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Optimization of ethanol production from fermentation of cassava mill effluent

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Abstract

The quantity of ethanol produced from fermentable waste is usually small. The aim of this research was to maximize ethanol production from cassava mill effluent (CME) using *Bacillus* and *Saccharomyces*. Effluent pH, inocula-effluent ratio, and fermentation duration were selected as the adjustable parameters. Ethanol concentrations obtained from different value combinations of these parameters were fitted using a polynomial model so as to obtain a predicting polynomial equation. The equation was used in generating prediction profile from which the combined values of the parameters that lead to the highest achievable ethanol concentration (AEC) was determined. The predicted combined values were implemented in a new setup, and the ethanol produced determined. The results showed that ethanol produced from different combinations of the parameters ranged from 8.2 – 18.2 % v/v. Prediction profile generated from the resolved polynomial equation showed that the highest ethanol concentration (18.0%) is achievable at pH = 7, Inoculum-Effluent ratio = 0.02, and Fermentation/Incubation duration = 3 days. Ethanol concentration and yield obtained using these values were 17.6±0.2% v/v and 48.6±0.6% respectively. It is concluded that optimization studies is advantageous, and should be applied in assessing fermentation of an agro-substrate for ethanol production before using such substrate.

Keywords: Box-Behnken experimental design; Prediction profile; Cassava mill effluent; Ethanol; Fermentation

1. Introduction

Ethanol can be produced from fermentation of wastes containing carbohydrates/starch [1, 2]. Such wastes include spoilt fruits such as banana, starch-based food leftovers, and starch-based agro-wastes such as cassava processing wastewater also called cassava mill effluent. Cassava mill effluent (CME) is the wastewater generated during the processing of cassava tubers into edible products like Garri. It is acidic (pH 3.2 – 4.6), and contains about 30 to 73 % carbohydrate/starch, about 1 to 50 mg/L cyanide, and little amounts of nitrate and phosphate [3-5]. CME contains little or no free sugars, and therefore its starch content has to be converted to free sugars before fermentation to ethanol. This conversion has been achieved through the use of acid and enzyme hydrolysis [6, 7], and can be achieved using starch hydrolyzing microorganisms.

Microorganisms that can hydrolyze starch include *Aspergillus fumigatus*, *Chaetomium globosum*, *Lactobacillus acidophilus*, and several species of *Bacillus* [8, 9]. On the other hand, microorganisms that can ferment sugars into ethanol include *Zymomonas mobilis*, *Saccharomyces cerevisiae*, and *Pichia kluyveri* [10, 11]. Hydrolysis of starch in CME, and subsequent fermentation for ethanol production can therefore be achieved through the combine use of microorganisms that can hydrolyze starch and ferment the sugars that are the end products of the hydrolysis. Microorganisms that hydrolyze starch occur in starch-based agro-wastes including CME [5, 12, 13]. Microorganisms,

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especially yeasts that ferment sugars into ethanol occur on some fruits [14, 15] and in sap from different species of palm trees [7, 16, 17].

The quantity and yield of ethanol produced from hydrolysis and fermentation of agro/food wastes is usually small ranging from 2 to 12% [18-20]. There is therefore need to maximize ethanol production from fermentable wastes, and this can be achieved through optimization. Optimization is the process of making something as effective, efficient, or functional as possible [21]. It is widely used in various fields to achieve the best possible outcome based on given constraints and objectives. During optimization, a polynomial equation is generated which can be used to predict the maximum output of a system resulting from combinations of values of adjustable factors or variables of the system [22]. A combination of adjustable factors that influence ethanol production from fermentable substrates that will lead to maximum ethanol production can therefore be predicted using such generated polynomial equation. Factors that have been shown to influence ethanol production during fermentation include nutrients, medium pH, fermentation duration, substrate amount/concentration, inoculating microorganism, inoculum size, and contaminating microorganisms [23-25]. Some of these factors such as medium pH, fermentation duration, and inoculum size or inoculum-substrate ratio, are adjustable variables.

The aim of this research was to maximize ethanol production from cassava mill effluent through simultaneous hydrolysis and fermentation using *Bacillus* and *Saccharomyces* species. Optimization was applied in the maximization process, and the optimization was focus on the following adjustable variables/parameters: Effluent pH, inocula-effluent ratio, and fermentation duration.

2. Materials and Methods

2.1. Sample Collection

Effluent from a Cassava Mill in Ahoda-West Local Government Area in Nigeria was collected using disinfected 10 L Jerrycan. The effluent was transported within 24 hours to the Microbiology laboratory of the Rivers State University, Nigeria, and stored at 4 °C in a fridge until required for use.

Stock cultures of *Bacillus infantis* and *Saccharomyces cerevisiae* were obtained from the Microbiology laboratory of Rivers State University (RSU), Nigeria. The source of the stock cultures were indicated as CME and sap from the palm tree *Raphia vinifera*, respectively. Plate cultures of the *Bacillus* were prepared from the *Bacillus infantis* stock culture by picking growth from it and inoculating onto nutrient agar plates. The inoculated plates were incubated at 35 °C for 48 hours. Similarly, plate cultures of the *Saccharomyces* were prepared. However, potato dextrose agar (PDA) plates were used and incubation was at ambient temperatures (24 – 32 °C) for 4 days.

2.2. Authentication of the Microorganisms

The identity of the *Bacillus* was verified through the following tests: Gram-stain reaction, acid fast reaction, endospore staining, catalase, oxidase, motility, methyl red, Voges-Proskauer, citrate utilization, starch hydrolysis, and fermentation tests using glucose, lactose, maltose, mannitol, and sucrose. On the other hand, the identity of the *Saccharomyces* species was verified through the following tests: Gram-stain reaction, catalase, oxidase, methyl red, Vogues-Proskauer, citrate utilization, starch hydrolysis, and fermentation tests using glucose, maltose, and sucrose. The tests were performed as described in Microbiology literatures [8, 26].

2.3. Physicochemical Analysis of the Cassava Mill Effluent

The cassava mill effluent (CME) was analyzed for pH, and glucose, starch, and cyanide concentrations. The pH was determined using a calibrated hand held pH meter (Hanna Instruments, Ltd, UK). The concentration of glucose in the CME was determined using the Phenol-Sulphuric acid method [27] with slight modification as follows: 5 ml of the CME was mixed with 5 ml hot ethanol (95% v/v), and the mixture centrifuged at 4000 rpm for 10 minutes. After centrifugation, the supernatant was decanted into a test tube, and the sediment kept aside for starch analysis. A volume of 9 ml of the supernatant was placed in 250 ml capacity beaker, followed by the addition of 500 µl of phenol solution (5% w/v), and 2.5 ml conc. Sulphuric acid. The resulting coloured (yellow-orange colour) hot solution was allowed to cool down, and the absorbance of the solution at 490 nm was measured using a 721 VIS Spectrophotometer (Huanghua Faithful Instrument Co. Ltd, China). The absorbance reading was used to extrapolate the glucose concentration from a previously obtained graph of concentrations of glucose in phenol-sulphuric acid solution versus absorbance. The concentration of starch in the CME was determined through acid hydrolysis and the Phenol-Sulphuric acid method described above. Acid hydrolysis was achieved as follows: 7.5 ml Perchloric acid was added to the sediment, and allowed for 1 hour at ambient temperature (32 °C). Afterwards, the mixture was filtered through a Whatman no. 4 filter paper

into 50 ml measuring cylinder, and then the volume made up to 10 ml with distilled water. The resulting suspension was then subjected to Phenol-Sulphuric acid treatment, absorbance reading, and derived glucose concentration determination as described above. The derived glucose amount was expressed as the starch concentration. The cyanide content in the CME was determined using the alkaline picrate method as described by Eke-Emezie *et al* [28].

2.4. Preparation of the Microorganisms

The *Saccharomyces cerevisiae* was proliferated by inoculation into 10 ml sterile potato dextrose broth, and incubated at ambient temperatures (24 – 32 °C) for 4 days. After incubation, the turbidity of the resulting broth culture was measured with the aid of a Spectrophotometer (721 VIS Spectrophotometer; Huanghua Faithful Instrument Co. Ltd, China) set at 600 nm, and then adjusted to the turbidity of a freshly prepared 0.5 McFarland standard [29] (CLSI, 2012) using sterile normal saline. Similarly, the *Bacillus infantis* was proliferated by inoculation into 10 ml sterile nutrient broth, and incubated at 35 °C for 48 hours. After incubation, the turbidity of the resulting broth culture was measured, and then adjusted to the turbidity of 0.5 McFarland standard using sterile normal saline. A suspension of the *Bacillus* and *Saccharomyces* in a ratio of 1:2 was then prepared and used for the fermentation optimization experiment. The ratio was selected based on findings of prior work [30].

2.5. Fermentation Optimization Experiment

Three fermentation parameters (effluent pH, inoculum-effluent ratio, and fermentation duration) were varied for the optimization experiment. Range of variation of the parameters and subsequent experimental design matrix are presented in Table 1 and Table 2. Range of variation and the experimental design matrix were based on the Box-Behnken coded value levels and Box-Behnken experimental design respectively [31]. In line with the experimental design matrix (Table 2), different fermentation medium were prepared as presented in Table 3. First of all, 8 g nutrient broth powder was added to 1.5 L of the CME so as to provide nutrients including nitrogen for the inoculum (*Bacillus* and *Saccharomyces*). The modified medium was then distributed into 13 labelled conical flasks (250 ml capacity), accordingly as depicted in Table 3. Also, the pH of the medium in each labelled flask was modified accordingly. Modification of pH was achieved using 5.0 M NaOH (aqueous). The flasks and its contents were sterilized in an Autoclave at 121 °C for 15 minutes. After sterilization, the flasks were allowed to cool and then inoculated with different volumes of the standardized suspension of the *Bacillus* and *Saccharomyces* accordingly. After inoculation, the flasks were incubated at ambient temperatures (24 – 32 ºC) in semi-anaerobic condition. On completion of the incubation/fermentation duration for each labelled flask, the ethanol concentration was determined using a Refractometer. Ethanol concentrations obtained and values of the fermentation parameters were fitted using the generalized polynomial model for 3-factor design (Eq. 1; [32]).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{1,2} X_1 X_2 + \beta_{1,3} X_1 X_3 + \beta_{2,3} X_2 X_3 + \beta_{1,1} X_1^2 + \beta_{2,2} X_2^2 + \beta_{3,3} X_3^2 \quad \text{...Eq. 1}$$

Where Y is the predicted response (ethanol concentration); X_1, X_2, and X_3 represent the values of the factors (pH, inoculum-effluent ratio, and fermentation duration); β_0 is the value of fitted response at the centre point of the design; β_1, β_2, and β_3 are the linear coefficients; $\beta_{1,2}$, $\beta_{1,3}$, and $\beta_{2,3}$ are the interaction coefficients; and $\beta_{1,1}$, $\beta_{2,2}$, and $\beta_{3,3}$ are the quadratic coefficients.

The various polynomial equations obtained from the model fitting were resolved through matrices to a single polynomial equation. The calculation was carried out using Microsoft excel®. The derived single polynomial equation was then used to generate prediction profile of ethanol concentrations obtainable from different theoretical combination of extended value ranges of the 3 parameters. From the prediction profile, the combined values of the 3 parameters that lead to the highest ethanol concentration were determined.

Table 1 Variation range of the parameters for the optimization experiment

Fermentation parameters	**Factor code**	**Coded Levels**		
		-1	**0**	**+1**
pH	X_1	6.0	7.0	8.0
Inoculum-effluent ratio	X_2	0.005	0.015	0.025
Fermentation duration (days)	X_3	2	3	4

Table 2 Experimental design matrix for the fermentation and optimization experiment

EN	pH		IER		FD (days)	
	CL	(X_1)	CL	(X_2)	CL	(X_3)
1	-1	(6.0)	-1	(0.005)	0	(3)
2	-1	(6.0)	+1	(0.025)	0	(3)
3	+1	(8.0)	-1	(0.005)	0	(3)
4	+1	(8.0)	+1	(0.025)	0	(3)
5	-1	(6.0)	0	(0.015)	-1	(2)
6	-1	(6.0)	0	(0.015)	+1	(4)
7	+1	(8.0)	0	(0.015)	-1	(2)
8	+1	(8.0)	0	(0.015)	+1	(4)
9	0	(7.0)	-1	(0.005)	-1	(2)
10	0	(7.0)	-1	(0.005)	+1	(4)
11	0	(7.0)	+1	(0.025)	-1	(2)
12	0	(7.0)	+1	(0.025)	+1	(4)
13	0	(7.0)	0	(0.015)	0	(3)

EN: experimental number, IER: inoculum-effluent ratio, FD: fermentation duration, CL: coded levels, X_1-X_3: factor codes.

Table 3 Media (100 ml) preparation, inoculation, and incubation

EN	MCE	PHM	IER	INV	IFD
	(ml)			(ml)	(days)
1	99.5	6.0	0.005	0.5	3
2	97.5	6.0	0.025	2.5	3
3	99.5	8.0	0.005	0.5	3
4	97.5	8.0	0.025	2.5	3
5	98.5	6.0	0.015	1.5	2
6	98.5	6.0	0.015	1.5	4
7	98.5	8.0	0.015	1.5	2
8	98.5	8.0	0.015	1.5	4
9	99.5	7.0	0.005	0.5	2
10	99.5	7.0	0.005	0.5	4
11	97.5	7.0	0.025	2.5	2
12	97.5	7.0	0.025	2.5	4
13	98.5	7.0	0.015	1.5	3

EN: Experimental number, MCE: modified cassava mill effluent, PHM: pH modification, IER: inoculum-effluent ratio, INV: inoculum volume, IFD: incubation/fermentation duration.

2.6. Experimenting the Predicted Optimized Combination

The combined values of the 3 parameters that lead to the highest ethanol concentration were used in a final ethanol production process. In the final process, fermentation medium volume was increased to 10 L. At the end of the optimized predicted fermentation duration, pH, residual amount of starch, glucose, and cyanide, and ethanol concentration was determined.

3. Results

3.1. Characteristics of the Microorganisms

The reactions of the *Bacillus* and *Saccharomyces* species to the tests used are presented in Table 4. Of note is that the *Bacillus* is rod shape Gram positive bacteria having endospores and able to hydrolyse starch, while the *Saccharomyces* is oval shape Gram positive yeast that is able to ferment starch, glucose, maltose, and sucrose.

Table 4 Morphological and biochemical characteristics of the microorganisms

Tests	BI	SC
GSR	+	+
MPL	Rods	Oval cells
AFR	-	ND
POE	+	ND
CTS	+	+
OXD	+	-
MTL	+	ND
MTR	+	+
VPR	-	-
CTU	-	+
SHD	+	+
STF	ND	AG
GLF	A	AG
LTF	A	ND
MLF	A	AG
MNF	A	ND
SUF	A	A

BI: *Bacillus infantis*, SC: *Saccharomyces cerevisiae*, GSR: Gram stain reaction, MPL: Morphology, AFR: Acid fast reaction, POE: presence of endospores, CTS: Catalase, OXD: Oxidase, MTL: Motility, MTR: Methyl red, VPR: Voges-Proskauer, CTU: Citrate utilization, SHD: Starch hydrolysis, STF, GLF, LTF, MLF, MNF, and SUF: Starch, Glucose, Lactose, Maltose, Mannitol, and Sucrose fermentation respectively, A: acid production, AG: acid and gas production, ND: not determined.

3.2. Derived Polynomial Equation, Prediction Profile, and Ethanol Yield from Optimized Combination

The amount of ethanol produced on completion of the incubation/fermentation duration for each labelled flask, values of the fermentation parameters, and matrix of the fitted fermentation parameters using Eq. 1 is presented in Table 5. From the Table it can be seen that the ethanol concentrations obtained from the different combinations of the selected parameters ranged from 8.2 – 18.2 % v/v. Resolving the 13 polynomial equations derived from the information in Table 5 through matrices revealed the values of the coefficients β_0, β_1, β_2, β_3, $\beta_{1,2}$, $\beta_{1,3}$, $\beta_{2,3}$, $\beta_{1,1}$, $\beta_{2,2}$, and $\beta_{3,3}$ to be -134.64, 28.45, -305, 33, 70, 0.3, 65, -2.08, -10250, and -5.93 respectively. The single derived polynomial equation is thus given as:

$$Y = -134.64 + 28.45X_1 - 305X_2 + 33X_3 + 70X_1X_2 + 0.3X_1X_3 + 65X_2X_3 - 2.08X_1^2 - 10250X_2^2 - 5.93X_3^2$$ …Eq. 2

Analysis of variance (ANOVA) of the polynomial equation indicated that at least 1 coefficient out of the 9 coefficients is significant. In other words, a regression model exists between amount of ethanol produced and one or more of the factors (pH, Inoculum-Effluent ration, and Fermentation/Incubation duration). A summary of the ANOVA is presented in Table 6. In the Table, it can be seen that F calculated is greater than F tabulated, indicating the significance of at least one of the coefficients.

The prediction profile of ethanol concentrations obtainable from different theoretical combination of the three parameters as derived using Eq. 2 is presented in Table 7. From the prediction profile, it can be seen that the theoretical highest ethanol concentration (18.0%) is achievable at pH = 7, Inoculum-Effluent ratio = 0.02, and Fermentation/Incubation duration = 3 days. Ethanol concentration and yield obtained after using the values of this optimized combination was 17.6±0.2% v/v and 48.6±0.6% respectively. The actual ethanol concentration obtained indicates that the developed equation can predict ethanol concentration at about 98 % accuracy.

Table 5 Matrix of amount ethanol produced and fitted fermentation parameters

	Y	**β_0**	**β_1**	**β_2**	**β_3**	**$\beta_{1,2}$**	**$\beta_{1,3}$**	**$\beta_{2,3}$**	**$\beta_{1,1}$**	**$\beta_{2,2}$**	**$\beta_{3,3}$**
			pH	**IER**	**FD**						
	AEP	**Matrix [X]**									
EN	(% v/v)		X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	X_1^2	X_2^2	X_3^2
1	14.8	1	6	0.005	3	0.030	18	0.015	36	0.000025	9
2	13.4	1	6	0.025	3	0.150	18	0.075	36	0.000625	9
3	15.4	1	8	0.005	3	0.040	24	0.015	64	0.000025	9
4	16.8	1	8	0.025	3	0.200	24	0.075	64	0.000625	9
5	8.8	1	6	0.015	2	0.090	12	0.03	36	0.000225	4
6	8.2	1	6	0.015	4	0.090	24	0.06	36	0.000225	16
7	11.6	1	8	0.015	2	0.120	16	0.03	64	0.000225	4
8	12.2	1	8	0.015	4	0.120	32	0.06	64	0.000225	16
9	9.4	1	7	0.005	2	0.035	14	0.01	49	0.000025	4
10	10.2	1	7	0.005	4	0.035	28	0.02	49	0.000025	16
11	11.0	1	7	0.025	2	0.175	14	0.05	49	0.000625	4
12	14.4	1	7	0.025	4	0.175	28	0.1	49	0.000625	16
13	18.2	1	7	0.015	3	0.105	21	0.045	49	0.000225	9

EN: Experimental number, AEP: Amount of ethanol produced, IER: Inoculum-Effluent ratio, FD: Fermentation/Incubation duration (in days).

Table 6 Summary of the analysis of variance of the polynomial equation

SV	**DF**	**SS**	**MS**	**$F_{Cal.}$**	**$F_{Tab.}$**
Regression	3	59.36	19.79	2.95	2.81
Error	9	60.32	6.70		

SV: Source of Variation, DF: Degree of Freedom, SS: Sum of Squares, MS: Mean Squares, $F_{Cal.}$: F calculated, $F_{Tab.}$: F tabulated at α = 0.1, DF1 (v1) = 3, DF2 (v2) = 9.

Table 7 Ethanol production (% v/v) prediction profile

X_2 (IER)	X_3 (FID, days)				
	1	**2**	**3**	**4**	**5**
At pH = 6.0					
0.005	-9.3	8.0	13.5	7.1	-11.1
0.010	-9.2	8.5	14.3	8.2	-9.7
0.015	-9.6	8.4	14.6	8.8	-8.8
0.020	-10.5	7.9	14.3	8.9	-8.4
0.025	-11.9	6.8	13.6	8.5	-8.5
At pH = 7.0					
0.005	-7.2	10.4	16.2	10.1	-7.9
0.010	-6.8	11.2	17.3	11.5	-6.1
0.015	-6.8	11.5	17.9	12.5	-4.8
0.020	-7.3	11.3	18.0	12.9	-4.1
0.025	-8.4	10.5	17.6	12.8	-3.8
At pH = 8.0					
0.005	-9.3	8.6	14.7	8.9	-8.8
0.010	-8.5	9.7	16.1	10.7	-6.6
0.015	-8.2	10.4	17.1	12.0	-5.0
0.020	-8.4	10.5	17.6	12.8	-3.9
0.025	-9.1	10.1	17.5	13.0	-3.3

IER: Inoculum-Effluent ratio, FID: Fermentation/Incubation duration.

3.3. Change in Physicochemical Characteristics of the Cassava Mill Effluent

The pH of the raw CME was 3.85 ± 0.07, glucose concentration 4.7 ± 0.3 mg/ml (0.47%), starch concentration 361±8 mg/ml (36.1%), and cyanide concentration was 3.45 ± 0.07 mg/L. This indicates that the CME was acidic, and it had adequate amount of carbohydrate (glucose + starch) required for ethanol production through fermentation. At the end of fermentation of the modified CME, using the optimized values, the pH dropped from 7.0 to 3.9±0.1, and amount of starch, glucose, and cyanide were 18±2 mg/ml, 0 mg/ml, and 0.037±0.002 mg/L respectively. This indicates that there was drastic reduction in cyanide concentration, and that carbohydrate was adequately consumed for fermentation/transformation into ethanol.

4. Discussion

The *Bacillus* and *Saccharomyces* exhibited the primary attributes of *Bacillus* and *Saccharomyces* species as revealed in Microbiology literatures [8, 33-35]. It is therefore verified that the stock cultures of *Bacillus infantis* and *Saccharomyces cerevisiae* obtained from the Microbiology laboratory of RSU, Nigeria were authentic. The use of these microorganisms in fermentation of CME through optimization procedures, whereby the factors of concern were pH, Inoculum-Effluent ratio, and fermentation duration, yielded an equation that can predict ethanol yield at about 98 % accuracy. In other related works [36-38], combine values of some parameters that affect fermentation of agro/food wastes and led to maximum ethanol production were deciphered through statistical optimization models. In the work of Uncu and Cekmecelioglu [38], the effects of solid load, inoculum volume of baker's yeast, and fermentation time on ethanol production from simultaneously hydrolyzing and fermenting mixed carbohydrate components of kitchen wastes were evaluated through response surface methodology (RSM). Statistical analysis of the polynomial equation developed by RSM was significant. The equation developed predicted ethanol concentration at >90% accuracy, and maximum ethanol concentration of 32.2 g/L (4.8% v/v) and yield of 0.40 g/g (40%) as suggested by the equation occurred at solid load =

20%, inoculum volume = 8.9%, and fermentation duration = 58.8 h (2.5 days). In the work of Sivamani and Baskar [37], cassava peels were subjected to saccharification and fermentation for ethanol production. Substrate and α-amylase concentrations, and simultaneous saccharification & fermentation (SSF) mixture containing glucoamylase and *Zymomonas mobilis* were optimized through Box–Behnken design to yield a polynomial equation. Based on the equation, maximum ethanol concentration and yield of 35 g/L (4.4% v/v) and 83% were obtained at substrate concentration = 69.82 g/L, α-amylase concentration = 24.74% (v/v), and SSF mixture = 5.22% (v/v). Izmirlioglu and Demirci [36] using RSM showed that a medium consisting of 40.4 g/L industrial waste potato, 50 g/L malt extract, and 4.84 g/L $MgSO_4 \cdot 7H_2O$ was an optimized combination and yielded 24.6 g/L (3.1 % v/v) ethanol at 30 °C, 150 rpm, and 48 h of fermentation. The high accuracy of the equation developed in this study for prediction of achievable ethanol concentration is comparable to what was obtained in the study of Uncu and Cekmecelioglu [38]. However, in terms of ethanol concentration in % v/v, the maximum ethanol concentrations obtained in the other studies were low compared to what was obtained in this study. This could be due to the difference in the type of waste used. In this present study the agro-waste used had readily available appreciable amount of starch which could be easily hydrolyzed by both microorganisms supplied as inoculum, whereas in the other studies the agro-wastes used had to be subjected to saccharification so as to obtain starch for hydrolysis. In the situation where saccharification was not required, as in the case of Uncu and Cekmecelioglu [38], only one microorganism (the baker's yeast) was subjected to the burden of both hydrolysis and fermentation. Likewise in the other studies, enzymes were used in place of microorganisms to hydrolyze the starch obtained through saccharification. It is likely that the enzymes became denatured or saturated during the fermentation process, thereby leaving extra burden of starch hydrolysis to the sugar-fermenting microorganism in the medium. In another thought, it could be that pH as part of the combined parameters played significant role in the optimization process. This variable parameter was not part of the combined parameters used in all the other studies. The higher ethanol concentration achieved in this study is an indication that the agro-waste and selected parameters are better than those used in the other studies.

The pH of the raw CME falls within the ranges that have been observed in previous studies [3, 5, 7], likewise for the carbohydrate content [4, 5], and the cyanide content [3-5]. At the end of fermentation of the modified CME, using the optimized values, the pH of the fermented medium was acidic, and there was drastic reduction in cyanide concentration (about 99 %). In other related studies [39-41], cyanide reduction of 68 - 87 %, and pH reduction from a range of 5.9 - 7.2 to a range of 3.0 - 3.8 has been shown to occur during fermentation of cassava tubers in water and cassava mash. Reduction in pH in this study was similar to what have been observed in the other studies. However, the cyanide reduction in this study was greater than what was observed in the other studies. This could be attributed to the small starting concentration of cyanide in the CME used in this study as compared to starting concentrations of cyanide in the cassava tubers and mash in the other studies.

5. Conclusion

The combined values of three variable parameters that will lead to maximum ethanol production from fermentation of treated cassava mill effluent can be deciphered through optimization experiments. Optimization studies are therefore advantageous, and should be carried out to assess the fermentation of agro-substrates for ethanol production before using such substrate. Further research could be directed towards enhancement of spontaneous fermentation of cassava mill effluent through optimization experiment. Maximum ethanol yield through this approach implies that there will be reduced work and cost associated with large scale sterilization of agro-wastes.

Compliance with ethical standards

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Disclosure of conflict of interest

We, L.P. Peekate, L.O. Amadi, and E. Amos, the authors of this manuscript declare that no conflict of interest exists.

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