Effects of methanogenic bacteria type on yield and quality of biogas from agricultural wastes.

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Abstract
In order to solve the challenges facing agricultural waste disposal and energy scarcity, several researches to convert agricultural waste to biogas and to increase biogas yield have been conducted. However, studies that establishes maximum biogas yield in conjunction with improved biogas quality are few. Therefore, in a quest to finding a more sustainable solution to the challenges facing agricultural waste disposal and energy scarcity in Nigeria, this study examined the effect of methanogenic bacteria type on yield and quality of biogas from effluents from palm oil processing (POME) and rubber processing (RPE). In this study, laboratory experiments with a sequence of 500 ml bio-digesters were performed using the different effluents inoculated with cow dung, methanogenic bacteria A and bacteria B, in seven different E:I ratios of: 5:0, 4:1, 3:2, 1:1, 2:3, 1:4 and 0:5 using the mesophilic technique. The biogas produced was captured and analysed for its composition using a biogas analyser. Results from the study, reveals that inoculation led to a significant (about 6-fold) increase in the yield of biogas from anaerobic digestion of POME and RPE. Inoculation also enhanced the quality of the biogas generated. The coci spp. gave higher biogas yield of 1610 cm^3 and 1985 cm^3 respectively from equal volumes of POME and RPE. Better quality biogas with methane content of 69.98 and 67.96% respectively for POME and RPE was also obtained with cocci spp. as compared to the bacillus spp. Anaerobic digestion of raw uninoculated RPE did not generate biogas. The best performance E: I ratio for POME in all the trials and cow dung inoculated RPE was 1:1, while RPE inoculated cocci and bacillus spp. was 3:2

Keywords: Anaerobic treatment, effluents, inoculums, biogas, POME, RPE

Introduction

Waste contribution to global anthropogenic methane emission is 20% (IEA, 2005), and methane is one of the greenhouse gases with a global warming potentials of 23 in a 100 years (IPCC, 2001). A greater portion of the waste arises from agricultural activities such as deforestation, biomass burning, rice cultivation, animal husbandry, unplanned agricultural waste disposal methods etc. Methane contribution to climatic change is around one third to half of that of carbon- dioxide (Hensen and Sato, 2001). The effect of climatic change will reduce economic development (Christopher, 2001) and increase poverty. Also, inability to effectively control the adverse effects of climatic change can ruin the possibility achieving the millennium development goal 9 (eradication of extreme poverty and hunger) (Aliyu, 2011) and goal 7 (ensuring environmental sustainability) (Hulme *et al.*, 1995). In Nigeria, agricultural sector feeds industries such as, food and beverage industries, soap and detergent, paper, plastics, textiles production and other related industries. The sector along with most of the industries it feeds do not practice proper waste disposal methods and often times, indiscriminately discharge their waste on roadside, in storm drainage canals, into nearby sea, stream, the outskirt area and surrounding land with little or no treatment. These methods are insufficient, unsustainable and harmful especially in a country like Nigeria where legitimate environmental laws are not strictly adhered to; the impact of such indiscriminate discharges may lead to gross contamination of the environment. So far, report from W.H.O estimated 335,200 deaths, occurring annually in Nigeria (a 16.70% of death cases) as a result of poor sanitation, water and hygiene related infection (Ojuri and Ola, 2010; Ojuri, *et al.*, 2014). However, the concept of waste as a useless material is rapidly changing to that of a valuable resource (Kwaghe *et al*., 2011); especially when these wastes contain a lot of valuable chemical components with nutritional, energetic and fertilizing properties (Liang- Qiao and Wu, 2009) that are usually lost by improper waste disposal methods. If agricultural wastes are properly managed, it will maximize the benefits of nutrients available from farm waste and minimize environmental impact. Among these agricultural wastes, wastewaters constitute a major challenge. Thus, in this study effluent from palm oil processing (POME) and rubber processing (RPE) will be evaluated for their biogas generating potentials.

Effluents from palm oil processing (POME) and rubber processing (RPE) are both organic waste which are capable of releasing carbon dioxide and methane into the atmosphere. These effluents are also high oxygen depleting (Yahaya and Lau, 2013) and can result to gross environmental pollution. Effluents released straight or some other ways into water environments will reduce their dissolved oxygen, degenerating them into unsightly, anoxia, foul-smelling and disease causing conditions thereby endangering the aquatic lives (Yahaya and Lau, 2013) and the lives of the nearby inhabitants. Thus the urgent demand for proper, sustainable and profitable waste management methods that minimizes environmental impact. Also, as a result of the limited fossil fuel reserve throughout the world, the fluctuations in their prices coupled with their combustion problems, Nigeria intends to reduce its dependence on oil and foster its economic growth through increased industrialization and agriculture (Federal Ministry of Power and Steel, 2000).

In this regard anaerobic digestion of organic waste for biogas production emerges as the best option (Sunarso *et al.*, 2012) which also in line with the United Nation Framework Convention on Climatic Change (UNFCCC) and the Kyoto protocol

Research studies conducted so far to increase the quantity of biogas produced from biomass are many, with results revealing that the rate at which biogas is produced is influenced by several factors. These factors includes; nature of the substrate, Carbon to nitrogen ratio, reaction temperature, reaction pH, agitation etc. (Bogudo *et al.*, 2011). However, studies that establishes maximum biogas yield in conjunction with improved biogas quality are few. Thus, the present research examines the effect methanogenic bacteria type on yield and quality of biogas from agricultural wastes.

Materials and methods

Collection of samples

Palm oil mill effluent (POME) was obtained from the Nigeria Institute for oil Palm Research (NIFOR) and rubber processing effluents (RPE) was obtained from Okomu. The wastewaters were collected from the discharge unit of their sewage systems in pre sterilized jerry cans. The anaerobic slurries that were used for the microorganism isolation and characterization were obtained from an existing anaerobic digestion plants, namely: Guinness PLC, Okomu Oil Mill and Presco Oil Palm. The cow droppings was obtained from a slaughter house in Oluku. All samples were collected in Benin City, Edo State in triplicates from each source in pre-sterilized bottles and stored in a refrigerator at 4^{o}C before use.

Analysis of the effluents

The effluents were analyzed for their physiochemical properties before and after anaerobic digestion using standard methods (APHA, 1999);

Preparation of the cow dung slurry

Fresh cow dung was mix thoroughly with distilled water in a ratio of 1:2 (10 Kg cow dung: 20 litre water).The slurry was filtered and allowed to pre-decay for three days to ensure bacteria multiplication.

Isolation and characterization of microorganisms

The standard dilution plate method was used for recovery of bacteria in nutrient agar (NA) (Kamil *et al.*, 2007) from the anaerobic slurries samples. Isolation and population of colony forming units (Cfu ml^{-}) was evaluated by the 10-fold serial dilution method (Sylvia *et al*., 2013). Four-fold (10^{-4}) serial dilutions were placed 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. Primary isolation of bacteria was effected by streaking sample on the surface of a NA plate and then incubated at 28 $\pm$ 2^{0}C for 72 h. Single colonies from these plates were sub-cultured for purification (Amna and GFozia, 2012; Saraswati *et al.*, 2012).

The pure isolates of bacteria cultures were maintained on NA. Bacterial isolates were inoculated on nutrient broth and incubated for 7 days at 28 $\pm$ 2^{o}C before use.

Identification of bacteria

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 by Bergey's Manual of Determinative bacteriology (Holt *et al.*, 1994).

Inoculated anaerobic digestion of samples

Samples of palm oil mill effluent (POME) and rubber processing effluent (RPE) were treated separately with inoculum at varying effluent to inocolum (E:I) ratios in a closed anaerobic digester at the same organic loading rates (OLR), using the mesophilic techniques (Vavilin *et al.*, 2008). The experiment consisted of three batches, with each batch consisting of fourteen feeding treatments. The treatments at varying effluent to inoculum ratios (E:I), respectively, for RPE and POME are shown in Table 1

Table 1: Varying effluent to inoculum ratios (E:I)

Serial no	1	2	3	4	5	6	7

Effluent: Inoculum ratio	5:0	4:1	3:2	1:1	2:3	1:4	0:5

Plate 1: Laboratory Scale Setup of Biogas Production

Semi-pilot scale inoculated biogas production from best performance effluent to inoculum ratios

Slurries of a mixture of 1:1 of POME and 3:2 RPE to cow dung, bacteria A and bacteria B (inoculum), respectively, were fed into the digester. A biogas outlet pipe connected tightly to a balloon served as the biogas receiver (Plate 2).

Plate 2: Setup of Biogas Production

Combustion test and compositional analysis of biogas

The combustion test was carried out according to the method previously used by Mokobia *et al.*, *(*2012). The gas evolved was captured and analysed for its components (CH_4, CO_2, and H_2S) using a biogas analyser (Geotech5000) (Plate 3).

Plate 3: Set Up Of Biogas Analyser Analysing the Synthesized Biogas

Statistical analysis

One way analysis of variance (ANOVA) was used to assess the significant differences in the data obtained. The mean of the data was compared using SPSS (Statistical package for Social Scientist).

Results and discussions

The physiochemical properties of the palm oil mill effluent (POME) under study (Table 2) showed that the pH value was in the acidic region (4.50) and not within the regulatory discharge limits for wastewaters. The low pH values for POMEs are consistent with many research findings; Osaigbovo and Orhue, (2011) reported pH value of 4.8 for POME. Ma (2000); Ahmed *et al*. (2003) reported pH value of 4.7. Also pH values as low as 3.8 was also reported (Abdurrahman, *et al*., 2011). Rupani *et al*., (2010); Bala *et al*., (2014) reported that low pH of POMEs that ranged between 4 – 5 is usually due to the organic acid produced in the fermentation stage during palm oil processing. The temperature was also very high (86^{0}C) and not within the regulatory standard for effluent discharge to the environment.

Other physiochemical properties of POME were not within the regulatory standard for effluent discharge (Ma, 2000; Ahmed *et al*. 2003). The suspended solid (18, 178 mg/l) was higher than the 400 mg/l of the regulatory standard. The COD of the effluents were also very high suggesting that they have high pollution potential when discharged into the environment without proper treatment. Abdurrahman, (2011) stated that high COD for POME could be as a result of residual oil on the fruits after extraction.

On the other hand, the rubber processing effluent under study had pH value of 6.60 suggesting that the effluent is slightly acidic and within the regulatory discharge limits for effluent (Ma, 2000; Ahmed, 2003). Acidic pH values are consistent with some previous studies on RPE. Pillai and Girish (2014) reported a pH value of 5.7. Also a pH value of 5.0 was reported by Orhue and Osaigbovo (2013). Rungruang and Babel, 2008; Tekasakul and Tekasakul, (2006) had pH value range of between 3.7 -5.5. However, Asia and Akpohonor (2007) had a contrary value of 8.1. Rubber processing effluent is characterized by a highly fluctuating pH (Pillai and Girish, 2014; Muhammadi *et al*., 2010). The total solids (TS), total suspended solids (SS) and COD for RPE was lower than that of POME. The most likely reason for this is because the latex exuded by a rubber tree has about 60 percent water (Asia and Akpohonor, 2007). COD values for the RPE was higher than results obtained by Asia and Akpohonor (2007) (3142 mg/l) but consistent with Orhue and Osaigbovo (2013).

Table 2: Characteristics of the Palm Oil Effluent (POME) and Rubber Processing Effluent (RPE)

PROPERTIES	NIFOR POME	OKOMU RPE	REGULATORY DISCHARGE LIMIT(Ma, 2000; Ahmed *et al*., 2003)
TEMPERATURE ^{0}C	86	35	45

pH	4.50	6.60	5.0 -9.0
COD	59,814.81	1801.74	-
CONDUCTIVITY (S/m)	15805.66	414.46	-
TOTAL SOLIDS	48,000	1,450	-
SUSPENDED SOLID	18,178	668	400
DISSOLVED SOLIDS	29,822	782	-
VOLITILE SOLIDS	42,000	143	-
OIL & GREASE	4,200	8.9	50

All values, except conductivity, pH and temperature are expressed in mg/l.

BACTERIA PRESENT IN THE THREE ANAEROBIC SLURRY SAMPLES

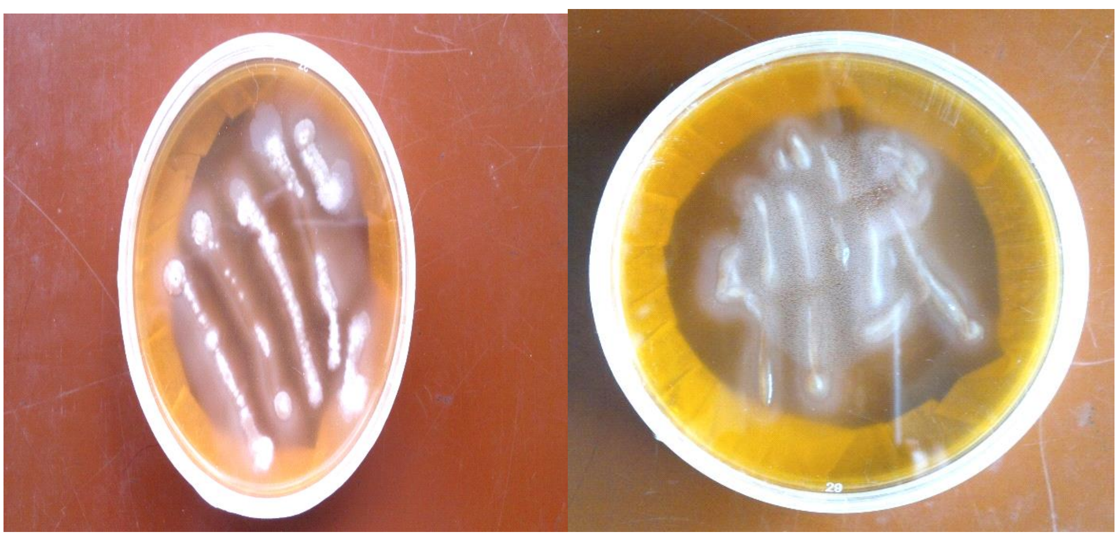

PLATE 1: Bacteria present in the three effluents -A, *Coccus spp*. and B, *Bacillus spp*

Table 3 shows the morphological characteristics of the bacteria present in the three anaerobic slurries samples. The Coccus type was Gram positive with smooth surface, filamentous in shape and edge. It was cream in colour on the agar plate. The Bacillus type was Gram negative with irregular shape, raised elevation, undulating edge, rough surface and white colour on the agar plate.

Table 3: Morphological Characteristics of the Bacteria

	MORPHOLOGY (FROM AGAR PLATES)						
	Shape	**Elevation**	**Edge**	**Colour**	**Surface**	**Cell Shape**	**Gram stain**
1	Filamentous	Raised	Filamentous	Cream	Smooth	Coccus	+
2	Irregular	Raised	Undulating	White	Rough	Rod	-

Table 4: Bacteria Population (Cfu/ml) of theAnaerobic Slurry Samples

Anaerobic slurry	Colony forming unit/ml (Bacteria)

Guinness	1.83 x 10^{8a}
NIFOR	1.86 x 10^{8a}
Presco	2.02 x 10^{8b}

Means with different alphabet remarks are significantly different at (P<0.05).

Table 4 shows the bacteria population (Cfu/ml) of the three anaerobic slurry samples. Effluent from Presco had the highest bacteria count (Cfu/ml) with values significantly different (P<0.05) from Guinness and NIFOR effluents. Guinness recorded the least Cfu/ml and its value was not significantly different (P<0.05) from the NIFOR effluent.

Table 5: Highest biogas yield from the inoculated POME and RPE

Effluent Samples	**Highest biogas yield (cm^3)**	**Effluent to Inoculum ratio (E:I)**
Cow dung inoculated POME	465.00 ± 47.097	1:1
Bacteria A inoculated POME	1610.00	1:1
Bacteria B inoculated POME	940.00	1:1
Cow dung inoculated RPE	690 ± 55.272	1:1
Bacteria A inoculated RPE	1985.00	3:2
Bacteria B inoculated RPE	1195.00	3:2

Means with different alphabet remarks are significantly different at (P<0.05).

Table 5 shows biogas yield from the inoculated POME and RPE. From the result, it is observed that RPE yielded more biogas in all the trials as compared to POME. The most likely reason for this could be because of the higher pH value (6.60) of the rubber processing effluents, providing an enabling environment for the bacteria to strive.

pH is an important factor in the anaerobic digestion of biomass. The importance lies on the fact that methanogenic microorganisms are very sensitive to changes in pH. Their growth and methane production are inhibited when the pH is not within 6.5 – 7.5 (Zupancic and Griliz, 2012). Ezekoye (2013) reported that both acid and methane formation bacteria cannot survive at pH values below 4 and above 10. Many cases of process failure are caused by the accumulation of volatile fatty acid concentration, leading to a drop in pH and subsequently inhibit methanogenesis (Parawira *et al*., 2006; Patel and Madawar, 2000).

TOTAL BIOGAS YIELD FROM COW DUNG INOCULATED NIFOR POME AT VARYING SUBSTRATE TO INOCULUM RATIOS

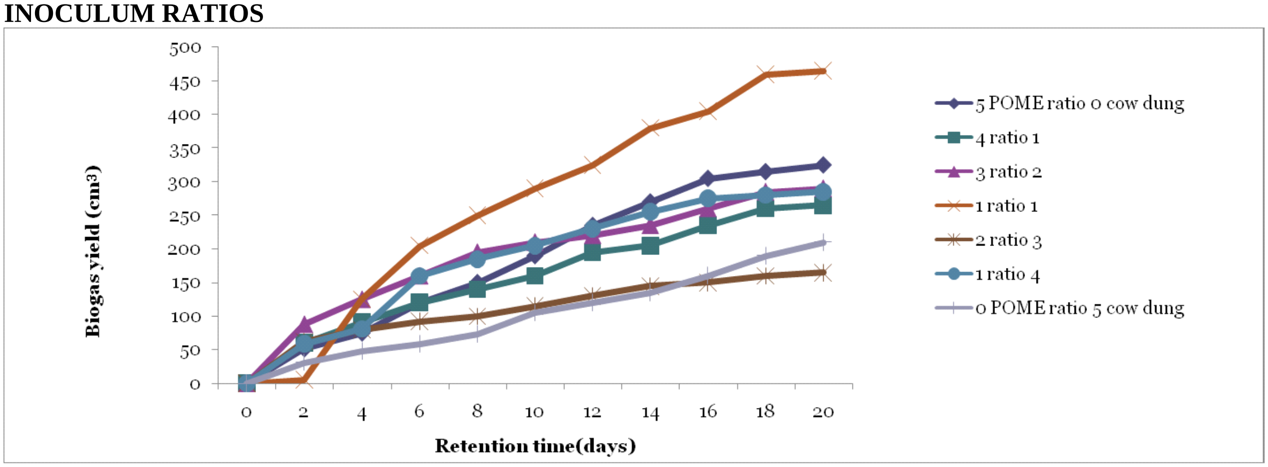

Figure 2: Total Biogas Yield from Cow Dung Inoculated NIFOR POME at Varying Substrate to Inoculum Ratios

TOTAL BIOGAS YIELD FROM COW DUNG INOCULATED OKOMU RPE IN SEVERAL DAYS RETENTION TIME AT VARYING SUBSTRATE TO INOCULUM RATIOS

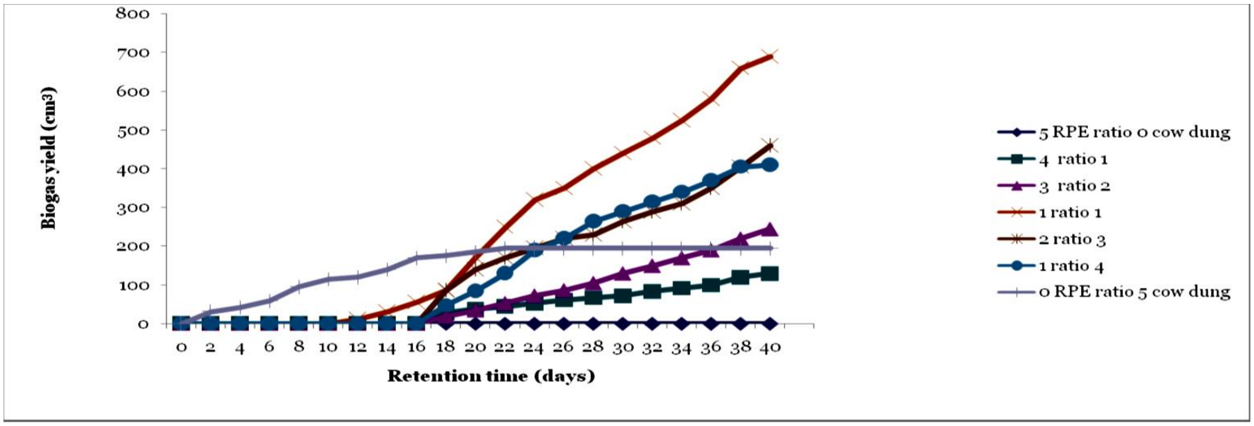

Figure 3: Total Biogas Yield from Cow Dung Inoculated OKOMU RPE at Varying Substrate to Inoculum Ratios

Figure 2 shows biogas yield from NIFOR POME at varying effluent to inoculums ratio. From the graph it was observed that the best performance effluent to inoculums ratio was the 1:1 (E: I) with a total biogas yield of 465cm^3 at 20 days retention time.

Figure 3 show the biogas yield from Okomu RPE. From the graph, it is observed that the highest biogas yield was 690 cm^3 which was attained at 40 days retention time,(E:I) ratio of 1:1. This yield was higher than for POME. The most likely reason for the high biogas yield from RPE could be because of the high pH value (6.60) of the rubber processing effluents, providing an enabling environment for the bacteria to strive. The methanogenes proliferate at pH range of between 6.5 –7.5 and their activities outside these values could result to system failure (Zupancic and Grilc, 2012). Low pH inhibits the activities, growth and population of the methanogens. It also leads to acid accumulation since the methanogens are responsible for the consumption of the acid formed. The final effect is low methane generation and system failure.

Also, higher biogas yield from inoculated effluents as compared to the raw effluent is consistent with literature (Ezeonu *et al*, 2002; Aigbodion *et al.,* 2014; Uzodinma *et al.,* 2007). Biogas production increases with increasing quantity of inoculums (Forster-Camero *et al*., 2008), feed to inoculum ratio influence biogas production rate (Sunarso *et al.*, 2012; Liu, et al., 2009); and inoculum content influences the performance of anaerobic reactor (Budiyono *et al*., 2009). Also, organic waste can be blended to improve biogas quantity and quality through synergistic effect (Uzodinma *et al.*, 2007; Ofoefule and Uzodinma, 2006). Biogas yield of over 400% increase was recorded from blending brewery spent grain with poultry dropping at a ratio of 4.1. (Ezeonu *et al.*, 2002).

BIOGAS YIELD FROM BACTERIA A INOCULATED NIFOR POME IN AT VARYING SUBSTRATE TO INOCULUM RATIOS

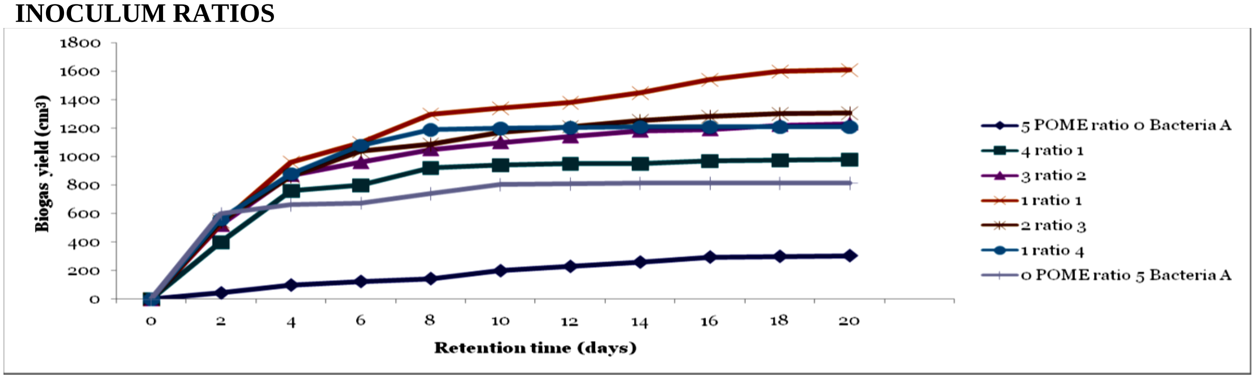

Figure 4: Total Biogas Yield from Bacteria A Inoculated NIFOR POME at Varying Substrate to Inoculum Ratios

TOTAL BIOGAS YIELD FROM BACTERIA B INOCULATED NIFOR POME IN TIME AT VARYING SUBSTRATE TO INOCULUM RATIOS

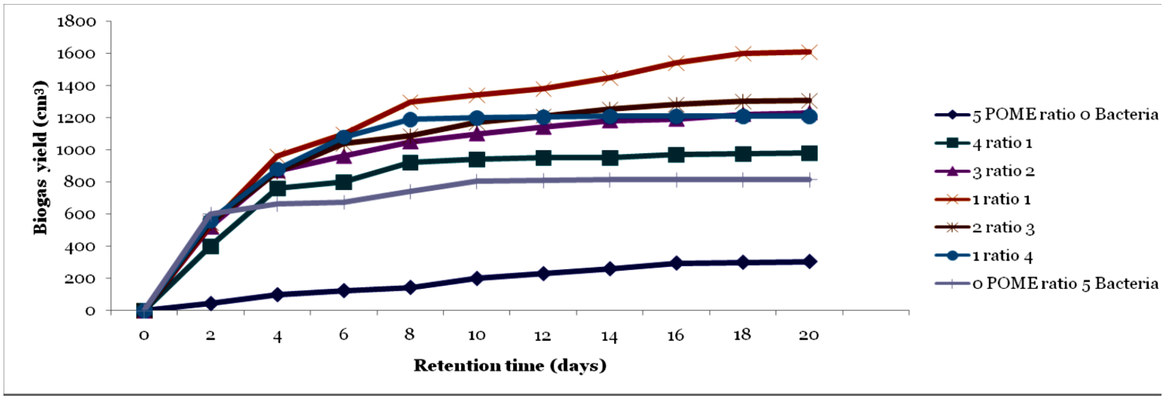

Figure 5: Total Biogas Yield from Bacteria B Inoculated NIFOR POME at Varying Substrate to Inoculum Ratios

Figure 4 shows the biogas yield from bacteria A inoculated NIFOR POME. From the graphs, it is observed that the best performance E: I ratio is 1:1, which gave the highest biogas yield of 1610cm^3 over the same retention time of 20 days. These results suggest that anaerobic digestion of pure culture bacteria inoculated POME does not reduce retention time. However, biogas yield was significantly increased (about six - times) as compared to raw POME.

Figure 5 shows the biogas yield from bacteria B inoculated NIFOR POME. From the graph, it is observed that the best performance E:I ratio is also 1:1, with the highest biogas yield of 940cm^3 over the same retention time of twenty days. The highest biogas yield from this experiment was lower than the one obtained from bacteria A, suggesting that bacteria A is more able to decompose the effluent, thus yielding more biogas in return.

TOTAL BIOGAS YIELD FROM BACTERIA A INOCULATED OKOMU RPE AT VARYING SUBSTRATE TO INOCULUM RATIOS

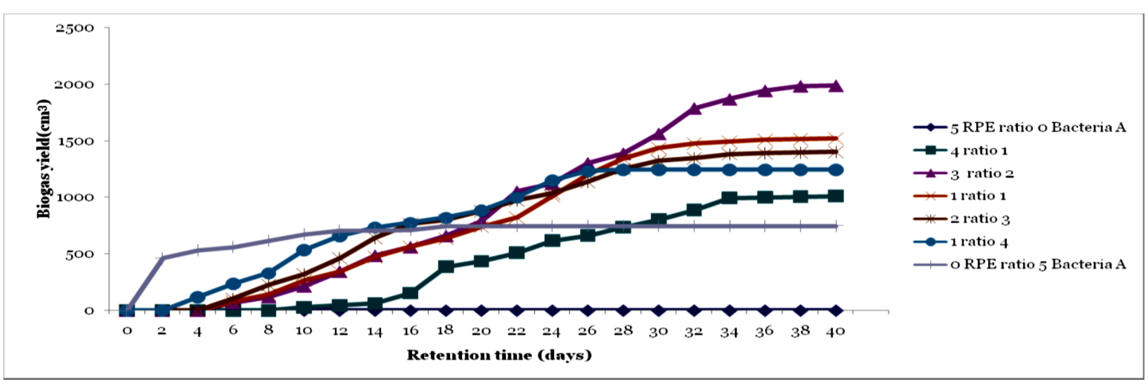

Figure 6: Total Biogas Yield from Bacteria A Inoculated Okomu RPE at Varying Substrate to Inoculum Ratios

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TOTAL BIOGAS YIELD FROM BACTERIA B INOCULATED OKOMU RPE AT VARYING SUBSTRATE TO INOCULUM RATIO

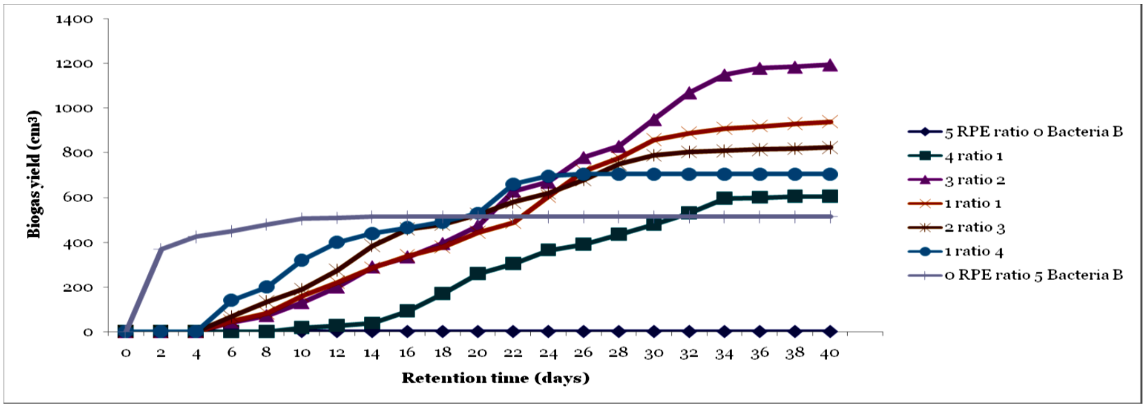

Figure 7 Total Biogas Yield from Bacteria B Inoculated Okomu RPE **at Varying Substrate to Inoculum Ratio**

Figure 6 shows the biogas yield from bacteria A inoculated Okomu RPE. From the graph, it is observed that the best performance E: I ratio is 3:2. This ratio gave the highest biogas yield of 1985cm^3 over the same retention time of forty (40) days. The biogas yield from bacteria A inoculated Okomu RPE is higher than the bacteria A inoculated NIFOR POME in spite of its high COD level. It is likely that the high pH of RPE provided an enabling environment for the bacteria to thrive which translated to a higher biogas yield.
Figure 7 shows the biogas yield from bacteria B inoculated Okomu RPE. From the graph, it is observed that the best performance E:I ratio is also 3:2 with a yield of 1195cm^3. The reduced yield of biogas in this experiment as compared to the bacteria A inoculation further confirms that bacteria A has a higher substrates degradation efficiency.

BIOGAS COMBUSTION TEST

There was a pop sound when a lit match was brought close to the mouth of the measuring cylinder, while the cover was partially removed. However, on controlled release of biogas, it burned with a blue flame.

Table 6: Compositional Analysis of Biogas

EFFLUENT: INOCULUM	TOTAL BIOGAS YIELD (Cm^3)	METHANE (CH_4)		CABON (IV) OXIDE (CO_2)		HYDROGEN SULPHIDE (H_2S)		OXYGEN (O_2)		TRACES OF OTHER GASES	
		(Cm^3)	(%)	(Cm^3)	(%)	(Cm^3)	(ppm)	(Cm^3)	(%)	(Cm^3)	(%)
NIFOR POME: Cow Dung	465	262.12	56.37	181.86	39.11	≈	448	-	-	20.97	4.51
NIFOR POME: Inoculum A	1,610	1,115.57	69.29	436.63	27.12	≈	246	2.09	0.13	55.54	3.45
NIFOR POME : Inoculum B	940	639.01	67.98	278.80	29.66	≈	174	-	-	22.09	2.35
Okomu RPE: Cow Dung	690	358.11	51.90	264.96	38.40	≈	612	1.38	0.20	64.86	9.4
Okomu RPE: Inoculum A	1,985	1,345.61	67.96	561.56	28.29	≈	273	-	-	74.24	3.74
Okomu RPE: Inoculum B	1,195	790.37	66.14	380.85	31.87	≈	125	-	-	23.66	1.98

Table 6 shows the compositional analysis of the biogas from NIFOR POME and Okomu RPE using the different inoculums. The results shows that the NIFOR POME inoculated with bacteria A (Coci spp) had the highest methane content. However, the Okomu RPE had the highest biogas yield. It was also observed that samples of POME and RPE both inoculated with cow dung had the lowest biogas yield, lowest methane content and highest carbon dioxide yields. This may perhaps be as a result of the fact that the bacteria inoculated effluents were pure culture and were more able to anaerobically degrade the substrates thus producing highest methane concentration and lowest carbon IV oxide content. The biogas yield from rubber processing effluents could be attributed to its high pH which creates enabling environment for the bacteria to strive. Also, previous study had shown that low pH (acidic) accounts for low volume of biogas (Uzodinma *et al.*, 2007). Also, comparing biogas yield and quality of the different inoculums in a particular effluent, it was observed that higher biogas yield and better quality was obtained with bacteria A (Coci spp).

Conclusion and recommendation

Inoculation led to a significant (about 6-fold and above) increase in the yield of biogas from anaerobic digestion of POME and RPE. Inoculation also enhanced the quality of the biogas generated. RPE had the highest biogas yield compared to POME. The coci spp. gave higher biogas yield and better biogas quality as compared to the bacillus spp. Anaerobic digestion of raw uninoculated RPE did not generate biogas. The best performance effluent to inoculums (E: I) ratio for both effluent was 1:1 Further investigation more blending ratios with the same or different effluents and inoculums to achieve higher biogas yield of better quality is recommended.

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