



RESEARCH ARTICLE

# Using cassava waste of the cassava starch processing as food for raising African Nightcrawler (*Eudrilus eugeniae*) to obtain vermicomposting and earthworm biomass

*Sử dụng chất thải sản của quá trình chế biến tinh bột sắn làm thức ăn nuôi giun quế African Nightcrawler (*Eudrilus eugeniae*) nhằm thu nhận phân trùn và sinh khối giun*

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The raising earthworms by cassava waste is a useful solution to reduce environmental pollution caused by cassava starch processing. In this study, cassava waste (including cassava peel, cassava pieces and soil) was used as a food source for raising African Nightcrawler (*Eudrilus eugeniae*) with three experiments: E1, earthworms were raised in crushed cassava waste right after being discharged; E2, earthworms raised in crushed cassava waste that had been incubated with organic matter after decomposing microbiological preparation for the previous two weeks; E3, earthworms were raised in crushed cassava waste that had self-decomposed naturally for the previous two weeks. The cassava waste was decomposed naturally for 4 weeks for control. The results showed that the content of organic matter, humic acid and total nitrogen in organic cassava humus, obtaining from experiments, increased compared to the control; the total organic matter content reached from 10.4%-15.7%, higher than the control (8.2%) from 1.27-1.92 times, humic acid content reached 0.6 - 0.8% and total nitrogen reached 0.3%. Experiment 3 had the highest quality of humus (organic matter content 15.7%, total nitrogen 0.3%, humic acid 0.7% and fulvic acid 0.5%). The experiment 3 also had the highest earthworm biomass (3.6kg), increasing 30.5% compared to experiment 1 and 19.4% comparing to experiment 2. Therefore, experiment 3 was proposed for application in treatment of cassava waste at larger scale. The organic humus obtaining from raising earthworms by cassava waste can be used as raw material for vermicompost production. The earthworm biomass can be used as protein-rich food for domestic animals (such as chicken, tortoise, eel, fish, etc) or used as nutritious fertilizer.

*Nuôi giun bằng phế liệu sắn là giải pháp hữu ích nhằm giảm thiểu ô nhiễm môi trường do chế biến tinh bột sắn gây ra. Trong nghiên cứu này, bã thải sắn (bao gồm vỏ, đầu mẩu sắn và bùn đất) được sử dụng làm nguồn thức ăn giun đất Châu Phi (African Nightcrawler (*Eudrilus eugeniae*)) với các thí nghiệm như sau: Thí nghiệm 1: Giun quế được nuôi trong bã thải sắn nghiền ngay sau khi được loại bỏ; Thí nghiệm 2: giun đất được nuôi trong bã thải sắn nghiền đã được ủ với chế phẩm vi sinh phân hủy chất hữu cơ trong hai tuần trước đó; Thí nghiệm 3: Giun đất được nuôi trong bã thải sắn nghiền được để tự phân hủy tự nhiên trong hai tuần trước đó. Đối chứng là bã thải sắn để phân hủy tự nhiên trong 4 tuần. Bên cạnh đó, bã thải sắn nghiền còn được ủ bằng chế phẩm vi sinh phân hủy chất hữu cơ trong 4 tuần để cung cấp thêm số liệu so sánh giữa các thí nghiệm (Thí nghiệm 4). Các thí nghiệm được theo dõi trong 4 tuần. Kết quả cho thấy: hàm lượng chất hữu cơ, axit humic và nitơ tổng trong mùn sắn hữu cơ thu được từ thí nghiệm đều tăng so với đối chứng: hàm lượng chất hữu cơ tổng số đạt từ 10,4% -15,7%, cao hơn đối chứng (8,2%) so với 1,27-1,92 lần, hàm lượng axit humic đạt 0,6 - 0,8% và nitơ tổng số đạt 0,3%. Trong đó, thí nghiệm 3 có chất lượng mùn cao nhất (hàm lượng chất hữu cơ 15,7%, đạm tổng số 0,3%, axit humic 0,7% và axit fulvic 0,5%). Thí nghiệm 3 cũng có sinh khối giun quế cao nhất (3,6kg), tăng 30,5% so với thí nghiệm 1 và 19,4% so với thí nghiệm 2. Do đó, thí nghiệm 3 đã được đề xuất ứng dụng để xử lý phế thải sắn ở quy mô lớn hơn. Chất mùn hữu cơ thu được từ việc nuôi trùn quế bằng phế thải sắn có thể được sử dụng làm nguyên liệu để sản xuất phân trùn quế. Sinh khối trùn quế có thể dùng làm thức ăn giàu đạm cho vật nuôi (như gà, ba ba, lươn, cá ...) hoặc làm phân bón dinh dưỡng.*

**Keywords:** African Nightcrawler (*Eudrilus eugeniae*), raising earthworm, earthworm biomass, cassava waste.

## 1. Introduction

Cassava starch processing is a developing industry in Vietnam. The annual production of cassava starch ranges from 1.6 - 2.0 million tons; about 70% is exported and 30% for domestic consumption. However, development of the cassava starch industry generates a large amount of solid waste, including mud, peeling, roots and cassava starch residues. The composition of cassava waste contains very high nutrients including 5.3% of protein, 56% of starch, 0.1% of fat, 2.7% of ash and 35.9% of fiber. If the cassava waste is not treated in time, the decomposition of organic matter takes place after 48 hours and create  $H_2S$ ,  $NH_3$ ,  $CH_4$ , causing unpleasant odor and environmental pollution (A. O. Ubalua (2007). In Vietnam, cassava waste is used as organic fertilizer (Nguyen Thi Hang Nga et al., 2016), animal feed (Bac Cam Thi Xiang, 2017), alcohol production (Truong Tat Hieu, 2008) and burial (Dương Phương Nhung, 2017). However, most of it is still discharged directly into the environment and enhancing pollution.

The African Nightcrawler (*Eudrilus eugeniae*) is a species of fecal worms living in environments that contains many nutrients and decomposing complex organic compounds through the digestive system. Earthworms play an important role in agricultural production. The crude protein content in earthworm biomass is very high. Accounting for 70% of dry weight, it is equivalent to the amount of protein in fishmeal and soybean meal commonly used in animal feed. In the earthworm biomass, there are 12 types of amino acids, several vitamins and necessary minerals for the growth and development of animals. Fresh worms are ideal food for chickens, fish, tortoise, eel, frogs, etc. (Edwards C.A., 1985). Earthworm farming is considered as one of the most effective and sustainable methods to treat organic waste and generate earthworm biomass to be used as animal feed (Han Quang Hanh et al., 2020; Nandeesha M.C. et al., 1988). Earthworms are capable of converting most of organic waste such as animal manure and other agricultural waste into humus, creating an ideal fertilizer source with useful nutrients and dissolved minerals such as nitrogen, phosphorus and potassium for plants (Ndegwa and Thompson, 2001).

The ability of producing earthworm biomass from using the waste is also very efficient. According to Edwards (1985), one ton of livestock waste can generate about 100kg of earthworm biomass with the metabolic rate for dry matter of about 10%. Earthworm biomass is a nutritious animal feed source with high protein content (60-70% of dry matter), lipid (6-11%), carbohydrate content (5-21%), mineral content (2-3%) and many vitamins, especially Niacin and B12. Therefore, the development of earthworm farming limits environmental pollution and reduce the production cost of animal feed (Ansari A.A. and Ismail S.A., 2012).

Lara Zirbes et al. (2011) studied composting organic matter by using different ratios of water hyacinth and pig manure as food for earthworms. The obtained compost was of high quality, increasing the growth of plants. Birundha M. et al. (2013) surveyed the growth and reproduction of earthworms in different types of organic waste such as straw and coir. According to the authors, straw is a good source for earthworms. The composition and function of the intestinal microbial communities of earthworms was also studied. Intestinal bacteria of the earthworms played an important role in the breakdown of organic matter, helping to metabolize and accelerate digestion, creating organic fertilizer with essential macronutrients and trace elements for plant growth (Singh A et al 2015).

In Vietnam, the model of raising earthworm by using cattle and poultry manure, domestic waste and agricultural waste has been of interested in research and application. However, the model of raising earthworm by using cassava waste as food has not been yet studied. Therefore, this study aims to use cassava waste including cassava peeling and pieces for raising African earthworm (*Eudrilus eugeniae*) with different formulas in order to obtain organic humus and produce organic fertilizers and earthworm biomass as food for domestic animals.

## 2. Materials and methods

### 2.1. Materials

Cassava waste, including cassava peel and pieces, were collected in the dumping area of Van Yen cassava factory, Yen Bai province, Vietnam. African earthworm (*Eudrilus eugeniae*), organic matter decomposing microbiological preparation containing *Bacillus subtilis*, *Streptomyces nigrescens*, *Saccharomyces cerevisiae* were provided by the Center for Culture Collection and Genetic Resource Conservation of Microorganisms, Institute of Biotechnology, Vietnam Academy of Science and Technology.

*Medium for culturing microorganisms:* LB agar (Luria Bertani) for culturing bacteria, Gause I for culturing actinomycetes, PDA for culturing fungi, Ashby for culturing *Azotobacter* bacteria, Baird Parker for culturing *Staphylococcus aureus*, TSA and VRB agar for culturing coliform and *E. coli*.

### 2.2. Methods

*Pre-treated cassava waste:* Cassava waste samples (cassava peel, cassava pieces and soil) were pre-treated by crushing. Based on the growth and development of earthworms and the amount of breed earthworms for each experiment, we added 30 kg of cassava waste in each experiment (and the control) and conducted as described below to ensure that the amount of feed was suitable to the growth of the

earthworms in 4 weeks. Foam boxes (size 60 \* 40 \* 30 cm) were used for the experiments and the control sample (duration of four weeks), the experiments were designed as follows:

Experiment 1 - Earthworms were raised in crushed cassava waste immediately after being discharged (600 g of Breed earthworms and 20 kg of cassava waste). From the 3rd week, feeding the earthworms 2 kg/3 days/time (total 10kg).

Experiment 2 - Earthworms were raised in crushed cassava waste that had been incubated with organic matter with a decomposing microbiological preparation (600 g of breed earthworms and 20 kg of cassava waste that were previously incubated microbiologically for 2 weeks). From the 3rd week, feeding the earthworms 2 kg/3 days/time (total 10 kg).

Experiment 3 - Earthworms were raised in crushed cassava waste that had been previously decomposed under natural conditions (600 g of breed earthworms + 20 kg of cassava waste that have been decomposed naturally for 2 weeks). From the 3rd week, feeding the earthworms 2 kg/3 days/time (total 10 kg).

Experiment 4 - Earthworms were not be raised in this experiment so cassava waste was not added weekly. Thus, 30 kg crushed cassava waste was incubated by organic matter decomposing microbiological preparation for 4 weeks.

Control - 30 kg crushed cassava waste was decomposed naturally for 4 weeks.

Experiments with microbiological preparation: mixing microbiological preparation and cassava waste at the rate of 10%, maintaining a humidity range of 50-60% and covering the compost pile.

*Experiments without microbiological preparation:* maintain a humidity rate of 50-60% and covering the compost pile. The experiments and control were repeated 3 times.

*Microbiological methods:* using routine microbiological methods (dilution method, counting CFU method). Identifying *Staphylococcus aureus* according to Vietnamese standards TCVN7927:2008, *Coliforms*, *E. coli* and *Salmonella* according to Vietnamese standards TCVN 11039-4:2015 and TCVN 11039-5:2015.

*Methods for determining organic matter in organic cassava humus:* total nitrogen (according to Vietnamese standards TCVN 8557: 2010), total phosphorus (according to Vietnamese standards TCVN 8563:2010), total organic carbon (according to Vietnamese standards TCVN 9294:

2012), content of humic and fulvic acids (according to Vietnamese standards TCVN 8561: 2010).

The statistical analysis was performed utilizing the R software. The experiments were performed at the laboratory of the Institute of Biotechnology (VAST) and the laboratory of the University of Technology (VNU).

## 3. Results and discussions

### 3.1 Analysis of organic matter content and microbiological population in cassava waste samples collected from production of cassava starch

The cassava residue including cassava peel, cassava pieces and soil discharged during the process of producing starch in Yen Binh Cassava Factory, Yen Bai province (figure 1). The mixture of cassava waste was crushed with a humidity range of 40-50%. Samples were analyzed for determining composition of some organic compounds and microorganisms.



**Figure 1. Cassava waste collected from Yen Binh Cassava Factory, Yen Bai province, Vietnam**

The protein content was low (3.9%) but the starch and cellulose contents were quite high (25.6% and 30.2%) in the collected cassava waste sample ( $p \leq 2 \times 10^{-16}$ ). Further, the cassava residue also contained useful microorganisms including aerobic microorganisms, nitrogen-fixing microorganisms, cellulose decomposing microorganisms, in approx.  $7.67 \times 10^1 - 5.93 \times 10^1$  CFU/g ( $p \leq 1.1 \times 10^{-5}$ ) and the pathogenic microorganisms (such as *Salmonella* sp, *Staphylococcus aureus*, *Coliform* and *E. coli*), the pathogenic microorganisms were detected in extremely small amounts ( $< 10$ ).

Therefore, the cassava waste was considered as a good source of raw materials for producing organic fertilizers (Table 1 and 2).

**Table 1. Composition of some organic compounds in the original cassava waste sample**

Composition	Content (%) $p \leq 2 \times 10^{-16}$
Protein	3.9
Starch	25.6
Cellulose	30.2

According to Gomez G. (1988), the cassava is rich in starch, energy, minerals and vitamin C among others but poor in fat and especially poor in protein. Content of amino acids are unbalanced, excess arginine but lack of sulfur-containing amino acids. The total of dry matter, content of protein, fat, minerals, fiber, sugar, and starch can change depending on the variety, the planting season, the number of months of growth. However, fresh cassava is rarely used directly as food for humans and animals because it also contains a type of HCN toxin (Cyanogenes). Thus, cassava is often processed before being used as food for humans and animals.

**Table 2. Density of microorganisms in the original cassava waste sample**

Microorganisms	Density (CFU/g) $p \leq 1.1 \times 10^{-5}$
Total aerobic microorganisms	$1.80 \times 10^7$
Nitrogen-fixing microorganisms	$5.93 \times 10^1$
Phosphate solubilizing microorganisms	-
Cellulose decomposing microorganisms	$7.67 \times 10^1$
Salmonella sp	-
Staphylococcus aureus	< 5
Coliform	< 10
E. coli	< 10

Notes: (-) not detected

### 3.2 Preparing microbiological preparation for treatment cassava waste

The cassava waste was incubated by microbiological preparation for decomposing organic matter and to reduce the amount of toxins in the waste. The strains of microorganisms capable of decomposing organic matter including *Bacillus subtilis*, *Streptomyces nigrescens* and *Saccharomyces cerevisiae* were cultured in suitable liquid

media. The preparation was made by mixing substances and microorganisms as follows: microorganisms 5%, rice husk 2%, bran 10%, sugar 2%, soybean powder 1%,  $\text{KH}_2\text{PO}_4$  0.1%,  $\text{MgSO}_4$  0.1%, peat 84.8% with 50% of humidity. The density of microorganisms in the preparation was analyzed after 7 days, the number of microorganisms reached  $7.6 \times 10^8 - 1.9 \times 10^9$  CFU/g ( $p \leq 1.6 \times 10^{-15}$ ). This preparation was used to incubate the crushed cassava waste.

**Table 3. The number of microbiological groups in microbiological preparations**

Density (CFU/g) $p \leq 1.6 \times 10^{-15}$		
Bacillus subtilis	Streptomyces nigrescens	Saccharomyces cerevisiae
$1.4 \times 10^9$	$1.9 \times 10^9$	$7.6 \times 10^8$

### 3.3 The content of organic matter in organic cassava humus

Four experiments and the control were taken for 4 weeks. The biochemistry ingredients of obtained organic cassava humus from each experiment was analyzed. The results in table 4 showed that the content of organic matter in obtained organic cassava humus in the experiments with earthworms (experiment 1, 2 and 3) increased comparing to the experiment without earthworms (experiment 4 and the control). The content of organic matter in four experiments reached 10.4% - 15.7%. Experiment 3 achieved

the highest rate (15.7%) higher than the control (8.2%) from 1.27 - 1.92 times. It was followed by experiment 2 (12.5%), experiment 1 (12.1%) and experiment 4 (10.4%). The total nitrogen content in all four experiments was 0.3%, higher than the control (<0.2%).

Humic acid contents of experiments 2, 3 and 4 (ranged in 0.6-0.8%) were higher than the control (<0.2%). Also, the total phosphorus and fulvic acid contents were equal to the control (total phosphorus <0.2%, fulvic acid <0.5%), except fulvic acid content of experiment 3 was 0.5%. This analysis was statistically significant with  $p \leq 4.2 \times 10^{-7}$  (Table 4).

In intestinal of the earthworms, there were a lot of microorganism and enzymes such as protease, lipase, amylase, cellulose and chitinase, which helped in the decomposing of organic matter into amino acids and minerals, creating high-quality organic humus (Edwards C.A. (1985). That is why the composition of the organic humus in the experiments with earthworms was higher than in experiments without earthworms. The difference in humus content between earthworms raising experiments, especially experiments 2 and 3 could be due experiment 2 was incubated with a microbiological preparation for two

weeks before using for raising the earthworms, so the organic matter (food for earthworms) in the cassava waste was be reduced partially by decomposition of microorganisms. Despite the cassava waste in experiment 3 was under natural condition for two weeks before being used for raising the earthworms, the organic matter was still abundant and through the digestion of earthworms created composition of the organic humus higher than experiment 2.

**Table 4. Content of organic matter in organic cassava humus after raising earthworm**

Content	Control	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Vietnamese Standard*
$p \leq 4.2 \times 10^{-7}$						
Total organic matter (%)	8.2	12.1	12.5	15.7	10.4	$\geq 15$
Total nitrogen (%)	< 0.2	0,3	0.3	0,3	0.3	$\geq 2.0$
Total Phosphorus (%)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	$\geq 2.0$
Humic acid (%)	< 0.5	< 0.5	0.8	0.7	0.6	$\geq 3.5$
Fulvic acid (%)	< 0.5	< 0.5	< 0.5	0.5	< 0.5	

Note: \*Regulations for organic fertilizers, organic traditional fertilizers, mineral organic fertilizers, micro-organic fertilizer and bio-organic fertilizer according to Government of Vietnam (No. 108/2017/NĐ-CP, September 20, 2017)

Out of four experiments, the experiment 3 (raising earthworms with crushed cassava waste that was decomposed naturally for 2 weeks) gave the best quality of humus (organic matter content 15.7%, total nitrogen 0.3%, humic acid 0.7% and fulvic acid 0.5%). Although the contents of total nitrogen, total phosphorus and fulvic acid of experiment 3 was lower than regulations for organic fertilizers, according to Vietnamese standards, the humus of experiment 3 can be still used for researching and development of organic fertilizers or microbial organic fertilizers.

The content of humic and fulvic acids are important indicators to evaluate humus quality. Furthermore, these acids play a very important role in the soil. They help maintaining soil fertility, improving soil moisture, increasing nutrient and water retention in soil. They are a nutrient source for beneficial microorganisms in the soil, reducing salinity. For plants, these acids increase the plant's ability of absorbing nutrients in the soil, increase germination and improving health of the root system (Jacoby R. et al., 2017). The quality of the cassava humus in this study showed that the cassava waste can be used as food to raise earthworm for treating cassava waste (Figure 2).

In Vietnam, the organic waste has also been studied as material source for raising earthworm for treating waste and to obtain earthworm biomass. Pig, buffalo and cow manure had been evaluated as very effective food source for raising earthworms. The organic trash was used for

raising earthworms. An amount of 100g - 200g of earthworms could use 300 kg of organic trash with treatment efficiency of 100%. (Hoang Anh Vu et al., 2017; Do Ngoc Bien, 2012; Dang Vu Binh, 2008).



**Figure 2. The organic cassava humus in experiments**

### 3.4 The density of microorganisms in organic cassava humus

The density of microorganisms was also analyzed from organic cassava humus of the experiments and the control (table 5). The total microbiological density, the density of useful microorganisms (nitrogen fixing bacteria and cellulose degradation microorganisms) in four experiments increased comparing the control sample. The number of total microorganisms ranged in  $1,92 \times 10^{15}$  CFU/g -  $3,63 \times 10^{15}$  CFU/g, especially the total microorganisms of experiment 2 (using cassava waste incubated by microbiological preparation for two weeks) and experiment 3 (using



cassava waste self-decomposed naturally for two weeks) were much higher than the control (experiment 2:  $3.63 \times 10^{15}$  CFU/g; experiment 3:  $2.23 \times 10^{15}$  CFU/g and the control:  $1.5 \times 10^{15}$  CFU/g). The number of nitrogen-fixing bacteria (approximately  $6.2 \times 10^3$  CFU/g -  $9.27 \times 10^3$  CFU/g) and cellulose degradation microorganisms (approximately  $6.78 \times 10^3$  CFU/g -  $2.57 \times 10^5$  CFU/g) were much higher than the control ( $9.3 \times 10^1$  CFU/g and  $1.07 \times 10^2$  CFU/g); this difference was statistically significant with  $p \leq 5.3 \times 10^{-5}$ . The phosphate solubilizing microorganisms were not detected at any experiment nor in the control. There were a lot of

microorganisms in the intestine of earthworms, so the process of digesting food of the earthworms creating high quality organic humus content with large number of microorganisms. That is why the number of microorganisms in the experiments was greater than the control. On the other hand, the number of microorganisms of experiment 2 was the highest because this experiment contained cassava waste that was previously incubated with microbiological preparation.

**Table 5. Microbiological analysis in organic cassava humus samples**

Content	Control	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Vietnamese standard*
<b>Useful microorganisms (CFU/g)</b>						
$p \leq 2 \times 10^{-16}$						
Total microorganisms	$1.5 \times 10^{15}$	$1.92 \times 10^{15}$	$3.63 \times 10^{15}$	$2.23 \times 10^{15}$	$2.17 \times 10^{15}$	
Nitrogen-fixing bacteria	$9.3 \times 10^1$	$6.20 \times 10^3$	$9.72 \times 10^3$	$9.05 \times 10^3$	$6.20 \times 10^3$	
Phosphate solubilizing microorganisms	-	-	-	-	-	
Cellulose degradation microorganisms	$1.07 \times 10^2$	$6.78 \times 10^3$	$2.57 \times 10^5$	$1.48 \times 10^5$	$1.36 \times 10^5$	
<b>Pathogenic microorganisms (CFU/g)</b>						
$p \leq 5.3 \times 10^{-5}$						
S. aureus	< 5	< 5	< 5	< 5	< 5	
Salmonella sp.	-	-	-	-	-	negative
Coliform	< 10	< 10	< 10	< 10	< 10	< $1.1 \times 10^3$
E. coli	< 15	< 10	< 10	< 10	< 10	< $1.1 \times 10^3$

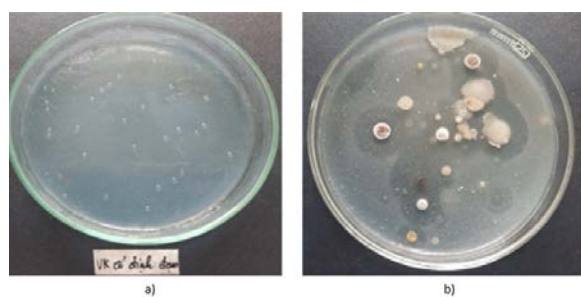
Note: \*Regulations for organic fertilizers, organic traditional fertilizers, mineral organic fertilizers, micro-organic fertilizer and bio-organic fertilizer according to Government of Vietnam (No. 108/2017/NĐ-CP, September 20, 2017).

The number of pathogenic microorganisms such as *Staphylococcus aureus*, *Salmonella sp.*, *Coliform* and *E. coli* were lower than the allowed threshold (comparing to regulations for organic fertilizers of the Vietnamese standard). The experimental results have proven that the organic cassava humus after farming earthworm can be used as raw material source suitable to producing of organic fertilizer, mineral organic fertilizer, microbiological organic fertilizer or biological fertilizer.

### 3.5 Obtaining earthworm biomass

The growth of earthworm is estimated through increasing of size. From a pair of breeding earthworm is possible to produce from 1.000 to 1.500 individuals per year in suitable living conditions. The hatched earthworms are small as needles (2 – 3 mm long). After 20 - 40 days they become mature worms with a genital belt.

The experiments were implemented within four weeks from adding the breeding earthworm to the harvesting earthworm biomass. The increasing in earthworm biomass also depends on climate factors such as the appropriate temperature and humidity. The experiments were conducted in the spring, a suitable season for the growth and development of the earthworms. African Nightcrawler (*Eudrilus eugeniae*) grows very fast. According to Ali and Kashem (2018), the earthworm's weight increased gradually from hatching to 50 days of age. Before maturity and after laying, the earthworm's weight increased slower. The biomass of the African Nightcrawler could reach 17.43 mg/individual for the first 7 weeks after hatching.



**Figure 3. The nitrogen fixing bacteria (a) and cellulose degradation microorganisms (b) from organic cassava humus**

Comparing to other types of earthworms, African Nightcrawler grows faster. Venter & Reinecke (1988) announced the growth of the earthworm *Eisenia fetida* gaining 2.5mg /individual/day. The growth of African

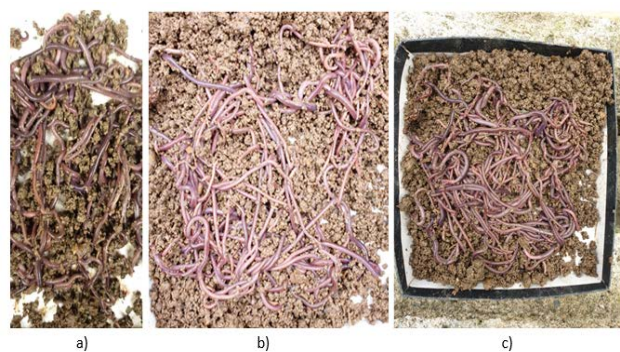
Nightcrawler (*Eudrilus eugeniae*) was also much higher than *Perionyx excavates* (reaching 3.48 mg/ individual/day (Birundha M., 2013).

**Table 6. Earthworm biomass was obtained from the experiments**

Content	Experiment 1 $p \leq 4.76 \times 10^{-3}$	Experiment 2	Experiment 3
Breeding earthworm biomass (kg)	0.6	0.6	0.6
Harvested earthworm biomass (kg)	3.1 ± 0,3	3.5 ± 0.2	3.9 ± 0.3
Increased earthworm biomass (kg)	2.5 ± 0,3	2.9 ± 0.2	3.6 ± 0.3

After four weeks of feeding, the earthworm grew best in medium containing cassava waste that was decomposed naturally for 2 weeks before feeding the earthworm; the average biomass reached 3.6 kg (experiment 3). This was followed by experiment 1 and 2 with average biomass reaching 2.5 kg and 2.9 kg, respectively ( $p \leq 4,76 \times 10^{-3}$ ). The weight of earthworm in experiment 3 increased 30.5% comparing to experiment 1 and 19.4% comparing to experiment 2.

The naturally decomposition of cassava waste for 2 weeks could be suitable time for activity of indigenous microorganism in cassava waste. That could be reason why the earthworm biomass of experiment 3 was the highest. This result was proportional to the result of analysis organic humus in Table 4. The cassava waste in experiment 2 might be reduced organic nutrients as it was incubated with the microbiological preparation for 2 weeks. That was why earthworm biomass of experiment 2 was less than earthworm biomass of experiment 3.



**Figure 4. Earthworm biomass from experiment formulas: experiment 1 (a), experiment 2 (b), experiment 3 (c)**

According to Han Quang Hanh et al. (2020), the growth of African Nightcrawler (*Eudrilus eugeniae*) gained 15.71 mg/individual in weight and 9.09 mm/individual in length for 4 weeks when they were raised with cattle and poultry manure. Because organic matter content of the cassava waste was lower than organic matter content of cattle and poultry manure, the growth of the earthworms raised with

cassava waste was not higher than the growth of the earthworms raised with cattle and poultry manure. However, the results of this study have proven that the cassava waste can be applied as food to farm earthworm for obtaining organic humus and biomass, reducing environmental pollution by cassava waste.

#### 4. Conclusion

Content of organic matter, humic acid and total nitrogen in organic cassava humus obtained from experiments increased comparing to the control. The total organic matter content raised from 10.4% to 15.7%, higher than the control (8.2%); this is from 1.27-1.92 times. Humic acid content reached 0.6 - 0.8% and total nitrogen reached 0.3%. Experiment 3 (raising earthworms with crushed cassava waste that was decomposed naturally for 2 weeks) had the highest quality of humus (organic matter content 15.7%, total nitrogen 0.3%, humic acid 0.7% and fulvic acid 0.5%). Especially, the organic matter contents of the experiments 1, 2 and 3 with African Nightcrawler earthworms (12.1%-15.7%) were higher than the experiment 4 without African Nightcrawler earthworms (10.4%).

The number of pathogenic microorganisms in four experiments were lower than those limits of Vietnamese standards for compost. African Nightcrawler (*Eudrilus eugeniae*) grew well in medium containing cassava waste, the average earthworm biomass gained 2.5 - 3.6 kg. The earthworm biomass of experiment 3 reached 3.6 kg, increasing 30.5% comparing to experiment 1 and 19.4% comparing to experiment 2.

The results of this study showed that the original cassava residue including cassava peel, cassava pieces and soil, contained amount of organic matter suitable to raising earthworm to obtain earthworm biomass and organic cassava humus for producing vermicompost and nutritious fertilizer. Also, the earthworm biomass can be used as protein rich food for domestic animals such as chicken, tortoise, eel and fish.

## 5. References

- [1] Ali S., Kashem M.A. (2018). Life Cycle of Vermicomposting Earthworms *Eisenia Fetida* and *Eudrilus Eugeniae* Under Laboratory Controlled Condition. Biomed J Sci. &Tech. Res. 10(5)-2018. BJSTR. MS.ID.002015. DOI: 10.26717/BJSTR.2018.10.002015.
- [2] Ansari A.A. and Ismail S.A. (2012). Role of Earthworms in Vermitechnology. Journal of Agricultural Technology, 8(2): 403-415.
- [3] A. O. Ubalua (2007). Review cassava wastes: treatment options and value addition alternatives. African Journal of Biotechnology 6 (18): 2065-2073.
- [4] Bac Cam Thi Xieng (2017). Study on using silage of cassava stems, leaves and tubers as food for beef cattle in winter in Phu Yen district, Son La province, Vietnam (Master). Thai Nguyen University of Agriculture and Forestry, Vietnam.
- [5] Birundha M., J.A. John Paul, P.Mariappan (2013). Growth and reproduction of *Perionyx excavatus* in different organic wastes. J. Curr. Microbiol. App. Sci. 2(2): 28 – 35.
- [6] Dang Vu Binh (2008). Evaluation of the growth of earthworm (*Perionyx excavatus*) on different food sources. Journal of Science and Development, Vietnam, 4: 321 – 325.
- [7] Do Ngoc Bien (2012). Reducing environmental pollution and climate change through waste treatment with earthworm raising models.: Techniques of raising earthworms. Synergies Project.
- [8] Duong Phuong Nhung (2017). Assessing the current status and solving some environmental problems of An Thai cassava starch processing factory, Cam Lo district, Quang Tri province, Vietnam (Master). University of Science and Technology, The University of Da Nang, Vietnam.
- [9] Edwards C.A. (1985). Production of feed protein from animal waste by earthworm. Philosophical Transactions of the Royal Society of London. 310: 299-307.
- [10] Gomez G., Valdivieso M., Santos J. (1988). Cassava whole root chips silage for growing finishing pigs. Nutr. Rep. Int., 37(5): 1081-1092.
- [11] Han Quang Hanh, Nguyen Thi Xuan, Vu Dinh Ton (2020). Biology, Growth and Reproductive Performance of African Nightcrawler (*Eudrilus eugeniae*) Raised in a Tray Vermiculture System and Supplemented with the Concentrated Feed Vietnam. J. Agri. Sci.,18(5): 323-331.
- [12] Hoang Anh Vu, Nguyen Thi Thanh Thuy, Le Khanh Vu, Vo Thi Nho, Diep Thi Le Chi. Researching on using some food sources for designing model of raising earthworm *Perionyx excavatus* in Quang Binh province, Vietnam. In Proceedings of 7th national science conference on ecology and biological resource, Hanoi, Vietnam: 2037-2042.
- [13] Jacoby R., Peukert M., Succurro A., Koprivova A., Kopriva S. (2017). The Role of Soil Microorganisms in Plant Mineral Nutrition-Current Knowledge and Future Directions. Frontiers in plant science, 8, 1617. <https://doi.org/10.3389/fpls.2017.01617>
- [14] Lara Zirbes, Quentin Renard, J. Dufey, P. Tu, Hoang Nghia Duyet, p.Leibailly, F. Francis, E. Haubruge (2011). Valorisation of a water hyacinth in vermicomposting using an epigeic earthworm *Perionyx excavatus* in Central Vietnam. Biotechnol. Agron. Soc. Environ., 15(1): 85 – 93.
- [15] Nandeesh M.C., Srikanth G.K, Basavaraja N., Keshvanath P., Varghese T.J., Bano K, Bano K., Ray A.K., Kale R.D. (1988). Influence of earthworm meal on the growth and flesh quality of common carp. Biological Wastes. 26(3): 189-198.
- [16] Ndegwa P.M. & Thompson S.A. (2001). Integrating composting and vermicomposting in the treatment and bioconversion of biosolids. Bioresource Technology. 76: 107-112.
- [17] Nguyen Thi Hang Nga, Nguyen Lan Huong, Tran Khac Hiep, Nguyen Kieu Bang Tam, Luong Huu Thanh (2016). Research on the production of microbiological preparation for the treatment solid waste after processing cassava starch to make bio-organic fertilizer. VNU J. of Science: The earth science and Environment, 32: 282-288.
- [18] Truong Tat Hieu (2008). Production of the alcohol by using the residue of the cassava starch industry. Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology.
- [19] Singh A., Singh D.P., Tiwari R., Kumar K., Singh R., Singh S., Prasanna R., Saxena A., Nain L. (2015). Taxonomic and functional annotation of gut bacterial communities of *Eisenia foetida* and *Perionyx excavatus*. J. Basic Microbiol., 56 (3), pp.48 – 56.