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ORIGINAL PAPER

Physical and Chemical Properties of the Agro-processing By-products Decomposed by Larvae *of Musca domestica* and *Hermetia illucens*

A. Y. Bloukounon-Goubalan[1] · A. Saïdou[1] · C. A. A. M. Chrysostome[2] · M. Kenis[3] · G. L. Amadji[4] · A. M. Igué[5] · G. A. Mensah[6]

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Abstract
The present study aimed to assess the physical and chemical properties of the agro-processing by-products decomposed by fly larvae and to be promoted as soil amendment. The biodegradation process was performed using larvae of *Musca domestica* and *Hermetia illucens* during 30 days on corn bran, a mixture of soybean bran and corn bran (1:1 ratio), and a mixture of soybean bran and corn hull (8:2 ratio). The biodegradation by fly larvae was obtained by exposed the substrates to naturally-occurring flies. Data on temperature, pH, fly larvae production, substrate weight, organic carbon, total nitrogen, nitrite and ammonia contents in the substrates were collected during the biodegradation process. Temperature ranged between 33 and 45 °C during the process and was higher in the mixture of soybean bran and corn hull while pH changed from neutral to alkaline. The substrate weight was reduced and a low carbon loss was recorded. The mixture of soybean bran and corn hull produced the highest amounts of fly larvae (38.28 g kg^{-1} dry matter). Significant reductions ($p < 0.05$) of total nitrogen, ammonia and nitrite contents were recorded in the substrates. An increase of nitrite content in corn bran and in the mixture of soybean bran and corn bran starting from day 12 was observed. The final products of the biodegradation have a high total N content (from 53.75 ± 1.35 to 55.85 ± 0.87 g kg^{-1}) and provide suitable C:N and NH_4-N:NO_3-N ratios, suggesting that they can be used as high quality soil amendment.

Keywords Soil fertility · Biodegradation · Organic residues quality · Crops' residues · Benin

Introduction

In Benin, in the last few decades, the interest in corn and soybean cultivation has increased among farmers. Corn production increased from 671,949 t in 2006 to 1 345 821 t in 2014 [1]. Also, the total land for soybean cultivation has increased from 10,173 ha in 2004 to 95,106 ha in 2013 [1]. As a result, many processing units of these products have been created at household level and by women's groups, which led to an important production of agro-processing by-products such as corn and soybean bran and corn hull [2]. Total corn bran production in the country was estimated at 6% of total sub-Saharan production. These by-products can be used as animal feed [2, 3]. However, if not valorized, they constitute a loss for women involved in the processing activity and for the households. They can also help spreading

✉ A. Y. Bloukounon-Goubalan
adinbloukounon@gmail.com

[1] Research Unit of Integrated Soil and Crops Management, Laboratory of Soil Sciences, School of Crop Production, Faculty of Agronomic Sciences, University of Abomey-Calavi, 03 BP 2819 RP Cotonou, Benin

[2] Laboratory of Aviculture and Zoo-Economy Research, School of Animal Production, Faculty of Agronomic Sciences, University of Abomey-Calavi, 03 BP 2819 RP Cotonou, Benin

[3] CABI, Rue des Grillons 1, 2800 Delémont, Switzerland

[4] Research Unit of Eco-Pedology, Laboratory of Soil Sciences, School of Crop Production, Faculty of Agronomic Sciences, University of Abomey-Calavi, 03 BP 2819 RP Cotonou, Benin

[5] Laboratory of Soil Sciences, Water and Environment, Center of Agriculture Research of Agonkanmey (CRA-Agonkanmey), Benin National Institute of Agriculture Researches, 01 BP 884 RP Cotonou, Benin

[6] Laboratory of Zootechny, Veterinary and Fishery Researches, Center of Agriculture Research of Agonkanmey (CRA-Agonkanmey), Benin National Institute of Agriculture Researches, 01 BP 884 RP Cotonou, Benin

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diseases by sheltering and feeding various disease vectors [4, 5].

The valorization or the recycling of these by-products will have a positive impact on the incomes of women and producers' households. Composting is usually offered for recycling organic waste but the labour intensity of manual application and the cost of the practice were the principal constraints highlighted by farmers [6]. At the same time, proteins sources for poultry diets become more expensive [7] and soils' organic matter is still low for supporting plant growth in southern Benin [8, 9]. In the perspective of crop-livestock integration, scientific research is turning increasingly towards less constraining systems that offer direct benefit both for agriculture and for poultry rearing. The use of fly larvae is increasingly explored since it constitutes a source of protein for poultry [10] and an organic material biodegrader [11]. It was demonstrated that fly larvae easily degrade organic material of different origins such as domestic waste, poultry, pig, cow manure, and even human excreta [12, 13], leading to a more stable and mature bio-product. While the quality of the animals' substrates biodegraded by fly larvae has been increasingly documented, that of crop organic residues such as the agro-processing by-products remains limited. In many cases, farmers throw away these organic residues after larvae production [14].

This paper aims to assess the ability of fly larvae to improve the physical and chemical properties of common crop organic residues and their contribution to the maturity and stability of these types of substrates. Specifically, it aims at (i) assessing the physical properties (temperature and weight) and pH of the agro-processing by-products during the biodegradation process by the fly larvae; (ii) evaluating nutrients (carbon, total nitrogen, $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$) dynamic in the agro-processing by-products during the biodegradation process; (iii) assessing the quality of the final product of the biodegradation process in the perspective of its use as soil amendment.

Material and Methods

Biodegradation Process and Experimental Design

The study was carried out from November to December 2016 at the experimental station of the Faculty of Agronomic Sciences of the University of Abomey-Calavi in southern Benin (2°21′8.58"E, 6°26′27.78"N, 10 m a.s.l.). Corn bran, a mixture of soybean bran and corn bran (1:1 ratio) and a mixture of soybean bran and corn hull (8:2 ratio) were chosen because they are the most important agro-industrial by products in Benin after cotton by-products [2]. These substrates were exposed to natural fly oviposition during 30 days.

Fig. 1 House fly larvae

Fig. 2 Black soldier larvae

In the first 12 days, the biodegradation process of the organic substrates was mainly done by *M. domestica* larvae (Fig. 1), which develop fast and usually pupate after 5–6 days. However, *Hermetia illucens* larvae (Fig. 2) appeared in the substrate from the fifth day and were the only responsible agent of the biodegradation process from the thirteenth to the thirtieth day duration.

The experiment was subdivided into four stages: For the first stage, a quantity of 3 kg of each type of substrate (65–75% moisture content) was weighted in plastic containers and placed in a ventilated shed for free oviposition by the flies. After 10 h, the containers were covered with mosquito net in order to obtain larvae of equal size. After 4 days, mature larvae of *M. domestica* were harvested using a sieve and the substrate remaining in each container was replenished with 1 kg of additional fresh substrate for a second and a third stage of 4 days each. At the end of the third stage, the

substrates were left for biodegradation by *H. illucens* until day 30 [15].

The experimental design was a randomized block design with seven replications and three substrates (corn bran, mixture of soybean bran and corn bran, mixture of soybean bran and corn hull) collected from the processors and in the household and fermented during 3 days before starting the experiment.

Sampling and Data Collection

The samples of each substrate were collected at the beginning and the end of each stage of the biodegradation process. They were immediately conserved at −4 °C for laboratory analysis. Substrates temperature, pH and weight were measured. The pH and temperature were measured daily during the first 12 days of the biodegradation process and then every 2 days from day 13 to day 30. The weights of the substrates were measured at the beginning and at the end of each stage of the biodegradation process.

Laboratory Analyses

A sample of each substrate was sent to the Laboratory of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT-Niger) to perform analyses on organic carbon (wet oxidation by sulfochromic acid, Walkley and Black method), total nitrogen (in a mixture of H_2SO_4–Se and salicylic acid method and quantify by auto-Analyzer using Bertholet reaction), ammoniac ($N\text{-}NH_4^+$) and nitrate ($N\text{-}NO_3^-$) (in 0.01 M $CaCl_2$ method). The C:N and NH_4^+:NO_3^- ratios were then calculated to assess the maturity of the degraded substrates [16]. The productivity of fly larvae during the biodegradation process was determined for each substrate using the Eq. 1

$$\text{Larva productivity (g/kg)} = \frac{\text{Larva weight (g DM)}}{\text{Manure weight (kg DM)}} \quad (1)$$

Statistical Analyses

The statistical analyses were performed using R 3.2.1 software. Temperature, pH, organic carbon, total nitrogen, $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ concentrations, the weight of the substrates, C:N and $N\text{-}NH_4$:$N\text{-}NO_3$ ratios and larva productivity were subjected to repeated measurement analysis of variance considering each stage of biodegradation. This analysis was performed following the GLM procedure with the substrate as factor. The significant level was set at 0.05 and post-hoc tests were carried out using the Student Newman–Keuls test.

Results

Change of Substrates' Temperature and pH During the Biodegradation by Fly Larvae

The changes of temperature and pH in the agro-processing by-products during the biodegradation by the house fly larvae and black soldier fly larvae are presented in Fig. 3. Significant differences ($p < 0.01$ to $p < 0.001$) in pH and temperature were found among the agro-processing by-products. The temperatures ranged between 33 and 45 °C during the biodegradation process (Fig. 3A). During the first stage of biodegradation, the temperature rose rapidly from day 1 and reached 45 °C, 43 °C and 41 °C respectively in the mixture of soybean bran and corn hull, corn bran and in the mixture of soybean bran and corn bran in day 4. During the second stage, after the partial renewal of the stock of the substrate, the mixture of soybean bran and corn hulls presented an increase of the temperature from 33 °C (day 5) to 41 °C (day 7) and decreased to 37 °C in day 8. The temperature in the corn bran rose from 34 °C (day 5) to 40 °C (day 6) and decreases to 35 °C (day 8). The same temperature trend was observed in the mixture of soybean bran and corn bran, showing an increase from 34 to 36 °C and then a decrease to 35 °C. During the third stage, the highest temperature was registered in corn bran (39 °C in day 9). The temperature did not change significantly ($p > 0.05$) during the fourth stage of biodegradation and was on average around 35 °C in each substrate.

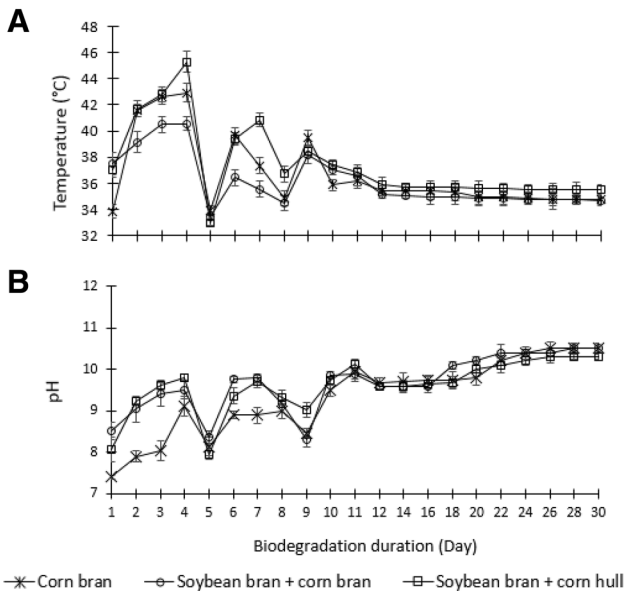

Fig. 3 Change of the temperature (**A**) and pH (**B**) in the vegetable substrates during the biodegradation process by the house fly and black soldier fly larvae

The pH of the substrates ranged between 7 and 10 during the biodegradation process. At the end of the third stage, the pH of the substrates were on average about 9 and increased to 10 during the fourth stage (Fig. 3B).

Change of Substrates' Weight During the Biodegradation Process

As shown in Fig. 4, the weight of the substrates declined significantly ($p < 0.05$) from the initial to the final time of each stage of the biodegradation process. An important weight loss was noticed during the first and fourth stages of biodegradation. At the first stage of biodegradation, weight was reduced from 510 to 298 g in the mixture of soybean bran and corn bran (42%), from 600 to 366 g in corn bran (39%) and from 630 to 412 g in the mixture of soybean and corn hull (35%). Against 5, 10 and 27% of weight loss during the second stage of biodegradation process and 34, 5 and 46% during the third stage respectively. The highest weight loss (61%) during the fourth stage was recorded in the mixture of soybean and corn hull (from 212.34 to 82.61 g) whereas the mixture of soybean bran and corn bran and corn bran recorded respectively 50 and 45% of weight loss. Generally, considering the supplies during the biodegradation process, the mixture of soybean bran and corn hull showed the highest weight loss (from 752.8 to 82.61 g) and was followed respectively by corn bran (from 816.96 to 202.39 g) and the mixture of soybean bran and corn bran (from 597.61 to 167.90 g).

Nutrients Dynamic in the Substrates During the Biodegradation Process

Significant differences ($p < 0.05$) were found in the organic carbon, total nitrogen, ammoniac and nitrite contents during the biodegradation process by the fly larvae (Fig. 5). The organic carbon content in the mixture of soybean bran and corn hull decreased by 10% against 7% for the corn bran and 4% for the mixture of soybean and corn bran (Fig. 5A). In general, the most important organic carbon decrease in the residues occurred between day 12 and day 30 with black soldier flies alone.

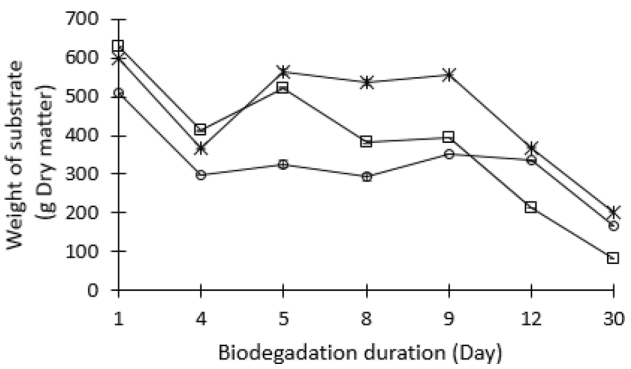

Fig. 4 Substrate weight reduction during the biodegradation by house fly and black soldier fly larvae

The total nitrogen content in the residues ranged between 61.96 ± 0.56 g kg^{-1} (mixture of soybean bran and corn bran) and 66.50 ± 1.00 g kg^{-1} (corn bran) at the beginning of the trial and between 53.75 ± 1.35 g kg^{-1} (mixture of soybean bran and corn hull) and 55.85 ± 0.87 g kg^{-1} (mixture of soybean bran and corn bran) at the end of the trial (Fig. 5B). The highest total nitrogen content in the residues was recorded during the first and the fourth stages of the biodegradation process. In general, at the end of the biodegradation process, the mixture of soybean bran and corn hull presented the highest total nitrogen content (53.03 ± 1.00 g kg^{-1}) against 48.99 ± 0.93 g kg^{-1} in the corn bran and 45.40 ± 0.43 g kg^{-1} in the mixture of soybean bran and corn bran. Moreover, the ammonia content was more important than the nitrite content (Fig. 5C, D). At the beginning of the trial, they ranged between 12.73 ± 1.45 and 16.43 ± 1.00 g kg^{-1} (for ammonia) and 4.56 ± 0.18 and 6.53 ± 0.25 g kg^{-1} (for nitrite) whereas, at the end of the process, they ranged between 4.57 ± 0.10 and 10.31 ± 0.16 g kg^{-1} (for ammonia) and 1.57 ± 0.05 and 5.14 ± 0.20 g kg^{-1} (for nitrite). Contrary to the trends of the ammonia content, an increase of the nitrite content was noticed in the corn bran and in the mixture of soybean bran and corn bran during day 12. That gain of nitrite was estimated at 3.60 g kg^{-1} in the mixture of soybean bran and corn bran and at 3.14 g kg^{-1} in the corn bran. When one compares the total N and the minerals N values, it appears that the organic N form was more important (66–70%) than the mineral N forms. Also, at the beginning of the trial, the organic N contents were average 66, 72 and 78% in mixture of soybean bran and corn bran, mixture of soybean bran and corn hull, and corn bran respectively whereas at the end there were 69, 87 and 79% respectively (Fig. 5B, C, D).

Table 1 presents the C:N and $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ ratios in the final product after the biodegradation process by the fly larvae. The C:N ratio was about 8 at the beginning of the biodegradation, then it increased to 10 in all the substrates at day 12 and to 12 and 11 in the mixture of soybean bran and corn bran and in the corn bran respectively at day 30. It decreased to 9 in the mixture of soybean bran and corn hull at day 30. At the beginning of the experiment, $N\text{-}NH_4^+$ contents were three times higher than the $N\text{-}NO_3^-$ contents in the mixture of soybean bran and corn bran and in the mixture of soybean bran and corn hull. They were similar in the corn bran at the same time. At day 12, the $N\text{-}NH_4^+$ contents were seven times higher in the corn bran, in the mixture of soybean bran and corn bran and four times higher in the mixture of soybean bran and corn hull than the $N\text{-}NO_3^-$ content. Whereas, at day

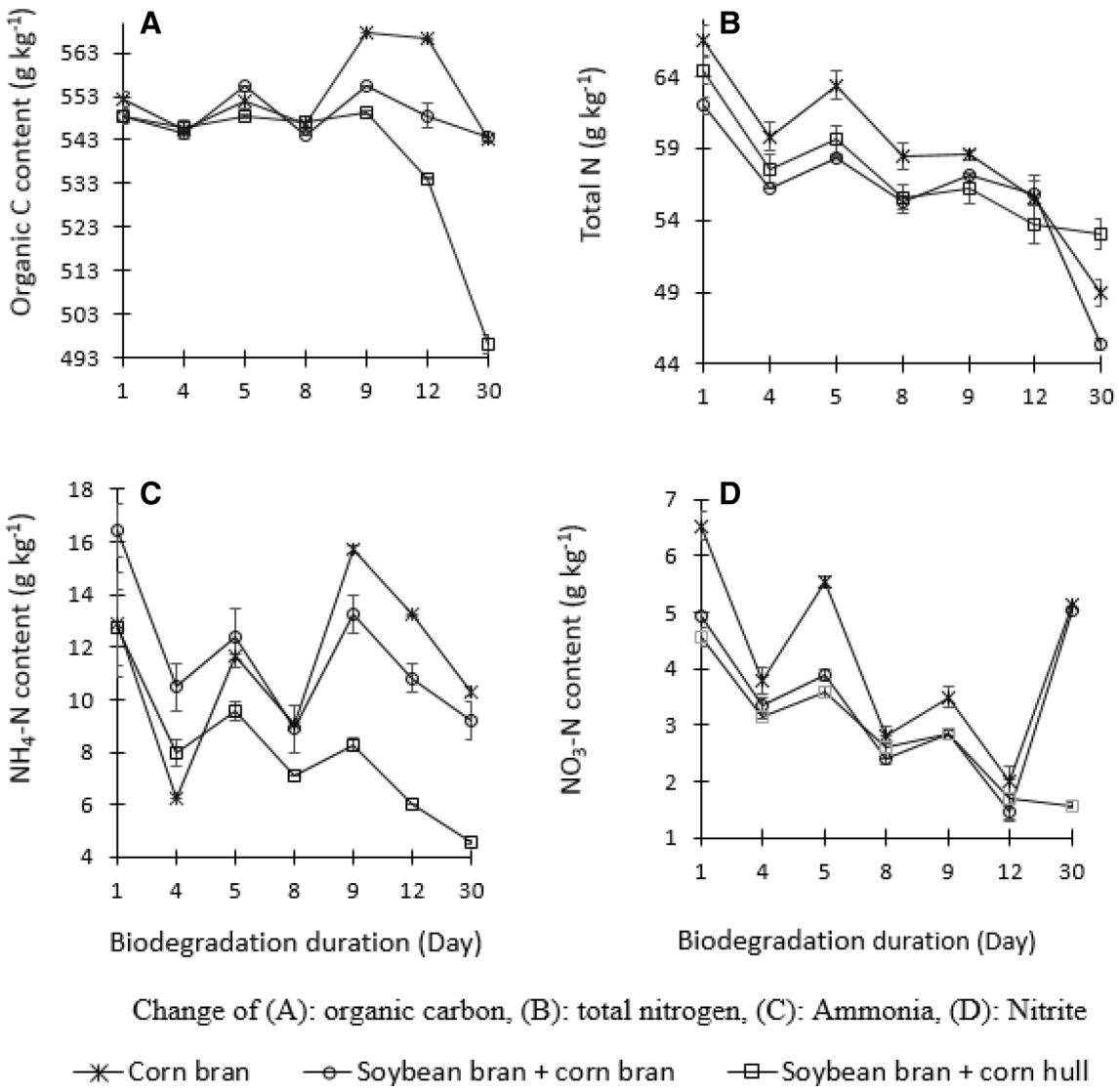

Fig. 5 Change of carbon, total nitrogen, ammonia and nitrite content in the agro-processing by-product substrates during the biodegradation by house fly and black soldier fly larvae

Table 1 C:N and N-NH_4:N-NO_3 ratios of the final product of the agro-processing by-product substrates after the biodegradation process by the fly larvae

Substrates	C:N ratio			N-NH_4:N-NO_3 ratio		
	Day 1	Day 12	Day 30	Day 1	Day 12	Day 30
Corn bran	8	10	11	1	7	2
Soybean bran + corn bran	9	10	12	3	7	2
Soybean bran + corn hull	8	10	9	3	4	3

30, in the substrates submitted to *Hermetia illucens* biodegradation, the N-NH_4^+ content were twice higher in the corn bran substrates, in the mixture of soybean bran and corn bran substrates and three times higher in the mixture of soybean bran and corn hull than the N-NO_3^- contents.

Quantity of Larvae Recorded with the Different Substrates

Figure 6 presents the amount of larvae of *M. domestica* and *H. illucens* produced with the different types of agro-processing by-products during the biodegradation process.

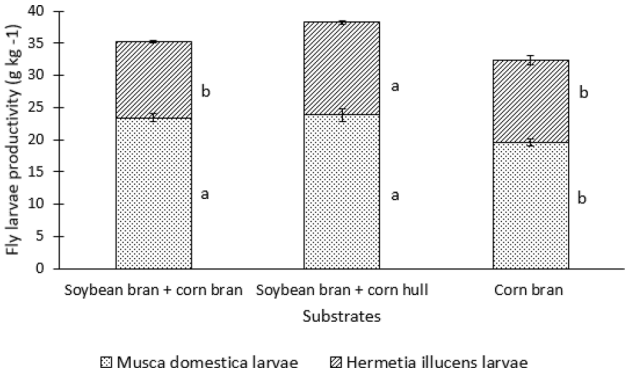

Fig. 6 *M. domestica* and *H. illucens* fly larvae produced with the substrates during the biodegradation process

The mixture of soybean bran and corn bran produced the highest quantity of fly larvae (23.83 ± 1.01 g DM kg^{-1} for *M. domestica* and 14.45 ± 0.29 g DM kg^{-1} for *H. illucens*), followed by the mixture of soybean bran and corn bran substrates, which produced 23.42 ± 0.65 g DM kg^{-1} of *M. domestica* and 11.84 ± 0.15 g DM kg^{-1} of *H. illucens*. The corn bran substrate produced the lowest amount of larvae (19.53 ± 0.57 g DM kg^{-1} for *M. domestica* and 12.86 ± 0.69 g DM kg^{-1} for *H. illucens*). In general, the substrates produced more *M. domestica* larvae than *H. illucens* larvae.

Discussion

Changes in the Agro-processing By-products Characteristics During the Biodegradation Process

Under the action of fly larvae, we observed an initial mesophilic phase lasting 1–3 days and a quick increase in temperature. In comparison with the composting process, this phase is characterized by the degradation of simple compounds such as sugars, amino acids, proteins by mesophilic bacteria and fungi [17–19]. The degradation of these compounds is necessary for the development of fly larvae [20]. The biodegraded substrates reached the thermophilic phase on day 4 with a maximum temperature of 45 °C (in the mixture of soybean bran and corn hull), 43 °C (in the corn bran) and 41 °C (in the mixture of soybean bran and corn bran). That reflects the intense microbial activity in the substrates and confirms the potential of the larvae to contribute to the breakdown of organic matter. During the biodegradation process, the thermophilic phase is characterized by the decomposition of fats and some recalcitrant compounds such as cellulose, hemicellulose and lignin by thermophilic microorganisms associated with the destruction of pathogens [21]. Unfortunately, the thermophilic phase occurred only in day 4 and therefore can be considered inefficient. However, we recorded a second mesophilic phase at 12–30 days. In comparison with the composting process, this second mesophilic phase refers to a slow degradation of lignin and other highly resistant compounds, and the formation of resistant organic mixtures [22]. The substrates' pH changed from neutral to alkaline. This result confirms those of several similar researches [12, 14, 24, 25] and indicates that fly larvae can transform the organic substrates. By comparison, the maximum of pH 10 recorded in the agro-processing by-products has been never reached in animal's manure biodegraded by fly larvae [12]. It reflects an important hydroxyl ions (OH^-) production through ammonification and mineralization of organic nitrogen compounds. Thus, the agro-processing by-products seem to be more favorable for fly biodegradation than that of animal's manure.

Moreover, the agro-processing by-products recorded a high mass loss during the biodegradation process. Similarly high mass losses in organic solid market wastes were reported by Saragi and Bagastyo [26] during the biodegradation by *Hermetia illucens* larvae. The authors explained that, the natural decomposition by the existing microorganisms also contributes to an increase of mass loss. The hypothesis of an interrelation between fly larvae activity and micro-organisms in the substrates during the biodegradation process was reported by Čičková et al. [27]. According to these authors, the fly larvae activity favors the growth of aerobic microorganisms by increasing water and ammonia losses through mechanical aeration. The reduction of substrates' mass by fly larvae is an important advantage for the adoption of the biodegraded residues as soil amendment. In fact, the key constraint of the adoption of organic fertilizer technologies in sub-Saharan Africa is the transport of the organic material since fields are often distant from the nutrient sources [28]. Also, fly larvae deodorize the substrates [29] which facilitates their utilization.

The agro-processing by-products produced more fly larvae than animal manures tested by Bloukounon-Goubalan et al. [12] in the same experimental conditions. In general, the high mass loss due to larvae is followed by a reduction of organic carbon [12]. The low reduction of organic carbon in the agro-processing by-products during the biodegradation process can be explained by a low breakdown of carbon chains in substrates during the process. In fact, corn bran and soybean bran contain a high amount of dietary fiber such as cellulose, hemicellulose and lignin, which are almost insoluble [3]. But they also contain a low fraction of proteins, starch and fat, which were probably decomposed and consumed by fly larvae. Indeed, from day 1 to day 12, the $N\text{-}NH_4^+$ contents in the substrates increased from three times to seven time more than the $N\text{-}NO_3^-$ content. This finding points out the decomposition of organic N (proteins) into $N\text{-}NH_4^+$ through aminization and ammonification with

the help of the fly larvae. The reduction of N-NH_4 content recorded in the substrates reflected not only the use of N-NH_4 by the larvae but also the nitrification process. In fact, the corn bran substrates and mixture of soybean and corn bran substrates recorded the gain of nitrite at the end of the biodegradation process. According to Bernal et al. [21], when the NH_4-N concentration decreases and NO_3-N appears in the substrate, this latter is considered mature and ready to be used as soil amendment. The apparition of nitrite in the substrates is also an advantage for plants nitrogen nutrition since they prefer the nitrite form of nitrogen than ammoniac. Also, the total nitrogen values of the final product of the biodegradation of the agro-processing by-products were higher than those recorded in the pig manure by Zhu et al. [25]. Since nitrogen is the limited nutrient in Benin soils [30], the agro-processing by-products decomposed by the fly larvae may better fulfil the plants nitrogen requirements than animal manure decomposed by fly larvae.

Use of the Agro-processing By-products Biodegraded by the Fly Larvae as Soil Amendment

The application of non-stable or immature amendments may inhibit weed seed germination, reduce plant growth, and damage crops by competing for their oxygen or causing phytotoxicity to plants due to insufficient biodegradation of organic matter [31, 32]. The C:N ratio has frequently been used to describe organic waste decomposition and it is widely accepted that a high C:N ratio in a substrate implies a low mineralization rate due to N deficiency. Furthermore, substrates with high C:N ratio can cause nitrogen immobilization upon amendment to soil and those with low C:N ratio can cause ammonium toxicity [33]. Zmora-Nahum et al. [34]; Benny [35]; Namkoong et al. [36]; Rosen et al. [37] suggested various ideal C:N ratios of substrates, ranging from 10 to 20, for use as soil amendment.

In our experiment, the C:N ratio of corn bran and the mixture of soybean bran and corn bran were 10 at day 12 and respectively 11 and 12 at day 30. The mixture of soybean bran and corn hull presented a C:N ratio of 10 at day 12 and 9 at day 30. The application of these substrates would reduce N_2O emissions due to the low amount of N released during the decomposition into the soil [38, 39]. However, the net N mobilization in the soil cannot occur with the corn bran substrate and the mixture of soybean bran and corn hull since corn hull and corn bran contain some stabilizing molecules such as cellulose and hemicellulose [40]. Mineralization studies of the substrates may elucidate these questions.

Moreover, the C:N ratio alone is not a sufficient criterion to evaluate the maturity and stability of a substrate to be used as soil amendment. Total N, which includes all forms of nitrogen, will normally range from 5 to 25 g kg^{-1} (dry weight basis) in the final substrates. Values exceeding 150 g kg^{-1} are considered to be high [41]. The values of total N in the final products obtained in this experiment ranged between 45 and 53 g kg^{-1}. These N values are higher than those found by Bloukounon-Goubalan et al. [12] in animal manures (ranging from 15.5 to 27.5 g kg^{-1}) after 12 days of biodegradation by *Musca domestica* larvae. They are also higher than those found by Zhu et al. [25] in pig manure (average of 41 g kg^{-1}) after 7 days of biodegradation by *Musca domestica* larvae.

The organic N form was more important in the substrates (66–70%) than the others forms of N. Also, the organic N in the substrates was more important after the biodegradation process by the fly larvae. While NH_4-N and NO_3-N are immediately available for the plants, organic N is only slowly available and will be converted into inorganic forms after mineralization in the soil before being used by the plants. This finding points out the potential contribution of the substrates biodegraded by the fly larvae in sustainable soil fertility management.

Conclusion

The biodegradation of the agro-processing by-products by *Musca domestica* and *Hermetia illucens* larvae is an efficient technology for the production of bio-fertilizers suitable for soil amendment. During the biodegradation process, the temperature of the substrates rose and pH changed from neutral to alkaline, substrates' weight and ammonia content decreased and the organic nitrogen content increased. All these changes were particularly remarkable during the biodegradation by *Hermetia illucens*. Fly larvae activity induced the conditions of aerobic microorganism's growth and the apparition of NO_3-N in the corn bran substrate and the mixture of soybean bran and corn bran substrate in day 12 of the biodegradation process. The final products obtained from the biodegradation process have a high organic N content and fulfil the N requirements of the plants. The present study suggests the use of corn bran or a mixture of soybean bran and corn bran decomposed by the fly larvae as soil amendment for sustainable soil fertility replenishment.

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