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Extraction of Bioethanol from Fermentation Broth of Cocoa Pod Husks and Downstream Processing of the Vinasse for Biogas Production

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Abstract
Extraction from fermentation broths is an integral part of ethanol biorefinery. During extraction, as much as 17 litres of vinasse is generated per litre of ethanol produced. Vinasse, the liquid waste from ethanol production, has the potential to cause devastating effect on the environment if not properly disposed. One way of treating vinasse is by reducing its organic components through anaerobic digestion (AD). In this study, ethanol was extracted from a fermentation broth of cocoa pod husks by liquid-liquid extraction (LLE) and batch AD of the waste stream was carried out for biogas production. Operation parameters for LLE were varied using a batch process after which continuous operation was carried out based on the determined parameters. Since substrate-to-inoculum ratio (S/R) can affect biomethane yield, it was varied at 0.5, 1.0, 1.5, 2.0 and 2.5 (gCOD basis) for the AD process using the automatic biomethane potential test system. Three kinetic models were used to fit the experimental biomethane potential from each reactor. Results show that a distribution coefficient, percentage efficiency and selectivity of 37.49, 89.27% and 109 respectively were obtained for ethanol during LLE. A maximum biomethane potential of 328 mL/gCOD was obtained at a S/R of 2.0. Although all models gave a reasonably good fit to the experimental data, the dual pool model gave the best fit.

Keywords: Anaerobic digestion**,** distribution coefficient, lignocellulosic biomass, liquid-liquid extraction, kinetic models.

1. Introduction

In 2018, the global ethanol production was 110 billion litres, which was projected to reach 140 billion litres in 2022 (Sharma et al. 2020). Factors like climate change, food security and energy security have been attributed to the continual increase in the production of bioethanol especially from lignocellulosic biomass. Indeed, bioethanol is the most produced biofuel (Singh et al. 2022) which, when blended with gasoline, can significantly reduce greenhouse gas emissions. For instance, as much as 493 million tons emission of CO_2 equivalent was avoided in the transportation sector globally between 2008 and 2018 due to the use of gasoline that was blended with ethanol (Sydney et al. 2019).

Despite the advantages associated with the use of ethanol as a fuel blend, there are still some challenges associated with its production especially as regards its separation from fermentation broths. Depending on the method used, ethanol separation can account for up to 80% of the total cost of production (Zentou et al. 2019) of the biofuel. The most frequently used method for separation of ethanol from fermentation broths is distillation due to its high recovery efficiency and ease of simulation. However, distillation is an expensive process due to the formation of Azeotropes which often necessitates the use of more unit operations. A good separation alternative is liquid-liquid extraction (LLE), whose efficiency depends largely on the type of solvent used. Also known as solvent extraction, LLE works on the principle of mass transfer of a desired component into another stream with which it is non-miscible, even at ambient temperature.

Beyond the separation of ethanol from fermentation broths, the disposal of the vinasse after ethanol extraction can be a source of concern. As much as 17 litres of vinasse is generated from the production of 1 litre of anhydrous ethanol (dos Reis et al. 2019). Due to its low pH as well as high biochemical and chemical oxygen demand, the improper handling of vinasse could result to serious environmental consequences. Such environmental issues can be avoided if vinasse is used for biogas production. During biogas production, an important parameter that affects the efficiency of the process is the substrate-to-inoculum ratio (S/R). The optimal S/R required for biogas production varies from one substrate to another. A S/R below or above the required value can lead to process

inhibition (Mpofu et al. 2021). It is therefore necessary to determine the optimal S/R required for the anaerobic digestion (AD) of vinasse from fermentation broths of cocoa pod husks.

Kinetic parameters are necessary tools for the evaluation of biochemical processes. Parameters obtained from kinetic models can be used to better understand and optimize biochemical reactions. Since research on the kinetics of biomethane potential of vinasse from fermentation broths of cocoa pod husks are limited, it is necessary to determine the kinetic parameters that can be used for predicting reactor performance as well as for the design, and optimization of biochemical reactors for the AD of vinasse from fermentation broths of cocoa pod husks.

Although there are available literature on the extraction of ethanol from fermentation broths as well as the AD of vinasse for biogas production, there is no evidence that a simultaneous ethanol extraction and AD of vinasse from a fermentation broth of cocoa pod husks has been investigated. In a previous study, bioethanol was produced (Undiandeye et al. 2022a) from hydrothermally pre-treated (Undiandeye et al. 2022b) cocoa pod husks. The aim of the present study is to (i) separate the produced bioethanol from the fermentation broth using LLE method, (ii) subject the vinasse to anaerobic digestion (AD) for biomethane production, (iii) investigate the effect of inoculum to substrate ratio on biomethane production (iv) investigate the kinetics of the AD process.

2.0 Materials and methods

2.1 Substrate and inoculum

The substrate used was a fermentation broth of cocoa pod husks from a previous study. The physicochemical parameters of the fermentation broth are shown in Table 1. Two solvents were used for the LLE process including 2-methyl pentanol and a 1:1 mixture of menthol and decanoic acid, all obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). The inoculum used for the AD process was obtained from an active biogas plant digesting maize silage.

Table 1: Mean values (± standard deviation) of Physicochemical parameters (g/L) of substrate

Parameter	Unit	FB	Vinasse	inoculum
Total solids	g/kg	69.27 ± 6.73	73.28 ± 5.39	162.05 ± 9.27
Volatile solids	g/kg	45.16 ± 3.28	49.72 ± 1.25	69.14 ± 4.66
Ethanol	g/L	33.21 ± 0.98		
Acetic acid	g/L	0.29 ± 0.264		
Butyric acid	g/L	0.76 ± 0.18		
Sugar	g/L	0.92 ± 0.63		
COD	g/L	-	106.73 ± 13.96	97.17 ± 5.13
BOD	g/L	-	44.72 ± 6.38	58.03 ± 2.26
pH	-		3.97 ± 0.48	6.94 ± 0.47

FB, fermentation broth; COD, chemical oxygen demand; BOD, biochemical oxygen demand

2.2 Liquid-liquid Extraction

To carry out the LLE process, the fermentation broth was passed through a single bag filter housing (Model 01, Dazhang filter Equipment, China) to remove solid particles (Figure 1). Thereafter, batch tests were conducted using baffled flasks to determine the distribution coefficient, extraction efficiency and selectivity of each component of the fermentation broth. Operating parameters (pH, 3.0, 4.0 and 5.0; temperature, 38 °C, and 55 °C; and mixing ratio of feed to solvent, 0.5, 0.6 and 0.7) were varied to determine the optimum. The optimal parameters from the batch test were then used for continuous operation. The total length of the extractor (Pfaudler GmbH, Waghäusel, Germany) for the continuous operation was about 1.6 m and consisted of two columns, each with 30 stages. One of the columns was used for water removal using 2-methyl pentanol as solvent while the other was for the refinement of ethanol using a solvent mixture of methanol and decanoic acid. The mixing zone was about 1.2 m at a speed of 500 rpm. The volumetric flow rate of the aqueous and organic phases were respectively set at the optimal mixing ratio from the batch test. Samples from the aqueous and organic phases were withdrawn for analysis after 30 minutes of mixing using gas chromatography.

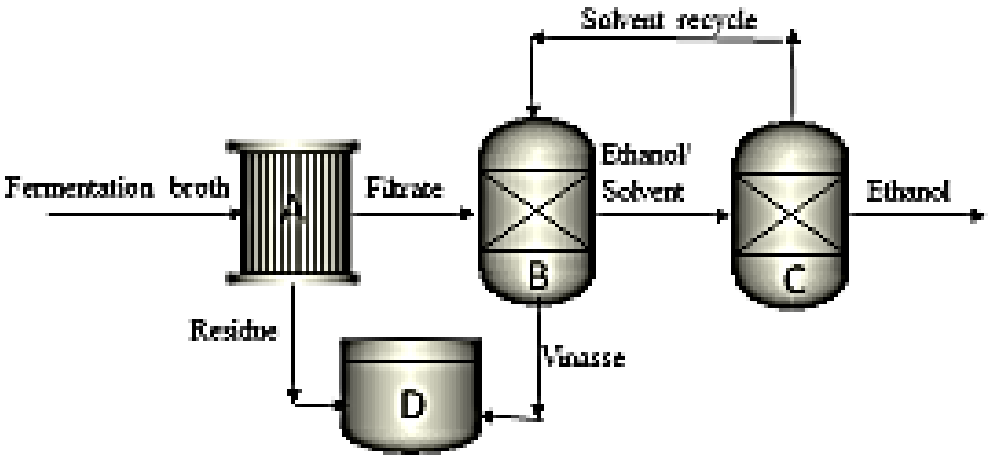

Figure 1: Schematic diagram of the LLE process (A, filter housing; B, LLE; C, distillator; D, biogas reactor).

2.2.1 Evaluation of Performance Parameters

The separation efficiency of the filter bag housing, S_E, was calculated using Equation 1. In addition, three parameters were evaluated to test the performance of the batch extraction process including distribution coefficient, k_d (Equation 2), extraction efficiency, %E (Equation 3) and selectivity, α (Equation 4).

$$S_E = 1 - \frac{m_{res}}{m_{fb}} \quad (1)$$

$$K_d = \frac{c_{org}}{c_{aq}} \quad (2)$$

$$\%E = \frac{c_{org}}{c_{feed}} \times 100 \quad (3)$$

$$\propto = \frac{K_{dE}}{K_{dW}} \quad (4)$$

where m_{res}, m_{fb}, c_{org}, c_{aq}, c_{feed}, k_{dE}, and k_{dW} are the moisture content in residue (%), moisture content in fermentation broth (%), ethanol concentration in organic phase (g/L), ethanol concentration in aqueous phase (g/L), ethanol concentration in feed (g/L), distribution coefficient of ethanol, distribution coefficient of water respectively.

2.3 Anaerobic Digestion

Anaerobic digestion of vinasse was carried out using the automatic methane potential test system (AMPTS II, Bioprocess Control, Sweden) shown in Figure 2. Five different S/R (gCOD basis) were used for the AD process including 0.5, 1.0, 1.5, 2.0 and 2.5 in triplicates. All reactors were operated at a mesophilic temperature of 37 °C (±1) for 23 days. A positive test made up of the inoculum and a microcrystalline cellulose as well as a negative test consisting of only inoculum and water were also set up. Experiments were terminated when the daily biomethane production was less than 1% of the cumulative biomethane produced for three consecutive days in accordance to the VDI guidelines (VDI 4630 2016).

2.4 Analysis

The aqueous and organic phases of the LLE process were analysed using an Azura HPLC system (LH 2.1, Knauer GmbH, Germany) equipped with degasser, binary pump system, auto sampler, column oven, and refractive index detector (RID) set at 40 °C. All other physicochemical parameters were evaluated using standard methods. Total solids, volatile solids, pH, chemical oxygen demand, biochemical oxygen demand, organic acid and ethanol concentration were determined as previously described (Undiandeye et al. 2023). All analysis were done in triplicates in order to determine experimental variation.

2.5 kinetic Modelling and Statistical Analysis

Three kinetic models including the first-order kinetic model (Equation 5), the dual pool model (Equation 6) and the modified Gompertz model (Equation 7) were used to fit the experimental biomethane potential. The models were evaluated using three statistical tools including the coefficient of determination (R^2), the root-mean-square-error, RMSE (Equation 8), and the Akaike Information Criterion, AIC (Equation 9). Comparison among parameters were performed using a one-way ANOVA followed by Tukey's post hock test at 0.05 significant level using SAS v 10.0 software (SAS institute, USA). Pearson correlation analysis was used to evaluate the interdependence of fermentation products using the same statistical software.

$$G_{(t)} = G_0(1 - e^{-kt}) \quad (5)$$

$$G_{(t)} = G_{(0)}[1 - \alpha e^{-k_1 t} - (1-\propto)e^{-k_2 t}] \quad (6)$$

$$G_{(t)} = G_{(0)}.\exp\{-\exp\left[\frac{R_{max}.e}{G_{(0)}}(\lambda - t) + 1\right]\} \quad (7)$$

$$RMSE = \sqrt{\frac{ss}{N}} \quad (8)$$

$$AIC = N \times ln\left(\frac{ss}{N}\right) + 2v \quad (9)$$

where $G_{(t)}$, $G_{(0)}$, k, α, k_1, k_2, R_{max}, λ, ss, N, and v are the ultimate biomethane potential (mL/gCOD), biomethane potential at any time, t (mL/gCOD), first-order kinetic constant (/day), fraction of degradable component, fast degradable first-order constant (/day), slow degradable first-order constant (/day), maximum rate of methane production (mL/gCOD/day), lag phase (day), difference of sum of squares, number of measurements during experiment, and number of parameters in a model respectively.

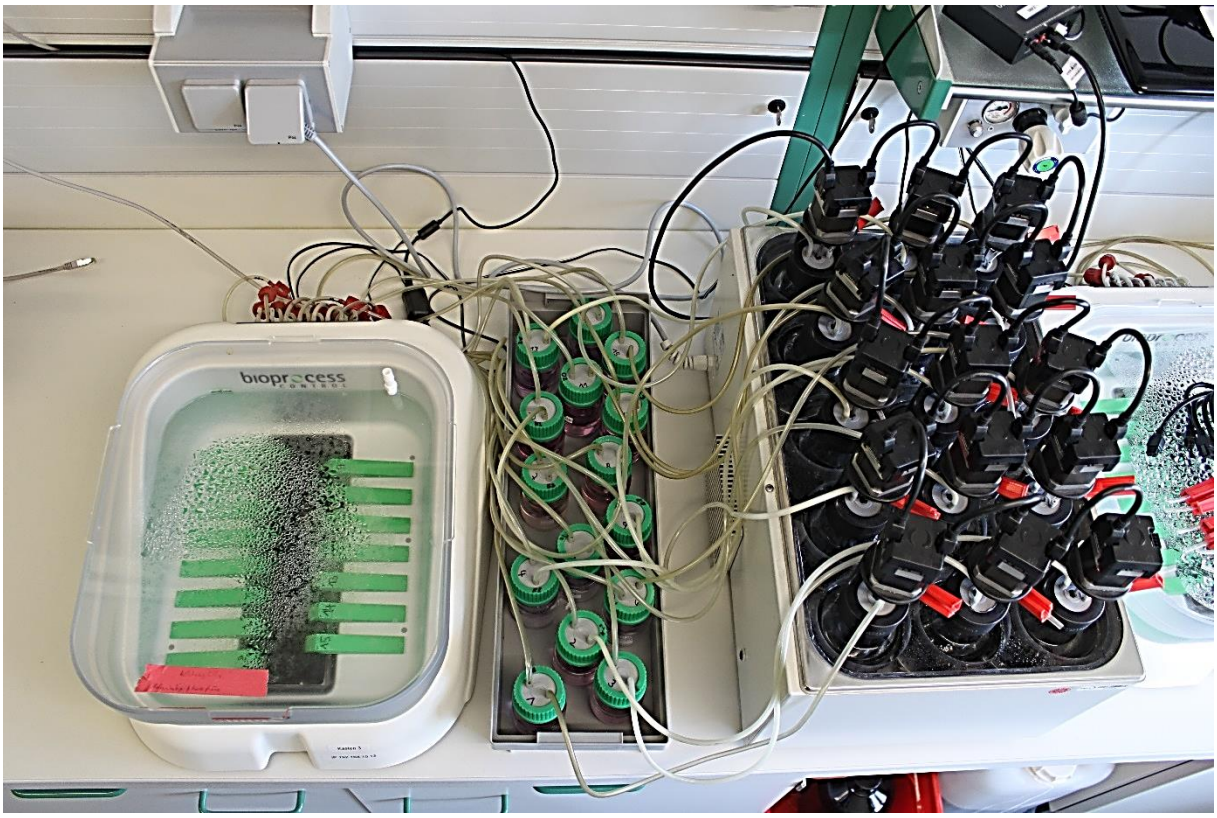

Figure 2: AMPTS for biomethane determination

3.0 Results and Discussions

3.1 Performance of Liquid-liquid Extraction

It was necessary to begin the extraction process by removing the solid content of the fermentation broth using a filter. The moisture content of about 93% in the fermentation broth was reduced to 15% in the residue, indicating that the efficiency of the filter bag was about 84%. In situations where the residue was to be discarded, there would have been the need to increase the efficiency of the filter bag by subjecting the residue to another round of compression in the filter bag. However, since the intention was to re-use the residue for biogas production, an efficiency of 84% was considered adequate. For the batch LLE process, a maximum ethanol recovery was obtained at 38 °C, pH 4.0, and mixing ratio of 0.6. In all operating conditions, the performance indicators were higher with ethanol than with other components (acetic acid and butyric acid) of the feed. The highest distribution coefficient, percentage efficient and selectivity for ethanol was 37.49, 89.27% and 109 respectively from the fermentation broth (Figure 3). The higher ethanol values of k_D, %E and α compared to those of acetic acid and butyric acid (not shown) is an indication that the chosen solvents were adequate for ethanol extraction from the fermentation broth. Performance values in literature are much lower than is reported in the present studies. For instance, Matsumura and Märkl (1984) reported ethanol distribution coefficients between 0.58 – 0.83 and selectivity ratios between 12.9 – 108.8 when five different solvents where used in the solvent extraction of fermentation broth of glucose while Avilés Martínez et al. (2012) reported an ethanol k_D of between 0.31 – 0.88 and α of 2.0 – 8.4 when ionic liquids were used as solvents. Clearly, the type of solvent used plays a key role in the determination of the overall performance of LLE. The solvent used in the present study falls into the category of solvents called deep eutectic solvents which have been reported to be environmentally friendly and efficient for bioethanol extraction (Lee et al. 2021). k_D values of up to 135.7 have been reported when such solvents were used to extract ethanol from aqueous solutions (Oliveira et al. 2013). About 0.07 litres of ethanol was recovered from each litre of feed that

was fed into the column during continuous extraction. Ethanol extraction efficiency was slightly lower at 88.97% compared to the batch process. The purity of ethanol was 99.47%, making it suitable for use as a fuel blend.

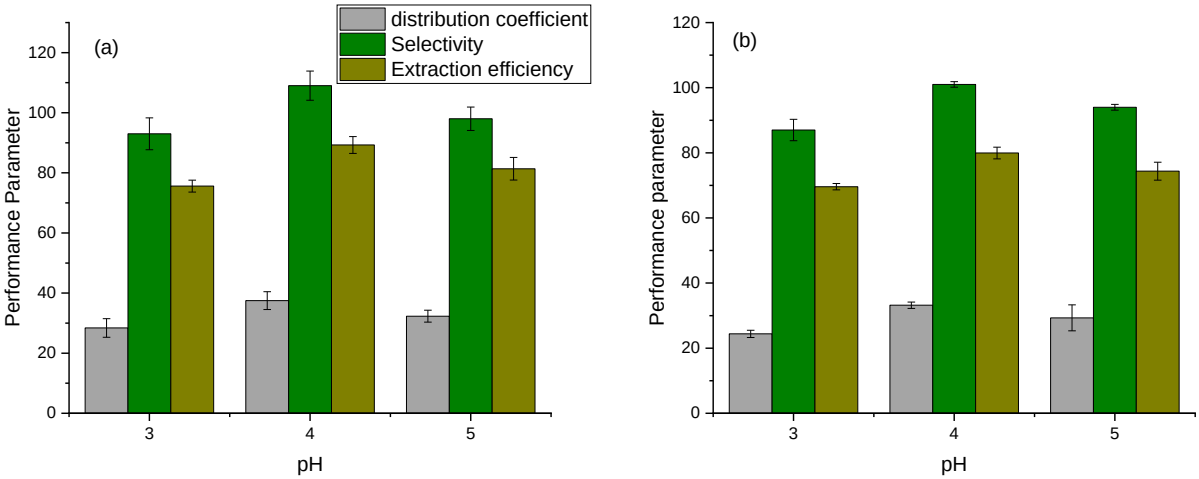

Figure 3: Performance parameters from the batch LLE at (a) 38 °C and (b) 55 °C (Error bars indicate standard deviation between replicates).

3.2 Results from Anaerobic Digestion

The cumulative biomethane potential (BMP) from each substrate is shown in Figure 4. Biomethane yield increased with S/R up to an S/R of 2.0, above which there was a reduction probably due to ammonia inhibition arising from the increase in vinasse to the digester as similarly reported by Estrada-Arriaga et al. (2021). Although there was an increase in BMP when S/R was increased, the increase in BMP was not significant ($p<0.05$). The maximum BMP of 328 mL/gCOD obtained in the present study is comparable to a biomethane potential of 242.13 mL/gVS obtained from cassava vinasse (Ibrahim et al. 2022a), 235 mL/gCOD obtained from sugar beet vinasse (Moraes et al. 2015) and 389 mL/gCOD obtained from sugarcane vinasse (Siqueira et al. 2013). In all substrates, the daily methane production fluctuated with time, reaching a maximum on day 7 and then decreased steadily thereafter (Figure 5).

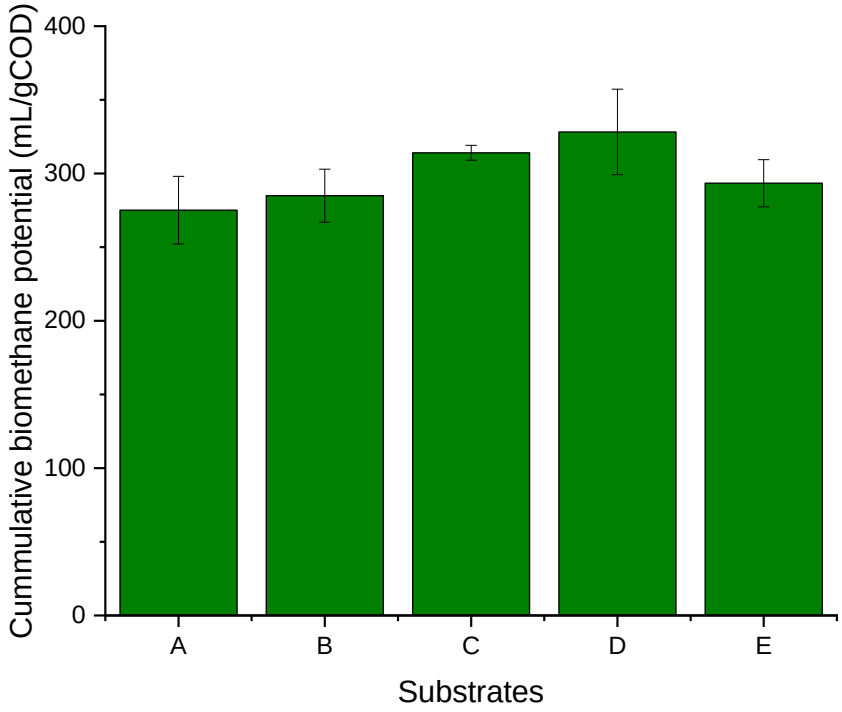

Figure 4: Cumulative biomethane potential of substrates (A, 0.5 gCOD; B, 1.0 gCOD; C, 1.5 gCOD; D, 2.0 gCOD; E, 2.5 gCOD. Error bars indicate standard deviation between replicates).

Certain factors like the type of inoculum used could affect the time at which maximum methane (or biogas) can be reached. For instance, Ibrahim et al. (2022b) reported a maximum methane production between day 17 and 19 during the AD of cassava vinasse using ruminal fluid as inoculum. The earlier time it took to reach maximum productivity in the present study could be due to the presence of more active methanogens in the microbial community of the inoculum consequent upon its source.

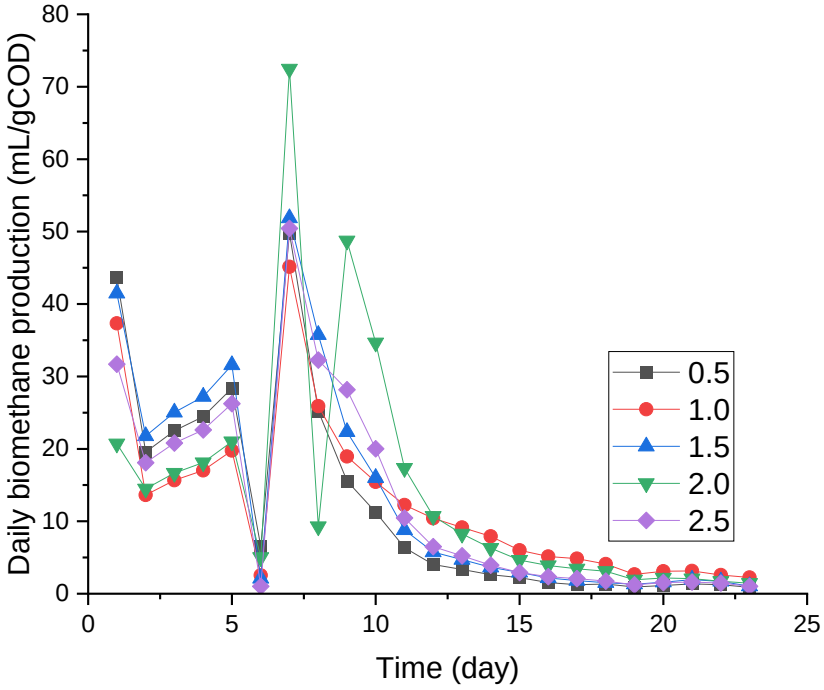

Figure 5: Daily methane production from the substrates

3.2 Kinetic Study

All three models provided a reasonably good fit to the BMP of the substrates as seen from the high values of R^2 ($\geq$ 0.956), an indication of a good consistency between the experimental data and the values predicted by the models. However, the dual pool model gave the best description as seen from the lower values of AIC and RMSE. Low RMSE values is an indication that the accuracy of prediction by the models is high. The fitness of the measured data to the dual pool model is shown in Figure 6. This model has also been reported to better fit the biomethane production from Elodea silage (Gallegos et al. 2018) and water hyacinth (Undiandeye et al. 2023), probably because, unlike other models, it was developed on the assumption that substrates that are used for AD contain both fast and slow degradable components (Gouveia et al. 2022).

As seen from Table 2, the first-order kinetic constant, k, from the first-order model was not significantly different ($p<0.05$) in all substrates, probably because all reactors were operated at the same temperature and pH. Although the concentration of inoculum was different, Fogler (2016) has reported that such variation in concentration has no significant effect on k. From the modified Gompertz model, the lag phase of the AD process in all reactors was negligible. The absence of a lag phase in the reactors can be attributed to the source of inoculum. Since the inoculum was obtained from an active reactor producing biogas, the microbial community quickly adapted to the new environment (AMPTS), which had similar temperature and pH with the source of inoculum. The maximum rate of methane production, R_{max} was also obtained from the modified Gompertz model and was seen to be consistent with the experimental data for all substrates. From the dual pool model, the fraction of fast degradable components, α, increased with an increase in S/R and may have contributed to the increase in BMP in the substrates with higher S/R (except in S/R 2.5). Such fast biodegradable components include organic acids and sugars whose digestion could be inhibited by ammonium as it may have been the case with the substrate with a S/R of 2.5. Also from the dual pool model, k_1 in all substrates were significantly ($p<0.05$) higher than k_2, a confirmation that the substrates contained both fast and slow degradable components. It was also necessary to determine the effective methane production time, t_{eff}. This is the time it will take for 90% of the cumulative biomethane to be produced and it is usually an indication of conversion efficiency (Mao et al. 2017). In the present study, the t_{eff} was 5, 10, 6, 8 and 7 days in the reactor containing a S/R of 0.5, 1.0, 1.5, 2.0 and 2.5 respectively. What this means is that there was a higher conversion efficiency of degradable components in the reactor containing a S/R of 0.5 compared to other reactors, an indication that S/R affects conversion efficiency and that not all degradable components were degraded during the AD process.

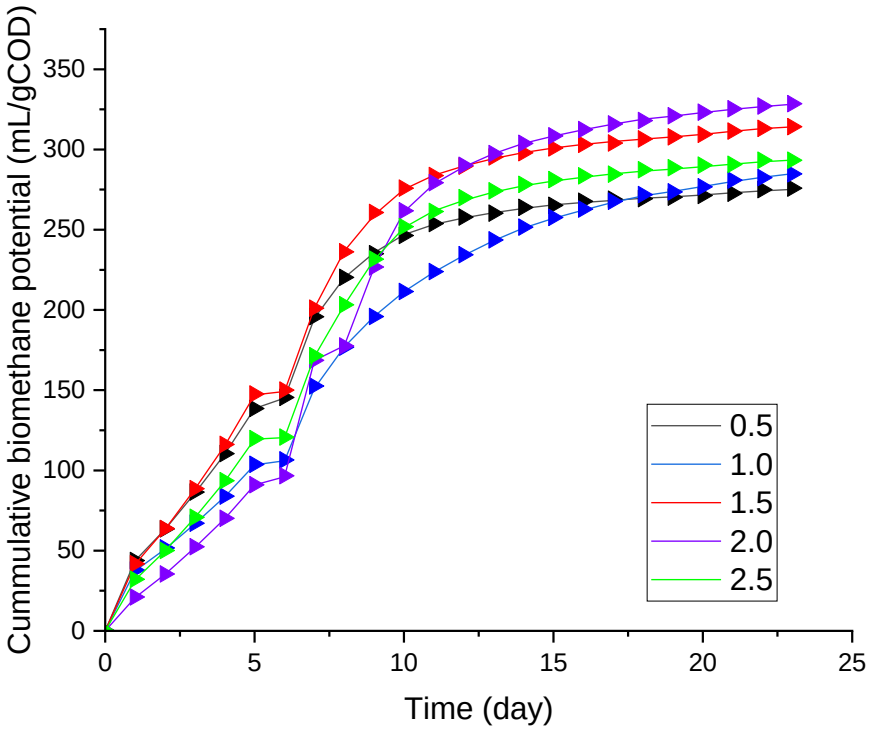

Figure 6: Kinetic fitting of the measured data to the dual pool model (Lines represent experimental data; shapes represent model data).

Table 2: Kinetic parameters and statistical indicators for the models

Model	Parameters	Substrates (S/R)				
		0.5	1.0	1.5	2.0	2.5
First-order	G_0	275.99	285.58	314.57	328.73	294.12
	k	0.28	0.25	0.32	0.31	0.26
	R^2	0.969	0.956	0.971	0.968	0.972
	RMSE	0.32	0.23	0.16	0.16	0.25
	AIC	-66.28	-24.57	-41.28	-47.26	-49.11
Dual pool	G_0	275.14	284.94	314.09	328.25	293.45
	α	0.51	0.57	0.68	0.73	0.77
	k_1	0.36	0.38	0.37	0.35	0.36
	k_2	0.007	0.008	0.006	0.008	0.007
	R^2	0.992	0.983	0.987	0.994	0.998
	RMSE	0.01	0.01	0.01	0.01	0.02
	AIC	-84.17	-33.71	-54.37	-53.42	-76.24
Gompertz	G_0	275.53	285.20	314.28	328.47	293.68
	Rmax	50.17	45.48	51.96	72.74	50.69
	λ	0.09	0	0.07	0.04	0.08
	R^2	0.989	0.980	0.979	0.985	0.981
	RMSE	0.15	0.10	0.07	0.08	0.10
	AIC	-70.29	-28.39	-46.49	-50.19	-53.28

4.0. Conclusion

With an extraction efficiency of 88.97%, the use of 2-methyl pentanol as well as an equal mixture of methanol and decanoic acid as solvents for ethanol extraction from a fermentation broth of cocoa pod husk can be considered successful. Ethanol extraction efficiency was higher at mesophilic temperature (38 °C) compared to at thermophilic temperature (55 °C). A pH of 4.0 also produced a higher ethanol efficiency compared to pH of 3.0 and 5.0. The overall bioethanol production process may be improved if the vinasse is used for biogas production especially if the substrate to inoculum ratio is set at 2.0 (gCOD basis). In designing systems for the optimization of the anaerobic digestion of vinasse from a fermentation broth of cocoa pod husk, the dual pool model is more appropriate compared to the first order model and the modified Gompertz model

Nomenclature:

K_d	distribution coefficient
c_{org}	ethanol concentration in organic phase (g/L)
c_{aq}	ethanol concentration in aqueous phase (g/L)
c_{feed}	ethanol concentration in feed (g/L)
α	selectivity
K_{dE}	distribution coefficient of ethanol
K_{dW}	distribution coefficient of water
%E	extraction efficiency
$G_{(0)}$	ultimate biomethane potential (mL/gCOD)
$G_{(t)}$	biomethane potential at any time, t (mL/gCOD)
k	first-order kinetic constant (/day)
α	fraction of degradable component
k_1	fast degradable first-order constant (/day)
k_2	slow degradable first-order constant (/day)
R_{max}	maximum rate of methane production (mL/gCOD/day)
λ	lag phase (day)
ss	difference of sum of squares
N	number of measurements during experiment
v	number of parameters in a model
m_{res}	moisture content in residure (%)
m_{fb}	moisture content in fermentation broth (%)

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