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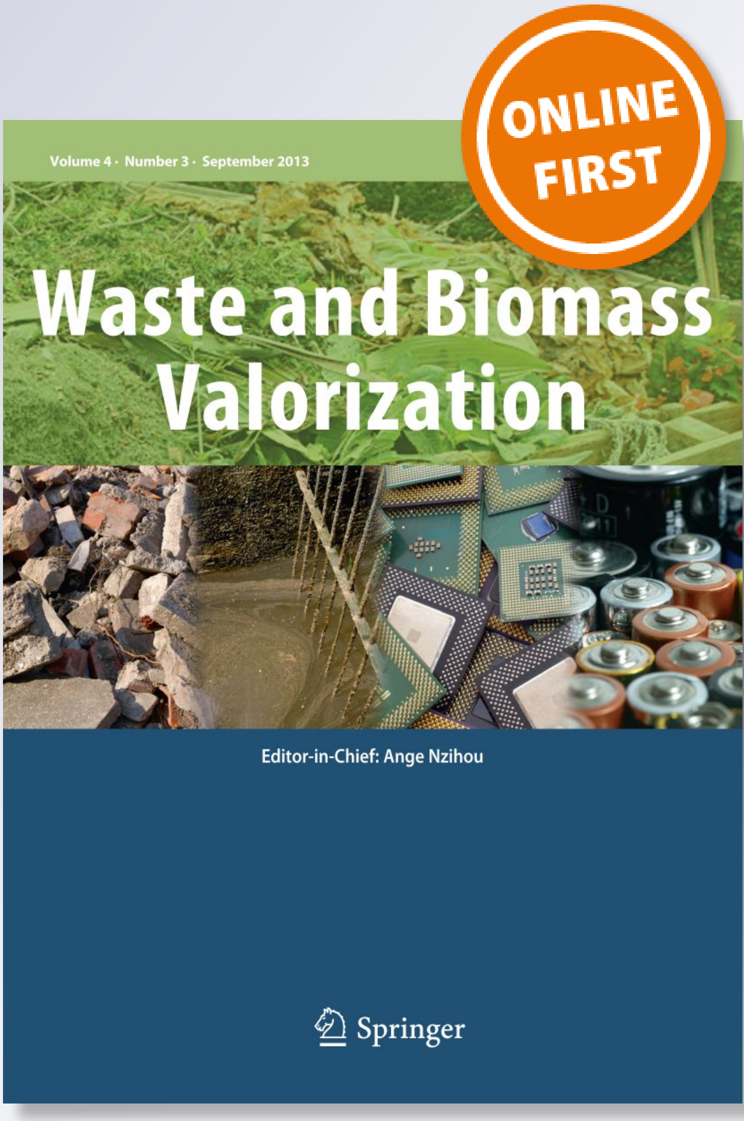

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ORIGINAL PAPER

Optimizing Methane Production from Co-digestion of Cassava Biomass and Winery Solid Waste Using Response Surface Methodology

Unathi Mkruqulwa[2] · Vincent Okudoh[1] · Oluwaseun Oyekola[2]

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Abstract

Renewable energy security for the future and better use of natural resources are key challenges that can be concurrently managed by a practical anaerobic co-digestion approach where substrates with a high carbon/nitrogen (C/N) ratio are combined with lower ones to create a balance of the nutrients during the production of methane. This study determined the optimal conditions for methane production from anaerobic co-digestion of cassava biomass (CB) and winery solid waste (WSW) using response surface methodology (RSM). A three-factor central composite design was used to set-up the anaerobic co-digestion experiments. The individual and interactive effects of temperature (25–45 °C), pH 6–8 and a range of substrate (CB/WSW) ratios (0–100) on the methane yield were explored. Optimisation using RSM showed a close fit between the predicted and experimental data as indicated by the coefficient of determination (R^2) value of 0.9521. The RSM model predicted a maximum methane yield of 346.28 mL CH_4/g VS_{added} for the optimal conditions of pH 7, temperature of 35 °C ± 0.5 and 70/30 ratio of CB/WSW. The verification experiment produced 396 mL CH_4/g VS_{added}, 12.6% higher than the predicted value at the same conditions. Although, there was a gap between the predicted and actual yield, the significance of the variables in the analysis of variance (ANOVA) shows the model could be relevant to similar research. The substrate ratio at 70 CB:30 WSW was the most significant factor during methane production. The RSM model proved successful in the optimisation process of methane yield.

Keywords Cassava · Co-digestion · Methane · Response surface method · Winery solid waste

Abbreviations

CB	Cassava biomass
WSW	Winery solid waste
CCD	Central composite design
RSM	Response surface methodology
AD	Anaerobic digestion
CH_4	Methane
GHG	Greenhouse gas
UN	United Nations
C/N	Carbon to nitrogen ratio
ZD	Zebra dung
ARC	Agricultural Research Council
RSA	Republic of South Africa
ANOVA	Analysis of variance
TSs	Total solids
VSs	Volatile solids
TOC	Total organic carbon
MC	Moisture content
ICP	Inductively coupled plasma
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
VFA	Volatile fatty acid
H_2SO_4	Sulphuric acid

✉ Vincent Okudoh
okudohv@cput.ac.za

[1] Department of Biotechnology, Faculty of Applied Sciences, Cape Peninsula University of Technology, Cape Town, South Africa

[2] Department of Chemical Engineering, Faculty of Engineering and Built Environment, Cape Peninsula University of Technology, Cape Town, South Africa

Statement of Novelty

There are no previous reports on optimisation of methane yield from anaerobic co-digestion of cassava biomass (CB) and winery solid waste (WSW) using response surface methodology (RSM). Substrate ratio was identified as the most significant factor in improving methane production from these substrates. The presence of phenolics in the WSW was suggested to be inhibitory to methane production. Remarkably, higher methane yield than RSM predicted was recorded under the interactive effects of pH, temperature and substrate ratio. This is an invaluable data in describing the optimal conditions for up-scaling of anaerobic digestion (AD) plants especially in agricultural and winery farms.

Introduction

In the past decades, fossil fuel have been the dominant source of energy for global economies. However, the demerits of its use far outweigh the merits due to its greenhouse gas (GHG) emission that contributes to global warming [1]. It also causes environmental pollution which is a major concern for environmental health practitioners. These problems have led the United Nations (UN) and many national governments to adopt strategies geared towards mitigating them. The UN sustainable goal no. 7 is clear on clean and affordable energy of which many renewable energy sources such as solar, wind, and bioenergy form part of the main energy mix for the future. Bioenergy from biomass is favoured due to the advantages of being a clean and sustainable energy supply source of which biogas is currently gaining more interest due to its production being carbon neutral [2]. Biogas is a gaseous fuel rich in methane (50–80%) and carbon dioxide, with traces of hydrogen sulphide (H_2S), ammonia (NH_3), hydrogen (H_2) and other gases [3]. It is produced through the biological transformation of a variety of organic substrates in the absence of oxygen, through a process called anaerobic digestion (AD) [4–6]. The quantity and quality of biogas produced usually depends on the type of substrates used [7].

Studies have shown that cassava (*Manihot esculenta* Crantz) tuber and its peels can be used for the high rate production of biogas/methane through AD, owing to their high carbohydrates composition [8, 9]. In most developing countries, cassava is used as food. However, in South Africa (SA) it is mainly used for animal feed and starch production (~20,000 tons per annum) and cultivated in Limpopo, Mpumalanga and northern KwaZulu-Natal [10]. Hence, the use of CB is sustainably available for energy production without affecting food security and other biomass applications in SA. On another note, the use of WSW as a substrate for the production of biogas/methane serves as an alternative waste disposal technique to the winery industry as it decreases pollution to the environment through its GHG emissions. SA is one of the largest wine producers (seventh in the world) with total annual harvest of approximately 1189 million litres in 2017 [11]. The wine making process generates huge amounts of both solid (grape pomace) and liquid waste [12]. Their disposal to the environment is detrimental through leaching of organic acids in pomace which poses a threat to soil and groundwater [12]. Therefore, the idea of converting the WSW into a renewable energy specifically biogas/methane was identified as a viable option in this study.

The main reason for co-digestion of substrates is to balance the carbon/nitrogen (C/N) ratio of the feedstock in the digester during AD and to alleviate inhibition [13]. The quality and yield of the biogas produced depends on the type and composition of the feedstock [14, 15]. Methanogens utilise the carbon present in a feedstock, 25–30 times faster than nitrogen, hence, a C/N ratio of 25–30:1 is generally recommended as optimal for methanogens in many AD studies [16–18]. This means that if a feedstock has a higher than normal C/N ratio, it will lead to rapid acidification as a result of decreased pH levels that is harmful to the methanogens. Similarly, if the C/N ratio is lower, it will lead to ammonia accumulation that is toxic to the organisms. Co-digestion then becomes a necessity to balance the C/N ratio when different feedstock are combined thereby helping to improve the yield of biogas [19]. According to Mkruqulwa et al. [20], CB has a higher C/N ratio than normal (129:1) as compared to WSW with C/N ratio of 29:1, hence the need for co-digestion to bring a balance in the digester operation [18]. Another reason for co-digestion is that co-substrates with high methane potential are usually digested to increase the methane yield [21–24]. The CB and WSW are rich in volatile solids (VSs, 83–98%) which aid in methane production [20]. Whilst CB is highly deficient in protein, nitrogen and iron content, the lack is compensated by higher amounts found in WSW which plays a stabilising role during AD [20].

The current commercial-scale methods of biogas/methane production, yield is in the range of 50–100% conversion of substrate to methane on a mass basis depending on the substrate. Carbohydrate-rich substrates yield higher amounts of methane than substrates with high concentrations of lignocellulose or nitrogenous materials. The input feedstock determines the composition of the biogas product. Carbohydrate substrates yield roughly 50% methane and 50% carbon dioxide, while more reduced (oxygen-poor) substrates yield higher proportions of methane [25]. The environmental conditions in which the biogas/methane production process takes place also influence the yield and rate of the reaction. Various factors such as temperature, pH, redox potential and the C/N ratio together with the ratio of co-substrates influences the yield of methane [26].

Several authors have used RSM to optimise the yield of methane produced during AD [27–29]. RSM is a statistical model conventionally used to understand the interactions between different parameters at varying levels and to evaluate the optimal level for each parameter for a particular response target [30]. It has lots of benefits such as enhancing the product yield, reducing the process variability, savings in the time and cost of running experiments, etc. [31, 32]. Sathish and Vivekanandan [28] used RSM for parametric optimisation for floating drum anaerobic biodigester to investigate the optimal conditions for methane yield from AD of agricultural waste and concluded that RSM was an efficient tool to develop models that could effectively predict methane production. Other studies have also demonstrated the reliability and advantage of employing statistical methods for the optimisation of AD. Yusof et al. [33] demonstrated that substrate ratio (poultry manure: food waste) had the most significant effect on the enhancement of methane production. Using RSM and artificial neural network, the study showed a strong interactive effect between substrate ratio and pH. Optimal methane production (535.13 mL/g VS_{added}) was obtained at initial pH of 7.11, substrate ratio 80:20 and temperature 35 °C. This emphasises other factors that have been suggested to have significant impact on co-digestion of substrates for the enhancement of methane production are food/microorganism ratio and C/N ratio [29]. Zhang et al. [34] investigated the enhancement of methane production from cassava residues using thermal-dilute sulphuric acid pretreatment by employing a three-factor central composite RSM. Their results showed that at 55 °C, untreated cassava residues generated methane yield of 158 mL/g VS while the pre-treated samples showed enhanced methane yield of 248 mL/g VS_{added} under optimal conditions (157.84 °C, 2.99% w/w TS H_2SO_4 and 20.15 min). The major challenge faced by several researchers is to guarantee the adequacy between the proposed theoretical model and the experimental data. In many co-digestion experiments, the chosen model that actually captures the relationships between the various environmental factors and methane production is either not available or very complex [35]. There is a necessity to investigate the interactive effects of these main reported factors (pH, temperature and substrate ratio) in optimising methane production from CB and WSW found in SA, via RSM approach.

The aim of this research was to optimise methane yield from co-digestion of CB and WSW in SA using RSM. Different pH, temperatures and substrate co-digestion ratios were evaluated and the optimum conditions for enhanced methane production identified.

Materials and Methods

Inoculum

Fresh zebra (*Equus quagga burchelli*) droppings (ZD) collected from a Stellenbosch Game (SA) Farm Reserve, were used as inoculum to start-up the experiment [36]. The inoculum was stored in a refrigerator at about 4 °C until utilisation. Before digestion, zebra dung (ZD, 0.5 kg) was soaked in warm water and incubated (36 ± 0.5 °C) for 7 days prior to loading in a digester and the water was sieved and used as the inoculum for all experiments.

Samples Collection and Preparation

Fresh cassava (*M. esculenta* Crantz) biomass was collected from plantation areas in Bizana, Eastern Cape, SA and stored in a refrigerator at 4 °C prior to utilisation. Some cassava samples were left to spoil under its natural flora (*Aspergillus niger* and *Rhizopus* sp.) to soften the tubers prior to digestion. Cassava was chopped into small pieces (1 cm^3) and oven dried at 105 °C for 48 h and then milled into powder. Similarly, fresh WSW was collected from a winery farm at the Agricultural Research Council (ARC), Stellenbosch, SA. It was sun dried and milled into powder form using a Hammer mill SER No. 400 (Scientific®, SA) equipped with 2 mm sieve mesh [8].

Experimental Design for Statistical Analyses and Optimisation

The RSM was used for the selected parameters. In order to determine the optimal combination of parameters equivalent to the highest methane production, statistical software Design-Expert® Software version 6.0.8 was used. A central composite design (CCD) was used to design the experiment for methane optimisation from co-digestion of CB and WSW. Table 1 shows the chosen parameters which were pH (*A*), temperature (*B*) and co-digestion ratio (*C*).

Each of the chosen variables was studied at five different levels on the CCD. The levels were allocated as − 2, − 1, 0, + 1, and + 2 respectively as shown in Table 1. The output variable was the methane yield in mL CH_4/g VS_{added}. Twenty

Table 1 Factor levels showing actual and coded values of the experimental plan

Variable	Parameters	Units	Level				
			− 2	− 1	0	1	2
A	pH	–	6.0	6.5	7.0	7.5	8.0
B	Temperature	°C	25	30	35	40	45
C	CB:WSW	%	40:60	60:40	70:30	100:0	0:100

trial runs were performed according to the design with six centre point replications as shown in Table 2.

The evaluation of lack of fit and inaccuracy in the experiment was measured by centre run. The experimental values obtained were subjected to response surface analysis so as to evaluate the relationship between the input variables namely pH (*A*), temperature (*B*) and co-digestion substrate ratio (*C*). Functional relationship was developed between the response variable (Y) which is the methane yield and the input parameters. The methane yield ($mL\,CH_4/g\,VS_{added}$) was determined by dividing the cumulative CH_4 production by the total amount of VS added to each digester. The experimental results were subjected to RSM to check the interactive effect between the chosen parameters and then fitted to a second order polynomial Eq. 1 by means of multiple regression analysis:

$$Y = b_0 + b_1A + b_2B + b_3C + b_xA^2 + b_yA^2 + b_zA^2 + b_iAB + b_jAC + b_kBC, \quad (1)$$

where Y is the response variable (methane yield $mL\,CH_4/g\,VS_{added}$), b_0, b_1, b_2, b_3 are coefficient of linear expressions, b_x, b_y, b_z are quadratic coefficients, b_i, b_j, b_k are interaction coefficients. A second order polynomial was fitted to the model so as to determine the optimal point. The regression model was calculated by analysing the analysis of variance (ANOVA), Fisher-test value dependent on the p of 95% confidence level as shown in Table 3. Coefficient of determination R^2 was used to express the adequacy of the model according to Chong et al. [37].

Analytical Method

The collected CB and WSW were analysed for pH, total solids (TSs), VSs, total nitrogen (TN), total organic carbon (TOC), calcium and phosphorus, ash content and moisture content (MC). The TS and MC were quantified by heating fresh biomass at 105 °C to a constant weight using Moisture Analyser Model MA35 (Sartorius GmbH, Germany). Merck Spectroquant Pharo® Spectrophotometer (Darmstadt, Germany) together with Merck cell tests or kits were used to determine the TOC (cat no. 14879), TN (cat no 14537), total phosphorous (TP, cat no. 14729), total ammonia nitrogen as NH_3–N (cat no. 00683). Ash content was determined by furnace method; crude protein and carbon by Dumas combustion method; starch and sugars by enzyme method. Elementary composition was also done on inductively coupled plasma (ICP). All the biomass analyses were performed according to standard methods [38] and has been published in our previous report [20]. A summary

Table 2 Experimental results for actual methane and predicted yield values for the composite design

Run order	Code values (pH, temperature and CB/WSW)			Actual values			Actual methane yield ($mL\,CH_4/g\,VS_{added}$)	Predicted yield (response)
	A	*B*	*C*	*A*	*B*	*C*		
1	0	0	0	7	35	70	353	346.28
2	−2	2	−2	6	45	40	121	105.12
3	0	0	0	7	35	70	346	346.28
4	−2	0	0	6	35	70	237	283.33
5	2	0	0	8	35	70	285	295.33
6	2	−2	−2	8	25	40	100	93.72
7	0	0	−2	7	35	40	189	235.33
8	0	0	0	7	35	70	358	346.28
9	−2	−2	1	6	25	100	101	94.72
10	0	0	0	7	35	70	378	346.28
11	2	2	−2	8	45	40	122	114.12
12	−2	−2	−2	6	25	40	115	98.72
13	0	0	0	7	35	70	396	346.28
14	0	2	0	7	45	70	250	279.53
15	2	2	1	8	45	100	136	138.12
16	−2	2	1	6	45	100	117	109.12
17	0	0	0	7	35	70	360	346.28
18	2	−2	1	8	25	100	108	109.72
19	0	−2	0	7	25	70	235	262.13
20	0	0	1	7	35	100	235	245.33

Table 3 Analysis of variance (ANOVA) of the model for methane production

Source	Sum of squares	DF	Mean square	F value[#]	P value*	Remark
Model	2.125E+005		23,608.41	22.08	< 0.0001	Significant
A	360.00	1	360.00	0.34	0.5745	
B	756.90	1	756.90	0.71	0.4198	
C	250.00	1	250.00	0.23	0.6391	
A^2	8920.51	1	8920.51	8.34	0.0161	Significant
B^2	15,656.82	1	15,656.82	14.65	0.0033	Significant
C^2	30,872.51	1	30,872.51	28.88	0.0003	Significant
AB	98.00	1	98.00	0.092	0.7683	
AC	200.00	1	200.00	0.19	0.6745	
BC	32.00	1	32.00	0.030	0.8661	
Residual	10,690.08	10	1069.01			
Lack of fit	8981.25	5	1796.25	5.26	0.0463	Significant
CV	14.40					Reliable
Predicted residual sum of squares	45,556.08					
Predicted R^2	0.7959					
Adequate precision	10.924					Desirable
R^2	0.9521					Good model
Adjusted R^2	0.9090					

DF degree of freedom, *CV* coefficient of variation

*Probability value (significant at $p < 0.05$), [#]Fisher's (F) precise test value

of our earlier feedstock report showed that dried CB contained 98.45% TS, 98.20% VS, 45.6% carbon, 359.75 mg/kg sodium, 2.25% protein, 1.15 mg/kg iron, 1.7% ash and trace amounts of nitrogen (0.36%), phosphorus (0.05%), and cyanide (0.88 mg/kg). Similarly, dried WSW contained 95.92% TS, 83.86% VS, 50.40% C, 1191.9 mg/kg Na, 28.05 mg/kg Fe, 15.95% ash and trace amounts of phosphorus (0.16%) and calcium (0.06%) [20]. The C/N ratio of dried CB was calculated as 127:1 compared to 29:1 for dried WSW. These results demonstrated the properties of the feedstock prior to AD and the need for co-digestion.

Experimental Procedure

The optimisation test system for this study was conducted under controlled and reproducible conditions. Twenty batch digestion tests experiments were conducted in Schott Duran® bottles (digesters) with each having a total capacity of 500 mL and a working volume of 300 mL. The CB and WSW were mixed inside the digesters at different ratios of 0:100, 40:60 and 70:30, followed by incubation in a water bath at different temperatures (25, 35, and 45 °C ± 0.5). The pH was adjusted using 32% hydrochloric acid (HCl) and 1 M sodium hydroxide (NaOH) when necessary. The production of biogas was measured daily by downward displacement of acidified water (pH 2 with H_2SO_4) and methane composition determined by using the state-of-the-art Geotech Biogas 5000 portable gas analyser (Geotechnical Instruments Ltd., UK). Each experiment was carried out for 15 days. To rid the bottles of the dissolved oxygen and other gases, each bottle was flushed with nitrogen gas for 3 min. The bottles were shaken manually before reading each biogas flow rate. This was done to achieve homogeneity inside the reactor, free trapped gases and to prevent scum accumulation.

Results and Discussion

Design of Experiments and Optimisation

The CCD with experimental results of the 20 runs together with the predicted value is shown in Table 2. Equation 2 was obtained based on mathematical regression models for methane production and was fitted in terms of coded factors:

$$\begin{aligned} \textit{Methane yield} = 346.28 + 6A + 8.7B + 5C - 56.95A^2 - 75.45B^2 \\ - 105.95C^2 + 3.50AB + 5AC + 2.00BC. \end{aligned} \qquad (2)$$

All the independent variables studied had statistically significant effects on the methane yield (Eq. 2). This is in agreement with previous reports [23, 27, 39] that showed that temperature, pH and substrate ratios are the parameters that significantly influence co-digestion process. The adequacy and statistical significance of the model were ascertained on the results obtained by ANOVA as shown in Table 3. ANOVA is a highly beneficial tool in finding the best fitted mathematical model. The Model F-value of 22.08 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. The "Lack of Fit F-value" of 5.26 implies the lack of fit is significant. There is only a 4.63% chance that a "Lack of Fit F-value" this large could occur due to noise. The p-value ($p < 0.05$) of the quadratic regression model indicates that the model terms are significant. In the observed response values, the correlation coefficient, $R^2 = 0.9521$, indicated that the sample variation of 95.21% in the methane yield response could be explained by the model. A good statistical model should have R^2 value in the range of 0.75–1 [27, 29]. The "Pred R-Squared" value of 0.7959 is in reasonable agreement with the "Adj R-Squared" value of 0.9090. "Adequate Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 10.924 indicates an adequate signal, meaning that this model can be used to navigate the design space. The percentage of coefficient of variation (CV %) is a measure of residual variation of the data relative to the size of the mean. Usually, the higher the CV value, the lower the reliability of experiment. Hence, a lower value of CV (14.4%) indicated a greater reliability of the experiment. The predicted residual sum of squares (PRESS) is a measure of how well the model fitted each point in the design. The smaller the PRESS statistics, the better would be the model fitting the data points. Here the value of PRESS was found to be 45,556.08 meaning the model fitted the data points. Table 3 also shows that the quadratic model terms (A^2, B^2, C^2) were signiificant ($p < 0.05$). However the linear model terms (A, B, C) and the interactive terms (AB, AC, BC) were insignificant ($p > 0.05$). Further, the actual methane yield values were close to the predicted values (Fig. 1).

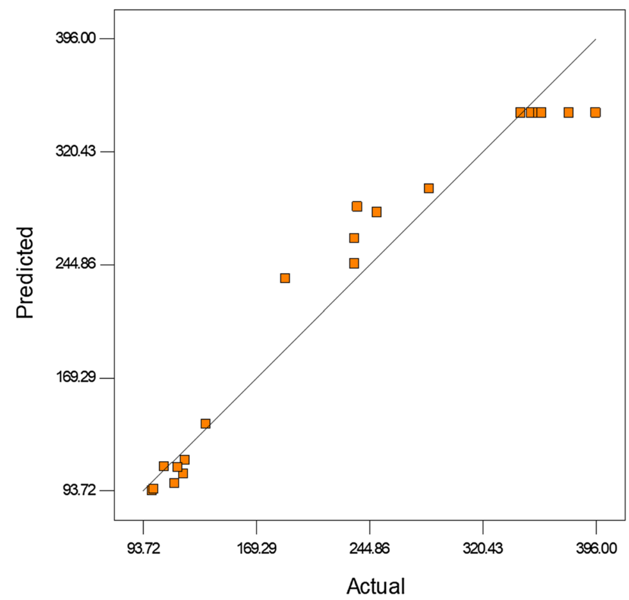

Fig. 1 Parity chart for the model employed: predicted versus actual methane yield values ($R^2 = 0.9521$)

Response Surface Analyses

Interaction Between pH and Temperature

The relationship between pH and temperature was demonstrated using the three dimensional response surface and the corresponding contour plot by keeping one variable at the central level of plot and contrasting the others inside the expected experimental range. Statistical difference was found to be non-significant ($p > 0.05$) for interactive effect of pH and temperature but individual effects of these factors were significant on the digester performance (Eq. 2). Carotenuto et al. [40] found that at high temperature of 55 °C (thermophilic conditions) and high pH > 8, the production rate of methane was almost double what was obtained at 37 °C, during the AD of buffalo manure. The study showed that for some animal manures like buffalo, the digestion process can still run without any pH manipulation. In the current study, the maximum methane yield (396 mL CH_4/g VS_{added}) was higher than the predicted maximum value (346.28 mL CH_4/g VS_{added}) at a temperature of 35 °C ± 0.5 and pH 7 (Fig. 2). These observations suggest the need for a robust acclimated microbial consortium for AD and the possible impact of inoculum source on microbial activity.

It was also observed that the production of methane decreased slightly when the temperature was at 25 °C ± 0.5 during co-digestion of CB and WSW. This could be due to the fact that the chosen micro-organisms existed at about 35 °C which is the body temperature for zebras therefore they are most active at that temperature and when the temperature drops to 25 °C it causes a decrease in methane production. Methanogens have been observed to be very sensitive to extremes of temperature and pH [41] and have a very limited activity range. The optimum pH for methane production has been reported between 7 and 7.2 [4, 40].

Interaction Between the Effect of pH and Substrate Ratio

The interaction between pH and different ratios of CB and WSW is shown in Fig. 2b. Both the pH and substrate ratios individually have significant impact (Eq. 2) on methane production ($p < 0.05$) but their interactive effect was statistically insignificant ($p > 0.05$). In this study, methane production reached its peak at substrate ratio of 70% CB and 30% WSW (Fig. 2b). Methane production was observed to decrease at 100% WSW digestion and also when the quantity of WSW was more than that of CB. This could mean that WSW has inhibitory properties on

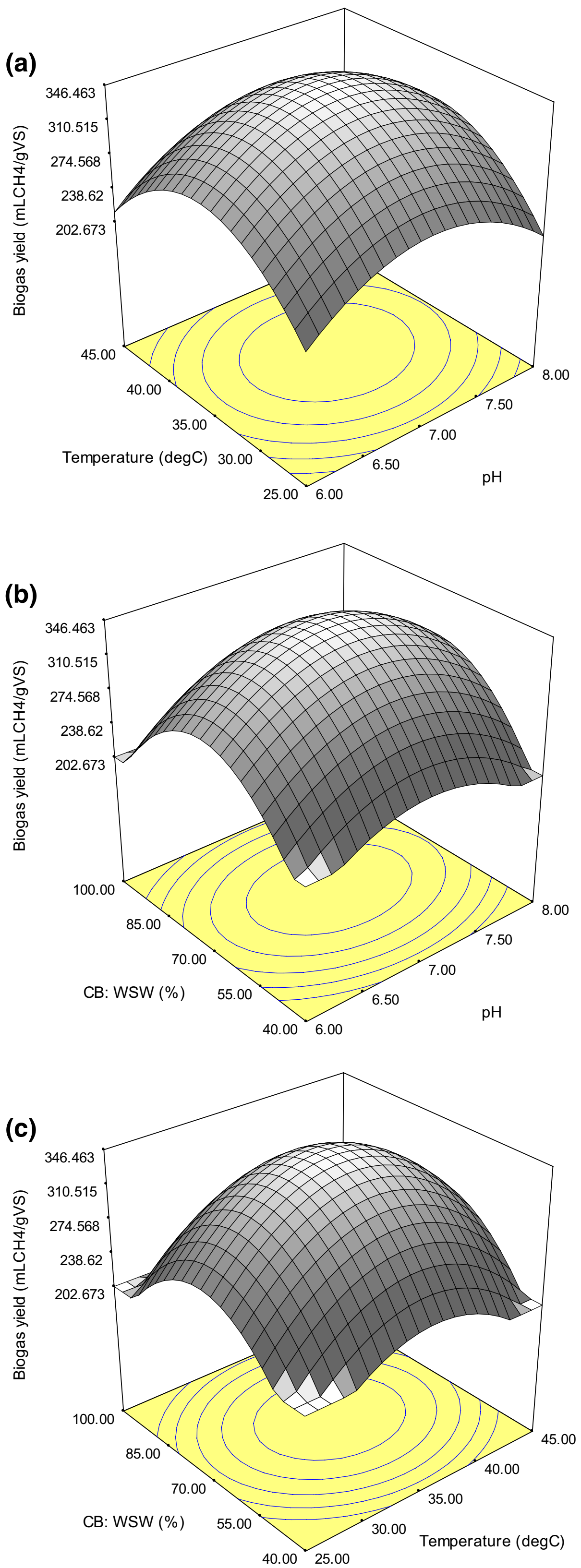

Fig. 2 Response surface and contour plots showing **a** interaction of pH (A) and temperature (B) on methane yield, **b** interaction of pH (A) and substrate ratio (C) on methane yield, and **c** interaction of temperature (B) and substrate ratio (C) on methane yield

methane production which could be attributed to a number of factors such as the presence of phenolic and the high C/N ratio when the two substrates were combined [39].

The optimisation of the pH in the digesters was selected to range from 6 to 8 [42, 43]. When the pH is less or greater than the optimum range (pH 7–8.5), the methanogenic process gets inhibited thereby decreasing the rate of methane production [44, 45]. One of the benefits of co-digestion is its buffering capacity in the digester during AD. A decrease in pH will lead to acidification and thriving acidogenic microorganisms due to the accumulation of volatile fatty acids (VFAs) in the early days of the anaerobic process and if not controlled can lead to digester failure [46]. In the present study, stable pH values at 7 favoured the production of maximum methane values at different yield combinations (Fig. 3a).

Interaction Between Substrate Ratio and Temperature

The interactive effects of substrate ratios and different temperatures are shown in Fig. 2c. It was observed that an increase in the CB/WSW ratio and temperature resulted in an increase in methane production and conversely the decrease in these two factors decreased methane production. The CB/WSW ratio individually affected methane production significantly ($p < 0.05$) but its interactive effect with different temperatures was insignificant ($p > 0.05$). The predicted maximum methane yield value (346.28 mL CH_4/g VS_{added}) of the substrate ratios and temperature are indicated by top of the surface (Fig. 2c) and was lower than the highest methane yield of 396 mL CH_4/g VS_{added} recorded experimentally in this study at the ratio of 70:30 (70% CB and 30% WSW) and at 35 °C ± 0.5 as shown in Table 2. This could be explained by the type of inoculum (ZD) used in this study where the methanogens are most active at the body temperature of 35 °C. At optimal organic load, as the temperature increases the degradation rate is proportional with the biomass (cells growth) [47]. The observed difference from the predicted value may be that the microbes were not allowed to adapt to a different range of temperatures before the optimisation experiments, as compared to what they are accustomed to in their zebra origin. The lowest methane production in the present study was at the ratio of 40:60 (40% CB and 60% WSW) at a temperature of 25 °C ± 0.5. This result could be attributed to the fact that WSW degradation compound/s in itself is inhibitory to the methanogens due to the presence of phenolic compounds [39] coupled with the low temperatures for methanogenic activity [40, 41]. The combination of these two negative factors will ultimately lead to a lower methane yield.

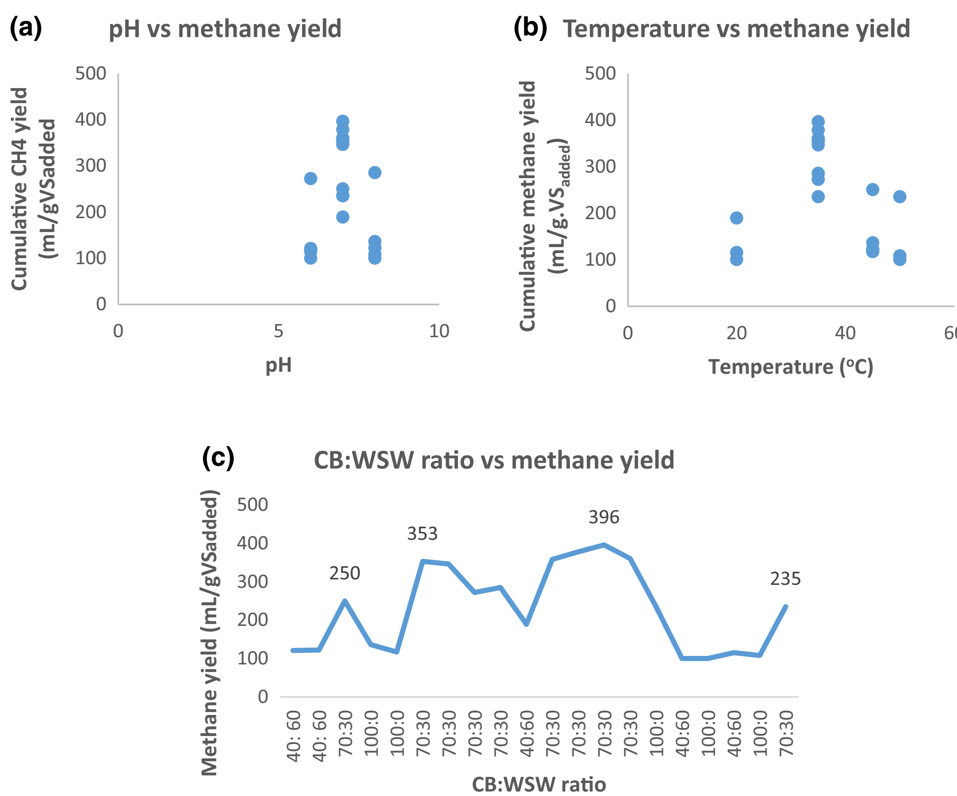

Fig. 3 Optimal conditions for maximizing methane yield **a** effect of pH, **b** effect of temperature, and **c** effect of substrate ratios

Conditions for Optimum Response and Model Validation/Verification

The specific optimal conditions for methane production were determined using the second order polynomial model (Eqs. 1 and 2). The model predicted a maximum methane yield of 346.28 mL CH_4/g VS_{added} for the optimal conditions. To ascertain the validity of the predicted values, experiments were carried out at the optimal conditions of pH 7, temperature of 35 °C ± 0.5 and 70/30 ratio of CB/WSW. The maximum methane production was recorded to be 396 mL CH_4/g VS_{added} (Fig. 3a–c). The obtained value was12.6% higher than the predicted value. Although, there was a gap between the predicted and actual yield, the significance of the variables considered as displayed in the ANOVA table shows the model could still be relevant to similar research.

The co-digestion of two substrates has been reported to produce more methane than a single substrate [23]. Zhang et al. [34] also reported a methane production of 259.46 mL CH_4/g VS_{added} when CB residues and distillery waste water were co-digested using a batch thermophilic reactor. Figure 3b shows that the optimum temperature for methane production from co-digestion of CB and WSW is 35 C ± 0.5. It was observed that at 25 °C and 45 °C, methane production decreased. This could be attributed to the sensitivity of methanogens to both low and high temperatures and hence the significant impact on the AD process [48, 49]. Most anaerobic treatment plants around the world are operated at mesophilic conditions [50].

Methane production presented several small peaks before production finally declined to very low levels (Fig. 3c). This could be related to the dynamic balance between the metabolism of acidogens and methanogens in the digester [51]. The highly concentrated CB, at the early stages of digestion, provided sufficient organic acid for faster growth of the methanogens and resulted in an increased methane production. As the organic acid concentration becomes less, the methanogenic activity is reduced hence allowing acidogens to thrive. This causes a decrease in methane yield. This cycle of acidogenic and methanogenic activities continues until a decline in the final substrate concentration occurs as compared to the initial concentration.

Based on the results presented in the current study, it can be seen that the optimum ratio for methane production between CB and WSW was 70% CB and 30% WSW. The high R^2 value of 0.991 showed that the RSM model was useful for methane yield prediction in agreement with Sathish and Vivekanandan [28], and could be effectively used to predict methane production from agricultural food waste and animal manure [33].

Conclusion

The ratio of CB to WSW proved to be the most significant factor during methane production. Increasing the CB quantity yielded more methane but increasing the WSW decreased methane production. This decrease in methane yield by WSW could be due to the chemical properties of WSW that contains phenolic compounds. The optimal conditions for methane production were found to be pH 7, temperature of 35 °C ± 0.5 and CB to WSW ratio of 70:30. The maximum response value for methane production was 346.28 mL CH_4/g VS_{added} while the actual maximum methane yield was close to the predicted value but higher at 396 mL CH_4/g VS_{added}. Co-efficient of determination, R^2, was 0.9521.

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References

1. Bessou, C., Ferchaud, F., Gabrielle, B., Mary, B.: Biofuels, greenhouse gases and climate change. A review. Agron. Sustain. Dev. **31**, 1 (2011). https://doi.org/10.1051/Agro/2009039
2. Vasavan, A., de Goey, P., van Oijen, J.: Numerical study on the autoignition of biogas in moderate or intense low oxygen dilution nonpremixed combustion systems. Energy Fuels **32**, 8768–8780 (2018). https://doi.org/10.1021/acs.energyfuels.8b01388
3. Balat, M., Balat, H.: Biogas as a renewable energy source—a review. Energy Sources A **41**(14), 1280–1293 (2009)
4. Demuynck, M., Nyns, E.J., Palz, W.: Biogas Plants in Europe. A Practical Handbook. Reidel Publishing Company, Dordrecht (1984)
5. Abdeshahian, P., Lim, J.S., Ho, W.S., Hashim, H., Lee, C.T.: Potential of biogas production from farm animal waste in Malaysia. Renew. Sustain. Energy Rev. **60**, 714–723 (2016)
6. Bundhoo, Z.M.A., Mauthoor, S., Mohee, R.: Potential of biogas production from biomass and waste materials in the Small Island Developing State of Mauritius. Renew. Sustain. Energy Rev. **56**, 1087–1100 (2016). https://doi.org/10.1016/j.rser.2015.12.026
7. Dobre, P., Nicolae, F., Matei, F.: Main factors affecting biogas production—an overview. Rom. Biotechnol. Lett. **19**, 9283–9296 (2014)
8. Okudoh, V., Trois, C., Workneh, T.: The potential of cassava biomass as a feedstock for sustainable biogas production in South Africa. J. Energy Power Eng. **8**, 836–843 (2014)
9. Hansupalak, N., Piromkraipak, P., Tamthirat, P., Manitsorasak, A., Sriroth, K., Tran, T.: Biogas reduces the carbon footprint of cassava starch: a comparative assessment with fuel oil. J. Clean. Prod. (2016). https://doi.org/10.1016/j.jclepro.2015.06.138
10. Department of Agriculture, Forestry and Fisheries (DAFF) Republic of South Africa: Cassava production guideline (2010). https://www.nda.agric.za/docs/Brochures/ProdGuideCassava.pdf. Accessed 6 Sept 2018
11. WOSA: World Statistics. https://www.wosa.co.za/The-Industry/Statistics/World-Statistics/. Accessed 26 Feb 2018
12. Zacharof, M.P.: Grape winery waste as feedstock for bioconversions: applying the biorefinery concept. Waste Biomass Valoriz. **8**(4), 1011–1025 (2017). https://doi.org/10.1007/s12649-016-9674-2
13. Zhang, Q., Hu, J., Lee, D.J.: Biogas from anaerobic digestion processes: research updates. Renew. Energy **98**, 108–119 (2016). https://doi.org/10.1016/j.renene.2016.02.029
14. Achina, S., Achinas, V., Gerrit, J.W.E.: A technological overview of biogas production from biowaste. Engineering **3**, 299–307 (2017)
15. Weiland, P.: Biogas production: current state and perspectives. Appl. Microbiol. Biotechnol. **85**, 849–860 (2010)
16. Okudoh, V., Trois, C., Workneh, T., Schmidt, S.: The potential of cassava biomass and applicable technologies for sustainable biogas production in South Africa: a review. Renew. Sustain. Energy Rev. **39**, 1035–1052 (2014). https://doi.org/10.1016/j.rser.2014.07.142
17. Debabrata, B.: Introduction to energy from toxic organic waste for heat and power generation. In: Debabrata, B. (ed) Energy from Toxic Organic Waste for Heat and Power Generation, pp. 1–6. Woodhead Publishing (2019). https://doi.org/10.1016/B978-0-08-102528-4.00001-8
18. Ning, J., Zhou, M., Pan, X., Li, C., et al.: Simultaneous biogas and biogas slurry production from co-digestion of pig manure and corn straw: performance optimization and microbial community shift. Bioresour. Technol. **282**, 37–47 (2019). https://doi.org/10.1016/j.biortech.2019.02.122
19. Sawatdeenarunat, C., Surendra, K.C., Takara, D., Oechsner, H., Khanal, S.K.: Bi anaerobic digestion of lignocellulosic biomass: challenges and opportunities. Bioresour. Technol. **178**, 178–186 (2015)
20. Mkruqulwa, U.L., Okudoh, V.I., Oyekola, O.O.: Biomethane potential from co-digestion of cassava and winery waste in South Africa. In: Proceedings of the 9th International Conference on Advances in Science, Engineering, Technology and Waste Management (ASETWM-17), 27–28 November, Parys, South Africa, pp. 107–122 (2017)
21. Riaño, B., Molinuevo, B., García-González, M.C.: Potential for methane production from anaerobic co-digestion of swine manure with winery wastewater. Bioresour. Technol. **102**, 4131–4136 (2011). https://doi.org/10.1016/j.biortech.2010.12.077
22. Ziganshin, A.M., Liebetrau, J., Pröter, J., Kleinsteuber, S.: Microbial community structure and dynamics during anaerobic digestion of various agricultural waste materials. Appl. Microbiol. Biotechnol. **97**, 5161–5174 (2013). https://doi.org/10.1007/s00253-013-4867-0
23. Al Seadi, T., Drosg, B., Fuchs, W., Rutz, D., Janssen, R.: The Biogas Handbook: Science, Production and Applications Biogas Digestate Quality and Utilization. Woodhead Publishing Limited, Cambridge (2013)
24. Fitamo, T., Boldrin, A., Boe, K., Angelidaki, I., Scheutz, C.: Co-digestion of food and garden waste with mixed sludge from wastewater treatment in continuously stirred tank reactors. Bioresour. Technol. **206**, 245–254 (2016). https://doi.org/10.1016/j.biortech.2016.01.085
25. Buckley, M., Wall, J.D.: Microbial energy conversion (2006). https://www.osti.gov/biblio/895093. Accessed 2 Feb 2018
26. Wang, X., Lu, X., Li, F., Yang, G.: Effects of temperature and carbon–nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition. PLoS ONE **9**, 1–7 (2014). https://doi.org/10.1371/journal.pone.0097265
27. Reungsang, A., Pattra, S., Sittijunda, S.: Optimization of key factors affecting methane production from acidic effluent coming

from the sugarcane juice hydrogen fermentation process. Energies **5**, 4746–4757 (2012). https://doi.org/10.3390/en5114746
28. Sathish, S., Vivekanandan, S.: Parametric optimization for floating drum anaerobic bio-digester using response surface methodology and artificial neural network. Alex. Eng. J. **55**(4), 3297–3307 (2016)
29. Kainthola, J., Kalamdhad, A.S., Goud, V.V.: Optimization of methane production during anaerobic co-digestion of rice straw and *Hydrilla verticillata* using response surface methodology. Fuel **235**, 92–99 (2019). https://doi.org/10.1016/j.fuel.2018.07.094
30. Reddy, P.R.M., Ramesh, B., Mrudula, S., Reddy, G., Seenayya, G.: Production of thermostable β-amylase by *Clostridium thermosulfurogenes* SV2 in solid state fermentation: optimization of nutrient levels using response surface methodology. Process Biochem. **39**, 267–277 (2003)
31. Elibol, M.: Optimization of medium composition for actinorhodin production by *Streptomyces coelicolor* A3(2) with response surface methodology. Process Biochem. **39**, 1057–1062 (2004)
32. Chen, X., Wang, J.H., Li, D.S.: Optimization of solid-state medium for the production of inulinase by *Kluyveromyces* S120 using response surface methodology. Biochem. Eng. J. **34**, 179–184 (2007)
33. Yusof, T.R.T., Che-Man, H., Abdul Rahman, N.A., Hafid, H.S.: Optimization of methane gas production from co-digestion of food waste and poultry manure using artificial neural network and response surface methodology. J. Agric. Sci. **6**, 27 (2014). https://doi.org/10.5539/jas.v6n7p27
34. Zhang, Q., Tang, L., Zhang, J., Mao, Z., Jiang, L.: Optimization of thermal-dilute sulfuric acid pretreatment for enhancement of methane production from cassava residues. Bioresour. Technol. **102**(4), 3958–3965 (2011)
35. Sarabia, L.A., Ortiz, M.C.: Response surface methodology. In: Comprehensive Chemometrics, vol. 1, pp. 345–390. Elsevier, Amsterdam (2009)
36. Ellis, G.V., Schmidt, S.: Anaerobic digestion of zebra, elephant, wildebeest and impala droppings——assessment of the presence of methanogens and biogas yields. In: International IWA Symposium on Anaerobic Digestion of Solid Waste and Energy Crops (ADSW&EC), 28 August—1 September, Vienna, Austria, p. 5 (2011)
37. Chong, M.L., Abdul Rahman, N., Yee, P.L., Aziz, S.A., Rahim, R.A., Shirai, Y., Hassan, M.A.: Effects of pH, glucose and iron sulfate concentration on the yield of biohydrogen by *Clostridium butyricum* EB6. Int. J. Hydrog. Energy **34**, 8859–8865 (2009)
38. American Public Health Association, APHA: Standard Methods for the Examination of Water and Wastewater. American Water Works Association and Water Environment Federation, Washington, DC (2005)
39. Da Ros, C., Cavinato, C., Cecchi, F., Bolzonella, D.: Anaerobic co-digestion of winery waste and waste activated sludge: assessment of process feasibility. Water Sci. Technol. **69**, 269–277 (2014)
40. Carotenuto, C., Guarino, G., Morrone, B., Minale, M.: Temperature and pH effect on methane production from buffalo manure anaerobic digestion. Int. J. Heat Technol. **34**, 623–629 (2016). https://doi.org/10.18280/ijht.34S233
41. Luo, G., Xie, L., Zou, Z., Zhou, Q., Wang, J.Y.: Fermentative hydrogen production from cassava stillage by mixed anaerobic microflora: effects of temperature and pH. Appl. Energy (2010). https://doi.org/10.1016/j.apenergy.2010.07.004
42. Henze, M.: Basic biological processes. In: Henze, M., Harremoes, P., Cour, Jansen J., Arvin, E. (eds.) Wastewater Treatment: Biological and Chemical Processes, pp. 65–129. Springer, Berlin (2002)
43. Chanathaworn, J.: Operating condition optimization of water hyacinth and earthworm bedding wastewater for biogas production. Energy Procedia **138**, 253–259 (2017)
44. Van Kessel, J.A.S., Russell, J.B.: The effect of pH on ruminal methanogenesis. FEMS Microbiol. Ecol. **20**, 205–210 (1996)
45. Adelekan, B., Bamgboye, A.: Comparison of biogas productivity of cassava peels mixed in selected ratios with major livestock waste types. Afr. J. Agric. Res. **4**, 571–577 (2009)
46. Ghaly, A.E., Ramkumar, D.R., Sadaka, S.S., Rochon, J.D.: Effect of reseeding and pH control on the performance of a two-stage mesophilic anaerobic digester operating on acid cheese whey. Can. Agric. Eng. **42**, 173–183 (2000)
47. Boontian, N.: Conditions of the anaerobic digestion of biomass. Int. J. Biol. Biomol. Agric. Food Biotechnol. Eng. (2014). https://doi.org/10.5281/zenodo.1096285
48. Lin, Q., He, G., Rui, J., Fang, X., Tao, Y., Li, J., Li, X.: Microorganism-regulated mechanisms of temperature effects on the performance of anaerobic digestion. Microb. Cell Factories **15**(1), 96 (2016)
49. Tait, S., Astals, S., Batstone, D., Jensen, P.: Enhanced methane bioenergy recovery at Australian piggeries through anaerobic co-digestion 4C-113. Final report prepared for the Cooperative Research Centre for High Integrity Australian Pork, Queensland (2018). https://porkcrc.com.au/wp-content/uploads/2018/02/4C-113-Project-Final-Research-Report.pdf. Accessed 6 Sept 2018
50. Schnürer, A., Jarvis, A.: Microbiological Handbook for Biogas Plants. Swedish Waste Management, Swedish Gas Centre, Malmö (2010)
51. Yan, Z., Song, Z., Li, D., Yuan, Y., Liu, X., Zheng, T.: The effects of initial substrate concentration, C/N ratio, and temperature on solid-state anaerobic digestion from composting rice straw. Bioresour. Technol. **177**, 266–273 (2015). https://doi.org/10.1016/j.biortech.2014.11.089

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