Research Article

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Microbial population of palm oil mill effluent (POME) and efficiency of selected isolates in biogas production

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Abstract

A study was conducted to determine the microbial population suitable for enhanced anaerobic digestion of industrial effluents from Nigeria Institute for Oil Palm Research (NIFOR), Presco Oil Palm Plc, and Guinness Nigeria Plc. The standard dilution methods were used for recovery of bacteria and fungi. Isolation and quantity of colony-forming unit per mL (cfu mL^{-1}) was assessed using the 10-fold serial dilution method. Significant differences were recorded with the bacterial concentrations in the three sampled effluents. Effluent from Presco had the highest bacteria cfu mL^{-1}. Effluent from NIFOR consistently had the highest fungal population (cfu mL^{-1}). Effluent from Guinness had the least fungal cfu mL^{-1}. Two types of bacteria were recorded from the three effluents sampled. Similarly, seven types of fungi were present in the effluents from the three different locations. Inoculation of POME digester with the pure culture of the two bacteria led to a significant (about 5-fold) increase in the yield of biogas from the anaerobic digestion of POME; while inoculation with a consortium of bacteria at 20 per cent application yielded higher volume of biogas thereby indicating a kind of synergy between the two bacteria.

Keywords: Anaerobic digestion, biogas, brewery effluent, micro-organism, palm oil mill effluent

Introduction

Agro-industrial effluents constitute one of the most prevalent pollutants particularly in developing countries. The rate of contamination of our natural water-bodies increases with increased human activities especially in the agro-industries and domestic spheres. It has been reported that during palm oil processing about 5 to 7.5 tonnes of water is required for the production of one tonne of crude palm oil; and between 0.5 and 0.75 tonne of palm oil mill effluent (POME) is discharged for every tonne of fresh fruit bunch (FFB) processed (Ahmaad *et al.*, 2003; Yacob *et al.*, 2005). POME is an acidic brownish colloidal suspension containing high concentration of organic matter, oil, COD and BOD values. Although POME is considered as non-toxic, it has been identified as a major source of aquatic pollution by depleting dissolved oxygen when discharged untreated into water-bodies (Hwang *et al.*, 1978; Wood *et al.*, 1979; Khalid and Mustafa 1992; Rakkoed *et al.,* 1999). If not properly treated and disposed, agro industry effluent can severely damage the environment. The high level of phosphate and ammonia in rubber processing effluent makes it a good medium for algal growth and can result in eutrophication of surface water if discharged without proper treatment (Iyabga *et al.,* 2008). Raw and untreated effluents affect many species of plants, animals and human beings (Plohl *et al.*, 2002). Long term effect on ecosystem may increase the concentration of toxicant in organism towards the top of the food chain (Samanta *et al.*, 2002).

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Several methods have been described by various authors for industrial effluent treatment. However, in recent times, anaerobic digestion of waste by microorganisms have been receiving tremendous attention due to their ability to degrade waste materials leading to production of biogas and bio fertilizer as end products (Boominathan *et al*., 2007). Production of methane-rich biogas is of increasing interest as a partial solution to the energy needs of many nations (Sewell *et al*., 1988). Anaerobic digestion of any complex organic matter to methane consists of a cascade of biochemical conversions catalyzed by different interacting microorganisms. These bacteria can be classified as fermentation, acetogenic and methanogenic bacteria. The last class of bacteria is believed to play a significant role in the terminal step of anaerobic digestion (Wilke *et al*., 1986; Bryant *et al*., 1988). The objectives of this study were to isolate, identify and characterize the microorganisms from POME and brewery effluent digesters, and exploit them for treating POME for efficient and increased production of biogas.

Materials and methods

Collection of samples

Samples of POME were collected from Nigeria Institute for Oil Palm Research (NIFOR), Benin City and Presco Oil Palm Plc, Benin City. Brewery effluent sample was collected from Guinness Nigeria Plc, Benin City. Effluent samples were collected in duplicate from each source in pre-sterilized bottles and stored in a refrigerator at 4 ^{o}C till use.

Isolation of bacteria and fungi species

The standard dilution plate method was used for recovery of bacteria in nutrient agar (NA) from different samples (Kamil *et al.*, 2007) and fungi in potato dextrose agar (PDA) amended with lactic acid to discourage bacterial growth (pH of 3.5 to 4). Isolation and population of colony-forming units (cfu mL^{-1}) was assessed using the 10-fold serial dilution method (Sylvia *et al.,* 2013). Four-fold (10^{-4}) serial dilutions were plated on the different media and incubated at room temperature. Observations and counting for bacteria colonies were carried out at 24 to 48 h after inoculation. Counting of fungi colonies were carried out at 72 h after inoculation and the plates were observed for seven days after inoculation for fungi identification before they were discarded. The different fungi colonies were purified by repeated sub-culturing. Primary isolation of bacteria was effected by streaking sample on the surface of a NA plate and then incubated at 28 ± 2 ^{o}C for 48 to 72 h. Single colonies from these plates were sub-cultured for isolation and purification (Amna and Fozia, 2012; Saraswati *et al.,* 2012).

The pure isolates of fungi and bacteria cultures were maintained on PDA and NA, respectively. Bacterial isolates were inoculated on nutrient broth and incubated for 7 days at 28 ± 2 ^{o}C before use.

Identification of bacteria and fungi species

Identification of bacterial species was done by recording macroscopic and microscopic characters. The purified colonies were subjected to gram staining and characterized using biochemical tests

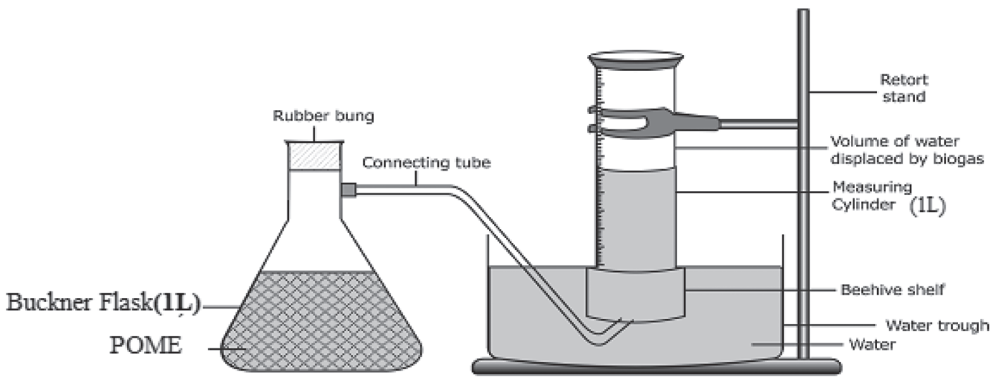


Fig. 1. Setup of biogas production

by Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994). Identification of fungi species were done by recording macroscopic and microscopic characters and were identified according to Gillman (1957).

Biogas production using the isolates

The experiment consisted of seven treatments. Six treatments were with two bacterial inoculants at different concentrations along with the un-inoculated raw POME.

Seven feeding concentrations of (I) 10 per cent bacteria A (coccus), (II) 20 per cent bacteria A, (III) 10 per cent bacteria B (rods), (IV) 20 per cent bacteria B, (V) 20 per cent bacteria A and B; (VI) 10 per cent bacteria A and B, and (VII) 100 per cent raw POME were used in the anaerobic digestion at ambient temperature of about 32 ^{o}C corresponding to mesophilic condition (Vavilin *et al*., 2008). The daily biogas productions for each run were recorded. The set-up used is as shown in Figure 1. It was first flushed with inert gas before the introduction of the samples for digestion. This is to ensure that it is free of oxygen.

Combustion test

The combustion test was carried out according to the method previously used by Chynoweth *et al*. (1982).

Statistical analysis was done by separating means of cfu mL^{-1} using Tukey's range test.

Results and discussion

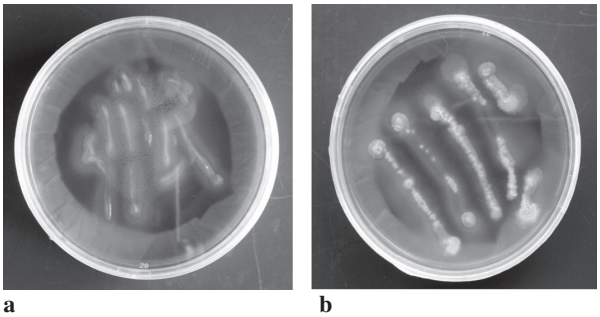
Colony-forming units of bacteria and fungi cultures per ml of the different effluents samples are summarised in Table 1. Significant differences were recorded with the bacteria concentrations in the three effluent samples. Effluent from Presco had the highest bacteria cfu mL^{-1}, while effluent from Guinness recorded the least cfu mL^{-1}. The cfu mL^{-1} recorded from the effluent from Presco was significantly different from the effluent from Guinness. The cfu mL^{-1} of effluent samples from Guinness and NIFOR were not significantly different.

Table 1. Microbial population (cfu mL^{-1}) of the effluent samples

Effluent	Colony forming unit per mL	
	Bacteria	Fungi
Guinness	1.83 x 10^{8a}	2 x 10^{8a}
NIFOR	1.86 x 10^{8a}	4.5 x 10^{8b}
Presco	2.02 x 10^{8b}	4 x 10^{8b}

Mean with the same letter along the same column are not significantly different from each other. P=0.05. All readings are mean of 6 plates.

Significant differences were not observed with the fungi populations from NIFOR and Presco effluent samples. Effluent from NIFOR recorded the highest cfu mL^{-1}, while the least cfu mL^{-1} was recorded from effluent from Guinness.



a b

Fig. 2. Types of bacteria present in the three effluent samples a) Coccus b) Bacillus

Two types of bacteria were identified from the three effluent samples. Bacteria identified were Coccus and Bacillus types (Fig. 2a and b; Table 2). The Coccus type was gram positive with smooth surface, filamentous in shape and edge and with

Table 2. Morphological characterisation of the bacteria isolates

Colony	Morphology (from agar plates)					Cell Shape	Gram stain
No.	Shape	Elevation	Edge	Colour	Surface		
1	Filamentous	Raised	Filamentous	Cream	Smooth	Coccus	+
2	Irregular	Raised	Undulating	White	Rough	Rod	-

cream colour on agar plate; while the Bacillus type was gram negative with irregular shape, raised elevation, undulating edge, rough surface and white colour on agar plate. These two bacteria were present in all the three effluents. Seven types of fungi were also observed to be present in the effluents from the three different locations. The fungi were *Aspergillus fumigatus*, *A. sclerotiorum*, *A. niger, A. versicolor, A. terreus, A. ochraceaus* and *A. flavus*.

Figure 3 shows the effect of inoculation with pure culture of methanogenic bacteria on biogas yield.

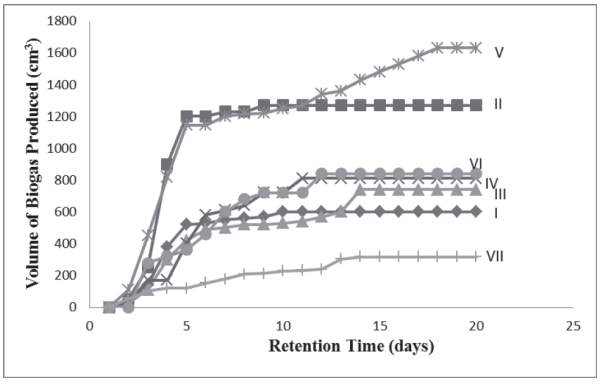


Fig. 3. Cumulative biogas production from anaerobic digestion of POME inoculated with pure culture of bacteria A & B and the consortium - I) 10 per cent bacteria A, II) 20 per cent bacteria A, III) 10 per cent bacteria B, IV) 20 per cent bacteria B, V) 20 per cent consortium of A & B, VI) 10 per cent consortium of A&B, VII) uninoculated raw POME

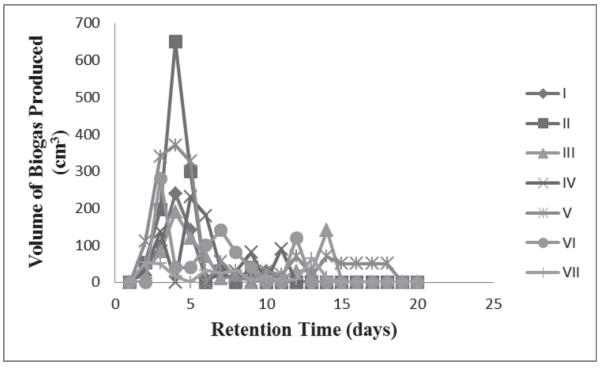


Fig. 4. Daily biogas production from anaerobic digestion of POME inoculated with pure culture of bacteria A & B and the consortium - I)10 per cent bacteria A, II) 20 per cent bacteria A, III) 10 per cent bacteria B, IV) 20 per cent bacteria B, V) 20 per cent consortium of A & B, VI) 10 per cent consortium of A&B, VII) uninoculated raw POME

It was observed from this experiment that highest yield of biogas was obtained from inoculation with 20 per cent bacteria A (II). However, inoculating with the consortium of bacteria A & B (V) at 20 per cent level gave highest yield of biogas over a longer retention time of about 20 days compared to retention time of less than 10 days for II. This result seems to show that there is a kind of synergy between the two bacteria when used in consortium.

Combustion test

A match lighted and placed close to the mouth of the measuring cylinder while the cover was partially removed produced a pop sound. However, controlled release of the biogas towards the lit match burned with a blue flame. This shows that the biogas produced has good combustion property. The result in this study is in line with earlier works reported by Chynoweth *et al*. (1982) and Mokobia *et al.* (2012), that combustion of biogas produced blue flame.

Conclusion

There is an increasing global concern on proper waste management in order to minimize and possibly eliminate their potential harm to public health and the environment. In order to do this, the pollutants need to be brought to permissible limits for safe disposal. In this study, two bacteria and seven *Aspergillus* spp were isolated from the three effluents after 72hr of incubation.

This study therefore provides baseline of isolates from three industrial effluents with a view to using them in the production of biogas and purification of waste water in future study.

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