In addition, the mixtures made with 40% and 50% *Typha* allowed us to understand that if we want to increase the proportion of *Typha* in the total amount of substrate, we would also have to increase the amount of water, which represents 3/4 of the mixture as a whole.

This would completely change the reasoning behind the behavior of the reaction medium. However, regardless of the variability of the environment's parameters, what attracts scientists and operators the most is the quantity of biogas produced and/or its elemental composition. To better understand the effect of salinity in the reaction medium and/or its effect on microorganisms, studies were conducted using varying amounts of table salt (NaCl). Quantities of 1, 2.4, 8 and 16 g/L were placed in different bottles. This showed that Euryarchaeota, Synergistetes, Firmicutes and Bacteroidetes were dominant at the phylum level. The proportion of Methanosaeta, the major genus among Euryarchaeota, was 16.46% after 70 days of acclimatisation to NaCl, which was higher than in the initial sample (22.08%). Methanosarcina also increased after acclimatization. Consequently, both Methanosaeta and Methanosarcina could adapt to high-salinity environments [12]. However, in our case, the salt comes from biomass due to the 2% salt content of the river water. This salinity depends not only on NaCl but also, and above all, on all the ions in solution. This explains the supersaturation effect when the density of *Typha domingensis* increases at the end of our experiment. It is important to remember that the same effects can be observed in our study since all ions undergo mineralization in the reaction medium. As for the concentration of Na⁺ above 4 g/L, it may cause the inhibition of mechanization due to the accumulation of volatile fatty acids (VFAs) [13].

3.6. Changes in CH₄, CO₂ and H₂S Concentrations

For an anaerobic digestion system operator, quantifying the biogas produced is crucial. However, for scientists, knowing the composition of biogas is even more important due to certain unwanted components. Indeed, knowing the composition of biogas provides a precise indication of each substrate's contribution to ensuring the facility's proper functioning. Thus, the variation in methane (CH₄) and carbon dioxide (CO₂) concentrations throughout our study will enable us to assign a specific range of values to each ratio. Referring to the composition of the biogas produced during the pilot system activation process reveals a clear difference in the current characteristics of the CH₄ and CO₂ curves. There was a particular instance of uncontrolled biogas use where the two curves converged. However, in this case, we observe a significant shift in the concentration of these gases throughout the study period (Figure 9). Overall, the CH₄ concentration is higher than the CO₂ concentration at all points, regardless of how the produced biogas was used. Examining the two curves more closely reveals the same scenario. When the concentration of CH₄ increases, the concentration of CO₂ decreases, and vice

versa. Furthermore, the decrease in pH observed during the winter period impacts the concentrations of CH_4 and CO_2 . In this case, however, the period of low CH_4 concentration extends from the introduction of 20% Typha to 30% Typha (**Figure 9**).

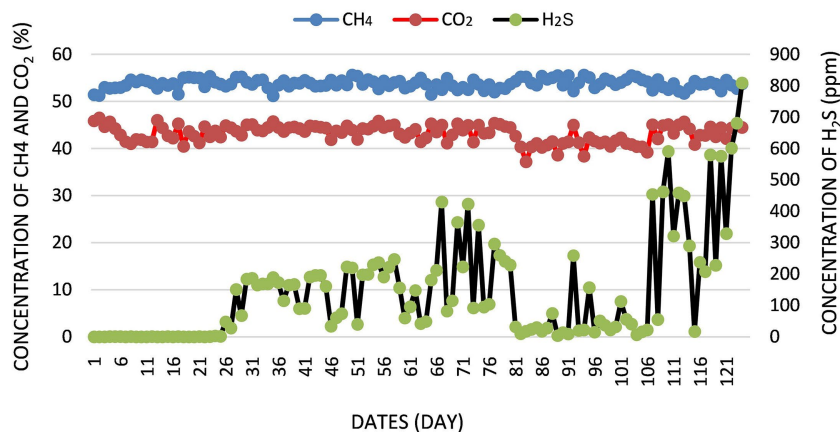


Figure 9. Variation in methane (CH_4), carbon dioxide (CO_2) and hydrogen sulfide (H_2S) concentrations over time. CH_4 : Concentration of CH_4 (%); CO_2 : Concentration of CO_2 (%); H_2S : Concentration of H_2S (ppm).

Recalling the assumptions about pH, we can infer that this decrease in CH_4 concentration is linked to changes in the cows' feed on the farm, which affects the nature of the cow dung.

In fact, when the initial mixture contains 10% - 30% Typha, the contribution of cow manure to the mixture's concentration predominates. However, as soon as the Typha content reaches 40%, it predominates in terms of both volume and concentration in the mixture of co-substrates. Thus, with 40% to 50% Typha, we see that the CH_4 concentration has increased considerably compared to previous ratios, at the expense of the CO_2 concentration. This shows that, despite the reaction medium being supersaturated, the bacterial consortium has become more active. Hence, biogas production is maintained, given that a sudden change in the concentration of the reaction medium often disrupts the growth of the bacterial population. This is good news, given that CO_2 significantly reduces the calorific value of biogas. After 50% Typha, we observe a slight decrease in CH_4 concentration and a clear increase in CO_2 concentration (**Figure 9**). This is the leaching period, characterized by the introduction of cow manure alone into the biodigester before the co-substrate is changed. In fact, leaching allows the microbial populations to be reset. The CH_4 concentration varies from 51.22% to 55.59%, while the CO_2 concentration ranges from 37.17% to 46.5%. The maximum CH_4 value of 55.59% reached here is much lower than the value of 72.2% obtained during the activation phase. The most interesting thing about the co-digestion of Typha and cow manure is that it maintains production. While an average of three loads of cow manure per week is needed for digestion, co-digesting Typha at 40% can maintain

production for a whole week with just one load. This is a particularly desirable feature when considering substrate availability in certain areas. This makes Typha one of the best substrates and/or co-substrates worldwide. In fact, despite the reaction medium being oversaturated with a 50/50 proportion, biogas production continues to be maintained. However, the medium's concentration is increasing, hence the need to change the water supply if we want to continue the study. In addition to these two parameters, we also have to consider hydrogen sulfide (H_2S). Indeed, changes in hydrogen sulfide concentration must be closely monitored throughout a mechanization test for safety reasons. According to the IAEA report, inhaling hydrogen sulfide beyond certain limits can lead to complications ranging from skin reactions to death by asphyxiation. This is why we used an H_2S filter in our case study. The advantage of using this filter is that it enables us to verify its maximum efficiency based on the H_2S concentration in the biogas. In fact, the supplier estimates that our filter has a maximum usage life of 6 months. However, experience has taught us that the effectiveness of filters always depends on the concentration of the component to be eliminated. Examining the H_2S characteristic curve more closely, we can see that the filter remained effective for over 7 months, given that it has been in operation since December (**Figure 9**). Ultimately, we can say with certainty that the filter remains effective as long as there is no oversaturation due to the biodigester producing a very high concentration of H_2S . The nature of the co-substrates determines these high concentrations of H_2S . In our case, these may come from Typha and/or the type of feed consumed by the cows (e.g., straw, concentrate, maize silage and fodder). Its range of variation is from 0 to 800 ppm.

From a microbiological point of view, the physicochemical parameters of the reaction medium do not reach the thresholds that would stress the microbial population. This explains why our reactor functions well despite the supersaturation conditions linked to the ratio of Typha australis to cattle manure. This makes *Typha domingensis* an ideal co-substrate for mechanization in northern Senegal, where it is plentiful all year round.

3.7. Changes in the Minor Components of the Produced Biogas

Beyond the main components of biogas (CH_4 and CO_2), there are also minor components, some of which we monitored: O_2 , N_2 and H_2S . Typically, the concentrations of N_2 and O_2 in biogas do not exceed 2% and 1%, respectively. In our case, however, the concentrations of N_2 and O_2 reached 6.37% and 1.55%, respectively (**Figure 10**). These values can be explained in different ways depending on the parameter concerned. In the case of oxygen, this can be explained by air entering the pipes, given that the measurement is taken three meters from the digester outlet. The maximum value reached by the N_2 concentration could be due to the type of soil in which the co-substrate was harvested. Examining the areas where Typha grows more closely, we can see that they are places where both rainwater and river water flow. Given this plant's absorption capacity, this could well explain the maximum value.

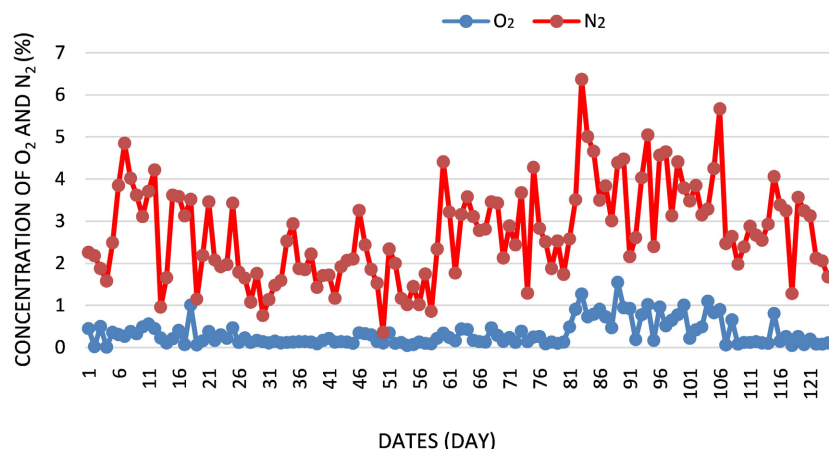


Figure 10. Variation in the concentration of N_2 and O_2 over time. O_2 : Oxygen concentration (%); N_2 : Nitrogen concentration (%).

3.8. The Evolution of the Higher Calorific Value (HCV) and Lower Calorific Value (LCV)

Whether the calorific value is lower or higher depends on the concentration of CH_4 over time. For verification purposes, we have plotted their characteristic curves alongside the CH_4 concentration curve in **Figure 11**.

Thus, it is clear that the curves vary in the same way at every point. In other words, when the CH_4 concentration increases, the HCV and LCV values also increase, and vice versa. In general, the HCV and LCV values range from 20.3 to 22.1 MJ/m^3 and from 18.3 to 20 MJ/m^3 , respectively (**Figure 11**).

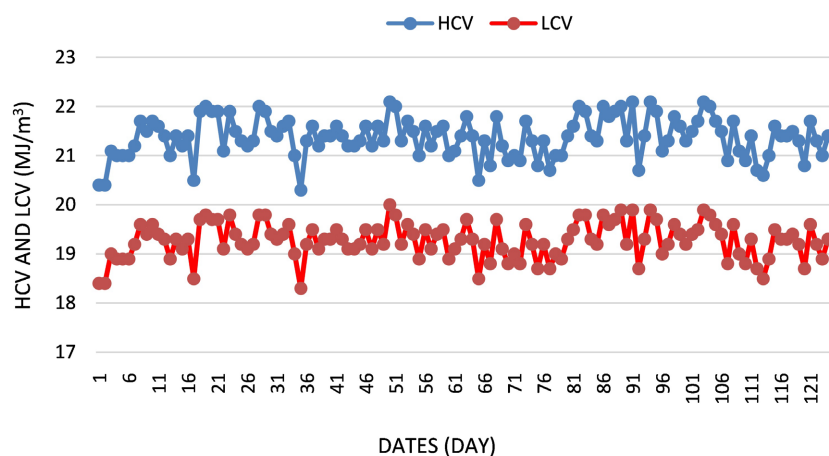


Figure 11. Variation in HCV and LCV over time. HCV: Higher calorific value; LCV: Lower calorific value.

4. Conclusion

Laboratory-scale anaerobic digestion tests conducted under controlled conditions require great care in developing countries, where power cuts and voltage drops

are common throughout the day. These disruptions have a negative impact on the system's operational parameters, such as temperature, alkalinity and reaction kinetics. The *in-situ* study carried out in the HOMEBIOGAS 2.0 system enabled us to characterize this system in Sub-Saharan Africa, providing an insight into the variation in its physical parameters. In order to move beyond the traditional framework of cow dung digestion, we co-digested it with *Typha domingensis*. By gradually increasing the Typha/cow dung ratio, we were able to understand the impact of Typha on maintaining production with two weekly feedings. It is important to note that despite the observable supersaturation in terms of bulk density when the *Typha domingensis* ratio in the mixture exceeded 30%, the growth and activity of the bacterial consortium were not disrupted. This resulted in good biogas production with a CH₄ concentration of 55.59%.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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