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Essay of Poultry Manures Hygienisation and Amendment Value Improving in South Benin

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ABSTRACT

Poultry manures are widely used in market gardening in South Benin. Several studies have shown that the use of raw poultry manures in market gardening has an impact on both the microbiological and chemical quality of the vegetables produced. Therefore, the hygienisation of these manures, through various composting processes, is necessary. The main objective of this research is to study the efficiency of aerobic composting and the anaerobic digestion of poultry manures in terms of reduction of pathogenic microbial load. To do this, poultry manure collected on a target farm in southern Benin was treated according to the two composting processes. Samples of the raw manure and humus obtained after composting were collected and analyzed in the laboratory for the count of the pathogenic flora. The quantum of *Escherichia coli* decreased by more than 90 % at the end of both processes, but aerobic composting was more effective than anaerobic digestion. On the other hand, there was a proliferation of coagulase-positive *Staphylococcus* and *Clostridium perfringens* whose quanta increased considerably at the end of the two processes, which can be justified by several factors whose redox potential of these two particular bacteria, conditions of the composting process and the hygiene of post-composting operations.

Introduction

Organic biodegradable wastes (vegetable matters, animal excrements, food-industry under-products, muds of purification stations, domestic rubbishes, etc.) can been transformed by biotechnological processes which represented a suitable solution in order to remedy environmental pollution problems, and secondarily, been incorporated to the soils to improve agricultural output in one hand and in another hand consolidate soils structure. For that, they are used as long as synthetical humus. According to Willey et *al.* [25], an humus resulted when the products of microbial metabolism have undergone chemical transformation within the soil. It represented natural achievement of organic matter transformation (recyclation) and constituted the precursor of that activity realyzed by controlled manner called composting.

According to TRAME [23], composting is a controlled biological process of organic wastes conversion and valorization (biomass under-products, organic wastes), a microbial processing of fresh organic matter under moist, aerobic conditions, resulting in the accumulation of a stable humified product which is suitable for soil improvement and stimulation of plant growth. The same author [22] described it as a biological technic of organic matter recycling which at the end of its evolution permitted to have some humus, factors of stability and fertility of soils. In Benin, poultry farms produced every year, some important quantities of poultry manures which piled up and polluted the environment if nothing had done to valorize them. Moreover, it has been noted through out many studies the utilization of crude poultry manures got an impact as well on microbiological quality as on the chemical one in the garden products obtained with it [11 ; 5]. So, the committee in charge of food microbiological standards at Quebec, has mentionned « the vegetables and fruits could be vectors of pathogenic microbes belonging to these organic fertilizers, and contaminated irrigation water » [3]. So, before their utilization in the garden culture, their hygienisation is essential.

There were two types of composting to know : the aerobic composting and the anaerobic one. In the aim to optimize this operation on poultry manures, we have used the two forms of composting. The main objective of this research was to study the performance of a aerobic composting and the anaerobic digestion of poultry manures in terms of reducing the load of pathogenic microbes (*Escherichia coli*, coagulase-positive *Staphylococcus, Clostridium perfringens and Salmonella spp*.). Specifically, it consisted to evaluate the microbiological salubrity and also the amendment value of the compostings through measurement of nitrogen and phosphorus contents present inside them.

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Material and Methods

Sampling and analysis of poultry manure collected on a South Benin farm

A preliminary investigation has permitted to choose a farm which was convenient to specific standards of livestock good practices. This farm was located at Pahou (a district of Ouidah) distant from some twenty five kilometers from Cotonou, the economical capital of Benin Republic. Poultry manure was purchased on the farm and transported to the Center of Waste Valorization in Renewable Energies and in Agriculture located on the main campus of the University of Abomey-Calavi.

Thanks to the quartage method [12 ; 24], five (5) samples about 500 g each, have been taken, preserved inside some isolated box at +4°C and transported to the laboratory. Bacteriological analyses were performed on it : mesophilic aerobic germs (ISO 4833-1 : 2013) [9], *Escherichia coli* (ISO 16649-2 : 2001) [8], coagulase-positive *Staphylococcus* (ISO 6888-2 : 2003) [7], *Clostridium perfringens* (NF V 08-056 : 1994) [17] and *Salmonella* spp. (ISO 6579 : 2002) [10].

Other five samples of 500 g each have been at the same time taken for the measurement of total nitrogen (Kjeldahl), the total phosphorus and bioavailable phosphorus [1] in order to appreciate its amendment value.

Realization of aerobic composting and anaerobic digestion of the poultry manure

The manure has been divided in two lots of same weight. The two lots have been treated by aerobic composting in one hand and by anaerobic digestion in the second hand. The study was conducted in the Center of Waste Valorization in Renewable Energies and in Agriculture.

Aerobic composting with « swath » method

The manure has been spread on a big plastic cover disposed on the soil. A pile of 3 meters in length, 2 meters in width and 20 centimeters in height) has been built up, realizing two layers of it. The two layers have been waterded the first after the second and heaped up in order to allow the infiltration of water till the profundity of the pile and, by this way, stimulate the metabolic activity of microorganisms. The pile so realized has been covered with a second plastic cover (Fig. 1), then the turnaround and the watering have been done all two weeks.

The turnaround has allowed the oxygenation of the pile as completely as possible in order to facilitate the survival of microorganisms. The temperature of the pile has been measured with an electrochemical thermometer which the probe has been introduced inside the pile, the temperature appeared on the screen five minutes after the introduction of the probe. The following up of the temperature has permitted to determine when the watering was necessary for microorganisms action, responsible of the decomposition.

Figure 1. Realization and covering of the swath.

The process of decomposition has lasted three months. The obtained humus has been spread on a new plastic cover and has been deshydrated in ambiant air during two weeks. And then the deshydrated composting has been sieved using a riddle with 10 millimeters of stitch.

The chips with big size resisted to the operation of sieving because of their non decomposition during the three months of experience.

Anaerobic digestion

The anaerobic digestion of the manure has been done with a metalllic domestic digestor, conceived on the model described by Lecesve [13]. It was an improved model of digestor with a capacity of 1 m^3 constituted of : a container to load the wastes, a fermentation tank, a pipe to let out biogas extented with a flexible coupling directing the biogas to a cooking burnth, an evacuation pipe of the effluent, a manometer and evacuation valves of the digestate and the biogas (Fig. 2). The system of this digestor was based on a continuous functioning, it means that after the initial period, the digestor could be supplied everyday with the animal wastes, vegetables or household wastes with water in the manner that the effluent and biogas have been daily obtained.

The manure has been introduced in the same time inside the loading container, and then homogenized with 125 liters of water, in order to make it pasty. The proportions are in accordance with the condition little solid or no of the manures to be used. This operation proved to be necessary to accelerate microorganisms action in this environment weak in oxygen. So the manure homogenized has been introduced inside the fermentation tank, by opening the gate of substrate introducing. The transformation of the manure has been achieved inside this tank according to the different steps of anaerobic digestion (hydrolysis, acidogenesis, acetogenesis and methanogenesis) [14] until the setting free of the biogas.

After a period of eleven (11) weeks of fermentation, the digestate obtained has been recuperated by opening the outlet valve located at the basis of the digestor. Then it has been spread over a big plastic cover to avoid the mixture with grains of sand and deshydrated in ambiant air during two weeks before it has been ground manually. So it was this powder from the digestate which will be used for the continuation of the experimentation.

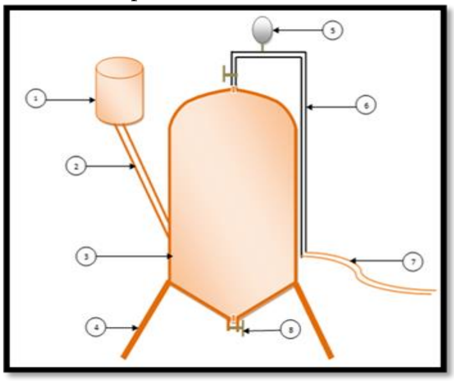

Figure 2. General scheme of the metallic domestic digestor.

1-Loading container, 2-Feed hose, 3-Fermentation tank, 4-Tripod, 5-Manometer, 6-Biogas outlet pipe, 7-Flexible coupling, 8-Evacuation valve of the digestate

Sampling and analysis of the compost and the digestate

After the deshydratation in ambiant air, the sieving in one hand and the grinding manually in anoher hand, respectively five samples of 500 g each, from the compost and the digestate have been sampled inside sterile bag and then took away to the laboratory, inside an isolated box at +4°C for determining mesophilic aerobic germs, *Escherichia coli*, coagulase-positive *Staphylococcus*, *Clostridium* *perfringens* and *Salmonella* spp. The content in fertilizing elements of the humus has also been measured. In this way, respectively five additional samples of 500 g each of compost and digestate have been taken analyzed in the laboratory for the measurement out of total nitrogen, total phosphorus and bioavailable phosphorus.

Results

The calculation of the results of all microbiological analyzes performed in the context of this research has been done according to the recommendation of the standard ISO 7218 of october 2007 [6].

Microbiological quality and amendment value of manure collected on the farm

According to the microbial quanta expressed in $\log_{10}$ CFU/g, the content in aerobic mesophilic germs was over 7.5, the one of *Escherichia coli* was more than 5.2 and it was enumerated an amount of 3.6 for *Clostridium perfringens* in the crude manure collected on the farm. On the other hand, the content of the manure in coagulase-positive *Staphylococcus* was lower than 1.0 and all samples were exempted from *Salmonella* spp. (Table 1).

By elsewhere, the contents (mgkg^{-1}) in total nitrogen, total phosphorus and bioavailable phosphorus of the manure were respectively 27880.2 ± 971.6 mgkg^{-1}, 15404.7 ± 72.6 mgkg^{-1} et 1168.2 ± 121.8 mgkg^{-1}.

Microbiological quality and amendment value of manure after aerobic composting and anaerobic digestion

Analyzes of compost and digestate have shown that the quantum of *Escherichia coli* has been considerably reduced at the end of the two processes of composting. It passed from more than 5.2 ($\log_{10}$ CFU/g) in the crude manure to 2.0 in the compost and to 4.0 in the digestate (Table 2). So there was a considerable reduction, about 99 % in the compost and about 92 % in the digestate for the quatum of *Escherichia coli*.

In contrast to *Escherichia coli*, quantities of coagulase-positive *Staphylococcus* and *Clostridium perfringen*s in both compost and digestate were significantly higher than the initial levels in crude manure (Table 2). The quantity of coagulase-positive *Staphylococcus* increased from less than 1.0 in crude manure to more than 5.2 in both compost and digestate. The number of *Clostridium perfringens* also increased to more than 5.2 at the end of the two composting processes.

The levels of total phosphorus and bioavailable phosphorus in the manure have increased at the end of the two processes of composting whereas total nitrogen amount has been reduced (Fig. 3). The content of bioavailable phosphorus was equal to 3180.8 ± 198.5 mgkg^{-1} in the compost and 2885 ± 104.0 mgkg^{-1} in the digestate. The one of total nitrogen was equal to 24591.7 ± 575.4 mgkg^{-1} in the compost and 21142.7 ± 575.0 mgkg^{-1} in the digestate (Table 3).

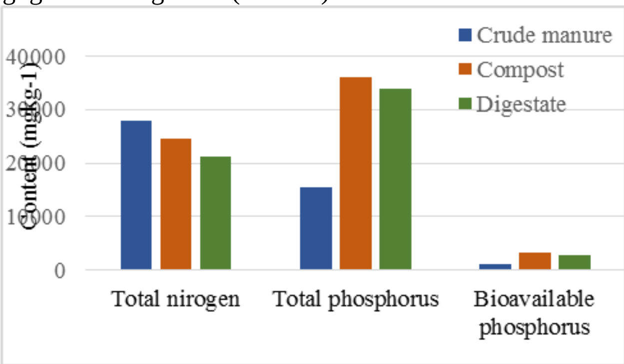

Figure 3. Content of the manure in fertilizing elements before and after aerobic composting and anaerobic digestion.

Table 3. Evolution of the contents in fertilizing elements (mgkg^{-1}) after aerobic composting and anaerobic digestion.

	Raw Manure	Compost	Digestate
Total nitrogen	27880.2 ± 971.6	24591.7 ± 575.4	21142.7 ± 575.0
Total Phosphorus	15404.7 ± 72.6	36131.3 ± 5635.5	33816.7 ± 3886.4
Bioavailable Phosphorus	1168.2 ± 121.8	3180.8 ± 198.5	2885 ± 104.0

Discussion

Microbiological quality and amendment value of raw manure collected on the farm

At present, there are no standards in the Republic of Benin concerning both the sanitary quality and the mineral content of organic fertilizers and amendments. The quantum of *Escherichia coli* was greatly higher than the three microbiological limits recommended by the regulation CE 1774/2002 [20], the standard NF U 44-051 [19] and Homologation ANSES concerning fertilizers and culture

Table 1. Bacterial load ($\log_{10}$ CFU/g) of manure collected on the farm and recommended microbiological limits.

	Manure (n = 5)	Microbiological limits [4] CE 1774/2002	NF U 44-051	Homologation ANSES*
Aerobic mesophilic germs	> 7.5	-	-	-
E. coli	> 5.2	3.4	2.0	2.0 (a) 3.0 (b)
Coagulase-positive Staphylococcus	< 1.0	-	-	< 1.0
C. perfringens	3.6			Absence/1g
***Salmonella* spp.**	Absence/25g	Absence/25g	Absence/25g[a] Absence/1g[b]	Absence/25g[a] Absence/1g[b]

*Certification of fertilizers and culture media by Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (France)(a) vegetable crops (b) All cultures.

Table 2. Evolution of bacterial loads ($\log_{10}$ CFU/g) in manure after aerobic composting and anaerobic digestion.

	Manure	Compost	Digestate
Aerobic mesophilic germs	> 7.5	> 7.5	> 7.5
E. coli	> 5.2	2.0	4.0
Coagulase-positive Staphylococcus	< 1.0	> 5.2	> 5.2
C. perfringens	3.6	> 5.2	> 5.2
***Salmonella* spp.**	Absence/25g	Absence/25g	Absence/25g

media. The quantity of coagulase-positive *Staphylococcus* conformed to Homologation ANSES whereas the one of *Clostridium perfringens* was not at all. There are as yet no normative specifications for aerobic mesophilic germs.

The quanta of *Escherichia coli and* of *Clostridium perfringens*, being out of the ordinary, thus compromise the hygienic quality of the manure collected on the farm of Pahou and consequently the livestock practices adopted in this farm. So it was necessary to hygienize this manure before using it as a fertilizer in the market gardening.

By elsewhere, considering their high contents in nitrogen and phosphorus, chemical composition of poultry manure was evaluated in accordance with the standard NF U 42-001 [18] which prescribed designations and specifications of mineral fertilizers [4]. So, the nitrogen and phosphorus contents in the analyzed manure could be compared to the recommendation of this standard.

Microbiological quality and fertilizing elements contents of manure after aerobic composting and anaerobic digestion

Commensal germs namely *Escherichia coli* and intestinal enterococci are acceptable indicators of composting effect, they are used to evaluate the effectiveness of composting hygienisation [16]. In the context of our study, only *Escherichia coli* has been researched and enumerated. Results have shown the two decomposition processes were effective because the quantum of *Escherichia coli* has been reduced in the manure more than 90 % as much after the aerobic composting as after anaerobic digestion. Aerobic composting was even more effective than anaerobic digestion because the decrease in the quantum of *Escherichia coli* was more significant in the compost than in the digestate.. This result is in accordance with Misra et *al.* [15] who considered aerobic composting more suitable than the anaerobic one for agricultural production.

The number of aerobic mesophilic germs staying very high after the composting is in accordance with the diverse microbial ecology of the humus, mainly composed of molds, bacteria and actinomyceta, which are the important mesophilic flora that have participated to the organic matter decompostion.

Considering the redox factor of coagulase-positive *Staphylococcus* (facultative anaerobic) and the one of *Clostridium perfringens* (microaerophilic), there have been a quick multiplication of these two particular flora, this could justify the high number of these flora both in the compost and in the digestate.

According to Misra et *al.* [15], in one hand, at the end of aerobic composting, the mesophilic flora recolonized the pile but the microbial activity is completly reduced in the mature compost, in the other hand, the pathogenic flora is not affected during the anaerobic digestion because this last one is carried out at low temerature ; this confirmed the increase of mesophilic pathogen flora (coagulase-positive *Staphylococcus* and *Clostridium perfringens*) in the manure at the end of the two decomposition processes.

During aerobic composting, the pile must have a water content of 40 to 65 % [15], excessive humidity leads to a temperature drop within the heap, which prevents the destruction of pathogens. Thus, the moisture content may well justify the increase in the quantities of coagulase-positive *Staphylococcus* and *Clostridium perfringens* after aerobic composting. Indeed, the experiment had coincided with the great rainy season of may to june, the pile probably had to absorb a lot of water during this period, despite the precautions that had been taken to avoid this.

The increase in coagulase-positive *Staphylococcus* and *Clostridium perfringens* can be explained by the post-composting operations conditions, namely the hygiene of the dehydration environment, the hygiene of the sieve and particularly the hands hygiene of the operator who crushed the digestate.

Indeed, the hands constitute an important natural reservoir for the staphylococci which are also found in the nasal cavities and the throat. In addition, as *Clostridium perfringens* is of land-based origin, there was probably a recontamination of humus due to the uplift of the sand by wind during dehydration in the open air.

The decrease in nitrogen at the end of the two decomposition processes is related to the succession of nitrification and relative denitrification phenomena (nitrate returned to the atmosphere in N_2 and N_2O forms) and bacteria such as *Pseudomonas denitrificans* could be present in both products and then to the phenomenon of dissimilatory reduction (conversion of nitrate to ammonia) by a variety of bacteria including *Geobacter metallireducens*, *Desulfovibrio spp*. and *Clostridium spp*. [25].

Unlike nitrogen, phosphorus levels increased in compost and digestate because there is no gas production during its biogeochemical cyclization. Organic phosphorus includes, not only that found in biomass, but in materials such as humus and other organic compounds. This organic phosphorus is recycled by microbial activity and then is available to plants and microorganisms between pH 6 and 7. The microbial transformation of phosphorus features the transformation of simple orthophoshate (PO_4^-), which bears phosphorus in the state of +5 valence, in more complex forms including polyphosphates. Finally, note that bioavailable phosphorus is the most useful form of phosphorus for plant survival because it is the part of the total phosphorus, directly assimilable by plants and necessary for their nutrition [2].

Conclusion

Both forms of composting are effective in terms of improved microbiological quality as well as in improving the amendment value of poultry manure but aerobic composting is better for producing humus of good microbiological quality and rich in nutrients for market gardening. However, the implementation of the two composting processes comprises some constraints related to the conditions of the composting process and to the hygienic conditions of the post-composting operations, which can affect the microbiological quality of the obtained humus.

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