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Original Research Article

Identication and morphology of pathogens in liquid effluent from a Cow dung biodigester

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Cow dung biodigester digestate and biogas are useful products in energy production and organic fertilizer for agricultural practice in Niger Delta area. This study focuses on the liquid effluents from the cow dung biodigester advocated for use as liquid fertilizer to investigate pathogen that might be harmful to human and animal in the cause of its use. The present study use liquid effluent collected from falcorp mangrove Nature Park owned by CMADI. Standard microbiological procedures isolated and identified the pathogen bacteria. The morphology and biochemical analysis could differentiate between Gram positive and negative, with identification of the genus of the pathogens. The pathogens isolated and identified in this study are *Klebsiella, Staphylococcus, Acinetobacter, Edwardsiella*, and *Alcaligenes* species respectively. Staphylococcus is Gram positive while others are Gram negative. *Acinetobacter* and *Alcaligenes* have been resistance towards the antibiotics, which means it will cause serious public health when exposed to population. Therefore, this study shows that liquid effluents got from cow dung biodigester are contaminated with pathogens, which may likely affect the population when used for agricultural purpose.

Keywords: Liquid effluent, biodigester, digestate Morphological identification, pathogens

INTRODUCTION

Waste management is an integral fabric of every society that needs urgent attention. It is a priority subject in many developed and developing countries because of the associated effect on environmental media and human health, and greenhouse effect that will add to global warming (Alkhalidi et al., 2019). The waste management interest over the decades has resulted in exploring reuse and recycling strategies that gave birth to the biogas production from a biodigester in the United Kingdom in 1895 (Alkhalidi et al., 2019). The anaerobic digestion of organic materials in a biodigester that come from animal manure and human waste produces biogas as renewable energy sources and bio-slurry as fertilizer for agricultural uses. In the same vein, it reduces the amount of non-useful waste material and increases their utilisation as renewable energy sources to increase the energy indexes of countries (Alkhalidi et al., 2019). Therefore, the biodigester could be described as a mechanical stomach, which breaks down feedstocks with the aid of microorganism through the anaerobic process to yield biogas, liquid extracts and bio-slurry (Nyirfa, 2014; Kemanusor et al. (2018) reported that over 35 million biodigester installation is available worldwide with the majority in Europe and North America. In recent years, China and India are making efforts to install larger biodigester plants for electricity and heat applications, while Africans are still building for household sizes to replace the traditional cooking with wood in the rural communities (Chen et al., 2014; Angelidaki et al., 2018). Moreover, Nigeria should generate 171 TJ of Energy from biogas by the year 2030 (Aliyu et al., 2015; Giwa et al. 2017).

Cow dungs have microbial composition of 60 species of bacteria, fungi and about 100 species of protozoa and yeast (Randhawa and Kullar, 2011). Their research reported that cow dung comprise of undigested fibre, sloughed off intestinal epithelium, some excreted products derived from bile (pigments), intestinal bacteria and mucus. Cow dung contains bacteria, fungi, and actinomycetes, namely, *Fecal streptococcus, Streptococcus, Pseudomonas* sp., *Sarcina, Nocardia, Mucor* spp., *Phizopusstolonifer, Rhizopus* sp., *Aspergillus, E. coli* sp., *Penicillium* microbes, *Acinetobacter*, *Pseudomonas*, *Bacillus* sp., *Corynebacterium* sp., *Lactobacillus* sp., *Serratia* and *Alcaligenes* sp., *Saccharomyces* and *Candida,* some of which are suitable for microbial degradation of pollutants, improve soil qualities and harmful to human health (Randhawa and Kullar, 2011).

A biodigester uses micro-organisms to produce methane gas from any kind of biodegradable material, which includes farm wastes. The system uses organic waste, particularly animal and human excreta, to produce fertilizer (liquid and bio-slurry) and biogas. It comprises of an airtight, high-density polyethylene container within which excretadiluted in water flow continuously and are fermented by microorganisms present in the waste. The fermentation process is anaerobic, because it takes place without oxygen, and the bacteria responsible for decomposition are methanogenic (i.e., they produce methane, also known as biogas). The processed manure is an organic fertilizer rich in nitrogen, phosphorus and potassium. The products are primarily for self-consumption on farms (Sherwin et al., 2014). The effluents discharged from a biodigester with cow dung feedstock may negatively affect both human and grazing animals because the pathogens remaining may survive for a long time in soil, air, water, and even in underground water (Nicholson et al., 2005, Islam et al., 2019). Gupta et al. (2016) discussed that animal manures are the significant source of human pathogens. The study reported that cattle, sheep, and deer dungs contain virulent pathogens such as E. coli O157: H7, Listeria, and Cryptosporidium, while poultry droppings are the potential source of Salmonella bacteria. In addition, Brisse et al. (2006) stated that the *Klebsiella* genus is responsible for a variety of diseases in animals and humans. As discussed by Caroline (2017), the cow dung manure because of runoff could contribute to algal blooms in water and make drinking water toxic, where they serve as organic fertilizer. There are other studies on health implication, contribution to greenhouse gas emissions and environmental risk associated with cow dung manures but minimal investigation is available on the microbial contents of cow dung biodigester discharge (digestate) on human health and environment (Zhou et al., 2005; Hozzein et al., 2007; Hozzein et al., 2011; Piccini et al., 2017; Brown et al., 2018; Islam et al., 2019).

The anaerobic digestion in the biodigester is a complex process influenced by many factors such as operational conditions which determines the biogas yield, quality of the digestate (liquid effluents and slurry) and the growth of many anaerobic microorganisms in the digester (Piccini et al., 2017).Many studies have highlighted plant nutrient and plant growth-promoting bacteria in disgestate (Khalid et al., 2009; Bonten et al., 2014; Makádi et al., 2012; Nkoa, 2014) to our knowledge no study has shown the availability of pathogens within the liquid effluents from cow dung biodigester. Emphasis on the use of the anaerobic digestion disgestate was broadly discussed in many studies with no references to the best of our knowledge on the pathogen content of the liquid effluents despite the obvious knowledge that the cow dung manure contains many harmful microbes (Burkholder et al.,2007; Piccini et al., 2017). Niger Delta has the largest mangroves in Africa,it experiences rainfall most time of the year and water table near the ground surface (Numbere et al., 2016; Nwankwoala and Ngah, 2014), which show a potential run off of the liquid effluents from farmland . The contaminants or pathogens have the potential to pollute land, water with no containment or treatment plan when they are flushed or wash into rivers, streams or seep into the underground water and find its way to well that human uses for domestic purposes. However, the current study focuses on analyzing the liquid effluent from a cow dung biodigester to isolate, identify and determine the morphology of pathogens present.

MATERIALS AND METHODS

Sample location

The study area is situated around falcorp mangrove Nature Park, owned by Coastal and Marine Area Development Initiative (CMADI), IjalaI kenren in Warri South Local Government Area of Delta State, Nigeria. The Global Positioning System (GPS) was used to determine the coordinates of the location which is (5°33’40” N and 5°41’56” E).

Samples collection

Liquid effluent

The sample of liquid effluent as shown in Figure 1 was collected from a cow dung biodigester at the falcorp mangrove nature park, Ijala Ikenren, Warri South Local Government of Delta State. Sampling was done with polyethylene bottle properly washed, with dilute HCI and rinsed with distilled water. (Okorhi-Damisa et al., 2020)

Identification and Enumeration of Total Heterotrophic Bacteria (THB)

The isolation and characterization of the pathogens in the

Figure 1: Showing the effluent collected from the cow dung biodigester

liquid effluents of the cow dung biodigester used ISO-6579, 2002, and methods as described in the study of Chikere and Ekwuba (2014) for the viable count of bacteria. The method of isolation and identification in this study is like method described by (Karoki et al., 2018) and (Mukhuba et al., 2018). Here, the culture plate was allowed to solidify and then inverted, followed by incubation at 37°C for 24 hours. Following incubation, Total Viable Count (TVC) were counted and calculated by multiplying average number of colonies in particular dilution with dilution factors and recorded as colony-forming unit per gram of samples. The total viable count isolates are *Klebsiella, Staphylococcus, Acinetobacter, Edwardsiella*, and *Alcaligenes* species, and their identifications with the biochemical tests was performed as described in Bergey's manual of determinative bacteriology (Holt et al., 1994).

Characterization and identification of microorganisms

Bacteria grown on different media were sub-cultured on agar plates to have pure isolate colony. The process ensures identification of all the isolates of the respective bacteria based on the cultural and morphological characteristics (colour, shape, size, surface, edges, margins and elevation). Standard procedure of biochemical test includes sugar fermentation, gram staining procedure, methyl red, Voges–Proskauer, indole, gelatin utilization test, catalase test, oxidase test, citrate utilization test, acid-fast stain, motility test (Cheesbrough, 1985; Cappuccino and Sherman, 1996).

RESULTS AND DISCUSSION

Biochemical identification of bacterial isolates

Agricultural husbandry generate animal wastes containing high concentrations of human pathogens, spilt feed, bedding material, fur, process-generated wastewater, undigested feed residues, faeces and urine. Therefore, improper discharge and usage of the waste could endanger or cause potential public health risk. (Erickson et al., 2005; Jenkins et al., 2007). Brown et al. (2016) confirms that Salmonellae infects approximately 1% to 3% of all domestic animal and their discharge will contain the pathogens.. Table 1 shows the biochemical identification of bacterial isolates (*Klebsiella, Staphylococcus, Acinetobacter, Edwardsiella*, and *Alcaligene*species) from the liquid effluents of cow dung biodigester.

Characterization of bacteria isolate

Table 2 revealed the morphological characteristics of the bacterial isolates in this study. They are grouped into A, B, C, D and E. It was observed that isolates A, C, D and E belong to the Phylum Proteobacteria and are all Gram-negative organisms, rod-shaped and cocci. In contrast, isolate B belongs to the phylum firmicutes and order Bacillales, Gram-positive cocci shaped with the appearance of grape-like clusters as revealed by morphological examination. Isolate A is a facultative anaerobe, small motile, gram-negative, straight rod with flagella that produces a heat-

Table 1. Morphology and biochemical identification of the bacterial isolates

Isolates	Gram	Shape	Motility	Catalase	Oxidase	Glucose	Lactose	Sucrose	Gas	Acid	Indole	Citrate	H2s	VP	MR	Urease	Probable identification
A	-	C	-	+	-	+	+	-	-	NA	-	-	-	-	-	-	*Acinetobacter spp.*
B	-	R	+	+	-	+	-	+	+	+	+	-	+	-	+	-	*Edwardsiella spp.*
C	-	R/C	+	+	+	-	+	-	-	+	-	+	-		-	-	*Alcaligenespp.*
D	_	R	-	+	-	+	+	+	+	+	-	+	-	+	-	+	*Klebsiellaspp*
E	+	C		+	-	+	-	-	-	+	+	+	-	+	+	-	*Staphylococcus spp.*

C =Chain/Cocci, + =Positive, H_2S = Hydrogen sulphide, - = Negative, MR = Methyl red, R= Rod shaped, VP= Vogesproskauer

Table 2. Morphological characteristics of bacterial isolates

Code	Probable isolates	Macroscopic characteristics	Microscopic characteristics
A	*Edwardsiella* Species	White, slimy irregular convex greyish colonies	Gram-negative rod
B	*Staphylococcus* Species	Grape-like clusters	Gram-positive cocci
C	*Klebsiella* Species	Small creamy flat and opaque colonies	Gram-negative rod
D	*Alcaligenes* Species	Smooth, creamy and convex colonies	Gram-negative rod/coccobacillus
E	*Acinetobacter* Species	Creamy irregular raised colonies	Gram-negative coccobacilli

stable enterotoxin (Janda and Abboth, 1993; Srinivasa- Rao et al., 2013). Isolate B is clustering Gram-positive cocci, non-motile, non-spore forming facultative anaerobes, it classifies them into two primary groups, and they are coagulase positive, because of their virulence factors and unique features (Von et al., 2002). Isolate C occurs in soil, water and is non-motile encapsulated, lactose fermenter, facultative anaerobes bacteria. They are thick when compared to others. The cells are rods in shape and measure 0.3 - 1.5 micro meters long (Lin et al., 2013). IsolateD is Gram-negative aerobic bacteria, while some strains are capable of anaerobic respiration in the presence of nitrate to nitrite. They are catalase and oxidase positive, short rods or cocci with the dimension of 0.5-10micrometer in diameter by 0.5 - 2.6micrometer, and the cells occur singly. Strains like *Alcaligene faecalis* (Austin, 2014). Isolate E is gram-negative, oxidase negative and exhibit twitching motility and occurs in pairs under magnification.

Isolates A, C, D and E are all Gram-negative organisms ranging from creamy irregular raised colony to small creamy flat and opaque colonies to white slimy irregular convex colonies. Isolate B was Gram-positive cocci shaped with the appearance like grape-like clusters as revealed by morphological examination.

DISCUSSION

This study revealed that liquid extracts collected from cow dung biodigester contain some pathogens identified as,*Klebsiella, Staphylococcus, Acinetobacter, Edwardsiella*, and *Alcaligenes* species. The experimental results of the liquid effluent, calls for caution before subjecting it for further use in agricultural practice, because of the presence of pathogens. Cow dung as reported by Hozzein et al. (2011) is a potential source of microbial contamination in agricultural but require further investigation *Klebsiella* genus are responsible for a variety of diseases in animals and humans (Brisse et al., 2006). Surface water, drinking water, soil, plants, sewage, and industrial effluent are the environmental reservoirs of *Klebsiella pneumoniae* (Stuve and Krogfelt, 2004). It causes pulmonary and urine diseases in human and animals, and sometimes exist as an endophyte in plants (Huang et al., 2016). Carbone et al. (2002) reported that the species were common in the environment naturally but there virulence potential in soil and aquatic environment requires further studies. *Edwardsiella sp*ecies can infect freshwater and marine organisms when the liquid effluents are drained into the aquatic environment. They are opportunistic pathogens to human and aquatic animals, and the leading cause of food-borne disease (Yamê et al., 2018).

Staphylococcus species act as a commensal of the human micro biota; it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and it can cause a range of illnesses from minor skin infections such as pimples to impetigo, boils cellulitis scalded skin syndrome and life-threatening diseases as well Pneumonia, meningitis, osteomylylitis, endocarditis, toxic shock syndrome bacteremia and sepsis (Hoffman and Barbara 2012). An important pathogenic *Staphylococcus* and *Escherichia coli* species act as an indicator and enteric organisms and occasionally causes an opportunistic infections and are frequently recovered from livestock manures, and most of them are pathogenic *E. coli* such as EHEC strains which produce cytotoxin (EFSA, 2011). *Alcaligenes* occasionally causes opportunistic infections such as nosocomial sepsis, enteric fever endocarditis and they are commonly resistant to antibiotics like *Acinetobacter* species (Kavuncuoglu et al., 2010).

Infections associated with pathogenic infections in animals and humans are common knowledge based on many research studies (Charles and James, 2005, Kavuncuoglu et al., 2010).

The paramount factor in the exposure's management risks requires an understanding of sources, concentration and removal processes in environment the wastes as the best mitigating process within exposed population (Charles and James, 2005). As discussed earlier that cow dung is home to many pathogens causing diseases in humans, they can persist from days to months in the environment depending on the pathogen types, media and environmental conditions (USEPA, 2003; Bagge et al., 2005; Albihn and Vinneras, 2007; Masse et al., 2011). Approximately 1% to 3% of all domestic animals are infected with *Salmonellae* (Brown et al., 2016). Furthermore, other non-bacterial pathogens that may be present with faecal material include protozoa (*Cryptosporidium* and *Giardia*) and viruses (Swine Hepatitis E- virus). The management and disposal of animal wastes harboring such pathogens can increase the risk of infections and diseases that threatens human health if these wastes are not properly treated (Erickson et al., 2005). Wastes from animals (poultry and livestock) often contain high concentrations of human pathogens, spilt feed, bedding material, fur, process-generated wastewater, undigested feed residues, faeces and urine, therefore, it must be effectively managed to minimize environmental and public health risks. However, the pathogens and microbial load depend on the waste and its composition (Jenkins et al., 2007). This study has revealed that the liquid effluents contain pathogens that have potential to cause health problems to human and animals. If the pathogenic organisms through run-off from farmland (because of excessive rainfall in Niger Delta), enters the environment and is exposed to human and animals, it will eventually lead to significant health challenge. This study however shows that further research need to be conducted on the bio-slurry and other biodigester using similar animal waste feedstock, and expand the work to cover many microbes that may be present in both liquid and bio-slurry digestate from the digester.

Conclusion

Although cow dung biodigester digestate is a good organic fertilizer in agricultural practice and strongly advocated over the inorganic fertilizer, they must not throw caution into the air with knowledge gained from this study. The liquid effluents from the cow dung biodigester shown to contain pathogens that belongs to *Klebsiella, Staphylococcus, Acinetobacter, Edwardsiella*, and *Alcaligenes*. In the meantime, Acinetobacter and Alcaligenes pathogens from literature,have been proved to be resistance towards antibiotics, which means it could cause serious public health when exposed to population. Therefore, liquid effluents should undergo further treatment before use.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the paper.

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