

Safety and Nutrient Values (NPK) of Bio-Effluent from Human Excreta Used to Feed Biogas Plants in Rwanda

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Abstract: Compared to cattle dung, human excreta have been used for methane generation in limited scale in most of developing countries. In Rwanda, institutions and households which have biogas plants connected to toilets are producing big quantity of bio effluent every day. It is not known whether such bio effluent is a rich fertilizer and safe for human health. This scientific research study was conducted in the four provinces of Rwanda where biodigesters connected to toilets are installed and operating. Field sites visits were made, samples were collected in sterilized bottles, transported in cooling boxes and taken to Laboratory for analysis. Laboratory results obtained revealed that bio effluent from human excreta digested in biogas plants connected to toilets is a rich natural fertilizer and it does not contain harmful bacteria (*Shigella* and *Salmonella*). Thus, use of this bio effluent could have a major impact on food security, agribusiness, agro-industries and may constitute a source of income.

Key words: Bio- effluent (NPK), Biodigesters, Human excreta, Toilets, Bacteria, Food security.

Résumé : Comparés à la bouse de vache, les excréments humains ont été utilisés pour la production du Méthane à petite échelle dans les pays en voie de développement. Au Rwanda, les institutions et foyers familiaux qui possèdent des installations de biogaz connectées aux toilettes produisent une grande quantité de fertilisants chaque jour. Il n'est pas connu si ces fertilisants sont riches et ne contiennent pas de bactéries (*Shigella* et *Salmonella*) qui causent des maladies à la population. La présente étude a été menée dans les quatre provinces du pays où les installations de biogaz ont été construites et fonctionnent correctement. Les échantillons ont été collectés dans des flacons stérilisés et ils ont été transportés au Laboratoire dans de petits frigos pour être analysés. Les résultats de Laboratoire obtenus ont montré que les excréments humains digérés dans des installations de biogaz constituent un riche fertilisant naturel et ne contiennent pas de germes pathogènes (*Shigella* et *Salmonella*) pour la santé de l'homme. Par conséquent, l'utilisation de ce fertilisant pourrait avoir un important impact sur la sécurité alimentaire, les affaires dans le domaine agricole, les industries agricoles et constituerait une source de revenus.

Mots clés: Fertilisants digérés (NPK), Installations de biogaz, Excréments humain, Toilettes, Bactéries, Sécurité alimentaire.

1. INTRODUCTION

All plants need NPK to grow well. Nitrogen (N) is responsible for the growth of leaves on the plant. Phosphorus (P) helps roots and flowers growth as well as fruits development. Potassium (K) is a nutrient that is responsible for the overall functions of the plant perform correctly. Many Rwandans population are handling and using bio effluent from biodigesters connected to toilets as fertilizer in the field and gardens, and they handle it without care with risk of being contaminated by bacteria. Currently, it is not known whether such fertilizer is safe for health of Rwandans. It is also not known whether such fertilizer contains enough NPK plants need. The research's purpose is to find out that fertilizer from biodigesters connected to toilets is harmful or not for farmers who are using it and to know whether it contains enough nutrient values (NPK). In the context of the crowded areas of developing country, various water borne diseases that spread through human excreta contamination such as worms (hook worms, round worms) bacterial diseases (typhoid, Para-typhoid, Cholera , Dysentery) and viral infections (gastro-enteritis) resulting into diarrhea and hepatitis are found in abundant. In Rwanda currently total household biogas plants fed with cow dung is 10,200 and total number of household biogas plants connected to toilets is 1157; their sizes are 4, 6, 8 and 10 m³ respectively. In Institutions particularly Prisons, Schools, Hospitals and Barracks all biogas plants are connected to toilets. Total number of community biogas plants connected to the toilets is currently 87; their capacities are 30, 50 and 100 m³ respectively; bio-digesters are connected in series. Human excreta is currently being used for feeding those biogas plants. It has been observed that during the anaerobic digestion process, stabilized manure called bio-effluent is obtained as by product. That bio-effluent from human excreta is currently produced and used in many households and institutions of Rwanda; it has deemed necessary to carryout research on that bio-effluent in order to find out whether it contains enough nutrients for plants and if it is safe for Rwandan population.

The overall objective of the research study is to check through Laboratory analysis the Safety and Nutrients content (NPK) of Bio effluent from bio digesters connected to toilets.

Districts covered by the research study are the following: Kicukiro, Gasabo and Nyarugenge in Kigali city province, Bugesera in Eastern province, Rulindo, Gakenke, Musanze and Burera in Northern province; Nyabihu and Karongi in Western province; Muhanga, Nyanza, Ruhango and Huye in Southern province. It was not possible to cover all the 30 districts of Rwanda due to the fact that biogas plants connected to the toilets in households are constructed in few districts, they represent 11% (1157 bio-digesters) compared to bio digesters not connected to toilets 89% (10,200 bio-digesters).

The results obtained have indicated that bio-effluent from human excreta is safe; it can be used as a natural fertilizer in addition those results have shown that bio-effluent is rich in NPK. The nutrient values (NPK) contained in this research study are similar to those obtained from compost and fresh cow dung.

The research team expresses its gratitude to the Sweden Government particularly Sida, the Government of Rwanda, the University of Rwanda and the Directorate of Research, Innovation and Post graduate studies for providing the funding required for the study. A special thanks to the owners of the biodigesters connected to toilets and their households who participated in the study, for their cooperation and contribution, which made this research possible.

2. METHODOLOGY

2.1. Scope of the study

To conduct the study, 13 districts out of 30 districts where biogas plants connected to toilets are installed were selected for samples collection. As mentioned in the introduction, the samples of bio effluent studied were collected from the following districts: Kicukiro, Gasabo and Nyarugenge in Kigali city province, Bugesera in Eastern province, Rulindo, Gakenke, Musanze and Burera in Northern province; Nyabihu and Karongi in Western province; Muhanga, Nyanza, Ruhango and Huye in Southern province. Bio digesters had to be connected to the toilets and operating well in order to be selected for this research study.

2.2. Study tools and samples collection

The main instruments of the study for samples collection were sterilized bottles and cooling boxes. 140 samples were collected in Kigali city and Eastern province; 80 samples in Northern Province and 90 samples in Southern and Western provinces. In total 310 samples were collected and taken to the Laboratory where plants nutrients (NPK) and pathogen bacteria were analyzed. Additional investigation tools include observations, photographs, of different of biogas plants connected to toilets and bio effluent pits.

2.3. Study components

The whole study was divided into three major phases: field study, laboratory testing, and samples analysis for determination of NPK and pathogen bacteria.

2.3.1. Field study

Household biogas plants and community biogas plants in schools and prisons were visited by the research team. Collection of samples was made in 13 districts of the country. From each site, samples were collected from the outlet tank and from the slurry pit.

Samples were placed in sterilized bottles, transported in cooling boxes and carried to Laboratory for analysis.

2.3.2. Laboratory testing

The bio effluent samples were tested for NPK and pathogens bacteria. The protocols applied for the laboratory testing are provided here below:

Determination of Nitrogen (N)

Procedure: Weight accurately 2grs of sample and dissolve in distilled water. Make up the volume to 100 ml in volumetric flask. Samples of well-mixed unfiltered water are digested in an autoclave for 45 minutes at 110°C with Potassium Persulphate to convert all Nitrogen forms to Nitrate. The Nitrate is then analyzed using the Cadmium reduction method that consists on reducing Nitrates in sample to Nitrite. The Nitrite ions produced is determined by diazotizing with Sulfanilamide and coupling with N-(1 naphthyl)-ethylene diamine dihydrochloride to form a colored dye that is proportional to Nitrates concentration.

Formulae used for calculation of Nitrogen is the following:

$$\text{N \% in bio-effluent} = (0.0014 \times T \times 100) / 10 \times 100 / 2$$

Determination of Phosphorus (P)

Procedure: Weight accurately 2 grs of sample and dissolve it in distilled water. Make up the volume to 100 ml in volumetric flask. Samples of well-mixed unfiltered water are digested in an autoclave for 2 hours at 121°C with Potassium Persulphate to convert all Phosphorus to Orthophosphate. The Orthophosphate is then analyzed using the Acid ascorbic method. The Ascorbic method consists on reducing the complex formed by Orthophosphate reacted with Molybdates in an acid medium to produce a Phosphomolybdate complex.

Formulae used for calculation of Phosphorus is the following:

$$\text{P}_2\text{O}_5 \% \text{ in bio-effluent} = F \times (V_1N_1 - V_2N_2), \text{ where, } N_1 = \text{Normality of the standard alkali,}$$

$$N_2 = \text{Normality of standard acid, } F = \text{Factor for P}_2\text{O}_5$$

Determination of Potassium (K)

Procedure: - Pipette out 10 ml aliquot in a 250ml beaker. Dilute it with 100ml distilled water.

- Add 5 ml of concentrated HNO₃ and 10grs of Ammonium nitrate.
- Heat this mixture on water bath at 55°C till it is just unbearable to hand.
- Add 3 per cent Ammonium molybdate solution in beaker drop by drop with the help of burette. Continue stirring with a glass rod till 50ml of molybdate solution have been added.
- Stir for another few minutes till the yellow precipitate appears to become granular.
- Cover the beaker with glass and allow it to settle for some time.
- Decant the clear solution through a Whatman No 44 filter paper
- Wash the precipitate with 2 per cent sodium nitrate solution agitating thoroughly and allowing the precipitate to settle.

- Transfer the precipitate to the filter paper and wash with NaNO_3 solution .
- The precipitate and filter paper are then transferred to the beaker and 10ml of 0.1 N NaOH is added at a time by pipette till the precipitate become soluble.
- Add 2 drops of 1 per cent Phenolphthalein and titrate the excess of alkali against 0.1 N Sulphuric acid.

Identification of *Shigella*, *Salmonella* and *E- Coli* in bio effluent

Identification of *Shigella* and *Salmonella*

The detection of *Shigella* and *Salmonella* requires four phases:

- Pre-enrichment in non-selective liquid medium : Buffered pepton water
- Enrichment in selective liquid medium: Selenite broth and Tetrathionate broth
- Plating out and identification: Xylose lysine desoxycholate (XLD), SS agar
- Confirmation of identity: TSI agar

Procedure:

Pre-enrichment in non-selective liquid medium: With pipette, transfer 25grs of the sample in a glass box containing 225 ml of pre-enrichment medium. Close the box but don't shake it. Let it to rest within an hour at room temperature

If after an hour the sample is not homogenous, homogenize it by shaking or by using a sterile spatula. Incubate the box at 37°C between 16 to 20 hours.

Enrichment in selective liquid medium: Transfer 10 ml of previously incubated culture in 100 ml of selenite broth . Incubate at 37°C for selenite broth in 24 hours.

Plating out and identification: By use of inoculum obtained in enrichment medium, inoculate with help of Platinum the surface of a petri dish containing XLD and SS agar so that well isolated colonies are obtained. Incubate at 37°C between 20-24 hours. Observe obtained colonies. If necessary, culture again on XLD and SS agar using observed colonies; so that you obtain isolated and typical colonies of *Shigella* and *Salmonella*. On XLD and SS agar, typical colonies are pink, red; either with or without a black center. No colony has been observed, the detection is nil.

Identification of *Escherichia coli*

- From typical colonies obtained from Violet Red Bile Lactose Agar (VRBL), plant each colony on own petri dish containing EMB agar. - Incubate at 37°C in 24 hours.
- Do identification by test Indol Methyl red Voges-Proskauer Citrate + H_2S for colonies obtained from EMB (Eosine Methyl Blue) agar count the number of *Escherichia*

coli per gram sample. For every retained petri dish, the number of *Escherichia coli* per g was calculated using the following formulae: $(Cfu/100ml) = (Ne \times Nd) / Np * 10x$

Where N: Number of *Escherichia coli*, Ne: Number of identified *E-coli* colonies Nd: Number of counted typical colonies of coliforms. Np: Number of typical colonies of thermo- tolerant coliforms used to search *E.coli*. 10 x: inverse of corresponding dilution rate.

2.3.3. Data analysis

Each household and community having biogas plant connected to toilet was visited by the survey team composed of one Laboratory technician and 1 researcher and 1 research assistant.

A total of 310 samples were collected from 13 districts. The collected samples were taken to the Laboratory for analysis. Laboratory results were checked to ensure that the samples and

the owner of bio digesters are consistent with the samples and owner of biogas plant recorded by the Laboratory technician and the researcher and the research assistant during field study. The results were grouped and analyzed and average per province was determined.

4. RESULTS AND DISCUSSION

4.1 RESULTS

The research study covered 13 districts.

140 samples were collected from districts of Kigali city and Eastern province; they were analyzed in the Laboratory; 80 samples collected from Northern Province and analyzed; 90 samples collected from Southern and Western provinces and they were analyzed.



Picture 1: Household Biogas plant connected to toilet



Picture 2: Lab. technician collecting samples of bio effluent



Picture 3: Bio effluent after anaerobic digestion process of human excreta

Laboratory results of Nitrogen (N), Phosphorus (P) and Potassium (K) values are respectively 0.80%, 0.93%, and 0.63% in Kigali city province; 0.79%, 0.90% and 0.58% in Eastern province; 1.76%, 1.12% and 1.01% in Northern province; 1.70%, 1.66% and 2.13% in Western province; 2.05%, 2.21% and 2.12% in Southern province.

Laboratory results for bacteria: *Shigella*, *Salmonella* and *Escherichia Coli* have revealed that no harmful bacteria contained in the bio effluent.

Laboratory results obtained for Bio effluent collected from the 4 Provinces of Rwanda are indicated in the table below:

Table 1: Average Laboratory results obtained for Bio effluent collected from the 4 Provinces of Rwanda.

Province	N%	P%	Average		
			K%	<i>Salmonella and Shigella</i> (cfu/25gr)	E.coli (cfu/gr)
Kigali City	0.8	0.93	0.63	0	3x10 ¹
Eastern Province	0.79	0.9	0.58	0	3x10 ¹
Northern Province	1.76	1.12	1.01	0	4x10 ¹
Western Province	1.7	1.66	2.13	0	5.5x10 ²
Southern Province	2.05	2.21	2.12	0	2.7x10 ³

The above results indicate that bio-effluents from biodigesters connected to toilets is safe/ not harmful to the Rwandan population and it constitutes a rich organic fertilizer. The same observations have been obtained in “A Scientific Comparative Performance Study of Fixed dome masonry, Fiber Glass and Flexibag biodigesters in Rwanda” (Rwigema, A. Lam, J. et al). For these reasons we recommend Rwandan farmers to use this type of natural fertilizer in their farms, gardens and fields because it rejuvenates the structure of the soil and helps the plants to provide high yield (Oppenoorth H., Warmans L.).

4.2 DISCUSSION

In all samples collected, no *Shigella* and no *Salmonella* were detected; *Escherichia coli* (E.coli) were identified but their colonies were scanty (3x10¹ – 5.5x10² cfu/gr). It is well known that most strains of E. coli are harmless compared to *Shigella* and *Salmonella*. As no *Shigella* and no *Salmonella* identified in all the samples collected and analyzed, it deemed not necessary to compare the results obtained to any standard or other findings for those pathogens in order to confirm that bio effluent from biogas plants connected to toilets is a natural fertilizer without harmful bacteria *Shigella* and *Salmonella*).

Nutrient values (NPK) obtained in bio- effluent r are acceptable; indeed they are in the same range as NPK in compost: %N:1.5-3.5; %P: 0.5-1; %K: 1-2 and in fresh cattle manure: %N:0.5-1.5; %P: 0.2-0.7; %K: 0.5-2 (Ross Penhallegon.). Fresh cattle manure and compost are natural organic fertilizer well known and used by farmers. Bio effluent is also a natural organic fertilizer. That is the reason considered in comparing the NPK values of fresh cattle manure and NPK values of compost with NPK of bio effluent. It has been observed that nutrient values of NPK in Kigali city province and Eastern province are low compared to those found in samples collected in Northern, Western and Southern provinces.

Results obtained in the Northern province and in the Western province are almost similar.

Laboratory results obtained in Southern province are higher than the results obtained in other provinces of the country.

The differences may be due to the climate in the Northern, Western and Southern provinces it is cold compared to Kigali city province and Eastern province, in cold climate people eat more food than people who live in hot climate where people eat little food. In Northern, Western and Southern provinces population consumes food produced from their land while in Kigali city province Eastern province population eat food bought at the market quantity is limited. In the Northern, Western and Southern types of food owners of bio digesters connected to the toilets consume are every day beans, cassava, Irish potatoes, Sweet potatoes, they eat big quantity and produce a big quantity of human waste after digestion process. In Kigali city province and Eastern provinces population consume rice, meat, chips, cowpeas, people do not eat a lot of food thus little quantity of human waste after digestion process is produced. The results obtained are similar to those obtained by other researchers ; they indicate that bio effluent from biodigesters connected to toilets is not harmful to the population and it is a rich organic fertilizer in nutrient values (NPK) plants need (Oppenoorth H., Warmans L.) and (Karki B. A., Jagan Nath). Based on the laboratory results obtained we strongly recommend Rwandan population involved in agriculture to use bio-effluent from biodigesters connected to toilets in their fields and gardens because it is a natural fertilizer which rejuvenates the structure of the soil and y help the plants to grow strong and develop well (Bonten, L. T. C., Zwart, K. B. et al).

It has been proved that bio- effluent does not have bad side effects to the soil, health and environment like chemical fertilizers; indeed chemical fertilizers have number of additional ingredients including dirt, sand and materials that are potentially hazardous to health and to environment ([http:// www.dirtdoctor.com](http://www.dirtdoctor.com)).

The Scientist Howard Garrett in his research on fertilizer fillers observed that Nitrogen found in chemical fertilizers dissolves very quickly in water meaning that excess Nitrogen may contaminate groundwater and freshwater sources. Phosphoric acid is used to produce a cheap Phosphorous (P) of high content. This Phosphorous (P) neutralizes other useful minerals from the soil that plants need such as Sulfur, Copper, Cobalt, Sodium, Boron, Molybdenum and Zinc ([http://www. utexas.edu](http://www.utexas.edu)). Furthermore Horward Garrett in his article on fertilizer fillers mentioned that Potassium (K) found in many chemical fertilizers is harsh form of Potassium (K) that can be harmful to plants.

Based to the above results of our research and the observations from Howard Garrett about chemical fertilizers, I advise Rwandan farmers to use bio- effluent from biodigesters connected to toilets in their fields rather than chemical fertilizers.

During digestion, about 25-30% of the total dry matter human waste will be converted into the residue of 70-75% of bio-effluent which is known as biogas slurry or bio-effluent. Bio effluent consists of 93% water and 7% dry matter of which 4.5% is organic and 2.5% inorganic matter Shrestha et al) . The percentage of NPK (Nitrogen, Phosphorous and Potassium) content of liquid slurry is (N) 0.25%, (K) 0.13% and (P) 0.12% (Karki B. A., Jagan Nath et al). In addition to the major plant nutrients, it also provides micronutrients such as Zinc, iron, Manganese and Copper that are also essential for plants but required in trace amounts (Bonten, L. T. C., Zwart et al).

An increase of 50% has been obtained by farmers who are using bio- effluent from biodigesters connected to toilets in Rwanda (information collected from owners of Biogas plants during this research study). In China use of bio effluent increases significantly agricultural yield as indicated here after: Maize 16.9 %, Rice 9.4%, Cotton 20.2%, Sweet potatoes: 18.8%, Vegetables: 25.0% (Karki B. A., Jagan Nath et al).

5. CONCLUSION

It has been observed that institutions and households which have biogas plants connected to the toilets are producing big quantity of bio-effluent every day. The results obtained have proved that bio-effluent from bio-digesters connected to the toilets is a safe and rich fertilizer with enough values of NPK ; its use will allow Rwandan population to avoid diseases and to increase agricultural yield thus famine will be avoided. Owners of biogas plants connected to the toilets will be able to use and sell that bio-effluent to the farmers who do have biogas plants without fear of being contaminated or contaminating other people thus bio-effluent will generate income for them. In fact, this study builds on the existing activities (use of compost and chemical fertilizers distributed by the Ministry of Agriculture to the farmers) in order help them increase agricultural products yield.

The results obtained are similar to those found by other scientists; indeed according to Oppenoorth H. and Warmans L., digested slurry called also bio-effluent is produced in the process of biogas production and it can be used as excellent organic fertilizer in the field, thus increasing the crops yield. The most responsive crops to bio-effluent are vegetables like carrots and radish, potatoes, fruit trees and rice. Bio effluent is the leftover of feeding raw material after biogas production, it constitutes a very good natural fertilizer containing enough values of NPK; compared to the compost 30 to 50 % of Nitrogen escape for compost while 30 to 50% of Nitrogen are kept in bio effluent; in addition there are about 25% excess nutrients in bio effluent which help plants to grow well and provide a high yield (Karki B. A., Jagan Nath et al).

6. REFERENCES

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