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COMPARATIVE STUDY OF EFFLUENT FOR POLLUTION INDICATORS AND INDICATOR PATHOGENIC ORGANISMS FROM ANAEROBIC DIGESTERS FROM HUMAN AND FRUIT WASTES

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ABSTRACT

The study examined comparatively the levels of pollution monitoring indicators (Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Conductivity), a selected pathogen (*Salmonella species*) and indicator microorganisms (Total Coliforms (TC), Faecal Coliform (FC), *Escherichia coli (E. coli)*) in the effluent of two anaerobic digesters treating Human Excreta (HE) and Fruit Waste (FW) (pineapple and mango peels). Both digesters were operated on mesophyllic temperature within short hydraulic retention time of 14 days. The mean, standard error (SE) and percentage reduction in effluent for each parameter were computed for both digesters. Except conductivity of HE, BOD, COD, TC, FC, *E. coli* and *Salmonella species* reduced between 50-75% in the effluent. The reduction in the digester treating HW was higher than the digester treating the FW except for COD, conductivity and *Salmonella species*. Despite the reduction in both digesters, the effluent quality exceeded the World Health Organization (WHO) or the Ghana Environmental Protection Agency standards for disposal or use in agriculture.

Keywords: human excreta, fruit waste, anaerobic digestion, pollution indicators, influent, effluent, pathogens,.

INTRODUCTION

The worldwide interest in Anaerobic Digestion (AD) is due to current issues such as global warming, demand for renewable energy, landfill tax on wastes and high fossil fuel prices (Lukehurst *et al*., 2010). In Ghana, the interest in AD has shifted from energy to on-site sanitation systems associated with faecal sludge management especially in relation to difficulties with emptying, transportation and disposal (Boot and Scott, 2008). Anaerobic digestion consists of several interdependent, complex sequential and parallel biological reactions in the absence of oxygen in which the products from one group of microorganisms serve as substrates for the next resulting in transformation of organic matter (Parawira, 2004). The products resulting from the transformation are biogas and nutrient rich effluent called digestate.

Characteristically, the nutrient rich effluent has lower BOD, COD, conductivity, turbidity, odour and pathogens compared to the influent. For instance, in a study to determine the biodegradability of human faecal matter using AD, Nwaneri *et al.* (2008) found that, COD values for faecal material present in the first layer were significantly lower than those measured for fresh faeces. Also, Arthur (2000) reported BOD reduction of anaerobic digested effluent at 60-80% compared to the influent.

In terms of pathogen reduction, anaerobic digestion can greatly reduce the number of pathogens at mesophyllic temperature (35^{o}C) as much as 95-98% and therefore limit the number entering the environment (Saunders and Harrison, 2011). For instance, *Salmonellae*, faecal coliforms, fungi, protozoan oocysts and viruses are completely destroyed at 50°C within 24 hours and reduced by 50-70% at 35°C. Reduction of fungi is close to 100% at 50°C and 95% at 35°C (FAO/CMS, 1999).

Though AD causes reduction in pollution indicators and indicator microorganisms, the reduction ought to meet regulatory standards to ensure the effluent environmental safety or agricultural utilization. According to the WHO (1989) guidelines for coliform bacteria, a limit of ≤1000FC/100ml is recommended for unrestricted irrigation and ≤ 10^5/100ml for restricted irrigation. Also, 10^3, 10^4 and 10^5 *E. coli /100ml* for leafy crops, root crops and drip irrigation, respectively and 10^4 - 10^5 for labour intensive high contact or highly mechanized agriculture (WHO, 2006). The Ghana Environmental Protection Agency (GEPA) (2010) nonetheless proposes stricter guidelines of 400 MPN/100ml TC, 10.0 E. coli (MPN/100 ml), 50-200mg/l BOD and 250-1000mg/l COD for effluent discharge into water bodies.

It is against this backdrop that this research sought to analyze the extent of changes in pollution indicators, indicator microorganisms and a selected pathogenic organism (*Salmonella species*) of anaerobic digested effluent with respect to the influent of anaerobic digesters treating HW and FW (pineapple and mango peels) to ensure that the changes in the effluent quality are within the World Health Organization (WHO) or Ghana Environmental Protection Agency (GEPA) limits for land disposal or agricultural utilization.

MATERIALS AND METHODS

The study area

The study was conducted in two anaerobic digesters treating Human Excreta and Fruit Waste in the

Greater Accra Region of Ghana. It is located on latitude 05°35'N and longitude 00°06'W and occupies a total land size of 200 km^2. The area falls within the dry coastal ecological zone with temperatures ranging from 20°C to 30°C. The annual rainfall ranges from 635 mm to 1,140 mm. In terms of waste management, the people of Greater Accra generate between 1,500-1,800 tonnes of waste daily and an average of 1,200 tonnes collected per day by waste management companies. The remaining wastes find their way into the drainage systems and other open spaces as the final destination.

Digester start up

For digester start up and acclimation, both digesters were loaded with about 1/3 digester volume slaughter house waste and 2/3 water for three weeks. After the acclimation stage, digester A was fed 120 litres / day HW plus 10 litres of water and digester B fed with 300 litres per two days interval FW.

Preparation of sampling containers and sampling procedure

In all, four (4) sampling plastic containers of 1.5 litres each were used for sampling from each digester (two for influent and the other two for effluent). The containers were washed with non-ionic detergent, rinsed with clean tap water and later soaked in 10% HNO_3 for 24 hours and finally rinsed with non-ionized water prior to usage (Akan *et al.*, 2008). Prior to usage, both substrates (influent and effluent) were thoroughly stirred to ensure even distribution of solids. Each container was filled and emptied with the respective substrate three times and finally dipped to about 10 cm below the respective substrates, filled to the neck level and firmly capped. The content were stored in a cool box containing ice blocks to maintain the temperature at 4^oC to reduce warming up and change in any biochemical activity and transported for analysis at the Water Research Institute (WRI) of the Council for Scientific and Industrial Research (CSIR) laboratory.

Analytical methods

Laboratory analysis of BOD, COD, Conductivity, Faecal Coliform, Total Coliform, *Escherichia coli* and *Salmonella species* were analyzed seven (7) times at 14 days interval following the procedures below.

Biochemical Oxygen Demand (BOD) was analyzed following procedure 5210B outlined in Standard Methods (1984). The sample to be analyzed was measured to ensure that the pH was between 6.5 and 7.5. The initial Dissolved Oxygen (DO) of the sample was then measured and recorded, and incubated at 20^oC for five days and the final DO measured.

$$BOD\ mg/l = (Initial\ DO - DO_5) \times dilution\ factor \quad (1)$$

Where;

Dilution factor = bottle volume / sample volume

Chemical Oxygen Demand (COD) was determined following a procedure outlined by APHA (1999). 10 ml of potassium dichromate (0.25N) was added to the sample and 20 ml of sulphuric acid rapidly added and digested for half an hour. 4-5 drops of ferroin indicator was added and titrated against ferrous ammonium sulphate solution (0.25N). The change in colour from blue green to wine red indicated the end point.

$$COD\ (mg/L) = (B-A) \times 8000 / C \quad (2)$$

Where; B = Volume of the titrant used against blank, A = Volume of the titrant used against sample, C = Volume of the sample.

Conductivity was determined following procedure outlined by Johnsson *et al*. (2005). 20g of the sample was put into a centrifuge bottle and 200 ml water with specific electrical conductivity not higher than 0.2µS/m at 20^oC added. The bottle was centrifuged for 30 minutes and content filtered. The electrical conductivity of the filtrate was then measured using a conductivity meter.

TC and *E. coli* was determined following procedure outlined by USEPA (2002), method 1604. 10g slurry was diluted to 100 ml and filtered through a 47 mm, 0.45-µm pore size cellulose ester membrane. The filter was then placed on a 5-ml absorbent pad saturated with 2-3 ml of MI broth and incubated at 35°C for 24 hours. All blue colonies on the MI plate under ambient light, were counted and recorded as *E. coli.* Also, each MI plate was exposed to long wave ultraviolet light at 366 nm, and all fluorescent colonies that appeared blue/green fluorescent were counted as *E. coli* and blue/white fluorescent as TC.

E. coli/100ml = [Number of blue colonies/volume of samples filtered (ml)] x100

TC/100ml = Number of fluorescent colonies + Number of blue, non-fluorescent colonies / volume of sample filtered] x 100

Faecal Coliform (FC) was determined using a procedure described by USEPA (2002), Method 1680: Five fermentation tubes containing 10 ml/tube of Lauryl Tryptose Broth (LTB) were inoculated with the sample and incubated for 24 hours at 35^oC and observed for the production of gas (presumptive test). Gas production at the end of 24 hours indicated positive for faecal coliform. The positive cultures were transferred to EC broth and incubated at 44.5°C for 24 hours. Tubes that produced gas confirmed positive for faecal coliform (confirmation test). All the positive fermentation tubes were then recorded on a test data sheet and faecal coliforms estimated and reported as Most Probable Number (MPN)/100 ml.

Salmonella species was determined following procedure outlined by (Thompson *et al.*, 2004). The sample was homogenously diluted and filtered through a membrane filter and incubated for 24 hours at 36°C on a sterile glass fibre filter saturated with tetrathionate broth resuscitation medium. After incubation, the membrane filter was further incubated at 36°C on chromogenic medium (Rambach agar). The membranes were examined

after 24 and 48 hours and colonies that appeared bright red when fermented with propylene glycol indicated positive for the presence of *Salmonella species.*

Statistical analysis

Means, standard error (SE) and percentage reduction for each parameter were computed for both digesters using Microsoft Office Excel (2007).

RESULTS AND DISCUSSIONS

Biochemical oxygen demand (BOD)

The results in Table-1 show that the process of anaerobic digestion reduces the level of BOD in the effluent of both digesters. In digester I (treating HE), the mean BOD of the influent was 12,460 mg/l and SE of 60.0. The mean effluent BOD recorded a value of 4250 mg/l and SE of 10.0 indicating a reduction of 65.9% with respect to the influent. In digester II (FW), the study found the mean BOD of the raw fruit waste at 12,845.7 mg/l and SE of 141. This decreased from 12,845.7 mg/l to 6594.3 mg/l with SE of 102.5 in the effluent representing 48.7%. BOD reduction in digester I (treating HE) was higher (12,460 mg/l to 4250 mg/l or 65.9%) than digester II (treating FW) (12,845.7 mg/l to 6594.3 mg/l or 48.7%). Though both digesters effluents had reduced BOD, the FW effluent was lower than the HW effluent. This might be due to the inability of microbes to degrade the fruit waste completely because of the insoluble fibrous content of fruit waste (Wensloff, 2011) coupled with short hydraulic retention time. In terms of disposal onto the environment, BOD reduction in both digesters did not meet Ghana Environmental Protection Agency (GEPA) (2010) maximum acceptable standard of 50 mg/l for discharge into water bodies or WHO (1989) standard of 20-100mg/l for irrigation or aquaculture.

Table-1. Results of pollution indicators, indicator microorganisms and pathogenic organism for human and fruit waste.

Digester I: Human excreta (HE)				**Digester II: Fruit waste** (FW)		
Parameter	**Mean influent**	**Mean effluent**	**% Reduction**	**Mean influent**	**Mean effluent**	**% Reduction**
BOD (mg/l)	12,460 ± 60	4,250 ± 10.0	65.9	12,845.7 ± 141	6,594.3 ± 102.5	48.7
COD (mg/l)	13,869 ± 1.0	5,794 ± 4.0	58.6	13,541 ± 163.6	4,971.4 ± 259.8	63.3
Conductivity (µS/cm)	6.35± 0.05	3.95± .05	37.8	3.9 ± 0.096	1.87 ± 0.037	52.1
*Faecal Coliform	3,635,000±15,000	1,295,000 ±5000	64.4	27,600±748.32	12,860± 492.56	53.4
*Total Coliform	5,135,000 ±5000	1,909,000±1000	62.8	37,980±265.3	18,380±330.8	51.6
**Escherichia Coli*	2,350,000±50,000	925,000±5,000	60.6	11,730±488.8	4,896±458.01	58.9
Salmonella species	6/25g sample	2/25g sample	66.7	4/25g	1/25g	75

* Faecal coliform, * Total coliform and **Escherichia coli* are in units of CFU/100

Chemical oxygen demand (COD)

The results of the study show that the process of anaerobic digestion similarly reduced COD in both digesters (Table-1). In digester I (treating HE), the research found COD of the influent at a mean of 13,869 mg/l with SE of 1.0 and 5,794 mg/l with SE of 4.0 in the effluent, representing a change of 58.6% which agrees with Nwaneri *et al.* (2008) study to determine the biodegradability of human faecal waste in a latrine. In digester II (treating fruit waste), COD of the influent was recorded at a mean of 13,541.9 mg/l and SE of 163.6 and 4971.4 mg/l with SE of 259.8 in the effluent after the anaerobic digestion process representing 63.3% reduction of COD. This shows that COD reduction in digester I (13,869 mg/l to 5,794 mg/l or 58.6%) was lower than digester II (13,541.9 mg/l to 4971.4 mg/l or 63.3%). The higher reduction of COD in digester II might be due to the acid content of the fruit waste that catalyses digester II content during the digestion process. Nevertheless, reduction of COD in the effluents of both digesters exceeded the Ghana Environmental Protection Agency (GEPA) maximum permissible level of 250 mg/l for discharge into water bodies or use for irrigation.

Conductivity

In terms of conductivity, the research results in Table-2 show a decrease from a mean of 6.35 µS/cm and SE of 0.05 in the influent to a mean of 3.95 µS/cm with SE of 0.05 in the effluent representing 37.8% reduction in digester I (treating HE). In digester II (treating FW), the mean value of the FW influent was 3.9 µS/cm with SE of 0.096. After the anaerobic digestion process, the conductivity of the effluent was 1.87 µS/cm and SE of 0.037 representing 52.05% reduction. Reduction in conductivity in the effluent of human excreta of digester I (6.35 µS/cm to 3.95 µS/cm or 37.8%) was found to be lower than digester II effluent of the fruit waste (3.9 µS/cm to 1.87 µS/cm or 52.05%). The low reduction in digester I effluent might be due to high salts in the influent (human excreta) of the people using the toilet facility. Nevertheless, the reduction of conductivity in the effluent of both digesters exceeded 1.50 µS/cm set by the GEPA

(2010) maximum for disposal onto the environment or use in agriculture.

Total coliforms (TC)

In digester I, mean TC in the influent of human excreta was enumerated at 5,135,000 CFU/100 ml with SE of 15,000 and 1,909,000CFU/100 ml with SE of 1,000 in the anaerobic digested effluent, representing 62.8% reduction in TC bacteria. In digester II, mean TC in the influent of the fruit waste was 37,980CFU/100 ml with SE of 265.3. After the process of anaerobic digestion, TC bacteria recorded a mean reduction of 18,380CFU/100 ml with SE of 330.8 in the digested effluent representing a reduction of 51.6%. Though, both digesters achieved more than half reduction in their respective effluents. This reduction agrees with Saunders and Harrison (2011) findings for pathogen reduction in mesophyllic digesters at 50 - 70% but the number of TC in the effluent were still above the GEPA maximum permissible level of 400 CFU/100 ml for discharge into water bodies and WHO (2006) guideline of ≤ 1000 for unrestricted irrigation (that is crops likely to be eaten uncooked, sports fields or public parks) or $\leq 10^5$/100 ml for restricted irrigation (that is irrigation of cereal crops, industrial crops, fodder crops, pasture and trees).

Faecal coliform (FC)

The mean FC enumerated in digester I influent of the human excreta was 3,635,000 CFU/100 ml with SE of 15,000. After the anaerobic digestion process, a mean of 1,295,000 CFU/100 ml with SE of 5,000 was found in the digested effluent, representing 64.4% reduction with respect to the influent. In digester II, FC in the fruit waste influent was enumerated at a mean of 27,600 CFU/100 ml with SE of 748.32. After the anaerobic digestion process, the effluent was found to contain 12,860 CFU/100 ml with SE of 492.56 FC in the effluent, representing a reduction 53.4%. The reduction of FC in the effluent similarly agrees with Saunders and Harrison (2011) findings for pathogen reduction in mesophyllic anaerobic digesters at 50-70%. Nevertheless FC numbers for the effluent quality exceeded the acceptable standards of 10^3- 10^6 CFU/100 ml set by the WHO (2006) for use in agriculture and aquaculture.

Escherichia coli

The mean *E. coli* enumerated in the influent of the human excreta in digester I was found at 2,350,000 CFU/100 ml with SE of 50,000. After anaerobic digestion process, the research recorded a reduction from 2,350,000 CFU/100 ml to 925,000 CFU/100 ml in the effluent representing 60.6% reduction. In digester I, *E. coli* in the fruit waste influent was 11,730 CFU/100 ml with SE of 488.8. After the process of anaerobic digestion, there was a reduction from 11,730 CFU/100 ml to 4,896 CFU/100 ml with SE of 458.01 in the effluent of the fruit waste. Nonetheless, the mean reduction in digester I of 925,000 CFU/100 ml and 4,896 CFU/100 ml in digester II were above WHO (2006) guideline of 10^3 *E. coli*/100 ml for use in irrigation of leafy crops, 10^4 *E. Coli* for root crops and 10^5 *E. coli* for drip irrigation but digester II effluent was within 10^4-10^5 *E. coli* /100 ml suitable for labour intensive high contact or highly mechanized agriculture (WHO, 2006).

Salmonella species

The absence of *Salmonella* in 25g of effluent or waste is considered the standard for its safe use as fertiliser as a guarantee of bacteria/pathogen absence (EC, 2001). However, the results of the study found that *Salmonella species* were detected in both influent and effluent of all the samples analyzed. In digester I, 6.0/25g *salmonella* were enumerated in the influent of the human excreta. This decrease from 6.0/25g to 2/25g in the effluent represents a 66.7% reduction. Also, in digester II, 4/25g *Salmonella species* were detected in the influent of the fruit waste. As a result of the digestion process, 1/25g *salmonella* was detected in the effluent, representing 75% reduction. The resistance of *Salmonella* to be inactivated completely in both digesters might have been due to the short hydraulic retention time together with the mesophyllic temperature.

CONCLUSIONS

Basically, the study revealed that, BOD, COD, conductivity, FC, TC, *E. coli* and *Salmonella species* were lower in the effluents of both digesters than the influent as a result of the anaerobic digestion process. The reduction nonetheless did not meet the WHO or GEPA recommended standards for disposal into the environment. The reason for the poor reduction might be due to short hydraulic retention time adopted at both projects sites. Therefore, for complete degradation of HE and FW, the hydraulic retention time can be prolonged for either digesters or both digesters be extended with anaerobic baffle reactors to prolong the hydraulic retention time to 100-120 days for further treatment before disposal into the environment or use for irrigation.

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