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Experimental Anaerobic Digestion of Poultry Droppings and Microbiological and Energetic Monitoring of the Process under Study

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Abstract*—Tunisia has limited primary energy resources and produces only fossil fuels. The technology of biogas production by anaerobic digestion or biomethanation is very little known and poorly applied in Tunisia. Indeed biogas is very neglected among the main sources of energy in the country. The use of biogas technology can solve a number of ecological and economic problems. Today, its prospects for the future appear promising, justifying the need for a judicious awareness, followed by pilot experiments with a view to the further popularization of biogas technology and its interests. In Tunisia, the poultry sector produces a large quantity of laying inches, abandoned without any recycling or recovery, which can constitute a potential source of an environmental contamination in general, although it is an organic source that can be valued and has important energy and agronomic interests.*

This study aims to optimize and to follow the experimental biometanation of the poultry litter. Physicochemical and microbiological evaluation of the substrates was carried out. As for the energy side, the anaerobic digestion chosen is in discontinuous (batch), mesophile (35 ° C) and wet (8% DM of digester) mode. The main results show that poultry excreta is rich in organic matter and bacterial load, which favors the production of biogas, in particular with the addition of inoculum and activator. It should also be noted that anaerobic digestion resulted in a decrease in microorganisms and that the activator (especially with 18%) improved the speed of the hydrolysis phase and favored the production of biogas and especially methane.

Keywords*—*Anaerobic digestion, biogas, energy recovery, microbiological monitoring, poultry litter.

I. INTRODUCTION

In intensive livestock systems in general and their effluents in particular can cause point and diffuse environmental pollution. This practice emit a large number of gaseous molecules, such as carbon dioxide (CO_2), ammonia, methane (CH_4), nitrous oxide and hydrogen sulphide (H_2S) [1]. Producers are trying to meet the demand for this modernization by recycling different types of wastes, especially organic and biodegradable wastes [2]. In this sense, some research programs such as ISARD have presented a general objective of "designing an integrated approach to decision-making [to] develop methods and tools to increase agricultural production through implementation of recycling practices" [3].

Along with this large generation of wastes on a global scale, energy demand is still growing, for example from 5,000 Mtoe in 1970 to 12,000 Mtoe in 2010 [4]. This demand, accompanied by the reduction of non-renewable resources and atmospheric emissions of greenhouse gases (GHG), is a major concern. Due to demographic and economic growth, mainly by emerging countries, it could double by 2050, according to (Laurent, 2015) [5]. This observation raises the question of the sustainability of such a model of dependence on fossil energies, given the difficulties inherent in their exploitation. Moreover, the challenge of climate change requires that carbon emissions be reduced on a global scale as soon as possible. These issues were the subject of the Kyoto Protocol in 1997, updated in 2012, to reduce global GHG emissions, or more recently from climate summits (in New York in 2014) [5].

Governments are constantly looking for technological solutions that allow for efficient and less costing waste treatment. One of the technologies used to treat the organic fraction of this waste is Anaerobic Digestion (AD), which can turn a waste problem into a source of wealth [6]. AD is best suited to convert organic wastes into energy and fertilizer. It has become popular in developing countries such as China, India and Nepal; however, in South Africa, biogas digesters are principally constructed and installed in the Western and Kwa-Zulu Natal provinces of the country [7]. Owing to the important roles demonstrated by rumen microorganisms in AD [8], animal manures have been established as suitable sources of biogas production in Africa although, they are co-digested with energy crops in Denmark and Germany [9,10]. The uses of biogas vary greatly from developing to developed countries. In Africa, biogas generated can be used as fuel for cooking,

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lightening and heating; it reduces the demand for wood and charcoal for cooking therefore helps preserve forests' areas and natural vegetation [11,12]. In Western countries (e.g., Germany & America), biogas is converted to electricity and heat for on-farm purposes by combined heat and power units after removing water and sulphur from its mixture [13].

Tunisia is currently suffering from poor waste management and limited primary energy resources, producing only fossil fuels. Our study will focus on waste generated by the poultry sector. The problem posed by the organic effluents of this sector in general and droppings of laying hens in particular could be solved biologically, especially by biomethanation and/or composting. The general objective of this work is to study and develop experimental AD applied to Poultry Droppings (PD) and to focus on the introduction of biogas technology as an innovative organic waste management solution and also as a source of clean and renewable energy. These biomasses available in large quantities will be studied on various levels: qualitative characterization, microbiological monitoring and energy optimization of this process on an experimental scale.

II. MATERIAL AND METHODS

2.1. Inputs used

In our research, the PDs are used as a substrate for energy recovery. These effluents are pasty to dry products, derived from the breeding of laying hens. They are bird droppings mixed with feathers and animal feed.

We also used an inoculum which recovered from an old digester fed by poultry manure, and a pure microbial activator called KT01 which exhibits gram$^-$ and catalase$^+$ stick-shaped microorganisms. It does not tolerate salinity (>1%).

2.2. Physico-chemical characterization

The collection of samples of poultry droppings from poultry farm buildings in laying hens is done according to the method described by [14]: Chicken droppings are very often recovered on carpets placed under rearing cages. In this case, it is sufficient to make several samplings at several points distributed along the length and level of each battery stage. In the case where the droppings are stored in pits, the height of the droppings may be large.

The parameters tested are mainly the potential Hydrogen (pH), the Electrical Conductivity (EC); which is an index of soluble salt content. This parameter was achieved by centrifugation (3000 rpm) of a suspension of the sample with distilled water in a ratio of 1: 5. The percentages of Dry Matter (DM), Mineral Matter (MM) and Organic Matter (OM) are determined after drying in an oven at 105 ° C. for 24 hours and then calcination at 600 ° C. [15].The dry bulk density is the mass of the dry volume unit. It consists in placing the samples in the oven at a temperature of 105 ° C. for 24 hours to determine the dry mass [15]. The porosity corresponds to the evaluation of the void spaces in relation to the total congestion of a substrate [16, 17]. Total Porosity (TP) is difficult to measure; there are several formulations that differ slightly from one another [16]. Among them, the one mentioned by [18,19] was used. Finally, for Suspended Matters (SM), they correspond to the set of mineral and / or organic particles present in natural or polluted water [20]. The analysis is based on the principle of quantifying all the materials that can be decanted after filtration and evaporation in the oven at 105 ° C.

2.3. Microbiological characterization

2.3.1. Different methods of microbiological enumeration

Several methods of enumeration can quantify the bacterial population. Molecular biology methods can also identify bacteria or have a representative profile of the total population. The quantitative and / or qualitative analysis of the biomass can be carried out according to different methods. According to [21], he distinguished three main types of methods to assess microbial biomass. These different approaches are detailed below:

- The determination of the total biomass by the ammonium ions resulting from the lysis of the bacterial cells called "fumigation and extraction" method [22, 23 and 24].
- The relatively recent technique of molecular biology: Polymerase Chain Reaction (PCR). Overall, direct extraction methods are more efficient than indirect methods. They generally allow the recovery of more DNA and are also faster and allow the simultaneous treatment of more samples [25,26].
- The enumeration after extraction:

- By cultures, solid phase; initially developed by [27], measurement of "CFUs, Colonies-Forming Units" and in liquid phase, measurement of "MPN, Most Probable Number", for example).
- By direct enumeration (the most appropriate technique of fluorescence microscopy).

In our work, the enumeration by cultures will be used to quantify the microbial biomass existing in the PD, inoculum, activator and fermentation medium used in our study.

2.3.2. Solutions and culture media used

The enumeration by cultures was chosen because it is easy, simple and the most available. It has considerable importance on the degradation of matter and assesses the hygiene of the tested substrates. The aim of enumeration is to determine the concentration of bacteria in order to estimate the quantity and the quality of the biomass that is involved in the AD (before and during the process) For the quantitative and qualitative enumeration of the

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microorganisms existing in the biotopesit is necessary to carry out the following four steps: Preparation of stock solutions, preparation of dilutions, incubation and finally reading the results. The following table lists all the solutions and media used for microbiological characterization.

Table.1: List of the solutions and media used for microbiological characterization

Solutions	Culture media	Conditions beforeseeding	Microorganisms sought	Incubation conditions
- Stock solutions: In each vial containing buffered peptone water (with a pH that promotes the multiplication of microorganisms), the biotope to be tested must be placed; The sample should be diluted 10 times.	- Nutrient Broth (NB): for 1L - 50g peptone - 10g meat extract - 20g yeast extract - 50g sodium chloride	- Restlessness - Autoclave sterilization at 120 ° C for 15 to 20 minutes - Adjusting pH to 7	- Mesophile Total AerobicFlora (MTAF)	- Incubation for 48 hours at 37 °C
	- YEG: for 1L - 10g glucose - 10g yeastextract - 18g agar	- Restlessness - Autoclave sterilization at 120 ° C for 15 to 20 minutes - Adjusting pH to 5	- Yeasts	- Incubation for 24 hours at 30 ° C
- Acid solution and basic solution for the pH adjustment of the culture media.	Chapman: for 1L - 10g peptone - 10g meat extract 75 g of sodium chloride - 10g manitol - 0.025g phenol red - 15g agar	- Restlessness - Autoclave sterilization at 120 ° C for 15 to 20 minutes - Adjusting pH to 7	- Staphylococci	- Incubation at 37 ° C and reading after 48 hours
	- Brillant Green Lactose Broth (BGLB): Ready-to-use medium		- Total coliforms - Fecalcoliforms	- Incubation 24 hours at 30 ° C - Incubation 24 hours at 44 ° C
	- PCA (Plat Count Agar): - 5g tryptone - 2.5g yeastextract - 1g glucose - 15g agar			Incubation at 30 ° C. for 24 hours.

2.3.3 Stages of microbiological characterization

2.3.3.1. Preparation of culture medium and the place of seeding

The culture medium (9 ml) was divided into each test tube using a pipette connected to a safety pipettor. Then, all prepared culture media, Distilled Water (DW) tubes and cone tips of the micropipette for the preparation of the dilution are sterilized by autoclaving at 120 ° C for 20 minutes. When culture media are obtained, the DW tubes and the tips are totally sterile; the preparation of a sterile and aseptic seeding site is used. The bleach is wiped off with the bench top of the hood and then the Bunsen spouts are lit inside the hood and the U.V light is switched on half an hour before sowing.

2.3.3.2. Preparation of decimal dilutions in series

Inside the sterile hood, UV light is extinguished and the first step consists in preparing several decimal dilutions (10^{-1} to 10^{-9}) by the following steps: One ml of the stock solution is introduced into the tube containing 9 ml of sterile distilled water. Using the vortex (used for homogenization), solution $10^{-1}$is obtained. Then, from solution 10^{-1} obtained, one ml was taken and placed in a new test tube containing 9 ml of sterile distilled water. Finally, it is necessary to continue the dilutions in cascade up to 10^{-9}.

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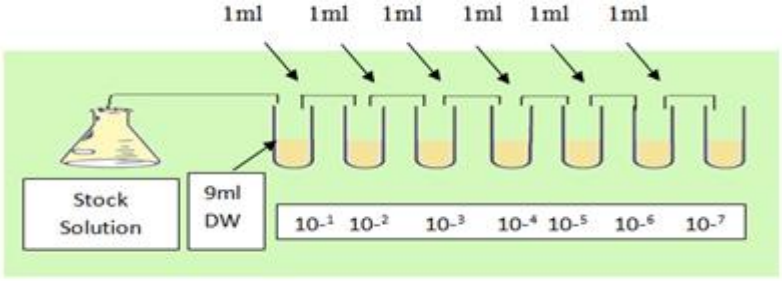

Fig.1: Preparation of dilutions

2.3.3.3. Seeding of dilutions and incubation

From each dilution one ml was inoculated into 3 tubes of nutrient broth.

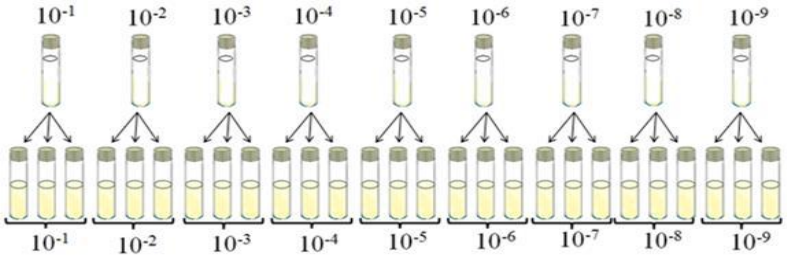

Fig2: Seeding of culture media

After seeding each tube of culture medium with a decimal dilution, these culture media are placed for incubation at the temperature and the duration favorable for each type of bacteria in order to search the number of colonies or the disorders.

In the case of liquid media, the characteristic number, which consists of three digits, is determined by: the first digit corresponds to the number of positive tubes (turbid presence) of the dilution which gives the maximum of the positive tubes, followed by two digits. This characteristic number, on the Mac.Grady table gives the Most Probable Number (MPN) of cell / seeded volume of the first digit dilution. Finally, bacterial biomass is determined. In the case of solid media, the number of colonies is that of the microbial flora present in the dejection.

2.4. Optimization of experimental AD of droppings of laying hens

The objective of this part is to study the effect of the use of an inoculum and an activator on the experimental AD of laying hen droppings and the suggestion that this combination helps to the degradation of the OM and the production of biogas. This experiment seeks to optimize AD by modifying the several parameters, and referring to the results obtained from PD with the inoculum without studying the addition of an activator [28].

2.4.1. Bioreactor preparation technology

The AD takes place in a reactor vessel in which the material to be treated is introduced either continuously or discontinuously. The bioreactor is a closed and sealed system in which bacterial biomass carries out several biological reactions of the methanogenic fermentation simultaneously and naturally, by consuming the OM present in the droppings of laying hens in liquid medium. The production of biogas within the bioreactor is the result of the metabolic activity of methanogenic bacteria. In our research, our bioreactor operates in discontinuous or batch mode, droppings and water are introduced at the start of processes in a tank (Flacon or Erlenmeyer) closed strictly to achieve total anaerobiosis. When organic poultry waste is under anaerobic conditions, all biodegradable material is converted to biogas and the remainder converts to humic. In order to exploit this waste, we are interested in biogas, mainly methane.

To carry out the experimental tests, the following device was used:

- Digesters used: Six Batchs (Erlenmeyers of 100 ml) linked to a gasometer consisting of an inverted graduated burette filled with a guard solution (5% citric acid, 20% Na Cl).
- The top of the gasometer is occupied with a valve and a syringe allowing the adjustment of the level of measurement. When the biogas is produced, it will push the guard solution down the gasometer.
- Each erlenmeyeris connected to a column of guard solution allowing the visual measurement of the volume of biogas produced daily.

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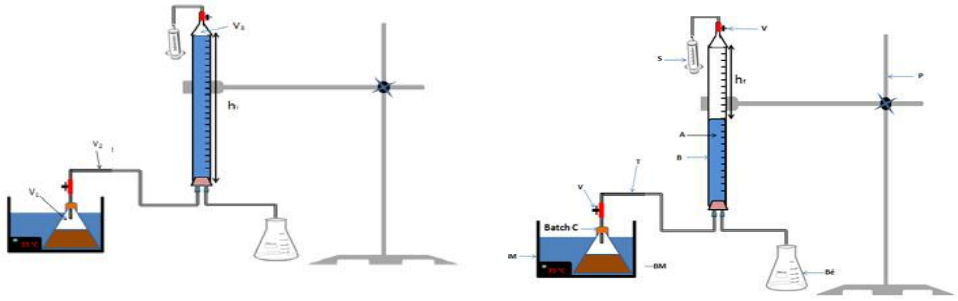

Fig.3: Device for measuring of biogas produced by droppings

The device chosen in this work uses the principle of measuring biogas, which is the displacement of liquid by biogas pressure. So, related to the density of moving liquid and dead volumes in each device. Every day, we note the volume of biogas called uncorrected volume (Vnc). This volume is given by the following equation (1):

(1) $\boldsymbol{Vnc = Vr + (hf \times œ)}$

With; Vr: real volume, hf: Measurement height (Height in the final state) and œ: Calibration coefficient of the measuring gasometer in ml / cm. Uncorrected biogas

2.4.2. Controlled parameters

In our study, AD was used in batch mode. At the end of the digestion, when the release of the biogas drops or becomes zero, the reactor is emptied and a new batch is introduced [30, 31]. It is wet-laid at 8% DM, when the solids content is less than 15%. This process is mainly used for the methanation of sewage sludge, slurry and manure or other liquid inputs. In the case of solid residues, their dilution must be done in order to obtain a concentration of 10 to 15% solids [31]. It is also in mesophilic mode between 30 and 40 ° C, with an optimal operating temperature of 35 ° C. It is the most used mode, because of its stability and good biogas production [32]. Moreover, mesophilic microorganisms are more robust and more tolerant of temperature variations, and are therefore more suitable for digesters with less controlled characteristics and localized in colder climates [31]. The monitoring of the pH value has been realized by taking a sample of the fermentation medium with a syringe (3 to

volumes must be corrected to temperature and pressure as standard conditions 0 ° C and 101.325 kPa [29]. According to the following equation (2):

(2) $\boldsymbol{Vc = Vnc \times Ts/Tamb \times (Ps-(hi-hf) \times d)/Pamb}$

With; Tamb: Laboratory ambient temperature at the time of measurement in K ° (° C +273.15), TS: Standard temperature in K ° (273.15 K °), Pamb: Laboratory ambient pressure at the time of measurement in hPa, PS: Standard pressure in hPa (1013.25 hPa), d: density of liquid, hf: Measurement height and hi: Initial Height (Reference Height);

5ml). The batch is equipped with a syringe to take up the samples to measure the pH and carry out the microbiological test. Among the practices used for the experimental trial; manual stirring twice a day, before each sampling, quantitative monitoring of the microbial load, daily monitoring of biogas production and the duration of measurements is 46 days.

2.4.3. Quantities of substrate

In order to determine the quantity to be introduced, it is necessary to know the percentage of the DM of the inputs to be used. The quantities used for digesters of 8% of DM are calculated by the following formula (3):

(3) $\boldsymbol{Vs \times \%DMi = Vd \times \%DMf}$

With; Vs: Volume of substrate, Vd: Digester volume and Vw (volume of added water) = V2 - V1.

Three tests were used; Test 1 with only the use of droppings and inoculum and 0% activator. For test 2, the activator was added by 9% relative to the total volume and this quantity was doubled for test 3 to reach 18%.

Table.2: Quantities of substrates used for the digesters 100 ml

	Substrate (g)	Inoculum (ml)	KT01 (ml)	Water (ml)	Real Volume (ml)
Test 1	10	46	00	32	88
Test 2	10	37	08	33	88
Test 3	10	28	16	34	88

2.4.4. Qualitative monitoring

For the qualitative monitoring (Composition of the biogas), three five-liter Bottles (B) were fed by the same components of the experimental digesters to 100 ml. The bioreactor (B); closed with a stopper connected to a flexible tube and

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completely empty thanks to a silicone pipe was placed in a water bath at constant temperature 35 ° C. along AD processes (fig4).The quantities of the substrates to be introduced are calculated by using the same method used for the quantitative monitoring (table 3).

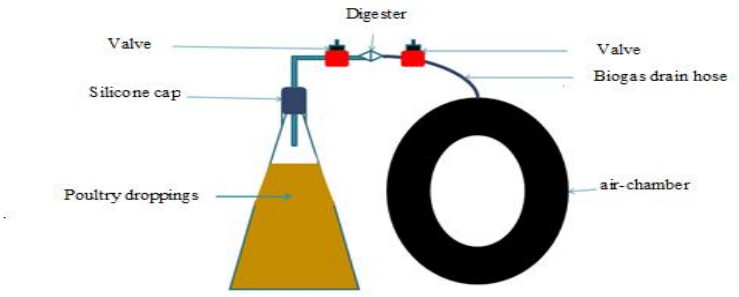

Fig.4: Installation of quantitative biogas monitoring

Table.3: Quantities of substrates used for the qualitative monitoring of biogas produced

	Substrate (g)	Inoculum (ml)	KT01 (ml)	Water (ml)	Real Volume (ml)
B 1	400	2243	00	1357	4000
B 2	400	2243	200	1157	4000
B 3	400	2243	400	757	4000

The KT01 used for the qualitative test is the remainder of the stock solution and that recovered from the boxes and tubes used for the microbiological characterization of this activator. The KT01 count of the bottles was made in the PCA solid medium and 200 10^2 CFU / ml was found. The qualitative monitoring was carried out by a portable biogas analyzer called Biogas 5000. This new biogas analyzer, specifically dedicated to measurement on anaerobic digesters, has real improvements compared to the previous biogas check generation. As standard, three gases can be tested: CH_4, CO_2 and O_2andit can be added to the control unit, an H_2S sensor.

III. RESULTS AND DISCUSSION

3.1. Evaluation of the physicochemical quality

Table 4summarizes the various physical parameters identified for each input considered.

Table.4: Results of the physico-chemical characterization of the studied poultry substrates

Parameter	Abbreviation (unit)	Average Value	Limitations	References
potentialHydrogen	**pH**	6.40	[6-8]	[33]
Electricalconductivity	**EC (mS/cm)**	12.44	-	-
Dry Matter	**DM (%)**	27.00	[20-40]	[34]
Organic Matter	**OM (%)**	75.35	[23.5-63]	[34]
Total Porosity	**TP**	94.5	-	-
Suspended Matters	**SM (%)**	8.70	-	-

The physicochemical characterization shows that the wastes tested are rich in OM (75.35%). Moreover, it can be deduced that the total porosity of the particles to be fermented is important, which will favor the degradation of the effluents and improve the microbial activity. For the SM, its determination makes it possible to estimate the bacterial biomass in the digester [35] that it seems important (table 4).

3.2. Microbiological evaluation

3.2. 1. Microbiological characterization of the constituents to be introduced

Microorganisms can use many substances as sources of energy, source of matter, and as final electron acceptors [36]. Their activity requires the presence of nutrients such as C, N, O, S, P, etc. as well as trace elements such as Fe, Cu, Mg, ... (brought by dissolution of materials) [37]. The micro-organisms multiply therefore from the nutrients available in their living environment. Overall, to develop,

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they have a number of common needs. These include water, a source of energy, a source of carbon, a source of nitrogen and other minerals. The very varied environmental conditions of the different give the microbial world a great diversity and a very wide metabolic capacity as well as a very great capacity of adaptation. Microbial life is thus possible even in extreme environments (high or low temperatures, alkaline pH, etc.). Microbial activity can include; variation in the quantity and composition of organic and inorganic matter, variation of the pH and also variation of the oxidation-reduction potential.

The identification of the microbial load of the test components can provide information on the number and nature of the bacteria to be introduced into the digester. Table 5 shows the bacterial load of each component CFU / ml.

Table.5: Microbiological enumeration of AD components

Type of microorganisms	**Microbial load of droppings in CFU / ml**	**Inoculum microbial load in CFU / ml**	**KT01 microbial load in CFU / ml**
MTAF	9400 10^6	2 10^6	860 10^6
Staphylococci	1250 10^6	0,07 10^6	
Total Coliforms	2250 10^6	0,008 10^6	
Fecal Coliforms	5000 10^6	0,004 10^6	
Yeasts	877 10^6	0,08 10^6	
Anaerobicbacteria	2,50 10^6	0,4 10^6	

The droppings of laying hens are laden with pathogenic bacteria flora. Mainly pathogenic staphylococci have a high content with (125 10^7 CFU / ml). The coliforms (total and faecal) are in first position with 250 10^7 CFU / ml). The microbiological enumeration shows that all the constituents are loaded with bacterial charge which can help the smooth progress of the AD and the production of Biogas.

3.2.2. Microbiological monitoring of Mesophilic Total Aerobic Flores (MTAF)

MTAF counts were conducted throughout the study (almost every 5 days). The following table shows the mean value found for each test and during each sampling.

Table.6: Microbiological enumeration of MTAFs

MTAF	**0% KT01**	**9% KT01**	**18% KT01**
I (t0)	25000 10^4	137500 10^4	7,15 10^4
1 (5days)	273 10^4	107,05 10^4	218,5 10^4
2 (10days)	2151 10^4	12250 10^4	7650 10^4
3 (15days)	1736 10^4	11350 10^4	1165 10^4
4 (20days)	7,03 10^4	3400 10^4	870 10^4
5 (25days)	2 10^4	0,9 10^4	2 10^4
6 (30days)	0,2 10^4	1,5 10^4	0,2 10^4
7 (41days)	4 10^4	4 10^4	0,2 10^4
Percentage Reduction (PR)	99.98	99.99	97.20

Mesophilic aerobic flora (also called total flora) represents all microorganisms developing in the presence of oxygen at an optimum temperature of 30 ° C. This term may therefore include both pathogenic and altering microorganisms. A high number of mesophilic aerobic floras represent a risk of the presence of pathogenic germs at potentially dangerous levels. It is noted that in general the number of MTAF is decreasing but it is still high compared to the nature of our study which takes place in anaerobiosis. This can be explained by taking samples from the syringe for microbiological monitoring and pH measurement. It is probable that during the sampling, there is oxygen intake which is the origin of the development of the aerobic bacteria.

3.2.3. Microbiological enumeration of staphylococci

It is found that the fermentation medium is loaded with staphylococci, but with AD, it is found that their values are decreasing. Anaerobic fermentation can be the cause of the decrease of pathogenic bacteria such as staphylococci.

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Table.9: Microbiological enumeration of staphylococci

Staphylococci	**0% KT01**	**9% KT01**	**18% KT01**
I (t0)	-	-	-
1 (5days)	-	-	-
2 (10days)	354 10^4	418 10^4	61 10^4
3 (15days)	51 10^4	38 10^4	7,4 10^4
4 (20days)	6 10^4	6 10^4	5 10^4
5 (25days)	36 10^2	64 10^2	73 10^2
6 (30days)	110	23	23
7(41days)	77	68	65
PR	99.99	99.99	99.99

3.2.4. Microbiological monitoring of Total Coliforms (TC)

Table 10 summarizes the numbers of TCs recorded for each sample and each test.

The results found in our experiment show that the fermentation medium is loaded with total coliforms. There was an increase observed on the 15th day and from day 20 onwards, a decrease was recorded until the end of our study.

Table.10: Microbiological enumeration of TCs

TC	**0% KT01**	**9% KT01**	**18% KT01**
I (t0)	-	-	-
1 (5days)	-	-	-
2 (10days)	19,71 10^4	1,33 10^4	0,77 10^4
3 (15days)	70,50 10^4	564 10^4	800 10^4
4 (20days)	12,62 10^4	1,55 10^4	0,19 10^4
5 (25days)	2,3 10^2	14 ,4 10^2	11 10^2
6 (30days)	23	23	20
7(41days)	25	24	27
PR	99.90	99.80	99 .65

The increase in total coliform numbers after two weeks can be explained by the presence of a large amount of degraded organic matter and the favorable conditions for the development of this type of bacteria: the pH is of the order of 7 (see curve 2) and the temperature is 35 ° C.

3.2.5. Microbiological monitoring of Fecal Coliforms (FC)

These coliforms are able to grow at 44 ° C. The main coliform bacteria, specifically of fecal origin are Escherichia Coli. This bacterium always appears in large quantities in animal excrement. Fecal or thermotolerant coliforms are a real danger to nature and especially to contamination of soils and waters. In our study, it is also noted that during AD, this type of bacteria are gradually decreasing.

Table.11: Microbiological enumeration of FC

FC	**0% KT01**	**9% KT01**	**18% KT01**
I (t0)	-	-	-
1 (5days)	-	-	-
2 (10days)	67,5 10^4	505 10^4	34,59 10^4
3 (15days)	95,0 10^4	37,0 10^4	18,68 10^4
4 (20days)	1,37 10^4	3,03 10^4	3,65 10^4
5 (25days)	9 10^2	20 10^2	11 10^2
6 (30days)	56	23	200
7(41days)	25	150	180
PR	99.99	99.99	99.99

3.2.6. Microbiological variation of Anaerobic Bacteria (AB)

Microbiological monitoring of AB has shown that our AD medium has exhibited a significant number

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of this type of bacteria. It is found that the enumeration results are close for the three tests with a slight increase for the test 3.

Table.12: Microbiological enumeration of ABs

Anaerobic bacteria	**0% KT01**	**9% KT01**	**18% KT01**
I (t0)	-	-	-
1 (5days)	-	-	-
2 (10days)	595 10^4	3550 10^4	6450 10^4
3 (15days)	80 10^4	315 10^4	450 10^4
4 (20days)	32 10^4	274 10^4	69 10^4
5 (25days)	20 10^3	56 10^3	74 10^3
6 (30days)	17 10^3	30 10^3	50 10^3
7(41days)	7 10^3	17 10^3	30 10^3
PR	99.88	99.95	99.95

The result of the microbiological analyzes show that the microbial biomassis decreased after AD. Among the main factors leading to the reduction of pathogens is the couple temperature and residence time. Indeed, the digestion temperature increases the shorter the residence time to obtain the same reduction rate. Mesophilia (condition of our study) allows reductions that are sometimes insufficient to make the digestate compliant with regulations. On livestock manure, it generally allows microbial hygienisation to be superior to simple storage. It often allows sufficient reduction of E. coli, with a reduction of about 10^2 [38]. Enterobacteria, Streptococci and Coliforms are only reduced by 10^2 CFU / g and are present at 10^5 [39]. For our case, the decrease recorded is greater than the values quoted above reported in bibliography. This may be due to the optimization of fermentation by the addition of inoculum and activator, which has improved the degradation of the OM and its stabilization and ink which has an important role in the activity of the microorganisms and hygienisation of final product.

The substrate used is very rich in organic matter (table 4) and microbial load (table 5) and is a good substrate for biomethanation. The addition of inoculum and activator has further increase the number of this load and also improved its quality. The bacterial biomass present in the inoculum and the activator is considered to be a more active and stable charge following the transformation of the already existing organic compounds into simple compounds which can be easily consumed by the microorganisms. This characteristic justifies the improvement of the degradation of the organic substrate material by the microorganisms. According to bibliographic research, AD reduces the amount of organic matter which may explain the regression of the bacterial load which requires OM to continue its life.

The AD of the droppings carried out in the present study had a destructive impact on the bacterial load. These results corroborate those of Couturier [40] who stresses that anaerobic digestion aims to reduce exposure by elimination or inactivation of pathogenic organisms. The main parameters for the elimination of pathogens are time and temperature. Overall, conventional mesophilic digestion makes it possible to remove 99% of pathogens as was the case in this study. The rate of reduction depends on many other parameters in practice. These are the initial concentration of pathogens, the feeding mode of the digester and competition with other microorganisms [40]. Digestion in anaerobiosis presents several fundamental advantages linked to the power of the biological mechanisms involved to ensure a high rate of degradation of organic matter, to the particular chemical conditions of anaerobiosis [41].

3.3. Follow up of the daily production of biogas

It can be seen from curve 1 that test 3 (with the highest% of KT01) showed the highest Biogas production, especially during the first 20 days. This can be explained by the nature of the pure microbial activator used (KT01) which favors the hydrolysis phase or also by the combination of the different constituents and the quantities used for this test with the increase of KT01 to improve the functioning of the inputs and the selected mixture and thereafter the improvement of the biogas production which started from the first test day and remained high and stable during the first period.. For the other tests, the same production was observed during the same period. From day 21, it is found that the production of three tests is close with a slight increase observed for test 2, but starting from the last week (from day 39 of study), it is noted that test 1 (With the lowest% of KT01) had the highest production. Quantitative monitoring showed that the most loaded activator test started with higher biogas production than the other two tests, but this

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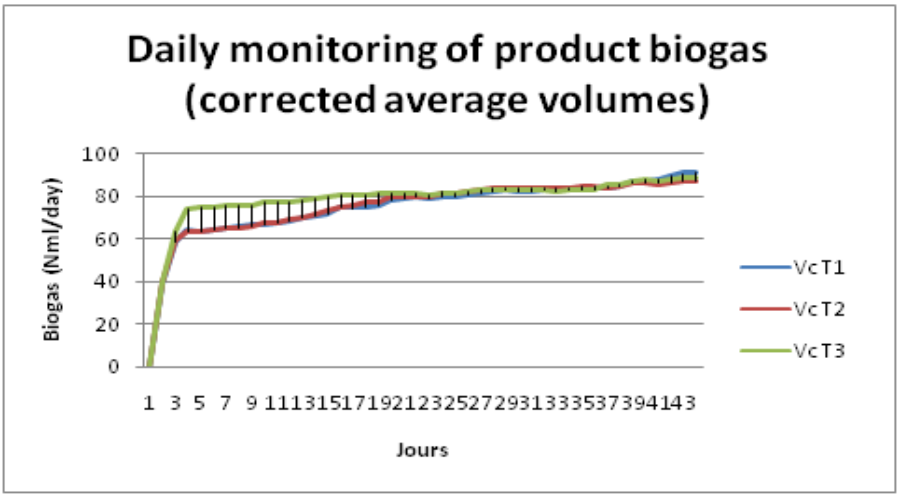
increase did not persist throughout the study. For the other two tests, it is noted that their productions are close almost throughout the study period. Several studies have shown that various parameters have influenced the production of biogas among others: the nature of the substrate, as in our case the PD. However, [42] attributed the higher biogas yield from the chicken dropping to the presence of native micro flora in this dropping while [43] attributed it to the low carbon-nitrogen ratio. According to [44], water content is one of the very important parameter effecting AD of solid wastes. Water make possible the movement and growth of bacteria, facilitating the dissolution and transport of nutrient. Furthermore, we find the addition of inoculum which is a good source of bacteria [43]:" inoculum is a biological active liquid or partially digested organic waste medium, rich in microorganisms" [45]. Microbial flora, elimination of lag phase and hence increased biogas production and methane contents especially where the synergy existed [46]. Finally, the addition of the activator has also a big influence on the amount of cumulative biogas.



Curve 1: Daily monitoring of production of biogas produced (corrected production)

3.4. Follow up of the composition of the biogas produced

Biogas is a mixture of CH_4 (40 – 75%) and CO_2 (15 – 60%) with small amounts of other gases and by-products, i.e. nitrogen (0 – 2%), carbon monoxide (< 0.6%), H_2S (0.005 – 2%), O_2 (0 – 1%) and ammonia (< 1%). Trace amounts of siloxanes (0 – 0.02%), halogenated hydrocarbons (< 0.65%) and other non-methane organic compounds as aromatic hydrocarbons, alkanes, alkenes, etc., are also occasionally present. Usually this mixed gas is saturated with water vapour and may contain dust particles [47].The quality of the biogas is evaluated essentially by measuring the percentage of CH_4 it contains. Indeed, a higher percentage of methane is better when it comes to biogas. However, it is also based on the determination of the percentage of CO_2 and the percentage of H_2S. In contrast to methane, the lower the percentage of these elements, the better the quality of the biogas produced. A good quality biogas is composed of circa 65% $CH_4$and 35% CO_2 [48].

Table.13: Monitoring the composition of the product biogas

Composition	T1= 12 days	T2= 28 days	T3=44 days	T4= 65 days
		B1 (0%KT01)		
CH_4	19,6%	21,2%	25%.	35 %
CO_2	78,2%	75,1%	67%	57%
H_2S	10ppm	17ppm	31ppm	900ppm
		B2 (9%KT01)		
CH_4	17,8	22,8%	20,8%	26%
CO_2	70,2	34,8%	67 ,2%	62%
H_2S	81ppm	22ppm	100ppm	110ppm
		B3 (18%KT01)		
CH_4	8,5	20,8%	79%	71,5%
CO_2	21,3	50 ,7%	10,9%	26,4%
H_2S	46ppm	31ppm	43ppm	900ppm

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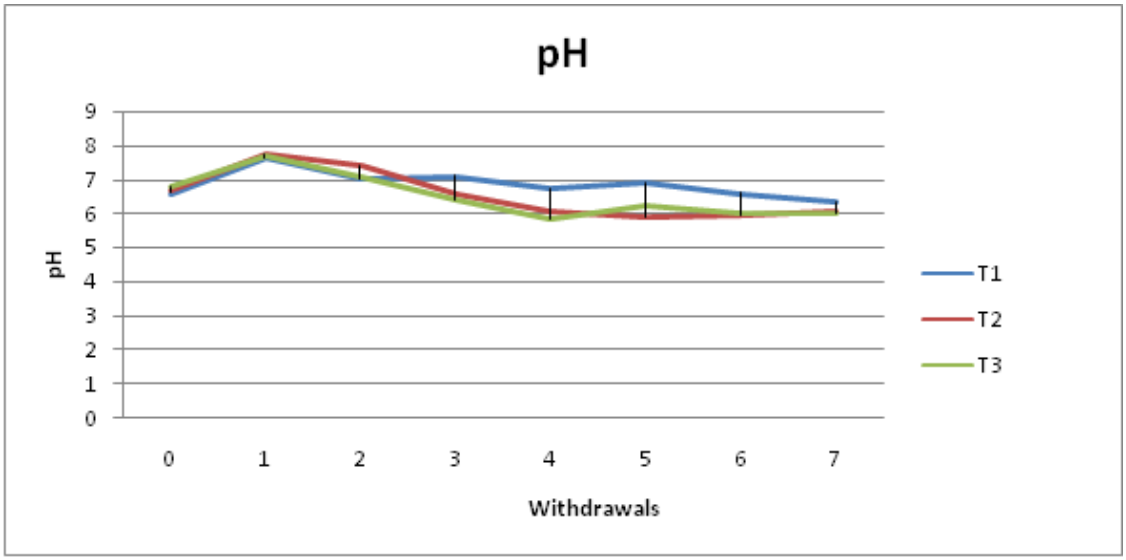
The qualitative follow-up shows that the good quality is observed at the level of test 3 and especially on day number 44 of the study with 79% of CH_4. However, it should be noted that during the first two weeks test 1 showed the highest amount 19.6% and the lowest one for test 3. After 28 days, it can be seen that the biogas percentages are close for the three tests. It can be concluded that the activator increased the amount of biomethane but with a residence time at the digester of the order of one and a half months. It is necessary to mention that the amount of biomethane found is very large with a low CO_2 percentage (for test 3). However, it must be said that the amount was obtained after a long period (after 44 days) and that the activator influenced the amount of biogas at the beginning of the test and not the quality. It is necessary to repeat other tests to validate the results found.

3.5. Evaluation of pH value change



Curve 2: Monitoring of the pH measurement during the different samples

The pH value in the digester is between 6 and 7 [49]. Low pH value inhibits methanogenic bacteria and methanogenesis [50]. The high pH value recorded in this study during the first week could be attributed to large ammonia losses resulting from C/N ratio of poultry waste [51].

The study of the influence of pH on the production of biogas from poultry waste showed that in general the production of biogas was always present because the pH remained throughout the period of the study in a range of 6 to 7.8. These values belong to the standards reported by several researchers from 6 to 8 and that the best production is observed for pH measurements of 7 to 7.2 [52]. For this reason, it is found that for test 3 the pH measurement has remained between 6 and 7.7 and that during the first 20 days the pH is 6.5 to 7.7, thus favoring the production of the biogas.

IV. CONCLUSION

According to the results of the microbiological evaluation showed that the constituents involved are loaded with microbial flora and especially the digester fed with the highest percentage of activator. It is necessary to say that this bacterial load was decreasing during AD.

The daily monitoring carried out of the experimental installation has a good functioning. However, it should be noted that the samples taken may have disrupted anaerobiosis (presence of aerobic bacteria).

The combined effect of the addition of inoculum and activator on the quantitative energy performance of the experimental biogas produced showed that gas productivity is higher in the case of digesters fed with 18% activator.

From the point of view of quality, it can be seen that test 3 showed generally the best portion of methane, especially after 44 days. It is also important to note that after the first month, test 3 showed the best gas productivity especially during the first 20 days, but the other two showed a higher amount of methane. For the percentages of CO_2 and H_2S, test 3 showed the lowest percentages. For this reason, it can be seen that the addition of activator (18%) has improved the quantity of biogas and, above all, the speed of productivity and quality.

In general, we can say that the addition of activator can influence the frequency and the rate of production of the Biogas. However, this interpretation can only be confirmed after evaluation of this activator alone without inoculum and with yet another economic evaluation on the price of this activator and its interest on the profitability side.

We can conclude that anaerobic digestion involves a very large number of bacteria and biological reactions, so it is

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a very complex process. Furthermore, it is impossible to generalize all the approaches because each digester is unique given the process involved, the substrates used, the bacterial populations present, etc.

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