



# Assesment of Bio-fertilizer Quality of Anaerobic Digestion of Watermelon Peels and Cow Dung

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**Abstract:** A study was carried out for assesment of bio-fertilizerquality of anaerobic digestion of watermelon peels and cow dung as substrates. Five kilogram (5 kg) each of water melon peels and cow dung were collected, pre-treated and mixed with water in a ratio 1:1 w/v to prepared 18 L slurry that was charged into the batch bio-digester and digested for 35 days at mesophilic temperature of between 26.2-30.8°C. The physicochemical and microbiological analyses of the substrates were determined before and after the digestion process using standard methods. The analyses showed that biochemical oxygen demand, total solids, organic carbon, carbon/nitrogen ratio reduced by 99.84%, 6.22%, 59.32% and 77.25% respectively, while chemical oxygen demand, total suspended solids and pH increased by 67.16%, 86.01% and 24.14% respectively after digestion. Bio-fertilizer yield of 83.35% and biogas yield of 16.65% were obtained. Plants macronutrients (N,P,K) content were substantially increased in the digestates by 78.57%, 89.09% and 84.62%. The presence of Clostridium (nitrogen fixer bio-fertilizer), Bacillus and Pseudomonas (phosphate solubilizing bio-fertilizers) revealed that the digestate was bio-fertilizer. Moreover, the implication of salmonella in the digestate is a major health concern, it is therefore recommended that further study to check if an extended retention period would ensure the removal of Salmonella.

**Keywords:** Bio-fertilizer, Biodigester, Anaerobic Digestion, Retention Time, Watermelon Peels, Cow Dung, Salmonella

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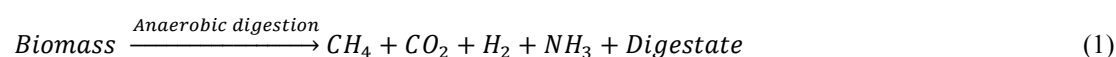
## 1. Introduction

Bio-fertilizers are environmentally friendly fertilizers that not only prevent damages to natural sources but help, to some extent in cleaning the nature from precipitated chemical fertilizers [1]. In addition, bio-fertilizers are one of the best modern tools for agriculture and it is a gift of our modern agricultural science. Bio-fertilizers are applied in the agricultural field as a replacement to our chemical fertilizers because they are not environment friendly and they may destroy the fertility of the soil in a long run [2].

The term 'bio-fertilizers' denotes nutrient supplement inputs for plant growth which are in biological origin. Bio-fertilizers accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants and also mobilizing

nutritive elements from non- usable form to usable form through biological processes. The role of bio-fertilizers in agricultural production assumes special significance, particularly in the present context of expensive chemical fertilizers. Moreover, it provides the farmers with a new strategy which is helpful for achieving the targeted goal of food security in Nigeria by increasing high productivity yield of food grains [3].

Anaerobic digestion is controlled degradation of organic waste in the absence of oxygen and in the presence of anaerobic microorganisms [4]. This process generates a product called biogas that is primarily composed of methane, carbon dioxide and digestate bio-fertilizer suitable as soil conditioners [2]. However, the biochemical reaction involved in the digestion process is shown in Equation (1);



Furthermore, the advantage of using anaerobic digestion in an urban areas is to treat organic waste as opposed to composting, anaerobic digestion produces biogas with a high percentage of methane which can be used as fuel whereas composting produces mostly carbon dioxide which could not be used as fuel and the demand for bio-fertilizer is dependent on compliance with quality standards [5].

The use of digestate bio-fertilizer to increase agricultural food production and soil improvement has been established by previous researchers, but its safety as determined by the amount of pathogens contained is still of public health concern to end users [2] and reports on the design of a bench scale biodigester for the production of bio-fertilizer using food wastes and cow dung are scanty in literature, despite the large volume of literature on biogas yield from various substrates [5]. Moreover, researches on biogas and bio-fertilizers in Nigeria have focused on the utilization of animal dung, human excreta, chicken droppings, and kitchen wastes as substrates while the use of plant wastes such as peels have been limited to water hyacinth, cassava peels, *cymbopogon citrus* and peels of water lettuce [5]. Watermelon (*Citrus latanus*) originated from western Kalahari region of Namibia and Bostwana in africa is now found in most tropical countries and Nigeria is one of the world's largest producers with over 347,000 metric tons in the year 2002 [6].

The objective of this study is to develop a bench scale bio-digester for the production of bio-fertilizer and to assess the quality parameters of the digestate of the anaerobic digestion of watermelon peels and cow dung.

## 2. Methods

### 2.1. Biodigester Design

A 23 liter bench scale batch anaerobic bio-digester of height 0.432 m and diameter 0.288 m was designed based Ajoy karki's biogas model [7] with some modifications, i.e

$$\text{Biofertilizer yield} = \frac{\text{mass of the digestates biofertilizer produced}}{\text{mass input of the substrate}} \times 100\% \quad (5)$$

### 2.3. Fabrication of Biodigester

An airtight batch anaerobic biodigester was fabricated using the appropriate machine tools and hand tools at the workshop of Mechanical Engineering Department of the Technology Incubation Centre (TIC) Bauchi, Nigeria. The theory behind is simply downward delivery and upward displacement. The biodigester consists of digestion chamber, inlet from the top cover, digestates outlet pipe, tap head for sampling taking unit and a stirrer as shown in isometric view figure 1 and detailed drawing figure 2 respectively.

### 2.4. Raw Materials Collection, Pretreatment, Blending, Dilution and Biodigester Loading

Watermelon peels and cow dung were collected in a water proof sack from Muda lawal market and cow market

separate floating gas holder system consisting of water jacket with inverted measuring cylinder was incorporated in the design and allowed the measurement of biogas volume. The design volume of the anaerobic biodigester was determined based on the substrate input quantity and chosen retention time. The cylindrical shape was adopted for better mixing. Stainless steel was used for fabrication of the biodigester because it is strong enough to withstand the weight and pressures of the substrates, good tensile strength and durability in an acidic environment.

### 2.2. Biodigester Design Consideration

The operating volume of the biodigester ( $V_o$ ) was determined based on the substrates input quantity ( $S_d$ ) and the chosen retention time ( $RT$ ) Equation (2) [8].

$$V_o = S_d \times RT [=] m^3 \quad (2)$$

The retention time is the interval of time during which the biomass remains to decompose in the digester. Normally, the retention time for anaerobic digestion of food wastes and cow dung at mesophilic temperature is between 20-40 days [8].

Substrate input is given as:

$$S_d = \text{Biomass (B)} + \text{Water (W)} [=] m^3/\text{day} \quad (3)$$

The total volume of the bio-digester ( $V_T$ ) should be greater than the operating volume. This is to give room for the expansion in volume of the slurry during fermentation. The operating volume of the digester must not exceed 90% of the total volume. Therefore, the working volume was chosen as 80% of the total volume [8].

The total volume is given as:

$$V_T = \frac{V_o}{0.8} [=] m^3 \quad (4)$$

Bio-fertilizer yield was given by Equation (5)

(Kasuwan Shanu) Bauchi, Nigeria. The raw materials were pretreated by removal of the unwanted non-biodegradable materials and watermelon peels were washed with distilled water in order to remove impurities. Moreover, cow dung was sundried for 3-days at an average mesophilic temperature of 28.5°C and watermelon peels were blended using standard BLG-401-18N blender. The elemental compositions of watermelon peels and cow dung were determined using DR/890 colorimeter and atomic absorption spectrophotometer.

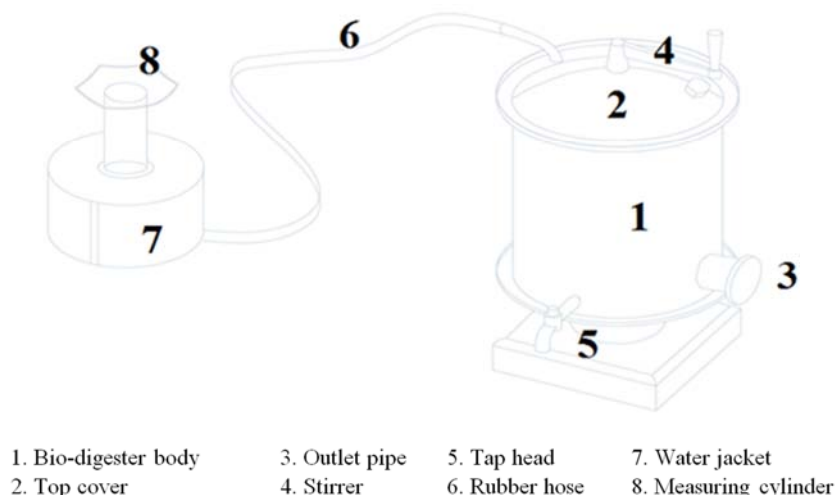
Five kilogram (5 kg) of the respective biomass (watermelon peels and cow dung) was measured using digital weighing balance. Ten kilogram (10 kg) of biomass were mixed with water in an equal proportion (1:1 w/v) weight by volume of the substrates and each co-substrate contributed 50% of the biomass. The dilution of the biomass with water

allowed the bacteria to move freely in the bio-digester. The substrate was homogenized for easy digestion and physicochemical and microbiological parameters of the substrates were analyzed before loading (charging) to the bench scale bio-digester.

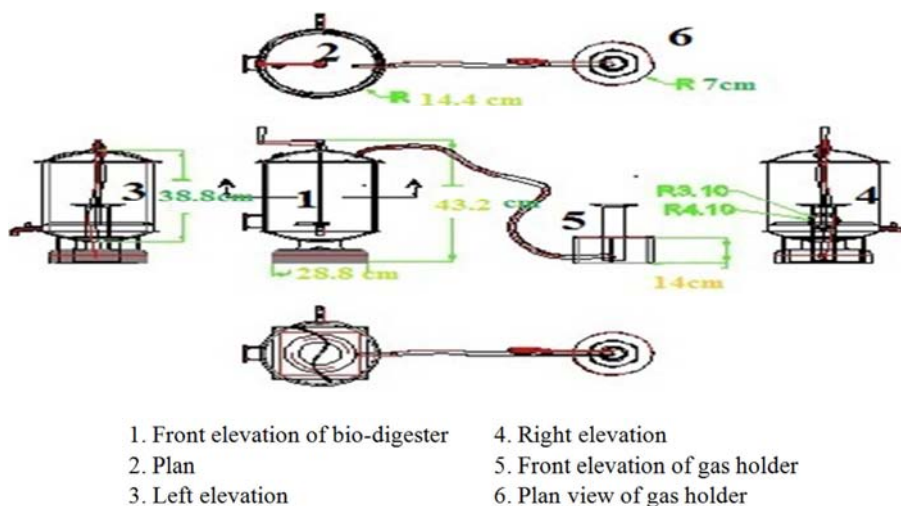
Twenty kilogram (20 kg) of the substrate was fed into the bio-digester from the top opening cover and closed after charging airtight condition of the digestion process was ensured. The substrate occupied 79% of the bio-digester volume leaving a clear space of 21% for biogas production and it was allowed to ferment for 35 days at mesophilic temperature, normally the retention period for the anaerobic digestion of food wastes and cow dung at mesophilic

temperature ranges between 20-40 days [8]. The experiment was allowed to run for 35 days in continuous fermentation during and after which the following was carried out:

- Physicochemical and microbiological analysis of feedstock before digestion.
- The temperature of the bio-digester was taken three times daily.
- Volume of biogas produced was measured daily.
- pH of the bio-digester content was taken daily.
- Weekly collection of samples for physicochemical and microbiological analysis.
- Physicochemical and microbiological analysis of the digestate.



**Figure 1.** Isometric projection of the developed bench scale bio-digester.



**Figure 2.** Detailed drawing of the developed bio-digester.

## 2.5. Physicochemical and Microbiological Parameters

The physicochemical parameters of the feedstock and digestate were evaluated using standard procedures [9]. Parameters analyzed includes biochemical oxygen demand (BOD), chemical oxygen demand (COD), total solids, total suspended solids (TSS), organic carbon, nitrogen content, carbon/nitrogen ratio,  $P_2O_5$ ,  $K_2O$  and pH respectively.

Microbial population in the bio-digester feedstock and

digestate produced were enumerated by standard plate count technique using 0.5 ml aliquots of appropriate dilution pour plated onto Nutrient agar, MacConkey agar, Eosin Methylene blue agar and Fastidious anaerobic agar for bacteria. Potato dextrose agar (PDA) plus chloramphenicol was used for fungal isolation and enumeration. Nutrient agar, MacConkey and Eosin methylene blue agar plates were incubated 37°C for 48 h, Potato dextrose agar plates were incubated at 25°C

for 5 days while Fastidious anaerobic agar plates were incubated in an anaerobic jar (oxoid) containing a moistened pack of gas generating kit (Bio-oxid) at 37°C for 7 days.

Purification and identification of individual colonies were carried out by morphological and biochemical tests while for fungi isolates, the microscopic and macroscopic features of the hyphal mass, morphology of cells and spores, nature of fruiting bodies were used for identification [10]. The colonies that appeared from the isolates were gram-stained, wire loops were sterilized using flame and they were allowed to cool. In addition, the portion of the colony was picked, emulsified and allowed to dry in the glass-slide. A drop of grams iodine and crystal violet were introduced in the slide and allowed to stay for 1 min. The slide was decolorized with 70% alcohol and flooded with water and it was further counter stained with safranin and allowed to stay for 1 min and further washed with water. The gram positive and gram negative organisms were identified by blue crystal and neutral red

respectively. Moreover, the slide was placed in the microscope and observed under x100 objective lens with a drop of oil emulsion for bacterial identification while fungi were identified at x40 objective lens with a drop of lactophenol cotton blue.

### 3. Results

A bench scale batch anaerobic bio-digester was designed and fabricated based on Ajoy Karki's kitchen biogas model [7] with some modifications as shown in figure 3. The estimation of the biodigester size as well as the biomass requirements was done on the basis of the substrate input quantity and the chosen retention time. Moreover, the design details of the biodigester are presented in Table 1 and the elemental analyses of watermelon peels and cow dung are presented in Table 2.

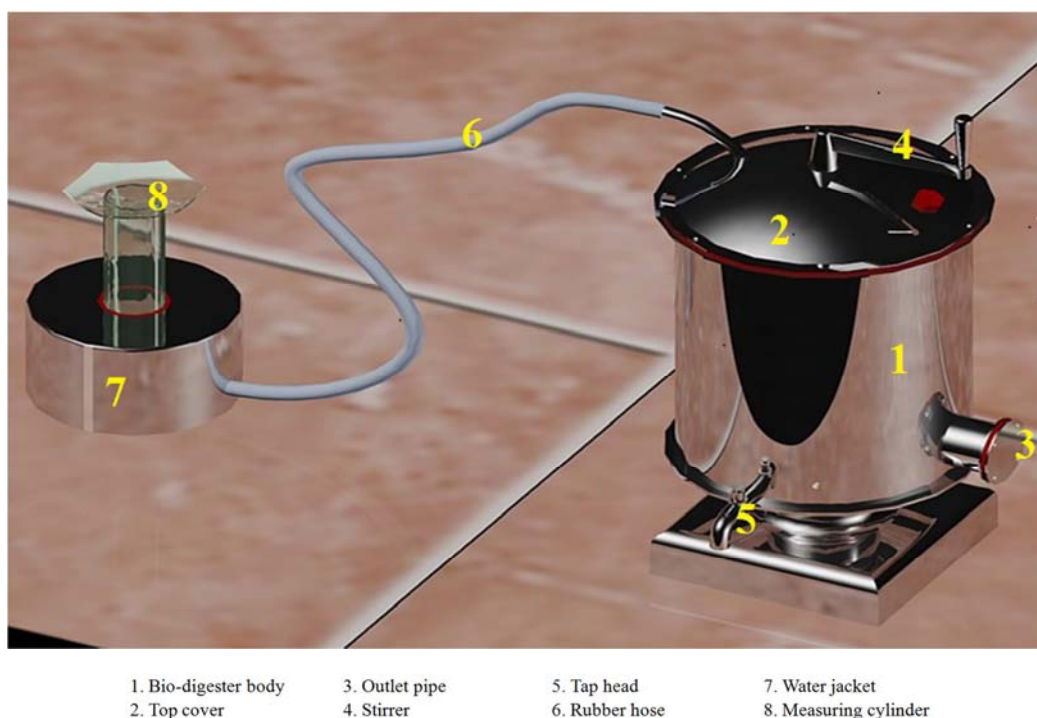


Figure 3. Pictorial view of the developed bench scale bio-digester.

The physicochemical characteristics of the bio-digester feedstock before and after the anaerobic digestion is shown in Table 1. The amount of BOD, total solids, organic carbon and carbon/nitrogen ratio in the feedstock was found to be reduced by 99.84%, 6.22%, 59.32% and 77.25% respectively. Moreover, there was an increase in total suspended solids (by 86.01%), COD (by 67.16%), nitrogen content (by 78.57%),  $P_2O_5$  (by 89.09%),  $K_2O$  (by 84.62%) and pH (by 24.14%) respectively. Table 3 reveals the mean microbial counts of the substrate before and after the digestion. The study reveals a significant reduction in total aerobic plate count, coliform count and fungal count from in the feedstock when compared with the digestate. Seventeen species of organisms were identified and isolated in the feedstock which reduced to nine

species of organism after the anaerobic digestion. However, Table 4 reveals the results of biochemical tests carried out on the isolates for the feedstock and digestate.

Table 1. Summary of design parameters of a bench scale bio-digester.

Design parameters	Calculated parameter
Digester Configuration	Cylindrical
Basis of bio-digester design	20 kg/batch substrate
Mixing ratio	1:1
Substrate input quantity ( $S_d$ )	0.018 ( $m^3/day$ )
Operating Volume ( $V_o$ )	0.018 ( $m^3$ )
Total Volume of the digester ( $V_T$ )	0.023 ( $m^3$ )
Height to radius ratio ( $h_d : r_d$ )	3:1
Height of bio-digester ( $h_d$ )	0.432 m (43.2 cm)
Radius of the bio-digester ( $r_d$ )	0.144 m (14.4 cm)
Digester diameter	0.288 m (28.8 cm)

**Table 2.** Physicochemical parameters of the substrates before and after digestion.

Parameter	Before digestion	After digestion	±%
BOD (mg/L)	88.29± 0.02	0.14± 0.01	99.84
COD (mg/L)	196.96±0.09	329.24± 0.25	67.16
Total solids (mg/L)	111.64± 0.98	104.70± 0.27	6.22
TSS (mg/L)	67.76± 0.98	126.04± 0.03	86.01
Organic carbon (mg/L)	590± 0.01	240± 0.00	59.32
Nitrogen (mg/L)	14± 0.001	25±0.001	78.57
P <sub>2</sub> O <sub>5</sub>	2.75±0.01	5.2± 0.06	89.09
K <sub>2</sub> O	2.6± 0.26	4.8 ± 0.04	84.62
C:N	42.76:1	9.73:1	77.25
pH	5.8±0.00	7.2± 0.00	24.14

Number of replicates= 3, ±% = percentage reduction/increase

**Table 3.** Microbial counts of bio-digester substrates before and after digestion.

WEEK	TAPC CFU/100ml	Coliform count	Fungal Count	Species of organisms isolated
Before	2.73 x 10 <sup>8</sup> ±0.02	4.73 x 10 <sup>8</sup> ±0.06	4.12 x 10 <sup>6</sup> ±0.07	Bacteria: <i>Escherichia coli</i> , <i>Citrobacter</i> , <i>Proteus</i> , <i>Bacillus</i> , <i>Pseudomonas</i> , <i>Enterobacter</i> , <i>Clostridium</i> , <i>Bacteroides</i> , <i>Staphylococcus</i> , <i>Streptococcus</i> , <i>Salmonella</i> , <i>Shigella</i> and <i>Klebsiella</i> . Fungi: <i>Rhizopus</i> , <i>Penicillium</i> , <i>Mucor</i> and <i>Aspergillus</i> .
After	1.34 x 10 <sup>4</sup> ±0.05	4.84 x 10 <sup>5</sup> ±0.02	2.30 x 10 <sup>3</sup> ±0.10	Bacteria: <i>Proteus</i> , <i>Bacillus</i> , <i>Bacteroides</i> , <i>Pseudomonas</i> , <i>Clostridium</i> and <i>Salmonella</i> . Fungi: <i>Penicillium</i> , <i>Aspergillus</i> and <i>Rhizopus</i> .

Number of replicate counts n=3;

**Table 4.** Biochemical tests of isolates from Feedstock and Digestate bio-fertilizer.

Source	Substrate	Isolates	Biochemical tests									Species of organism
			Urea	VP	Lact	Glu	Suc	Ind	Cit	H <sub>2</sub> S	Mot	
Feedstock	CD + WMP	A <sub>1</sub>	-	-	+	+	-	+	-	-	-	<i>E. Coli</i>
		B <sub>1</sub>	-	-	-	+	-	-	+	+	+	<i>Salmonella spp</i>
		C <sub>1</sub>	-	-	-	+	-	-	-	-	-	<i>Shigella spp</i>
		D <sub>1</sub>	+	+	+	+	+	-	+	-	-	<i>Klebsiella spp</i>
Digestate	CD + WMP	B <sub>2</sub>	-	-	-	+	-	-	+	+	+	<i>Salmonella spp</i>

Key: VP = Voges prospkeur, Ind = indole Lact = lactose H<sub>2</sub>S = Hydrogen sulfide M= motility

## 4. Discussion

The elemental analyses of the raw materials revealed that, cow dung has high nitrogen, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O, and micronutrient elements content when compared with watermelon peels. This might be due to high organic matter available in the cow dung a [5]. In terms of moisture content, the results showed watermelon peels have higher moisture content when compared with cow dung and this might be due to the high water content of watermelon peels this agrees with similar findings [11].

The study indicates 99.84% reduction in BOD of the digestate with respect to the feedstock and the results revealed that BOD of undigested feedstock was higher than in anaerobic digested bio-fertilizer. In addition, the soluble BOD was readily degradable by healthy microbes in a medium while the insoluble BOD requires longer retention time to degrade [12]. However, the study showed 67.16% increase in COD in the digestate when compared to the feedstock. This might be due to the biodegradation of the organic matter in the substrates resulted because of the activities of mesophilic micro-organisms at anaerobic condition [13].

The reduction in total solids by 6.22% and increase in total

suspended solids by 86.01% in the digestate when compared to the feedstock could be due to the very low solid content associated with water melon peels contrary to that of cow dung. In practice, the results showed that digestate has lower total solid content than the undigested substrates and this reduction in total solids agrees with similar findings [5]. Furthermore, the removal of organic carbon in form of CH<sub>4</sub> and CO<sub>2</sub> during the digestion process might have accounted for total solid reduction in the digestate. The reduction in carbon/nitrogen ratio from 42.14:1 before digestion to 9.6:1 after anaerobic digestion showed carbon was highly consumed in the digestion process. Furthermore, the C/N ratio of the digestate does not exceed the acceptable limit of 20:1 for biogas and bio-fertilizer production as reported by Owamah *et al* [2].

The study revealed 78.57% increase in nitrogen content of the digestate when compared to the bio-digester feedstock and the increased in nitrogen content from 14 mg/L in the feedstock to 25 mg/L in the digestate agrees with similar findings [14]. The increased in nitrogen content after the anaerobic digestion process might be due to high organic matter present in the cow dung as reported by [13]. The P<sub>2</sub>O<sub>5</sub> content of the feedstock was 2.75 mg/L and after the digestion, the content increased to 5.2 mg/L which showed

89.09% increase. This increased might be due to the released of organically bound phosphorous in watermelon peels and cow dung during the fermentation process as reported by [13]. Moreover, The study showed 84.62% increase in K<sub>2</sub>O content of the digestate when compared with the feedstock. The increase observed in K<sub>2</sub>O content from 2.6 mg/L in the feedstock to 4.8 mg/ L in the digestate agrees with similar findings [13, 11].

The results presented in Table 3 indicates a significant reduction in TAPC of the feedstock from  $2.73 \times 10^8 \pm 0.02$  to  $1.34 \times 10^4 \pm 0.05$  in the digestate. This agrees with [5] that TAPC decrease during the digestion process and possibly due to the decrease in carbon/nitrogen ratio which leads to the supply of low nutrients for micro-aerophilic organisms. Also, The study revealed a significant reduction in total coliform in the digestate as against the value in the feedstock and within the week of digestion. This agrees with [2, 16] that microbial population has a tendency to decrease within the period of digestion at mesophilic temperature. Though anaerobic digestates can be used to efficiently improve soil fertility and boost crop production, its safety still remains a source of concern to end users due to pathogens [5]. The reduction in fungal counts from  $4.12 \times 10^6 \pm 0.07$  in the feedstock to in the digestate  $2.30 \times 10^3 \pm 0.10$  might be due to the increase in bio-digester temperature because most of the fungi survive between the temperature of 21 -25°C in anaerobic digestion. This agrees with similar findings [5, 2].

The biochemical tests revealed several species of bacteria and fungi were found and isolated as presented in Table 4. Species of bacteria and fungi isolated from the digestate includes; *Bacillus*, *Pseudomonas*, *Penicillium*, *Clostridium*, *Bacteroides*, *Rhizopus*, *Aspergillus*, *Proteus* and *Salmonella*. *Clostridium* is known to be free-living nitrogen fixing bio-fertilizer [17] and the digestates would enhance the fertility of soil for crop production. *Bacillus* and *Pseudomonas* are phosphate solubilizing bio-fertilizers and furthermore, *Bacillus* act as solubilizers for trace elements like silicates and zinc as well as plant growth promoters [18]. Species of *Aspergillus* and *Penicillium* are also phosphate solubilizing fungi [5]. In the feedstock, four pathogenic microorganisms were detected (*E. Coli*, *Shigella*, *Salmonella* and *Klebsiella*) which reduced to only one (*Salmonella*) after digestion (Table 4), this might be due to the significance decrease in carbon/nitrogen ratio and high increase in plant macronutrient of the bio-digester content. In addition, these pathogenic microorganisms could not survive at very low carbon/nitrogen and at high macronutrient content. Furthermore, the presence of *salmonella* in the digestate calls for concern in its use on plant that can be eaten raw, since *Salmonella* it is pathogenic and could be transmitted to man and animals via contaminated food [18].

Moreover, from the results obtained for physicochemical (NPK) content and the type of microorganisms identified in the digestate, it can be concluded that the digestate is bio-fertilizer with following compositions;

- Nitrogen content: 25 mg/L
- P<sub>2</sub>O<sub>5</sub>: 5.2 mg/L

- K<sub>2</sub>O: 4.6 mg/L
- *Clostridium* (nitrogen fixer bio-fertilizer)
- *Bacillus* and *Pseudomonas* (phosphate solubilizing bio-fertilizer)

The anaerobic digestion of cow dung and watermelon peels at mesophilic temperature gave a high yield of digestates bio-fertilizer. Bio-fertilizer yield of 83.35% (16.67 kg) and biogas yield of 16.65% were obtained.

## 5. Conclusion

The study has shown that nitrogen fixer bio-fertilizer could be produced through the anaerobic digestion of cow dung and watermelon peels at mesophilic temperature. The developed bio-digester was effective for the production of bio-fertilizer with yield of 83.35% (N: 25 mg/L; P<sub>2</sub>O<sub>5</sub>: 5.2 mg/L and K<sub>2</sub>O: 4.8 mg/ L). The presence of *Clostridium* (nitrogen fixer), *Bacillus* and *Pseudomonas* (phosphate solubilizers) revealed that the digestate is bio-fertilizer. Moreover, the presence of *Salmonella* in the digestate is a major health concern, it is therefore recommended further study to check if an extended retention period would ensured the removal of *Salmonella* pathogen from the digestate and the digestate should only be applied through soil application and root dipping and should not be spread on crops that can be eaten raw.

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