SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF HYDROTHERMALLY PRETREATED COCOA POD HUSKS AND UNRIPE PLANTAIN PEELS FOR BIOETHANOL PRODUCTION; OPTIMIZATION AND KINETIC STUDY

Supported by tetfund TERTIARY EDUCATION TRUST FUND

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Received: September 21, 2022 **Accepted:** November 12, 2022

Abstract

Cocoa pod husks (CPH) and unripe plantain peels (UPP) are some of the agricultural residues that are improperly disposed in Nigeria with serious environmental consequences. In this study, CPH and UPP that were hydrothermally pretreated in a previous study were used for the production of bioethanol. An optimization of the simultaneous saccharification and fermentation of the substrates was carried out using the Box-Behnken design of the Response Surface Methodology of the Design Expert software. The process parameters that were varied include temperature, pH and solid loading. The study showed that these parameters were optimal at 40 °C, 6 and 17.5 %w/v respectively. These optimal parameters were used to scale-up bioethanol production that resulted in a maximum ethanol concentration of 19.89 and 36.08 g/L from the CPH and UPP respectively. The logistic kinetic model was used to fit the growth rate of biomass while the dual pool model and the modified Gompertz model were used to fit the experimental bioethanol production. It was observed that the dual pool kinetic model described the bioethanol production from the substrates better than the modified Gompertz model.

Keywords: Ethanol yield, fermentation, kinetic fitting, kinetic models, Lignocellulosic biomass, process parameters

Introduction

One of the aims of the sustainable development goals of the United Nations is to make clean energy affordable and accessible to everyone (UNO 2022). This has become necessary considering the negative impact of fossil fuel combustion on man and the environment. One of the sources of clean energy is agricultural residues like cocoa pod husks (CPH) and unripe plantain peels (UPP). CPH are by-products of cocoa processing. In 2019, the top eight cocoa producing countries in the world generated about 13 million tons of CPH with Nigeria generating about 6% of that quantity (Ouattara *et al.* 2020). In most cases, CPH are left to decompose on the farms with serious environmental, health and economic consequences. These consequences have motivated interests in the conversion of CPH to useful products, with more than 35 publications on CPH reported in 2018 alone (Ouattara *et al.* 2020). However, only very few of these studies are on the use of CPH for bioethanol production. Similarly, large quantity of unripe plantain peels are usually improperly disposed to the environment after plantain processing. One way of limiting the impact of these mass flows on the environment is by using them as substrates for bioethanol production. Although these substrates are recalcitrant to enzymatic hydrolysis and fermentation due to their lignin structure (Hernández-Mendoza *et al.* 2021), their potential as substrates for bioethanol production can be enhanced after pretreatment.

The production of ethanol from lignocellulosic biomass involves four major stages including pretreatment, hydrolysis, fermentation and separation (Balat *et al.* 2008). Pretreatment breaks down the lignin structure of a lignocellulosic biomass, making the structural carbohydrate of the biomass accessible to hydrolytic and fermentative enzymes (Betiku and Taiwo 2015). Alkaline pretreatment of CPH has been reported to produce an ethanol concentration of 18.06 g/L (Hernández-Mendoza *et al.* 2021). However, alkali pretreatment require the use of expensive catalysts (Badiei *et al.* 2014). Lower ethanol concentration of 2 g/L from CPH has also been reported after acid hydrolysis (Shet *et al.* 2018), probably because acid pretreatment have been reported to produce a high concentration of inhibitors that have significant impact on the overall yield of the process. Since pretreatment makes up about 40 % of the total cost of bioethanol production (Ingle *et al.* 2019), it is important to use a pretreatment process that is cost effective and efficient.

The next two stages of bioethanol production could be carried out separately or simultaneously. Compared to separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) is more economical (Berlowska *et al.* 2018) and more efficient (Valles *et al.* 2020). For example, Szambelan *et al.* (2018) reported more than 97% increase in the volume of ethanol produced from the SSF of a corn cultivar compared to when SHF was used. Process parameters like pH (Chohan *et al.* 2020), temperature (Ünal *et al.* 2022), yeast concentration and fermentation time (El-Mekkawi et al. 2019) significantly affect ethanol yield from biomass fermentation. Different pH has been reported as being optimal for the fermentation of biomass for bioethanol production. Nnaemeka *et al.* (2021) has reported an optimal pH of 7.0 in the fermentation of colocynthis vulgaris Shrad seeds shell while a pH of 5.0 has been reported as optimal in the fermentation of palm wood (Sathendra *et al.* 2019) for bioethanol production, indicating that the optimal pH or other process parameters for bioethanol production may depend on the nature of the biomass. To the best of our knowledge, there is limited literature on the optimization of the simultaneous saccharification and fermentation of CPH and UPP despite their huge mass flow.

In order to enhance the efficiency of a fermentation process, it is necessary to understand the rate of substrate utilization, products formation and microbial activities. One of the ways of studying these processes is by the use of kinetic models. Kinetic models enables a fermentation process to be adequately predicted, optimized and controlled (Fogler 2016). The suitability of a kinetic model in predicting a fermentation process is a function of substrate type, microorganisms and process conditions (Tan *et al.* 1996). The objectives of this study was to (i) optimize process parameters for the simultaneous saccharification and fermentation of CPH and UPP and (ii) carryout a kinetic study of bioethanol production from CPH and UPP.

Materials and methods

Substrates

CPH and UPP were obtained from Boki, Southern Nigeria and hydrothermally pretreated as previously reported (Undiandeye *et al.* 2022a). Briefly, the CPH and UPP were hydrothermally pretreated at an optimal temperature and time of 140 °C and 10 minutes respectively. The glucose concentration was 35 g/L and 43 g/L in the pretreated CPH and UPP respectively.

Enzyme and yeast inoculum

Cellic CTec2, as cellulase was obtained from Novozymes (Bagsvaerd, Denmark). The enzyme activity was 159 (± 2) FPU/mL, determined as described by Afzali *et al.* (2020). A dry form of commercially available 5% (w/v) *Sacchromyces cerevisiae* was obtained from the department of microbiology, university of Port Harcourt. A mixture of 10 g/L of yeast extract, 20 g/L of glucose, 20 g/L of peptone and 20 g/L Agar was dissolved in distilled water and then sterilized in an autoclave for 30 minutes at 121 °C according to the method described by the national Renewable Energy Laboratory (NREL). The final cell count of *Sacchromyces cerevisiae* was 6.95×10^7 cells/mL determined using a Hemocytometer (Electron Microscopy Sciences, Hatfield, United States).

Simultaneous saccharification and fermentation

Simultaneous saccharification and fermentation of the substrates was performed in sterilised 200 mL Erlenmeyer flasks with a working volume of 80 mL in order to maintain a 2:5 working volume to total flask volume as recommended by the NREL. Each flask contained the substrates, enzyme, yeast (1% w/v) and peptone (2% w/v). Enzyme loading was kept constant at 10 FPU/g following the study of Tang *et al.* (2019). Substrates loading and pH were set according to the experimental design. Flasks were incubated in shakers with set temperatures according to the experimental design and at 110 rpm. About 0.5 mL of aliquots was extracted after 72 hours for analysis.

Optimization of the simultaneous saccharification and fermentation process

By using the Box-Behnken design method of the Response surface methodology, three process parameters were optimized for the maximum yield of bioethanol from the substrates. These parameters were pH (5-7), temperature (30 – 50 °C) and solid loading (5 – 10%, w/v). A total of 17 runs were generated and carried out. Experimental data were fitted into the polynomial equation (Equation 1) generated by the Design Expert software (Stat-Ease Inc., United states).

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad 1$$

where Y is the response variable (ethanol), β0 = intercept, and other parameters are the coefficients of interactions.

Table 1. Box-Behnken Design

Parameter	Symbol	Low	Centre	High
Temperature (°C)	X_1	30	40	50
pH	X_2	5	6	7
Solid loading (w/v)	X_3	5	7.5	10

Scale-up of bioethanol production

Using the optimal process parameters, bioethanol production was carried out in 12 L bioreactors with working volumes of 5 L. The fermentation process lasted for 120 hours with samples taken out for analysis at 0, 8, 24, 48, 72, 96, and 120 hours as recommended by the NREL.

Kinetic modelling of bioethanol production

Kinetic models each were used to describe the change in yeast growth and bioethanol production during the SSF process. The changes in cell growth were described by the logistic model (Equation 2), while the dual pool model (Equation 3) and the modified Gompertz model (Equation 4) were used for the study of ethanol production rate.

$$X = \frac{x_0 \exp(\mu_{max}.t)}{1-\left[\left(\frac{x_0}{x_{max}}\right)(1-\exp(\mu_{max}.t))\right]} \quad 2$$

$$c = c_{max}[1 - \alpha e^{-k_1 t} - (1-\alpha)e^{-k_2 t}] \quad 3$$

$$c = c_{max}.exp\left\{-exp\left[\frac{R_{max}.e}{c_{max}}\right].(\lambda - t) + 1\right\} \quad 4$$

where X = biomass (g/L), x_0 = initial cell concentration (g/L), x_{max} = maximum cell concentration (g/L), μ_{max} = maximum specific growth rate (g/L/hr), c = ethanol concentration (g/L), c_{max} = maximum ethanol concentration (g/L), α = fraction of degradable materials, k_1 = first-order kinetic constant for the fast degradable material, k_2 = first-order kinetic constant for the slow degradable materials, R_{max} = maximum rate of ethanol production (g/L/hr), λ = lag phase (hr), t = fermentation time (hr), and e = Euler's constant.

Analytical methods

Ethanol concentration was measured using a 7890A gas chromatography with a flame ionization detector as previously described (Undiandeye *et al.* 2022b). Glucose concentration was measured using an Azura HPLC system (Knauer GmbH, Berlin, Germany) following the method previously described (Undiandeye *et al.* 2022c). Extracted samples were centrifuged at 10 000 rpm for 10 minutes and at 4 °C, and then used for dry cell weight determination by oven drying to a constant weight following the method described by NREL (Dowe and Mcmillan 2008). Ethanol yield, Y, was calculated using Equation 5 (Fogler 2016).

$$Y_{Ethanol} = \frac{mass\ concentration\ of\ ethanol\ formed}{mass\ concentration\ of\ glucose\ consumed}\left(\frac{g}{g}\right) \quad 5$$

Kinetic model evaluation and statistical analysis

Parameters from Equations 2, 3 and 4 were estimated using the Solver tool in Microsoft Excel. Three statistical parameters including the coefficient of determination (R^2),

the root-mean-square-error (Equation 6) and the Alkaike Information criterion (Equation 7) were used to select the most appropriate model that described the experimental data. Experimental data were analysed statistically using analysis of variance (ANOVA) and the response surface plots generated from the Design Expert.

$$RMSE = \sqrt{\frac{SS}{n}} \quad 6$$

$$AIC = nLn\left(\frac{SS}{n}\right) + 2c \quad 7$$

where ss = squared sum of residuals, n = number of data set, c = number of parameters in the model.

Results and discussion

Effect of process parameters on bioethanol production

The concentration of bioethanol produced from the substrates is shown in the Box-Behnken design matrix in Table 2.

Table 2. Box-Behnken design matrix for optimization of process parameters

	Process parameters			Concentration (g/L)	
Run	Temp (°C)	pH	SL (%, w/v)	CPH	UPP
1	40	5	5.00	5.89	9.89
2	40	6	17.50	20.78	37.07
3	40	6	17.50	18.09	31.94
4	50	7	17.50	0.30	0.20
5	40	6	17.50	15.13	27.11
6	40	7	30.00	16.00	27.79
7	40	7	5.00	4.94	8.03
8	30	7	17.50	8.08	13.87
9	50	6	30.00	0.35	0.41
10	50	5	17.50	0.29	0.29
11	30	6	30.00	0.16	0.12
12	40	6	17.50	16.89	28.21
13	50	6	5.00	0.28	0.30
14	40	6	17.50	15.96	27.93
15	30	5	17.50	7,96	14.28
16	40	5	30.00	0.50	0.70
17	30	6	5.00	8.00	15.00

SL, solid loading; CPH, cocoa pod husk; UPP, unripe plantain peels.

The multivariate regression equations generated by the Design Expert software with only the significant terms are shown in Equation 8 for CPH and in Equation 9 for UPP. These models were significant (Table 3 and Table 4) and could predict the production of ethanol from the respective substrates.

$$Y = 17.37 - 2.87X_1 + 4.11X_2X_3 - 8.92X_1^2 - 4.30X_2^2 - 6.25X_3^2 \quad 8$$

$$Y = 30.45 - 5.26X_1 + 7.24X_2X_3 - 15.47X_1^2 - 7.82X_2^2 - 11.03X_3^2 \quad 9$$

where Y= ethanol production, X_1 = temperature, X_2 = pH and X_3 = solid loading.

The optimal conditions for bioethanol production from both CPH and UPP were 40 °C, pH 6 and solid loading at 17.5 % (w/v). An optimal temperature of 40 °C compares with 38.87 °C and 39 °C reported by Zhang *et al.* (2015) and Jugwanth *et al.* (2020) in the SSF of pre-treated water hyacinth and sugarcane bagasse respectively. An optimal pH of 6 has also been reported in the SSF of pineapple peels. A process temperature of 50 °C significantly reduced ethanol concentration in both CPH and UPP probably because the yeast was inhibited at that temperature (Robak and Balcerek 2020).

Table 3. ANOVA for quadratic model of bioethanol production from CPH.

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	814.23	9	90.47	8.34	0.0053	Significant
X_1	66.01	1	66.01	6.09	0.0430	
X_2	26.93	1	26.93	2.48	0.1591	
X_3	0.56	1	0.56	0.05	0.8275	
X_1X_2	0.00	1	0.00	0.00	0.9877	
X_1X_3	15.64	1	15.64	1.44	0.2689	
X_2X_3	67.62	1	67.62	6.24	0.0412	
X_1^2	335.31	1	335.31	30.92	0.0009	
X_2^2	77.48	1	77.48	7.14	0.0319	
X_3^2	164.40	1	164.40	15.16	0.0059	
Residual	75.92	7	10.85			
Lack of Fit	56.54	3	18.85	3.89	0.1112	Not significant
Pure Error	19.38	4	4.84			
Cor Total	890.15	16				

Table 4. ANOVA for quadratic model of bioethanol production from UPP.

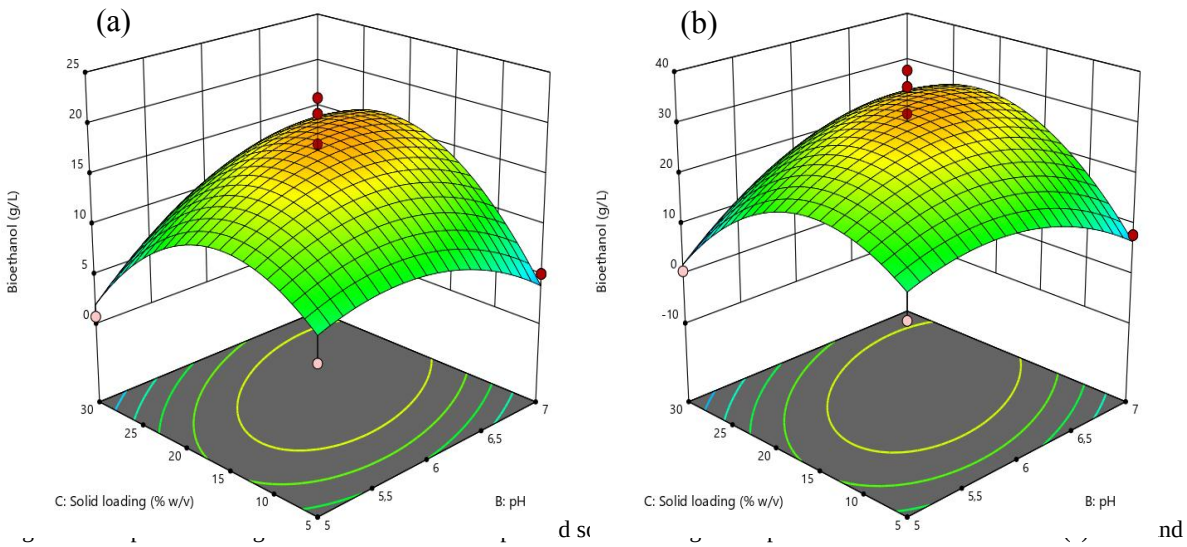
Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	2531.00	9	281.22	7.76	0.0066	Significant
X_1	221.27	1	221.27	6.11	0.0427	
X_2	76.43	1	76.43	2.11	0.1897	
X_3	2.20	1	2.20	0.06	0.8122	
X_1X_2	0.03	1	0.03	0.00	0.9797	
X_1X_3	56.18	1	56.18	1.55	0.2531	
X_2X_3	209.53	1	209.53	5.78	0.0471	
X_1^2	1007.52	1	1007.52	27.81	0.0012	
X_2^2	257.74	1	257.74	7.12	0.0321	
X_3^2	511.85	1	511.85	14.13	0.0071	
Residual	253.56	7	36.22			
Lack of Fit	184.99	3	61.66	3.60	0.1240	Not significant
Pure Error	68.57	4	17.14			
Cor Total	2784.56	16				

Figure 1 shows the 3D plots of the significant 2-factor interactions. For all process parameters, the middle points interactions produced the maximum concentration of ethanol from both substrates. A simultaneous increase in solid loading and pH from the lowest values to the middle values significantly increased ethanol yield by up to 256 % in both substrates. When these parameters were further increased, bioethanol concentration reduced by 22 %. A higher solid loadings above 17.5 % w/v may have reduced the agitation and consequently the mass transfer and aeration in the system, leading to a reduced production of bioethanol.

The interaction between temperature and pH appears to affect the production of bioethanol from both substrates (Table 2). Ethanol concentration increased from 7.96 g/L to 20.78 g/L in CPH and 14.28 to 37.07 g/L in UPP when there was a simultaneous increase in temperature and pH from 30 °C to 40 °C and 5 to 6 respectively at constant solid loading. When the temperature and pH were simultaneously increased to 50 °C and 7 respectively, ethanol concentration significantly dropped. However, as seen from Equations 7 and 8, the interaction effect of temperature and pH on bioethanol production was not significant. Therefore, the change in ethanol concentration as a result of a simultaneous change in both temperature and pH may largely be due to the influence of temperature.

Ethanol concentration was also affected by the interaction between temperature and solid loading, with the lowest

concentration produced when temperature was increased to 50 °C and solid loadings was increased to 30 %(w/v). At a temperature and solid loadings of 40 °C and 17.5 %(w/v) respectively, a maximum ethanol concentration of 20.78 g/L and 37.07 g/L was obtained from CPH and UPP respectively. Again, this combined effect may be due largely to the effect of temperature.



d s nd (b) UPP.

Experimental and theoretical ethanol yield under optimal conditions

During the scale-up of the SSF process, ethanol production from both substrates increased gradually until 72 hours after which the concentration started dropping, probably because of the depletion in glucose concentration (Figure 2). Glucose consumption was rapid within the first 24 hours in the fermentation of both substrates, reaching an ethanol yield of 0.50 ± 0.27 g/g and a maximum concentration of 20.78 g/L after 72 days from the CPH. An ethanol concentration of 20.78 g/L from CPH is comparable to 18.06 g/L reported by Hernández-Mendoza *et al.* (2021) in the fermentation of CPH. If a modified yeast is used for the fermentation, the concentration of ethanol may be improved. For instance, Igbinadolor (2012) obtained an ethanol concentration of 29.7 g/L from CPH using a genetically modified yeast and 14.0 g/L when an unmodified culture was used. For the UPP, a maximum ethanol concentration of 37.07 g/L was produced after 72 hours using the optimal conditions, which is comparable to 38.81 g/L reported by Alonso-Gómez *et al.* (2020) in the fermentation of UPP. The yield of ethanol production from UPP in the present study was 0.53 g/g.

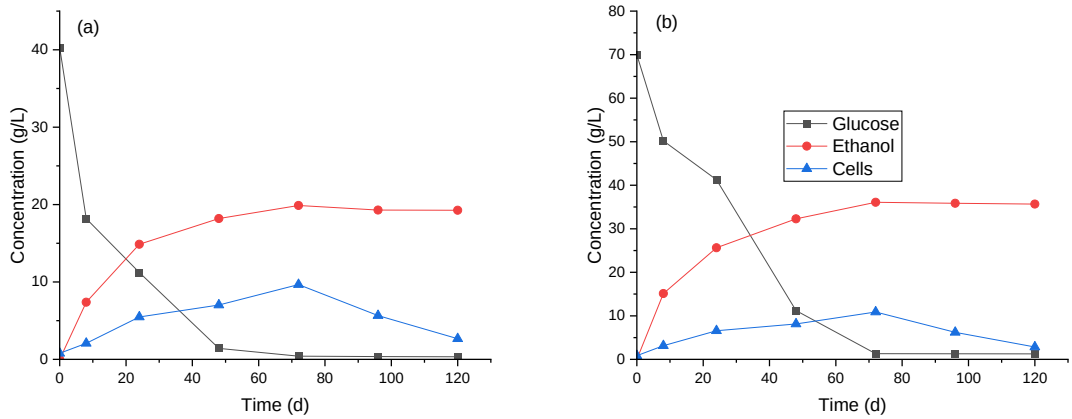


Figure 2: Dynamics of glucose, ethanol and yeast concentration in (a) CPH and (b) UPP during fermentation.

Model fittings to experimental data

Kinetic models were used to fit the experimental data of ethanol production and cell growth. As seen in Figure 3, all models gave a good fit to the experimental data as seen from the high values of the coefficient of determination (R^2) in Table 5. However, concerning ethanol production, the smaller values of RMSE and the AIC from the dual pool kinetic model makes it a better model compared to the modified Gompertz model. The kinetic values obtained in the present study are comparable to those obtained from the fermentation of other agricultural residues like potato peel wates (Chohan *et al.* 2020), sugarcane leave waste (Moodley and Kana 2019), bamboo and corn cobs (Laltha *et al.* 2021).

Table 5. Kinetic parameters and statistical indicators from the models

	Logistic model						
	X_0	X_{mass}	μ_{max}	R^2	RMSE		
CPH	0.90	7.74	0.213	0.978	0.023		
UPP	0.97	9.99	0.288	0.929	0.018		
	Dual pool model						
	c_{max}	α	k_1	k_2	R^2	RMSE	AIC
CPH	19.99	0.673	0.136	0.008	0.9739	0.057	12.98
UPP	36.18	0.697	0.172	0.012	0.9863	0.107	14.23
	Modified Gompertz model						
	c_{max}	R_{max}	λ	R^2	RMSE	AIC	
CPH	20.17	0.943	3.05	0.9706	0.069	17.21	
UPP	36.42	1.137	2.93	0.9791	0.129	21.47	

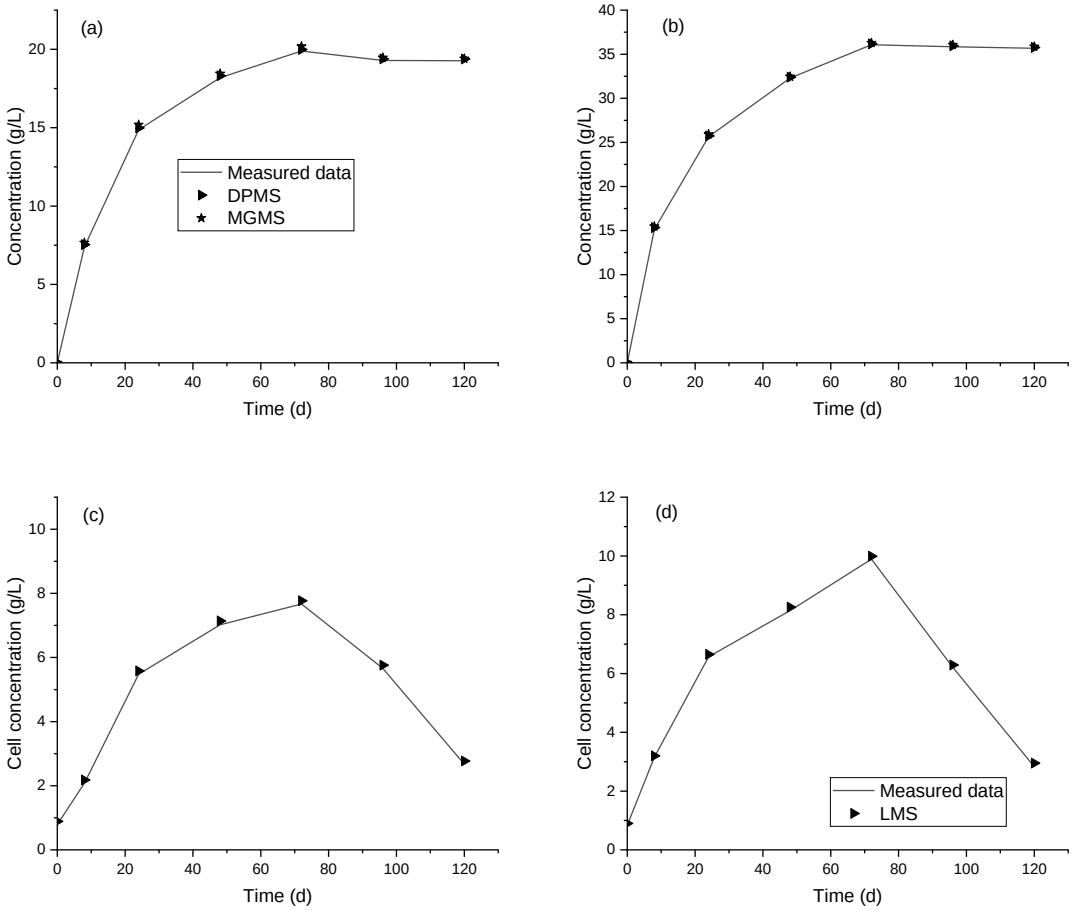


Figure 3. Kinetic fittings of the experimental data of (a) bioethanol production from CPH, (b) bioethanol production from UPP, (c) yeast growth during SSF of CPU and (d) yeast growth from SSF of UPP (DPMS, dual pool model; MGMS, modified Gompertz model; LMS, Logistic model).

Conclusion

Cocoa pod husks and unripe plantain peels have shown to be potential substrates for bioethanol production especially if they are hydrothermally pretreated. The simultaneous saccharification and fermentation of these substrates should be carried out at a temperature of 40 °C, pH 6 and a solid loading of 17.5 %w/v in order to obtain a maximum yield. The dual pool kinetic model gave a better description of ethanol production from CPH and UPP better than the modified Gompertz model, and may therefore be used for the study of the fermentation process in order to enhance the design and optimization of the process.

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