

# Characterization of Digestates from Anaerobic Co-Digestion of Manioc Effluent, Human Urine and Cow Dung

Kpata-Konan Nazo Edith<sup>1</sup>, Kouamé Yao Francis<sup>2</sup>, Kouamé Kouamé Martin<sup>2</sup>, Konan Koffi Felix<sup>2\*</sup>

<sup>1</sup>Department of Agroforestry/University Jean Lorougnon Guédé, Daloa, Côte d'Ivoire <sup>2</sup>Department of Environment/University Jean Lorougnon Guédé, Daloa, Côte d'Ivoire Email: \*konanfelix@yahoo.fr

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### Abstract

This study focused on the characterization of digestates resulting from anaerobic digestion of manioc effluents from attiéké factories. Two types of digestate were characterized, one consisting of manioc effluent + urine and another composed of manioc effluent + urine + cow dung. As a result, these residues of bio-digestion rich in nutrients (NPK) can be used as agricultural fertilizer. Moreover, the determination of some microorganisms and heavy metals digestates allowed to better appreciate its fertilizing quality. These parameters remained in accordance with the quality standards of a digestate prescribed. These results show that digestates from anaerobic co-digestion of manioc effluents, urine and cow dung can be used without fear as an agricultural biofertilizer.

### **Keywords**

Manioc Effluent, Human Urine, Cow Dung, Anaerobic Co-Digestion, Digestate

### **1. Introduction**

Anaerobic digestion is now widely used around the world to reduce the pollutant loads of various types of organic waste [1] [2] [3] [4] [5]. Indeed, it is a natural process by which organic matter is transformed into gas by the action of microorganisms in the absence of oxygen. Realized in confined spaces still called digesters or reactors, the reactions of fermentation can be optimized and checked while reducing at least of half pollution load constituted by numerous biodegradable by-products. These include municipal or industrial solid waste, agro-food effluents and livestock waste [6] [7].

These anaerobic treatment systems have the advantage of producing biogas, mainly composed of methane [8] [9] [10]. But, the parameters influencing the digestate's fertilizing properties as well as the mechanisms involved are not yet sufficiently specified.

This technology is increasingly recommended for the treatment of waste agro-industry, including factories of attiéké which develops largely in recent years in West Africa.

In addition, new agricultural production policies encourage green agriculture. The reuse of residues or digests obtained after anaerobic digestion is stable, deodorized, largely free of pathogens and is rich in nitrogen compounds [8] [11] [12]. This digestate can be used as an amendment or biofertilizer [13] [14] for green and sustainable agricultural production.

Indeed, the composition and the quality of the digestates depend on the substrate used as well as the efficiency of the anaerobic digestion. Also, it would be necessary to analyze the digestate in accordance with quality standards for healthy and efficient use in agriculture as biofertilizer.

The present work aims to determine the fertilizing quality of digestates from the anaerobic co-digestion of cassava effluents, human urine and cow dung through monitoring of physicochemical parameters, metallic trace elements and the identification of pathogenic bacteria.

### 2. Material and Methods

#### 2.1. Material

The co-substrates used are manioc effluent, human urine and cow dung. Cassava effluent is a mixture of peeled cassava wash water and crushed cassava juice. The human urine used to enrich manioc effluent with nitrogen and to correct the pH of these effluents. Cow dung was used to inoculate the digester. All these co-substrates were collected in Daloacity (Côte d'Ivoire). The pH and temperature were measured using a Hanna Instrument pH meter.

### 2.2. Sampling and Analysis of Digestates

Two experimental reactors consist of metal drums, reactor 1 (R1), reactor 2 (R2), were used for anaerobic digestion. Reactor 1 were fed with a mixture of manioc effluent, urine and cow dung (E + U + C). As for reactor 2, it was seeded with a mixture of manioc effluent and urine (E + U).

The samples to be analyzed were collected using a syringe with a capacity of 60 ml, collected in polyethylene 70 ml flasks and stored in an ice-cold box. This cooler is then transported to the laboratory for physicochemical, microbiological and heavy metals analyzes. Samples are made initially once a month and in a second time after 3 months of operation of the digesters.

The Chemical Oxygen Demand (COD) was determined by the potassium dichromate oxidation method (NF T 90-101). Biochemical oxygen demand (BOD5) were obtained by the manometric method [15]. Total Kjeldahlnitrogen (TNK)

was assayed by titrimetry after distillation of the selenium mineralized sample (NF T 90-010). Total phosphorus was determined after mineralization of the sample in the presence of sulfuric acid and sodium persulfate (NF T 90-023). Ammonium ( $NH_4^+$ ) was determined according to the AFNOR NF T90-015 standard [16]. The potassium (P) analysis protocol used is the flame photometry method fed by an air-butane mixture [17].

The metallic trace elements (Copper, Iron, Nickel, plumb) are determined by argon plasma ionization source mass spectrophotometry [17]. The bacterial germs were determined according to the agar incorporation method. EMB agars, Hektoen, Rapid medium, MOSSEL medium were used respectively for the enumeration of total fecal coliforms (NF T90-414) and (NFT 90-416), *Salmonella, Escherichia coli* and *Bacillus cereus* [18].

Statistical analyses were performed by the software Paleotological Statistic (PAST) version 3.21 [19].

### **3. Results**

### **3.1. Physicochemical Parameters**

### 3.1.1. pH and Temperature

For both reactors, the pH values increase strongly before stabilizing. pH values for the E + U digestate were ranged from 6.70 to 10.29 with an average of 8.29 (**Figure 1(a)**). For this reactor, the highest value was recorded on the  $62^{th}$  day of the experiment. Concerning the E + U + C digestate, the pH ranged from 6.50 to 9.76 with an average of 8.14 (**Figure 1(b)**). The highest value of pH for this reactor was observed on the 98<sup>th</sup> day of operation.

The temperatures recorded in the digestate varied between  $23^{\circ}C$  and  $34.4^{\circ}C$  for the E + U digestate (**Figure 2(a)**) with an average of  $28.66^{\circ}C$ . At the E + U + C digestate recorded, temperatures fluctuated between  $23^{\circ}C$  and  $34.9^{\circ}C$  (**Figure 2(b**)) with an average of  $29.40^{\circ}C$ .

## 3.1.2. Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD)

On the whole, COD values decreased from 8610 to 272 mg/L for E + U digestate (**Figure 3(a)**) with a reduction rate of 96.84% and 7290 to 254 mg/L for E + U + C digestate with a reduction rate of 96.51%. The COD values of the E + U + C digestate have significantly more important decreases than the E + U digestate (Anova test; p < 0.05).

Concerning BOD, the concentrations dropped from 3444 to 109 mg/L (**Figure 3(b)**), is a reduction of 96.84% for the E + U digestate and from 2916 to 50 mg/L, is a fall of 98.28% for the digestate E + U + C (**Figure 3(b)**). The concentrations of BOD did not show a significant difference (Anova test; p > 0.05) from one digestate to another.

## 3.1.3. Concentration of Total Nitrogen, Ammonium, Total Phosphorus and Potassium

In the E + U digestate, the concentration of Total Nitrogen (TN) (Figure 4(a))



**Figure 1.** pH variation of digestates. (a) Reactor 1: E + U + C (mixture of manioc effluent, urine and cow dung); (b) reactor 2: E + U (mixture of manioc effluent and urine).



**Figure 2.** Variation of digestates temperatures. (a) Reactor 1: E + U + C (mixture of manioc effluent, urine and cow dung); (b) reactor 2: E + U (mixture of manioc effluent and urine).



**Figure 3.** Variation of the COD (a) and BOD (b) of the digestates during the anaerobic digestion. Reactor 1: E + U + C (mixture of manioc effluent, urine and cow dung); reactor 2: E + U (mixture of manioc effluent and urine).

failed from 3251 to 2101 mg/L with an average of 2618.2 mg/L. The values of Total Nitrogen in E + U + C digestate decreased from 3617 to 3145 mg/L with an average of 3412.4 mg/L (**Figure 4(a)**). For this parameter, no significant difference (Anova test; p > 0.05) was observed between the two types of digestates.



**Figure 4.** Evolution of nutrients in digestates during the anaerobic digestion. Reactor 1: E + U + C (mixture of manioc effluent, urine and cow dung); reactor 2: E + U (mixture of manioc effluent and urine): (a) Total Nitrogen (TN); (b) ammonium ( $NH_4^+$ ); (c) Total Phosphorus (TP); (d) potassium (K).

Concerning the ammonium values, the concentrations increased in E + U digestate from 321 to 2487 mg/L with an average of 1651.6 mg/L (**Figure 4(b)**). For the E + U + C digestate, the growth observed varies from 624 to 2472 mg/L (**Figure 4(b)**), with an average of 2207 mg/L. Statistical analyses revealed no significant difference (Anova test; p > 0.05) of  $NH_4^+$  in digestates.

As for Total Phosphorus (TP), concentrations evolved from 232 to 217 mg/L for E + U with an average of 217.6 mg/L (**Figure 4(c)**). In the digestates from E + U + C, the phosphate values were changed from 267 to 230 mg/L (**Figure 4(c)**), with an average of 250.4 mg/L. There is no significant difference in total phosphate concentrations (Anova test; p > 0.05) between digestates.

Regarding potassium (K), a decrease in concentrations was observed during anaerobic digestion. The E + U digestate shows concentrations ranging from 2710 to 1520 mg/L with an average value of 2413.6 mg/L (**Figure 4(d)**). For the E + U + C digestate, the values decreased from 2710 to 2770 mg/L with an average of 2588 mg/L (**Figure 4(d)**). No significant difference (Anova test; p > 0.05) was observed between digestates for this parameter.

### **3.2. Concentrations in Metallic Trace Elements**

Concerning Nickel, the analysis showed a slight variation in concentrations of

0.77 to 1.53 mg/L in E + U and 2.78 to 2.48 mg/L in E + U + C (Figure 5(a)). For iron, a decrease was recorded from 5.89 to 2.69 mg/L in E + U and 4.45 to 0.62 mg/L in E + U + C from day 1 to day 122 (Figure 5(b)). Copper levels observed in the E + U digestate dropped from 0.01 to 0 mg/l until the end of the experiment (Figure 5(c)). For the E + U + C digestate, a slight decrease of Copper from 0.04 to 0.02 mg/L from day 1 to day 122 was recorded. As for plumb, the results showed values of 0.22 mg/L in E + U digester and 0.03 mg/L in E + U + C at day 1. These concentrations decreased to below the detection limit from day 19 to day 122 (Figure 5(c) and Figure 5(d)). Overall, heavy metals did not show a significant difference between the two types digestates.

### 3.3. Microbiological Parameters

Results on the microbiological quality of digestates (Figure 6(a) and Figure 6(b)) show that at day 1 samples contain a high concentration of germs. Fecal coliforms showed respective concentrations of  $58.10^5$  and  $145.10^5$  CFU/100 mL for the E + U and E + U + C digests. For total coliforms, concentrations of  $22.10^5$  CFU/100 mL (digestate E + U) and  $134.10^5$  CFU/100 mL (digestate E + U + C)



**Figure 5.** Contents of digestates in metallic elements during the anaerobic digestion. Reactor 1: E + U + C (mixture of manioc effluent, urine and cow dung); reactor 2: E + U (mixture of manioc effluent and urine): (a) Nickel (Ni); (b) Iron (Ir); (c) Copper (Cu); (d) Plumb (Pb).



**Figure 6.** Variation of bacterial loads of digestates. Reactor 1: E + U + C (mixture of manioc effluent, urine and cow dung); reactor 2: E + U (mixture of manioc effluent and urine). (a) Fecal coliforms; (b) Total coliforms; (c) *Escherichia coli*; (d) *Bacillus cereus*.

were recorded. With regard to the germs of *E. coli*, the analyzes revealed concentrations of  $18.10^5$  CFU/100 mL and  $115.10^5$  CFU/100 mL respectively in the E + U and E + U + C digestates. High levels of *Bacillus cereus* with values of 5.105 CFU/100 mL for E + U and 1.106 CFU/100 mL for E + U + C were observed. The presence of *Salmonella* was also observed at  $1^{st}$  day in the different substrates.

A reduction of the microbial load was observed from the 19<sup>th</sup> day to the 122<sup>nd</sup> day. Fecal coliforms decreased by  $58.10^5$  at 00 CFU/100 mL for E + U digestate and  $145.10^5$  at  $16.10^5$  CFU/100 mL for E + U + C digestate. At the level of total coliforms, an elimination of these germs from 22.10<sup>5</sup> to 00 CFU/100 mL for E + U and from  $134.10^5$  to  $115.10^4$  CFU/100 mL for E + U + C was noted. As for *E. coli*, the reduction was respectively  $18.10^5$  at 00 CFU/100 mL and  $115.10^5$  at 00 CFU/100 mL for E + U and  $115.10^5$  at 00 CFU/100 mL for E + U and  $115.10^5$  at 00 CFU/100 mL for E + U and  $115.10^5$  at 00 CFU/100 mL for E + U and  $115.10^5$  at 00 CFU/100 mL for E + U and  $1.10^6$  at 7.105 CFU/100 mL for E + U and  $1.10^6$  at 7.105 CFU/100 mL for E + U + C. A total absence of *Salmonella* was also reported.

### 4. Discussion

For pH, analysis of the results obtained for the two types of digestates shows an average of 8.29 for E + U and 8.14 for E + U + C. These values could be explained by the desorption of proteins or the volatilization of acidic compounds or CO<sub>2</sub> during anaerobic digestion, as noted by [12] and [20]. These observations were also reported by [21] during their studies on the qualitative characterization of a liquid avicolic digestates derived from industrial biomethanization and use in fertigation of tomato plants.

The temperature of the digestates fluctuated in the range of mesophilic fermentation (23°C and 35°C). This temperature is favored by the tropical climate characterized by strong sunshine and average annual temperatures of more than  $26^{\circ}C$  [9] [22] [23]. In this study, the reactors are displayed to the open air. According to [24], mesophilic fermentation is the most common and the best controlled systems.

Concerning COD and BOD, high levels are observed in digestates of the first day. The analysis of results show a major elimination of these pollutants in the digestate during the process. According to [25], the reduction of the pollutant load would be linked to the potential consumption of organic matter by the purifying microflora during its natural evolution in the digesters.

For the TKN, a slight decrease is observed in all digestates. This weak elimination indicates the conservation of TKN in reaction as also observed by [26]. The nitrogen contents of the digesters are similar to the values reported by [27]. According to this last author, a digestate designed to be used for the agricultural amendment must have a nitrogen content of between 1.5 and 6.2 g/Kg. For ammoniacal nitrogen, the increase observed in the reactors could be related to the mineralization of the organic nitrogen contained in the TKN in ammonium by the bacterial flora present in the digester [24] [28]. This transformation increases the fertilizing power of the digestate because plants much more easily assimilate it as source of nitrogen [28]. The elevation of  $NH_4^+$  is also thought to be due to the large production of amino acids after protein hydrolysis by proteolytic bacteria [24]. For [29], proteins allow specifying the fate of nitrogen in the anaerobic digester.

Regarding total phosphorus, the values revealed are slightly lower in the digestate. According to [30], this decrease could be explained by the solubilization of phosphorus to form mineral substances such as struvite and calcium phosphate (hydroxyapatite). Anaerobic digestion increases the availability of phosphorus and its efficiency in digestate [31]. However, for [32], no significant monitoring data can show an increase in soluble phosphorus in the digestate and [33] observes that the phosphorus values of a digestate are between 0.2 and 2.6 g/Kg. Presence of phosphorus in the digestate promotes root development, stiffness of the tissues, reproduction and the quality of the plant products [34].

As for potassium, the results showed a slight decrease for all digestates. Overall, co-digestion of manioc effluent, urine and cow dung produce a material rich in potassium. In fact, the average potassium contents of the digestate produced by the methanation are 2413.6 mg/L for the E + U digestate and 2588 mg/L for the E + U + C. These levels are higher than the minimum value reported by [27].

Concerning the metallic trace elements, the values recorded on the 1<sup>st</sup> and the 122<sup>nd</sup> day are in conformity with the standards described by the European Commission. According to [25], the heavy metals contents of digestates would be influenced by the origin of the substrates treated by anaerobic digestion. The heavy metal content of biofertilizers is one of the major concerns in their use in agriculture. Indeed, [25] believes that the use of a high metal biofertilizer can lead to soil contamination with the potential for metals to accumulate in plant roots, remain in the soil and pollute groundwater.

Regarding the microbiological characterization, the results showed a significant reduction of germs in the digestates. For total and fecal coliforms, their levels in digestates are lower than the standards set by the [35] for irrigation water. Concentrations of E. coli were below the thresholds set by European Union regulations indicating a value of less than 1000 CFU/g in 4 samples or less than 5000 CFU/g in a sample [36]. Bacillus cereus has lower rates than the threshold values of the European Commission regulations. Also, an absence of Salmonella has been recorded at the end of the experimentation period. This elimination of germs in the digestates would be related to the temperature and residence time pair. Indeed, the anaerobic digestion took place for 122 days in this study at an average temperature of 23°C corresponding to the mesophilic phase. According to [36], the time to reduce the population of pathogens by 90% is several days or even months at mesophilic temperatures. Because for a good hygienization of the digestates, it is necessary that the anaerobic digestion is done at pH close to the neutrality (6.8 to 8.5), at mesophilic temperatures (20°C to 45°C with an optimum towards 35°C) with residence times of 20 to 60 days [14] [25].

### **5.** Conclusion

The digestates treated during this study are rich in nutrients and can be used as agricultural fertilizer. Moreover, the determination of certain microorganisms and heavy metals in the studied digestate allowed to better assess its fertilizing quality. These parameters remain in accordance with the quality standards of a biofertilizer prescribed by the European Commission. The results of this study show that digestate from anaerobic digestion of manioc effluent, urine and cow dung can be promoted in agriculture as a biofertilizer.

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### **Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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