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Valorization of *Pennisetum purpureum* (Elephant grass) and piggery manure for energy generation

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ABSTRACT

This study investigated the biogas production potential of *Pennisetum purpureum* (Elephant grass) (El-g) co-digested with piggery manure (PM) under mesophilic condition in order to combat the menace of weed in cropping systems as well as pollution problems emanating from disposal of PM. Prior to anaerobic digestion (AD), El-g was subjected to a combination of mechanical, thermal and alkaline pretreatments. Using cattle rumen content as inoculum, the pretreated El-g was anaerobically co-digested with PM while the raw El-g was also co-digested with PM and served as control experiment. The physicochemical characteristics of feedstock were evaluated before and after the digestion period using standard methods. The initial high concentrations of chemical oxygen demand (COD) reduced significantly after digestion indicating efficiency of the digestion process. Also, there were reductions in concentrations of calcium and other parameter needed for microbial growth after the digestion which indicated their utilization by microbes to generate biogas. Biogas production began on the 5th and 7th days and was progressive until 30th and 24th days in both digestions after which a decline was observed until the end of the experiment. For the digestion period of 37 days, the total biogas recorded from the pretreated and untreated experiments were 409.5 and 184.1 m^3 CH_4/kg VS with average of 11.07 and 4.98 m^3 CH_4/kg VS/day respectively. The study concluded that co-digestion with piggery dung enhanced the biogas producing capacity of El-g hence advocated.

1. Introduction

The problem of waste management and stable power supply are parts of major challenges facing several developing countries. Wastes are indifferently abandoned as heaps to pollute the environment thereby constituting public health threat. Also, under-development and technological backwardness currently witnessed in many developing nations have been linked to energy crises being faced in these parts of the world [1]. Although, the use of fossil fuel has been the major source of energy supply right from time, apart from its possible depletion in the nearest future, the rise in the amount of fossil fuels and the scourge of its usage is undeniable causing negative impact on the environment, economy, also on man's health [2]. Therefore, the consequences of improper waste management and global over reliance on fossil fuel with their adverse effect in terms of spread of disease, environmental degradation and climate change have necessitated the quest for the utilization of waste as a source of renewable and sustainable energy [3–7]. Several studies therefore have reported the utilization of different agricultural, industrial and domestic waste materials for the generation of environmentally friendly renewable energy [8]. One major efficient approach for energy recovery from several organic wastes especially agricultural waste is anaerobic digestion. Anaerobic digestion is a biochemical process for the treatment of biodegradable matter, which involves bacteria degradation of biological material in the absence of oxygen to produce biogas and a

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stabilized sediment that can be used as organic fertilizer [9–10]. The process is not only adjudged as a commercially proven method for the treatment of organic waste, biogas produced can serve as panacea to energy crises being faced in several parts of the world, while the bio-fertilizer can be used to improve soil nutrients and plant growth, thereby increasing agricultural productivity [11–12].

Continuous increase in the market demand for pork meat has led to the upsurge in swine herds which in turn has resulted in generation of large swine manure worldwide [13]. In developing countries, poor management of piggery operation continues to take its hard toll on humanity in terms of environmental pollution arising from poor swine waste disposal [14]. The effects of the large quantum of the pig manure on environmental and public health are becoming a growing concern in many developing nations [15]. The utilization of pig manure for biogas generation is thought to alleviate its disposal problem as well as energy crises. Previous experiments using pig manure alone as a substrate in anaerobic digestion process have suffered some setbacks due to excess nitrogen content relative to available organic carbon [14–16]. This high nitrogen content may lead to toxic high ammonia level. Thus, materials rich in organic carbon must be added to the pig manure to provide the required organic carbon.

Elephant grass (*Pennisetum purpureum)*, commonly regarded as a very stubborn weed of crop, originated from Sub-Saharan tropical Africa from where it has dispersed to most tropical and subtropical regions worldwide [17]. It is currently found occupying large expanse of land in the United States of America, Central and South America, Australia, West Indies and several other parts of the world [18]. Although, it can withstand harsh condition, it does well in locations where temperature ranges from 25 to 40 °C [19]. It requires a comprehensive environmental impact assessment for large-scale deployment as a resource for cellulosic bioenergy or fodder in some countries particularly the United States [20]. Elephant grass is abundantly available in Nigeria and always constitutes environmental menace due to its high yield of biomass per hectares of land. Elephant grass yields about 30 to 40 metric tons of biomass per hectare under local environmental and climatic conditions [21]. Despite its abundant availability, its use has been relegated to limited applications such as folder, as a cover material for soil erosion control, as bedding for mushrooms cultivation, as a raw material in the production of paper and as a feed for cattle and buffaloes [18,21]. In Nigeria, El-g is widespread across many localities as a serious weed in crops, thereby posing a serious threat to agricultural activities. Urgent attention is therefore required to arrest its negative impacts on agricultural activities. This formed the basis of study in utilizing El-g as a feedstock for biogas generation. Being a cellulosic biomass, its high sugar content, high growth rate and short lifecycle will not only make it suitable substrate for biogas generation through anaerobic digestion but also enhance its constant availability all round year.

Some of the reported high energy-yielding biomasses include maize, sugar beets, switch grass and Sudan grass [4,9,22–23]. Though, the mono-digestion of each of PM and El-g have been documented [15,18], energy generation from El-g has been largely limited to its co-digestion with other substrates such as cow dung and chicken/poultry manure [24–27]. The co-digestion of El-g with PM has been scantily reported and, in that instance, El-g silage and not the raw grass was co-digested with PM [28]. To the best of our knowledge therefore, this is the very first reported attempt to co-digest PM and El-g for enhanced bioenergy generation accompanied with kinetic modeling of important process parameters. The aim of this study is to evaluate the biogas producing potential of PM co-digested with El-g as alternative renewable energy source. This is thought to reduce their indifferent abandonment to pollute the environment and serve as constraints to food production, thereby enhancing waste-to-energy strategy.

2. Materials and methods

2.1. Material collection and pretreatment

Elephant grass (El-g) and piggery manure (PM) used for the study were collected at Landmark University Teaching and Research farm. Cow rumen content obtained from a commercial abattoir in the city was also added to provide microbial floral for anaerobic digestion. The PM and rumen content were rid of pebbles, stones and other impurities before being refrigerated at 4 °C prior to use. Considering the lignocellulosic nature of El-g, sample of the biomass was pretreated using both mechanical and thermo-alkaline (NaOH) pretreatment methods as described by Dahunsi et al. [9]. This involves milling the biomass into mesh sizes $\leq$20 mm using harmer mill. This is necessary to achieve a smaller size and a larger surface area through which microbes can act as quick as possible [29].

The milled sample was then thermally treated at 90 °C for 1 h in a water bath. Pretreatment at higher temperature has been reported to cause chemical reaction and formation of protein inhibition [9,30]. The sample was then alkaline pretreated using 5 g NaOH/1 kg sample at 50 °C for 24 h. Only analytical grade reagents were used, the NaOH (98% W/W minimum) used in the alkaline pretreatment was procured from Panoli Intermediates, India.

2.2. Digester

The Computer Controlled Anaerobic Digester (Edibon PDANC 0007/144) was used for the study. The digester consists of two reactor vessels both having a heating water circuit with valves to regulate the appropriate temperature at every stage of the process. Water tank and water collector were attached to the back of the digester. Biogas produced entered the water tank through one of the tubes from the digester and discharged equal volume of water to the water collector.

2.3. Analytical procedure

All samples of El-g were analyzed so as to determine the fixed and extractive solids (untreated sample) and the three major structural components i.e. lignin, cellulose and hemicellulose (L-C-H) [31–32]. Evaluation of the extractable materials was carried out in all samples using the Soxhlet apparatus for 6 h while the fixed solid was determined after burning sample of El-g using a furnace [32]. Determination of total L-C-H was done using 0.3 g dried sample of El-g with 72% H_2SO_4 (3 mL $v.v^{-1}$) at 30 °C for 1 h while the filtrate from this process was employed for carbohydrate determination [33]. Compositions of sugars and acetic acid were determined by liquid chromatography method i.e. LC-MS. This was done in a DIR-10A refractive index detector operated with a BIORAD HPX87H column with 0.005 mol. L^{-1} H_2SO_4 as mobile phase. Other parameters were 45 °C, 20 μL injection volume and flow of 0.6 $mL.min^{-1}$. Each compound was then determined using calibration curves with corresponding Sigma-Aldrich LC-MS standards [34]. In determining the composition of furfural and hydroxymethylfurfural (HMF), same procedure was used except that to the LC-MS was an attached diode array detector while a C18 column used with 1:8 acetonitrile/water as the mobile phase. An oven temperature of 30 °C was used with 20 μL injection volume and flow of 0.8 $mL.min^{-1}$. Calibrations curves were used to determine concentrations as earlier explained.

For the determination of the physicochemical properties of the samples, an inductively coupled plasma mass spectrometry was used as earlier described [35]. For chemical oxygen demand (COD) measurement, the standard method [36] was used. Concentrations of volatile fatty acids (VFAs) were determined by gas chromatography (Clarus 580GC, PerkinElmer, USA) to which was attached a flame ionization detector. For determination of total solids (TS) and volatile solids, a standard method by the Finnish Standard Association (1990) was used

while total phenolic content of the samples were determined by using a microtube test which was followed by a 4-amino antipyrine colourimetric test [37].

2.4. Biochemical methane potential (BMP) tests

In order to measure the BMP value of the substrate (El-g + PM), a setup that mimics the Automatic Methane Potential Test system (AMPTS II, Bioprocess engineering, Lund, Sweden) was employed. The same cattle rumen content intended for the anaerobic digestion was used as inoculum in which the test was carried out using 500 mL bottles each having a total of 300 mL volume with a VS load of 3 g VS/100 mL liquid. Addition of substrate (El-g + PM) was done at a ratio of 1:2 (g VS Substrate/g VS inoculum) [7,38–41], after which the bottles were flushed with pure nitrogen in order to make the environment anaerobic. Continuous stirring was applied to the content of the bottles at 150 rpm and at temperature of 37 °C. Gas was produced at a steady rate until the 35th experimental day and it was terminated. After termination, measurement of pH was carried out using a pH meter (WTW pH 320, Christian Berner AB, Partille, Sweden) in order to ascertain the potential drop in pH and its possible effects on the activities of methanogens.

2.5. Anaerobic digestion

About 4 kg pretreated El-g was thoroughly mixed with equal mass of PM and further diluted with water to form slurry. Cattle rumen content (1 kg) was then added to provide adequate microbial floral for the anaerobic digestion [42–43]. The slurry was then fed into the automated digesters through the inlet to occupy two-third of the digestion tanks in order to leave space for gas build-up and collection. To serve as control, another experiment involving the combination of raw (Not pretreated) El-g and PM in equal mass i.e. 4 kg each was set up along with the pretreated experiment each of which was carried out in duplicate. The total solid content of the co-substrates i.e. El-g and PM were set at 50% prior to the digestion. In order to monitor the anaerobic treatment efficiently, different process parameter such as temperature, pH as well as chemical analysis of the substrate were evaluated at interval [35,42–44]. The digester was allowed to run for a 40-day period under mesophilic condition. The volume of biogas generated from the process was measured daily by water displacement method.

2.6. Energy balance assessment

In this study, an energy assessment of the co-digestion of El-g and PM was carried out in order to assess the feasibility of alkaline pretreatment on El-g based on yield of methane after digestion using a standard method proposed by Veluchamy and Kalamdhad [45]. In order to calculate the energy, input for alkaline pretreatment, the formula used is found below:

$$Qin = \rho VC(Tp - Ta) + w(trise + thold) - \mho\rho VC(Tp - Tr) \quad (1)$$

Where Q_{in} = energy input for alkaline pretreatment in kilojoule (kJ);
ρ = density of El-g (kg/m^3);
V = reactor's working volume in meter cube (m^3);
C = specific heat capacity (kJ/kg. °C);
T_p = temperature applied for pretreatment (37 °C);
T^a = Room temperature (27 °C);
w = heater's power requirement in kilojoule per hour (kJ/h);
t_{rise} = required time to attain pretreatment temperature (h);
t_{hold} = entire pretreatment time (h);
$\mho$ = heat recovered from El-g;
T_r = temperature for digestion (°C).

The energy output from the co-digestion of El-g and PM was calculated from the methane yield using the formula below:

$$Qo = RCH4\mho V\rho\eta \quad (2)$$

Where Q_o = output energy from the co-digestion of El-g and PM (kJ);
R_{CH4} = methane yield (m^3 CH_4/kg VS);
$\mho$ = lower gross calorific value of methane (kJ/m^3 CH_4);
V = reactor's working volume (m^3);
ρ = density of El-g (kg/m^3);
η = percentage energy conversion capacity assumed to be 90%.

2.7. Kinetic studies

The fitness of the results obtained from the anaerobic co-digestion of El-g and PM were confirmed using four different kinetic models including the Gompertz model [46–51], cone model [46,51], Fitzhugh model [46] and the logistic kinetic model [52] as shown below:

Gompertz model:

$$\boldsymbol{S} = T[1 - exp\ (-vt)], \quad (3)$$

Cone model:

$$S = \frac{T}{1 + (vt)^{-n}}, \quad (4)$$

Fitzhugh model:

$$S = T[1 - exp\ (-vt)^n], \quad (5)$$

Logistic kinetic equation:

$$S = \frac{a}{1 + b_{exp}(-vt)}, \quad (6)$$

Where:
S = cumulative biogas production (ml/g VS);
T = substrate's biogas potential (ml/g VS);
v = rate constant of biogas production (d^{-1});
n = shape factor dimensionless;
a & b = constants.

In all the models, assumption was made that the biogas production kinetics is proportional to the growth rate of bacteria and archaea responsible for the bioconversion of substrates inside the reactors [53]. Thus, the determination coefficient (R^2) and root mean square error (RMSE) which is a description of the standard deviation value between estimated and observed biogas yield were employed for the evaluation of all models and comparison of their fitness [51].

2.8. Analysis of microbial community

In order to determine the diversity of microorganisms (Bacteria and archea), samples were taken from the raw substrates, mixed sludge and digestates for the analyses of microbial community of the digesters. A total of 45 mL was taken from each sample at 5-day interval starting from the fifth experimental day and refrigerated at −20 °C. Extraction of the total genomic DNA from all samples was carried out using a standard method [54] followed by a conventional polymerase chain reaction (PCR) in order to capture the whole bacteria in each sample by using specific primers i.e. P338f and P518r [55–56]. After DNA extraction, the qualities of the DNA and products of the PCR were verified using gel electrophoresis after which a real-time PCR was conducted employing a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Carlsbad, CA). This gave room for the analyses of the entire bacteria and methanogens which include members of *Methanobacteriales, Methanomicrobiales* and *Methanosarcinaceae* [57]. The real-time PCR products were checked for quality by examining all parameters obtained from the software.

2.9. Statistical analysis

All regression analyses were carried out on the Microsoft excel software package 2010 while the Solver function of the excel was used for the models' kinetic non-linear regression determination [49]. For the

values obtained from physicochemical and structural analyses, comparison of mean was carried out by Analysis of variance (ANOVA) and the Tukey's test.

3. Results

3.1. Physicochemical characteristics of feedstock

The results of the physical and chemical analysis of the El-g (Raw and pre-treated) and that of the mixed substrates (before and after the digestion) are presented in Tables 1–3 respectively. The automated digester was set at a temperature range of 35 °C to 40 °C while the pH was set between 6.5 and 8.0 throughout the experiments. Table 1 reveals significant components of *Pennisetum purpureum* before and after pre-treatment as Total Nitrogen, Total Alkalinity, Total Ammonia, Chemical Oxygen Demand (COD), Total Phosphorus, Potassium, Phosphate, Sulfate, Calcium, Magnesium, Manganese, Iron, Zinc, Aluminium and Copper. While some of these physicochemical properties reduced after pretreatment, others especially Calcium and nitrogen which are required for microbial metabolism increased after pretreatment. However, calcium and nitrogen contents in the substrates reduced after anaerobic digestion which shows utilization of the nutrients for microbial activity during the digestion process. Table 1 also show the values obtained for fatty acids, sugars and phenols which all increased after the application of pretreatment due to the enormous solubilization of the structural components of El-g.

Table 1
Physicochemical characteristics of raw and pretreated *Pennisetum purpureum* and that of Piggery dung.

Parameters	Raw Elephant grass	Pre-treated Elephant grass	Piggery dung
Total solids (%)	68.2 ± 3.01[a]	55.6 ± 2.01[b]	18.7 ± 1.11
Fixed solids (%)	1.2 ± 0.01[a]	1.3 ± 0.02[a]	16.8 ± 0.02
Volatile solids (%)	51.5 ± 2.02[a]	75.2 ± 3.01[b]	83.2 ± 4.03
Moisture content (%)	94.22 ± 0.10[a]	96.31 ± 4.10[a]	81.3 ± 5.10
Calcium (Ca) (g/kg TS)	170.00 ± 0.10[a]	200.00 ± 0.10[a]	249.40 ± 5.11
Aluminium (Al) (g/kg TS)	2.2 ± 1.01[a]	2.15 ± 0.10[a]	3.05 ± 0.10
Copper (Cu) (g/kg TS)	1.50 ± 0.10[a]	1.25 ± 0.01[a]	3.10 ± 0.11
Manganese (Mn) (g/kg TS)	0.00 ± 0.10[a]	0.006 ± 0.00[b]	0.008 ± 0.01
Magnesium (Mg) (g/kg TS)	21.00 ± 0.01[a]	24.00 ± 0.12[a]	27.20 ± 0.12
Potassium (K) (g/kg TS)	7.5 ± 0.01[a]	9.10 ± 0.11[b]	11.61 ± 0.11
Sulphate (SO_4) (g/kg TS)	47.00 ± 0.12[a]	44.00 ± 0.01[a]	31.71 ± 0.01
COD (g COD/g VS)	0.00 ± 0.10	ND	680 ± 8.22
Total Nitrogen (N) (g/kg TS)	22.5 ± 4.02[a]	27.10 ± 2.01[b]	26.9 ± 3.11
Total Carbon (C) (g/kg TS)	354.3 ± 9.02[a]	467.7 ± 8.01[b]	276.2 ± 2.01
C/N	16:1	17:1	10:1
Total Phosphorus (P) (g/kg TS)	6.5 ± 0.03[a]	7.69 ± 0.01[a]	9.03 ± 1.01
Total Ammonia (NH_3) (g/kg TS)	0.44 ± 0.11[a]	3.40 ± 0.10[b]	7.33 ± 0.10
Iron (Fe) (g/kg TS)	4.70 ± 0.10[a]	5.50 ± 0.01[a]	7.50 ± 0.01
Nickel (Ni) (g/kg TS)	1.70 ± 0.01[a]	1.92 ± 0.10[b]	3.20 ± 0.21
Zinc (Zn) (g/kg TS)	2.53 ± 0.10[a]	1.95 ± 0.10[b]	25.0 ± 2.02
Chromium (Cr) (g/kg TS)	0.72 ± 0.01[a]	1.27 ± 0.01[b]	0.67 ± 0.01
Phosphate (PO_4) (g/kg TS)	ND	0.80 ± 0.10	1.83 ± 1.10
Total alkalinity (g/kg TS)	175.00 ± 0.11[a]	187.80 ± 0.10[b]	280 ± 6.41
Total Lignin (% m.m^{-1})	29.6 ± 0.02[a]	9.2 ± 0.01[b]	7.2 ± 0.01
Cellulose (% m.m^{-1})	27.3 ± 0.30[a]	40.6 ± 1.02[b]	5.0 ± 0.01
Hemicellulose (% m.m^{-1})	24.3 ± 1.01[a]	10.3 ± 0.11[b]	1.5 ± 0.01
Extractives (% m.m^{-1})	9.7 ± 0.01[a]	4.2 ± 0.10[b]	0.4 ± 0.01
Acetate (g COD/g VS)	0.08 ± 0.10	0.11 ± 0.01	1.07 ± 0.10
Propionate (g COD/g VS)	0.07 ± 0.11	0.11 ± 0.01	1.06 ± 0.02
TVFAs (g COD/g VS)	0.12 ± 0.10	1.14 ± 0.10	2.46 ± 0.10
Uronic acids (% VS)	1.61 ± 1.10	2.04 ± 0.10	1.73 ± 0.01
[@]Soluble sugars (% VS)	4.32 ± 1.00	8.96 ± 1.10	4.30 ± 0.01
Phenols (mg L^{-1})	0.001 ± 0.01	0.003 ± 0.10	4.65 ± 0.10

ND = Not detectable; Values shown in table are means of triplicate analyses; superscripts with same letters are not significantly different.

Table 2
Physicochemical characteristics of mixed substrate (Pretreated) before and after digestion.

Parameters	Before digestion	After digestion
pH	6.50 ± 0.02[a]	7.80 ± 0.01[b]
Calcium (Ca) (g/kg TS)	185.00 ± 0.10[a]	100.00 ± 0.12[b]
Aluminium (Al) (g/kg TS)	1.22 ± 0.10[a]	0.23 ± 0.10[b]
Copper (Cu) (g/kg TS)	3.30 ± 0.10[a]	2.90 ± 0.01[b]
Manganese (Mn) (g/kg TS)	0.008 ± 0.10[a]	0.009 ± 0.10[a]
Magnesium (Mg) (g/kg TS)	26.00 ± 0.02[a]	22.00 ± 0.12[b]
Potassium (K) (g/kg TS)	9.70 ± 0.11[a]	3.40 ± 0.10[b]
Sulphate (SO_4) (g/kg TS)	49.00 ± 0.01[a]	41.00 ± 0.02[b]
COD	151.00 ± 3.12[a]	82.50 ± 0.10[b]
Total Carbon (C) (g/kg TS)	371.3 ± 6.02[a]	193.1 ± 4.05[b]
Total Nitrogen (N) (g/kg TS)	21.50 ± 0.01[a]	14.90 ± 0.02[b]
Total Phosphorus (P) (g/kg TS)	8.21 ± 0.01[a]	2.30 ± 0.10[b]
Total Ammonia (NH_3) (g/kg TS)	3.70 ± 0.10[a]	5.30 ± 0.11[b]
Iron (Fe) (g/kg TS)	8.10 ± 0.11[a]	4.90 ± 0.01[b]
Volatile Solids (%)	79.04 ± 0.10[a]	47.20 ± 0.10[b]
Total Solids (%)	49.40 ± 0.22[a]	38.7 ± 0.20[b]
Moisture Content (%)	90.00 ± 0.12[a]	92.31 ± 0.10[a]
Nickel (Ni) (g/kg TS)	4.80 ± 0.10[a]	4.50 ± 0.10[a]
Zinc (Zn) (g/kg TS)	11.27 ± 0.10[a]	8.45 ± 0.10[b]
Phosphate (PO_4) (g/kg TS)	0.92 ± 0.10[a]	0.37 ± 0.12[b]
Total alkalinity (g/kg TS)	220.00 ± 0.10[a]	270.00 ± 0.10[b]
C/N	17/1	13/1
Ash Content (%)	6.76 ± 0.12[a]	5.23 ± 0.11[b]
Total Lignin (% m.m^{-1})	9.2 ± 0.01[a]	6.2 ± 0.01[b]
Cellulose (% m.m^{-1})	40.6 ± 1.02[a]	27.8 ± 1.00[b]
Hemicellulose (% m.m^{-1})	10.3 ± 0.11[a]	5.6 ± 0.01[b]
Extractives (% m.m^{-1})	4.2 ± 0.10[a]	1.8 ± 0.00[b]
Acetate (g COD/g VS)	0.11 ± 0.01	0.09 ± 0.01
Propionate (g COD/g VS)	0.11 ± 0.01	0.08 ± 0.01
TVFAs (g COD/g VS)	1.14 ± 0.10	1.12 ± 0.10
Uronic acids (% VS)	2.04 ± 0.10	2.01 ± 0.10
[@]Soluble sugars (% VS)	8.96 ± 1.10	8.97 ± 1.10
Phenols (mg L^{-1})	0.003 ± 0.10	0.002 ± 0.10

Values shown in table are means of triplicate analyses; superscripts with same letters are not significantly different.

The chromatographic analyses for the determination of structural components of the raw El-g showed its composition to be 29.6 ± 0.02, 27.3 ± 0.30, 24.3 ± 1.01 and 9.7 ± 0.01 for total L-C–H and extractives respectively (Table 1). The composition in the pretreated El-g sample however was 9.2 ± 0.01, 40.6 ± 1.02, 10.3 ± 0.11 and 4.2 ± 0.10 for the three structural components i.e. L-C–C and the extractives respectively. As shown in table 1, the composition of L-C–H ad extractives in PM are 7.2 ± 0.01, 5.0 ± 0.01, 1.5 ± 0.01 and 0.4 ± 0.01 respectively. After the alkaline pretreatment, lignin and hemicellulose contents of El-g were reduced by 69% (from initial value of 29.6 to a final value of 9.2 (% m.m^{-1}) and 58% (from initial value of 24.3 to a final value of 10.3 (% m.m^{-1}) respectively while the content of cellulose was increased by 33% (from initial value of 27.3 to a final value of 40.6 (% m.m^{-1}) as a result of the application of the pretreatment.

Table 2 reveals the characteristics of the pretreated mixed substrates (El-g + PM) before and after anaerobic digestion as seen in this study.

Table 3 reveals the characteristics of the untreated mixed substrates (El-g + PM) before and after anaerobic digestion as seen in this study.

Table 3
Physicochemical characteristics of mixed substrate (Untreated) before and after digestion.

Parameters	Before digestion	After digestion
pH	6.55 ± 0.01[a]	7.75 ± 0.01[b]
Calcium (Ca) (g/kg TS)	161.6 ± 3.00[a]	106.12 ± 0.12[b]
Aluminium (Al) (g/kg TS)	1.12 ± 0.10[a]	0.21 ± 0.10[b]
Copper (Cu) (g/kg TS)	2.36 ± 0.01[a]	2.04 ± 0.01[b]
Manganese (Mn) (g/kg TS)	0.005 ± 0.10[a]	0.003 ± 0.10[a]
Magnesium (Mg) (g/kg TS)	24.5 ± 0.02[a]	18.50 ± 0.12[b]
Potassium (K) (g/kg TS)	7.61 ± 0.01[a]	3.22 ± 0.10[b]
Sulphate (SO_4) (g/kg TS)	44.50 ± 0.01[a]	35.60 ± 0.02[b]
COD	133.41 ± 2.10[a]	81.80 ± 0.01[b]
Total Carbon (C) (g/kg TS)	296.61 ± 4.01[a]	150.14 ± 5.01[b]
Total Nitrogen (N) (g/kg TS)	18.60 ± 0.01[a]	11.51 ± 0.02[b]
Total Phosphorus (P) (g/kg TS)	6.51 ± 0.05[a]	2.11 ± 0.10[b]
Total Ammonia (NH_3) (g/kg TS)	2.90 ± 0.10[a]	1.66 ± 0.11[b]
Iron (Fe) (g/kg TS)	8.10 ± 0.11[a]	5.05 ± 0.01[b]
Volatile Solids (%)	71.44 ± 0.11[a]	36.50 ± 0.10[b]
Total Solids (%)	40.11 ± 0.12[a]	25.7 ± 0.20[b]
Moisture Content (%)	79.13 ± 0.12[a]	93.51 ± 2.11[a]
Nickel (Ni) (g/kg TS)	3.18 ± 0.10[a]	2.33 ± 0.10[a]
Zinc (Zn) (g/kg TS)	10.70 ± 0.10[a]	6.51 ± 1.10[b]
Phosphate (PO_4) (g/kg TS)	0.81 ± 0.10[a]	0.17 ± 0.12[b]
Total alkalinity (g/kg TS)	200.45 ± 0.10[a]	146.51 ± 0.10[b]
C/N	16/1	13/1
Ash Content (%)	6.06 ± 0.10[a]	5.23 ± 0.11[b]
Total Lignin (% $m.m^{-1}$)	29.6 ± 0.02[a]	23.1 ± 1.01[b]
Cellulose (% $m.m^{-1}$)	27.3 ± 0.30[a]	19.4 ± 1.01[b]
Hemicellulose (% $m.m^{-1}$)	24.3 ± 1.01[a]	14.7 ± 0.01[b]
Extractives (% $m.m^{-1}$)	13.7 ± 0.01[a]	8.2 ± 0.01[b]
Acetate (g COD/g VS)	0.08 ± 0.10	0.05 ± 0.10
Propionate (g COD/g VS)	0.07 ± 0.11	0.06 ± 0.01
TVFAs (g COD/g VS)	0.12 ± 0.10	0.10 ± 0.10
Uronic acids (% VS)	1.61 ± 1.10	1.58 ± 0.01
[@]Soluble sugars (% VS)	4.32 ± 1.00	4.21 ± 1.00
Phenols (mg L^{-1})	0.001 ± 0.01	0.001 ± 0.01

3.2. Gas production

Biogas generation commenced on days 5 and 7 for the pretreated and untreated experiments respectively and continued at an increasing rate till days 30 and 24 respectively for both experiments after which the production of gas began to diminish till the end of the digestion period as shown in Fig. 1. The cumulative biogas recorded from both pretreated and untreated experiments were 409.5 and 306.2 m^3 CH_4/kg VS respectively giving an average of 11.07 m^3 CH_4/kg VS/day for the experiment with prior pretreatment before digestion. This further showed a biogas yield increase of 25.2% by the pretreated biomass over the untreated one in co-digestion with PM. The produced biogas from the alkaline pretreated experiment showed composition ranging between 63 ± 1.4 and 69 ± 1.8 methane, 24 ± 2.6 and 32 ± 1.5 carbon dioxide while lower hydrogen sulfide value ranging between 19 ± 1.4 and 23 ± 0.2 were recorded. For the untreated experiment, methane content of between 56 ± 2.1 and 61 ± 1.5; carbon dioxide of 25 ± 1.6 and 30 ± 2.5 and hydrogen sulfide value ranging between 15 ± 1.4 and 20 ± 0.2 were all recorded.

3.3. Kinetic model and regression study

In order to predict biogas yield in this study, four major kinetic models were employed with various kinetic parameters based on the anaerobic co-digestion of El-g and PM. Results obtained from each kinetic model based on the R^2 and RMSE values are all in the acceptable range compared to previous studies.

4. Discussion

4.1. Physicochemical component of biomass

The pH of the digesters throughout the digestion period was in the range of 6.5 and 8.0. Microbes responsible for biogas generation operate optimally at a pH range of 6.5 to 7.5, any pH above or less than this is hazardous to the survival and actions of these organisms [58]. Research has it that the population and activities of most methanogens tend to increase at alkaline pH. The pH range recorded in this study agrees with the reported range between 6.5 and 8.0 for efficient functioning of methanogens. This is so critical that pH of <6.5 or >8.5 is capable of causing failure of the methanogenic stage of anaerobic process [3,59]. The Mesophilic temperature range was maintained throughout the digestion process. Temperature is a key factor in anaerobic digestion, which directly affects the activities of microbes. Therefore, deviating from the appropriate temperature may affect the efficiency of the digester and optimal production of biogas [60]. Maintenance of adequate temperature range increases process stability and abundance of microbes in the fermentation medium [61].

The composition of structural materials i.e. total L-C-H and those of

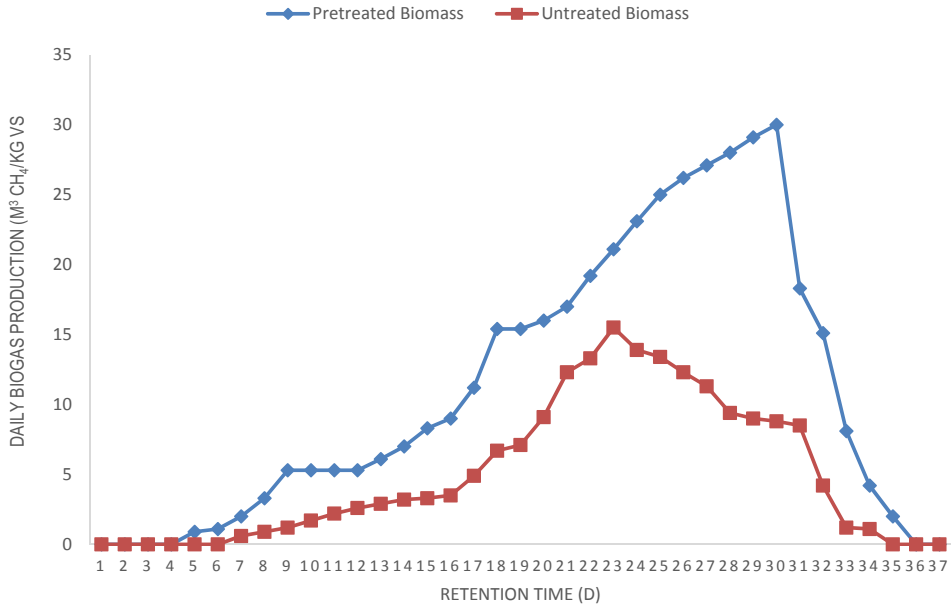

Fig. 1. Graph showing daily biogas yield from co-digestion of *Pennisetum purpureum* and piggery dung.

fixed solids and extractives reported in this study are similar to values obtained in their studies [62–63]; Venturin et al., 2018). Cai et al. [64], reported values of 21.4, 43.4 and 19.5% respectively for total lignin, cellulose and hemicelluloses from the analyses of different corn parts. Similarly, Li et al. (2016) analyzed evaluated the structural composition of the different parts of corn and reported 20.0, 34.0, 24.0 and 2.0% respectively for total lignin, cellulose, hemicellulose and fixed solids. In the reports of Venturin et al. (2018) on corn stalk, values of 18.9, 32, 23.5 and 3.8% were respectively obtained for lignin, cellulose, hemicellulose and fixed solids. All these values show slight similarities between the structural composition of El-g and those of corn stalk which is also a lignocellulose.

The nutrient characteristics of the El-g is comparable with those of other rich succulent plants earlier harnessed for biogas production. The nutrient content and rich elemental composition in the combination of El-g and PM makes it highly digestible for microbes ensuring availability of diverse microbes and with the ultimate effect being increased yield of biogas. The results obtained from the physicochemical analysis of substrate shows that El-g and PM is rich in vitamins and minerals. The results revealed that after the application of pretreatment to El-g, lignocellulosic bonds were broken hence important components of the substrate increased which includes; Calcium (Ca), Manganese (Mn), Potassium (K), Total Nitrogen (N) and Total Ammonia (NH_3), each of which was in sufficient amount, for microbes to proliferate, thrive, and grow. The results also indicated that the digestate is rich in vitamins and minerals hence would be a good source of fertilizer (used as biofertilizer) especially on nutrient depleted soils. The table also revealed the significant components of the digestate such as phosphorus (P), potassium (K), sulfate (SO_4), magnesium (Mg), iron (Fe), zinc (Zn), aluminium (Al), copper (Cu), manganese (Mn) and nickel (Ni) all of which increased in the digestate than the raw biomass hence making it a rich biofertilizer. Also, components such as calcium (Ca), nitrogen (N), ammonia (NH_3), phosphate and COD were utilized by microbes to generate biogas hence their reduced concentrations after the digestion period. In the experiment with prior pretreatment, gas production commenced on the 5th d, at a constant temperature of 35 °C and steadily increased daily until the 30th d whereas, production commenced on the 7th d and peaked on the 24th d in the experiment without biomass pretreatment. There are several factors that could be responsible for the delay in biogas generation observed in the two experiments which include acclimatization of the microbes to the new environment and components or nature of the substrates. Besides, the first two stages of digestion i.e. enzymatic hydrolysis and acidogenesis are acid formation stages of anaerobic digestion which makes the pH value slightly acidic. During this period, microbial growth is hindered because of inability of the microorganism to catabolize the acid being produced. However, as the methanogenic stage begins much later, the pH value continues to increase until the substrate becomes alkaline and this marks the commencement of methane generation. After the 30th 24th d for the pretreated and untreated experiments respectively, biogas generation began to decline until it finally ceased. This is due to the reduction in the action of biogas-producing microorganisms which is possibly consequential of the diminishing available nutrients. The total gas production at the end of the experiment is comparable to yields from other prominent sources used for gas production such as food waste, sewage sludge, Mexican sunflower, poultry droppings, water melon waste etc. [3–4,15,60,65] but higher than that of maize silage and lemon grass, hence the combination of El-g and PM is a good substrate for biogas production [66]. This high yield is an evidence of synergy between *Pennisetum purpureum* and PM in terms of carbon–nitrogen (C/N) balance and availability of required nutrient. High C/N ratio is an indication of deficiency in the available nitrogen for microbial activity, while low C/N ratio also suggests insufficient energy source for microbial environment. Among other factors, efficient anaerobic digestion depends on the provision of adequate C/N ratio for efficient microbial activity [65]. The C/N ratio recorded in this study is similar to some previously reported values [4,67] but lower than value documented by Riggio et al. [68].

4.2. Energy balance assessment results

From the results obtained from the laboratory experiments, the energy balance for pretreated El-g was determined. For the pretreatment of El-g, a total of 2822 kJ energy was estimated to be required, while a total of 4913 kJ was given as the output energy after NaOH alkaline pretreatment. The net energy obtained therefore was 2091 kJ which is reasonably high but can still be increased if heating used for pretreatment can be obtained via solar system. Being a renewable and sustainable energy source, this will greatly cushion the effect of energy cost during biomass pretreatment thereby improving the overall net energy gain. Even though it is often difficult to accurately quantify the economic feasibility of applying alkaline pretreatments to lignocelluloses based on biomethane alone, the inclusion of other by-products of the AD system in the overall calculation of the profitability of the process could be a veritable way of justifying the investments into pretreatment using alkali. These by-products include bio-hydrogen (A rapidly emerging alternative energy source), digestate and carbon dioxide.

4.3. Gompertz model

The cumulative biogas yield predicted by the Gompertz model were 419.4 and 317.3 m^3 CH_4/kg VS for the pretreated and untreated experiments respectively which were both higher than the observed biogas yields from the laboratory experiments. For both experiments, the biogas production rate (v) were within the ranges 0.0245 d^{-1} to 0.0746 d^{-1}. Prior to now, the v-values reported for the AD of different waste materials for Gompertz model has been below 0.15 d^{-1} [51]. For the R^2, values of 0.9141 to 0.9778 were obtained for the pretreated and untreated experiments respectively which are similar to previous value of between 0.911 and 0.966 [50] while the RMSE values ranged between 0.8958 and 6.4788 which are also similar to some previous findings where values less than 19.2 were obtained [51].

4.4. Cone model

The biogas yield prediction by the cone model was also within acceptable limits with yield of 426.2 and 322.1 m^3 CH_4/kg VS. Both v and n values were within the ranges 0.0562 to 0.1244 d^{-1} and 1.0326 to 3.4559 respectively which both corroborates earlier submissions of less than 0.24 d^{-1} as v-value [51] and 3.17 for n-value [46]. The R^2 and RMSE values both ranged between 0.9720 to 0.9941 and 0.9522 to 5.2144, respectively agreeing with previous values of 0.9592 to 0.9929 for R^2 and below 12.1 for RMSE [51].

4.5. Fitzhugh model

The results obtained from the Fitzhugh model showed predicted biogas yield to be 439.9 and 331.4 m^3 CH_4/kg VS while the v values ranged between 0.0244 and 0.0541 d^{-1} with corresponding n values of between 0.9324 and 1.4481. These values are in the same range with a previous v and n values of less than 0.30 and 4.81 respectively [46]. The obtained R^2 and RMSE values were between 0.8949 and 0.9879, and 0.9994 to 9.8657 respectively which agrees with previous values of 0.738 and 0.992 obtained for R^2 when Fitzhugh model was applied [62].

4.6. Logistic kinetic equation

The logistic model predicted biogas yield in both pretreated and untreated experiments to be 413.2 and 311.5 m^3 CH_4/kg VS with v values ranging from 0.1433 to 0.463 d^{-1} while the R^2 values were between 0.9778 and 0.9905 which are highly comparable with v-values of between 0.1249 and 0.1766 d^{-1} and R^2 of between 0.9775 and 0.9859 [52].

In all the kinetic models used in this study, lower values for both R^2 and RMSE gives the best fit with the results of the BMP experiment. In both pretreated and untreated experiments, the cone model gave the best fit in comparison with observed cumulative biogas yield with R^2 of 0.9941 and RMSE of 0.9522.

4.7. Proposed regression equation

For the results of this study, a regression analysis was done with the aid of a commercial software package (Data fit 9.0) while employing the observed values from the cumulative biogas yield in both experiments i.e. pretreated and untreated El-g in co-digestion with PM taking into action the number of experimental d. Equation 7 summarizes the biogas yield (D_O) which depends on the substrates i.e. El-g and PM, their combinations and the hydraulic retention time.

$$D_O = A + BX_1 + CX_2 + DX_1^2 + EX_2^2 + FX_1X_2 + GX_1^3 + HX_2^3 + IX_1X_2^2 + JX_1^2X_2 7$$

Where: D_O = Biogas yield

X_1 = the hydraulic retention time in d,

X_2 = the ratio of the reactor which range between 1 and 2 for Reactor 1 given by X2 = 1, and for Reactor 2 given by X2 = 2

A to J = Constant values

Equation (2) gives the cumulative biogas yield for both pretreated and untreated El-g in co-digestion with PM. Based on this, a proposed biogas yield of 432.5 and 327.3 m^3 CH_4/kg VS was made for from the co-digestion of pretreated and untreated El-g and PM. From this, the R^2 value for the proposed equation was 0.9533 with RMSE value of 2.6416. This shows high stability of the proposed model since the R^2 value is above 0.9 which is equivalent of 90% [50]. Figs. 2 and 3 shows the graphical representations of the model's prediction for the biogas yield from both pretreated and untreated co-digestion of El-g with PM.

5. Conclusion

The study showed that Elephant grass (*Pennisetum purpureum*) is a suitable candidate for biogas generation in co-digestion with piggery manure. The chemical and structural composition of the grass present enormous nutrients and elemental composition needed for microbial fermentation for the production of bioenergy. The application of alkaline pretreatment caused a pronounced solubilization of the structural materials as lignin and hemicellulose contents of El-g were reduced by 69% (from initial value of 29.6 to a final value of 9.2 (% $m.m^{-1}$) and 58% (from initial value of 24.3 to a final value of 10.3 (% $m.m^{-1}$) respectively while the content of cellulose was increased by 33% (from initial value of 27.3 to a final value of 40.6 (% $m.m^{-1}$) as a result of the application of the pretreatment. The pretreated experiment produced 25.2% more biogas than the untreated experiment. The study also reveals that co-digestion with piggery dung enhanced the biogas-producing capacity of *Pennisetum purpureum*, as a high quantity of gas was produced resulting from the synergy of both substrates in terms of carbon–nitrogen ratio and availability of required methanogenic bacteria. Therefore, the use of alkaline pretreatment for El-g is hereby solicited in its biotechnological conversion to biogas which can be used for various energy supplies especially in regions where the grass is abundant and where it is been regarded as a weed. The co-digestion of the plant with poultry manure which is another abundant and cheap bioresource is therefore advocated especially in developing world

CRediT authorship contribution statement

O.J. Ojediran: Carried out the project administration and supervision. **S.O. Dahunsi:** Conceptualization, Supervision, Resources, Visualization, Writing and Editing. **V. Aderibigbe:** Formal analysis, investigation and Resources. **S. Abolusoro:** Data curation. **A.T. Adesulu-Dahunsi:** Methodology, Investigation and Visualization. **E.L. Odekanle:** Formal analysis, writing, reviewing and final editing. **O.J. Odejobi:** Validation, Visualization. **R.A. Ibikunle:** Investigation, Software. **J.O. Ogunwole:** Writing – review and editing.

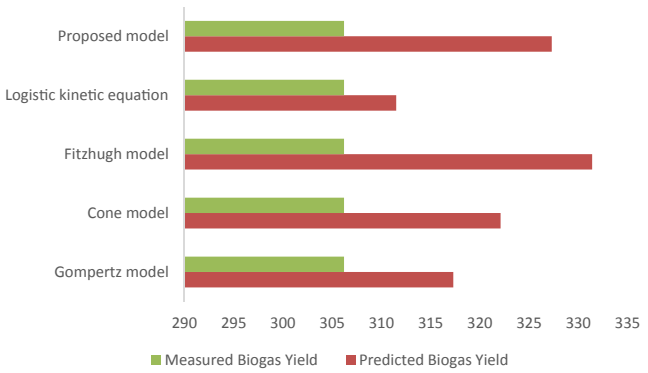

Fig. 2. Observed and predicted biogas yield from the anaerobic co-digestion of pretreated Elephant grass and poultry manure.

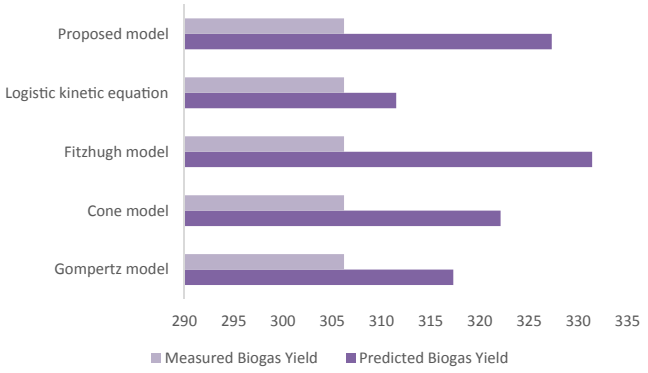

Fig. 3. Observed and predicted biogas yield from the anaerobic co-digestion of untreated Elephant grass and poultry manure.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Further reading

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