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Research Article

Optimization of Pretreatment and Hydrolysis Steps and Estimation of Theoretical Bioethanol Potential from Cattle Manure

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Abstract: The depletion of fossil fuels, the pollutant related issues,insecure supply chains of biomass, and the increasing energy prices leads to finding alternative energy sources. The goal of this study was to optimize bioethanol production from cattle manure and to estimate the theoretical bioethanol potential. To achieve this goal, both acid/and alkaline pretreatments and hydrolysis steps were optimized using H_2SO_4 and NaOH. A centered central composite design was used for acid pretreatment optimization, and a central composite design was applied for alkaline pretreatment and diluted acid hydrolysis with respect to physico-chemical parameters. The existing kinetic models were used to estimate bioethanol potential and to follow the degradation of macromolecules to bioethanol. The alkaline pretreatment offered 35% as the highest cellulose content for the time of 5 h and the NaOH concentration ranged between 2 M to 2.3 M. Meanwhile, the highest phenolic compound content was 800 mg/100 g for time varying between 3.7 h and 5 h and NaOH concentration ranged from 1.3 M to 2 M. The acid pretreatment revealed that the optimum area reached 45.5% sugar-free for acid concentration between 3.5 M and 4 M and time ranged from 9 min to 10 min. The results from acid hydrolysis pointed out that the maximum value of sugar-free was 78% for time ranging from 1.9 h to 2 h and the sulfuric acid concentration between 2.9 M and 3.0 M. The amount of cellulose per year was estimated at 593,271 t/year and the amount of bioethanol potential was evaluated at 461,433,000 L/year. The kinetic hydrolysis model based on sulfuric acid concentration showed that the maximum time of hydrolysis and the cellulose decreased when the temperature increased while the quantity of glucose followed the evolution of the temperature. Hence, temperature plays a very important role during the hydrolysis of cellulose. The satisfactory results obtained from this work display that, the production of bioethanol from cattle manure could permit improved environmental sustainability.

***Keywords*:** cattle manure valorization, pretreatment/hydrolysis steps, acid kinetic model, bioethanol potential

1. Introduction

Renewable energy from biomass is a promising solution to face fossil fuel depletion and environmental degradation caused by the use of gasoline in the transportation domain[1-3]. Thus, bioethanol is considered a suitable alternative to gasoline. In fact, Gupta and Verma[4] and Zabed et al.[5] reviewed that the advantages of using bioethanol compared to gasoline are in the reduction of CO and aromatic compound emissions due to its O_2 content (35%), bioethanol contains more combustible elements than gasoline, it is easy to integrate the fuel distribution logistic systems, the octane rating

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of the fuel mixture and its compression ratio is better, and the higher volumetric efficiency as a result of a higher vaporization heat increase the power output of the internal combustion engine.

The bioethanol used as biofuel is mainly produced from plants and agricultural residues[6-8]. However, their use in the production of bioethanol has become competitive with human and animal food[9,10]. Hence, it is necessary to find alternative substrates for the production of bioethanol. Thus, Lee et al.[11]; Azevedo et al.[12]; Hafid et al.[13] proposed the use of animal manure for bioethanol production. In addition, Azevedo et al.[12]; Hafid et al.[13] pointed out that, animal manure is rich in cellulose and hemicellulose thus, can be converted into sugar for subsequent use in bioethanol fermentation. Also, Ho et al.[14]; Azevedo et al.[12] revealed that bioethanol production from animal manure provides several advantages in terms of less energy consumption to depolymerize the crystallinity of holocellulose. In fact, manure contains the pre-depolymerized holocellulose materials via acid-hydrolysis reaction provided by herbivores digestion system for bioethanol conversion thus, energy used in a pretreatment step could be saved. Moreover, the content of N-source in manure is beneficial to ethanologens during the fermentation process. N-source is indeed essential for the growth of ethanologens. In the northern part of Cameroon and particularly in the Adamawa region, cattle manure is abundant, estimated at 1,900,000 dry bones tons[15] and can be a local solution to the gasoline scarcity observed.

The literature review shows four steps for the production of bioethanol: mainly pretreatment, hydrolysis, fermentation and distillation[16,17]. Among them, the choice of the pretreatment method is essential to improve yield and is related to compositional characteristics. More so, Tian et al.[18] and Brodeur et al.[19] presented that typical goals of pretreatment include producing highly digestible solids to enhance sugar yields during hydrolysis, avoiding the degradation of sugars including those derived from hemicellulose, and minimizing the formation of inhibitors for subsequent fermentation steps. These pretreatment methods are chemical, physical, biological and combined. Thus, Sigüencia et al.[20] showed that each of these pretreatments has advantages, disadvantages and scope. Nevertheless, Tayyab et al.[21] and Alvarez-Barreto et al.[22] suggested that the combined pretreatment seems to be promising.

Compositional characteristics of cattle manure from the Adamawa region of Cameroon have been carried out by Samomssa et al.[15] and are presented in Table 1 and can be used to predict suitable pretreatment. This feedstock is rich in cellulose, followed by lignin and hemicellulose. Lignin is a recalcitrant part of the fermentation process. Regarding the value of lignin from cattle manure (18%), alkaline, biological and organosolv pretreatments are suitable to remove it, thus enhancing the porosity, and surface area of biomass, and therefore improving the hydrolysis process. Among them, the alkaline method seems to be suitable for developed countries like Cameroon due to its low cost production. Nevertheless, this pretreatment could be combined with acid pretreatment due to high hemicellulose content (15%) which leads to the hydrolysis of hemicellulose to fermentable sugars and amorphous cellulose conversion to crystalline. However, Preshanthan and Kana[23] and Chang et al.[24] revealed that some physico-chemical parameters such as alkaline/acid solution concentration, heated time, heated temperature and biomass/solution ratio influence pretreatment yield.

Concerning the hydrolysis step, Solarte et al.[25] reported that diluted acid hydrolysis has been widely studied and applied industrially, due to its simplicity and low costs. Chang et al.[24]; Alio et al.[26] revealed that reaction time and acid concentration influenced the yield. The previous works using experimental design to obtain optimum conditions to produce bioethanol from biomass have been done either on the pretreatment step or on the hydrolysis step but there are a few studies on successively optimization on pretreatment and hydrolysis steps of the whole process. In addition, Saeman[27] and Thompson[28] formulated a simplified model in terms of two first-order irreversible pseudo-homogeneous reactions in series to estimate the potential for bioethanol production from feedstock. They were used to establish the amount of sugar obtained from the cellulose and hemicellulose present in the biomass. These models have been widely used in literature to estimate the amount of bioethanol[20,29-35]. To the limit of our knowledge, this estimation has not been done on cattle manure from the Adamawa region of Cameroon. The goal of this study was to optimize pretreatment and hydrolysis steps and to estimate the theoretical bioethanol potential from cattle manure from the Adamawa region of Cameroon.

2 Materials and methods

2.1 *Sampling*

Cattle manure was collected freshly in the Adamawa region of Cameroon which is situated in the northern part and located between the Guineo-Congolian rain forest and the Sudanian savannah. Then it was directly conducted in the lab and was dried at 50 °C until at constant mass and grounded to reduce the particle size. The compositional characteristics were reported by Samomssa et al.[15] and are presented in Table 1.

Table 1. Compositional characteristics of cattle manure on dry basis[15]

Structural analysis (%)	
Cellulose	32.21 ± 0.02
Hemicellulose	15.67 ± 0.06
Lignin	18.50 ± 2.12
Proximate analysis (%)	
Volatile matter	64.67 ± 2.08
Ash	22.31 ± 2.52
Fixed Carbon	13.02 ± 4.06
Ultimate analysis (%)	
Carbon	37.72 ± 1.84
Hydrogen	4.69 ± 0.12
Oxygen	34.74 ± 0.63
Nitrogen	0.80 ± 0.04

2.2 *Scanning Electron Microscope (SEM) and FTIR characterization of cattle manure*

The morphological studies were conducted using a Scanning Electron Microscope (SEM) Hitachi S4800 based on the interaction between electron and sample. The preparation of the sample consisted of dispersing a small quantity on a double-sided carbon adhesive glued to an aluminum sample holder. After that, the sample holder was introduced into the analysis chamber and the image was shown and collected.

FTIR spectra were conducted by means of iS50 RAMAN which is connected to the computer. Accordingly, 10 mg of cattle manure was placed on a sample holder and introduced into the analysis chamber. The results were displayed by computer and then, the data were collected and treated using OMNIC software.

2.3 *Optimization of alkaline/acid pretreatment and diluted acid hydrolysis*

2.3.1 *Establishing the test matrix*

The physico-chemical parameters investigated in this work were sodium hydroxide concentration, boiling time and biomass/solution ratio for alkaline pretreatment; acid concentration, residence time and temperature for acid

pretreatment; reaction time and acid concentration for diluted acid hydrolysis. The studied intervals and experiment design were chosen based on literature review and primary tests. Thus, face centered central composite design was used for acid pretreatment optimization and central composite design was used for alkaline pretreatment and diluted acid hydrolysis. Table 2 illustrates the studied intervals.

Table 2. Studied intervals based on previous works and primary tests

Alkaline pretreatment			
Levels	X_1 : Time (h)	X_2 : NaOH concentration (M)	X_3 : Cattle manure mass/Solution
-α	0.97	0.48	17.61
-1	2.00	1.00	20.00
0	3.50	1.75	23.50
+1	5.00	2.50	27.00
+α	6.02	3.01	29.38
Acid pretreatment			
Levels	X_1 : Time (min)	X_2 : Acid concentration (M)	X_3 : Temperature (°C)
-1	1.00	0.50	140.00
0	5.50	2.25	177.50
+1	10.00	4.00	215.00
Diluted acid hydrolysis			
Levels	X1 : Time (h)	X2 : acid Concentration (%)	
-α	1.80	0.18	
-1	2.00	0.50	
0	2.50	1.25	
+1	3.00	2.00	
+ α	3.20	2.31	

2.3.2 *Determination of responses*

The investigated responses were sugar-free, cellulose yield and phenolic compound. The determination of sugars-free was performed using 3,5-dinitrosalicylic acid (DNS) methodology described by Miller[36], while cellulose was conducted according to Kurschner Hoffer nitric acid method described by Samomssa et al.[15] and cellulose yield was calculated using equation (1). The phenolic compound was determined based on Marigo[37] methodology.

$$\text{Cellulose yield} = (\text{Cellulose content in liquid solution}/\text{Amount of residue}) \times 100 \quad (1)$$

2.3.3 *Validation of model*

Samomssa et al.[38] reviewed that the conditions for the validation of the model are adjusted R^2 has to be more than 95%, absolute average deviation (AAD) has to be nearer to zero, exactitude factor (Af1) and bias factor (BF) have to be between 0.75 and 1.25. They are obtained from the equations (2), (3), (4) and (5).

$$R^2 aj = \frac{\sum_{i=1}^{n}\left(y_{cal} - \overline{y_{\exp}}\right)}{\sum_{i=1}^{n}\left(y_{\exp} - \overline{y_{\exp}}\right)} \tag{2}$$

$$AAD = \left[\sum_{i=1}^{n}\frac{\left(\left|y_{\exp} - y_{cal}\right|\right)}{y_{\exp}}\right]/n \tag{3}$$

$$Af1 = 10^{\frac{1}{n}\sum_{i=1}^{n}\left|\log\frac{y_{cal}}{y_{\exp}}\right|} \tag{4}$$

$$BF = 10^{\frac{1}{n}\sum_{i=1}^{n}\log\frac{y_{cal}}{y_{\exp}}} \tag{5}$$

Where: y_{exp}: experimental value; y_{cal}: calculated value; n: number of experiments.

2.4 *Estimation of bioethanol potential from cattle manure*

The theoretical estimation of bioethanol from cattle manure was determined in three steps. Firstly, the amount of cellulose per year was determined using equation (6) based on cattle manure cellulose content. After that, the quantification of the glucose generated during the hydrolysis step was calculated by applying the kinetic model of Saeman[27] described by Sigüencia et al.[20] as shown by equations (7) and (8). For zero initial conditions, $C_A = C_B = 0$, and is illustrated by equations (9), (10) and (11). The maximum glucose concentration ($C_{B(max)}$) and the time taken to occur ($t_{(max)}$) were obtained from equations (12) and (13) respectively. The term diluted acid concentration was illustrated by equation (14). Finally, the estimation of the theoretical amount of bioethanol by fermentation of the glucose generated was deduced using equation (15).

$$\text{Cellulose (t/year)} = \%\ \text{cellulose} \times \text{Amountofcattlemanure (t/year)} \tag{6}$$

$$\text{Cellulose} \rightarrow \text{Glucose} \rightarrow \text{Bioethanol} \tag{7}$$

$$C_A \xrightarrow{K_1} C_B \xrightarrow{K_2} C_C \tag{8}$$

$$C_A = C_{A0}e^{-k_1 t} \tag{9}$$

$$C_B = C_{A0}\frac{K_1}{k_2 - k_1}\left[e^{-k_1 t} - e^{-k_2 t}\right] \tag{10}$$

$$C_c = C_{A0} - C_A - C_B \tag{11}$$

Where k_1 is the rate constant in glucose production (s^{-1}); k_2 is the rate constant in the breakdown of glucose (s^{-1}); C_A is the cellulose concentration (%); C_B is the glucose concentration (%), C_{A0} is the initial cellulose concentration (%) and C_c is amount of bioethanol.

$$C_{B(\max)} = C_{A0}\left(\frac{k_1}{k_2}\right)^{(k_2/k_1 - k_2)} \tag{12}$$

$$t_{(\max)} = \frac{\ln\frac{k_1}{k_2}}{k_1 - k_2} \tag{13}$$

$$K = k_0 C_s^n e^{-E/RT} \tag{14}$$

Where K is the rate constant takes into consideration the effect of temperature and acid concentration; C_s is the concentration of diluted sulfuric acid (%); k_0 is frequency factor at $c = 0$.

From all these results, volume of bioethanol (VE) is deduced by equation (15)

$$VE\left(\frac{L}{\text{year}}\right) = \frac{Cp\left(\frac{t}{\text{year}}\right) \times Rtp \times 10^6}{\rho\left(\frac{g}{L}\right)} \tag{15}$$

Where Rtp is theoretical yield of glucose of 0.49 g ethanol/g glucose; VE is the ethanol volume, L/year; bioethanol density, 790 g/L; 10^6, the conversion factor from grams to tons, and Cp is cellulose from cattle manure of Adamawa region per year, t/year.

3. Results and discussion

3.1 *Analysis of cattle manure characteristics*

3.1.1 *Functional group using FTIR*

FTIR spectra of cattle manure reveal five main groups as evident from the absorption bands presented in Figure 1. These absorption bands are between 3,000-3,600 cm^{-1}, 2,918 cm^{-1}, 1,634 cm^{-1}, 500 cm^{-1} and 1,600 cm^{-1}. The absorption band between 3,000-3,600 cm^{-1} is wide hence, it is attributed to OH bonded alcohol thus, cellulose, hemicellulose and lignin could be present. The fine absorption band at 2,918 cm^{-1} indicates the presence of C-H bonds in macromolecules, while the fine absorption band at 1,634 cm^{-1} is associated with carbonyl groups such as carboxylic bond and stretching band of aromatic ring and the absorption band at 1,000 cm^{-1} shows the presence of KCl and $CaCl_2$ due to the inorganic halogen compounds. The average absorption band is observed at 600 cm^{-1} which is characteristic of mineral components associated with phosphate (PO_4^{3-}) and carbonate (CO_3^{2-}).

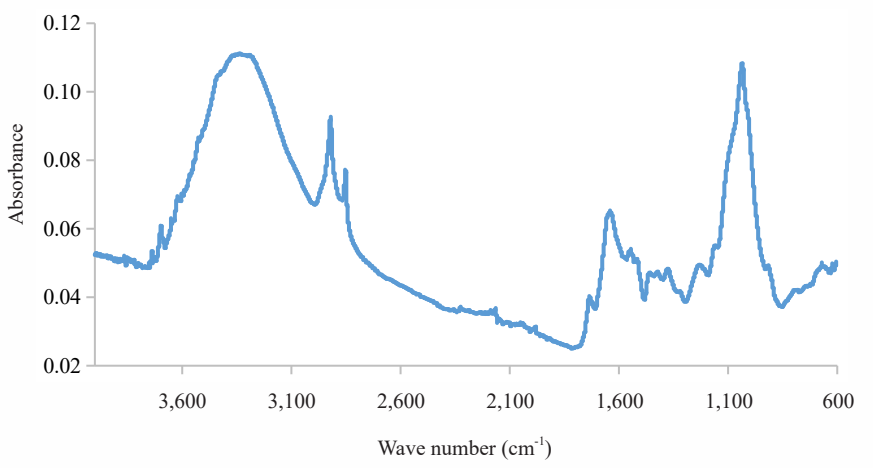

Figure 1. Functional group of cattle manure

3.1.2 *Microstructural (SEM) of cattle manure*

Figure 2 presents microstructures of unpretreated (Figure 2a) and pretreated (Figure 2b) cattle manure revealing the porous structure of cell walls illustrated by broken stems with inner structures and heterogeneous particles size. Pretreated cattle manure pores are wide compared to unpretreated cattle manure probably due to the fact that the lignin is removed after alkaline pretreatment and hemicellulose is converted to sugar and amorphous cellulose to crystalline during acid pretreatment. Concerning unpretreated cattle manure, Samomssa et al.[38] and Tsai and Sii-Chew[39] reviewed that the porous structure is shown by the presence of lignin on the surface of the cellulose which decreases the available surface. In fact, the space between the bright cellulose is filled with lignin which distributes across the different layers of the cell wall. Ormaechea et al.[40] justified the heterogeneous particle size by the varying proportion of lignocellulosic constituents such as cellulose, hemicellulose and lignin in the cattle manure. Besides, Tsai and Sii-Chew[39] reported that lignocellulosic constituents in addition to C and O contain fewer amounts of mineral elements, including Mg, Si and K.

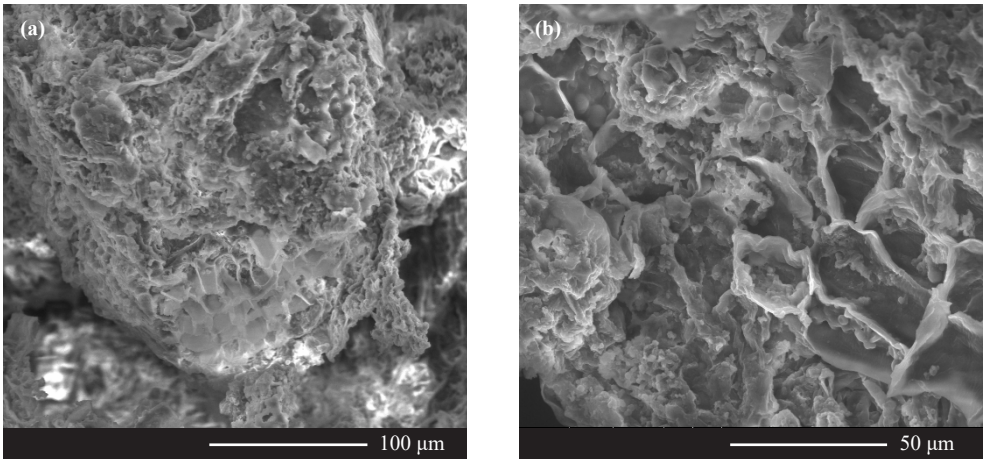

Figure 2. Micrograph of unpretreated cattle manure (a) and pretreated cattle manure with both alkaline and acid (b)

3.2 *Akaline pretreatment optimization*

The goal of optimizing alkaline pretreatment is to find the optimum conditions to obtain the highest value of

cellulose and phenolic compound. This optimum is obtained from alkaline pretreatment responses matrix, models and iso-curves response of cellulose and phenolic compound.

3.2.1 *Response matrix*

Table 3 presents the response matrix from the centered composite design. The results from the table revealed that cellulose content varies from 10.20% to 36% while phenolic compound ranges from 313.46 mg/100 g to 849.227 mg/100 g. The corresponding variations are 25.4% and 535.767 mg/100 g for cellulose and phenolic compounds respectively. This variation indicates that the physico-chemical parameters namely time, NaOH concentration and cattle manure mass/NaOH solution ratio influence responses.

Table 3. Alkaline pretreatment response matrix

Experiments	Time (h)	NaOH concentration (%)	Ratio	Cellulose yield (%)	Phenolic compound (mg/100 g)
1	3.50	1.75	23.50	22.22	535.41
2	2.00	1.00	20.00	32.10	599.80
3	5.00	1.00	20.00	10.20	686.14
4	2.00	2.50	20.00	34.30	952.20
5	5.00	2.50	20.00	29.20	697.58
6	2.00	1.00	27.00	33.00	569.03
7	5.00	1.00	27.00	16.20	826.99
8	2.00	2.50	27.00	30.20	812.08
9	3.50	1.75	23.50	25.40	481.15
10	5.00	2.50	27.00	26.60	653.89
11	0.978	1.75	23.50	31.24	705.51
12	6.03	1.75	23.50	12.10	543.10
13	3.50	0.49	23.50	23.40	651.91
14	3.50	3.01	23.50	36.01	683.29
15	3.50	1.75	17.61	28.40	848.45
16	3.50	1.75	29.39	29.15	822.78
17	3.50	1.75	23.50	25.50	443.21

3.2.2 *Validation of model*

The conditions used to validate the models are set in the literature. Thus, Joglekar and May[41] reported that adjusted R^2 should be closer to 100%; Bas and Boyac[42] presented that absolute average deviation (AAD) must be closer to zero;

Dalgaard and Jorgesen[43] showed that bias and exactitude factors should range between 0.75 to 1.25. From the intervals set in the literature, it is evident from Table 4 that these models are validated.

Table 4. Condition to validate the models

Validation Index	Cellulose yield	Phenolic compound yield
R^2	0.98	0.94
Adjusted R^2	0.87	0.87
AAD	0.02	0.04
Bf1	0.99	1.00
Af1	1.02	1.04

3.2.3 *Modeling*

Equation (16) illustrates cellulose yield model from residence time (X), NaOH concentration (Y) and ratio (Z). This Equation reveals that taking individually, X and Z influence negatively cellulose yield while, Y increase it. The influence of Y is 53 time the influence of Z and 33 time the influence of X. The interactions XY and XZ positively influence cellulose yield while YZ negatively influence the response. Quadratic effect of X and Z increase response and quadratic effect of Y decreases cellulose yield.

$$\text{Cellulose content (\%)} = 37.46 - 16.66 \times X + 54.01 \times Y - 1.73 \times Z + 1.26 \times X^2 + 3.36 \times X \times Y + 0.02 \times X \times Z - 16.14 \times Y^2 - 0.04 \times Y \times Z + 0.02 \times Z^2 \quad (16)$$

Equation (17) points out phenolic compound model from time (X), NaOH concentration (Y) and ratio (Z). This Equation shows that X and Y positively influence phenolic compound yield while Z affects it negatively. The influence of Y is around two times for that of X, while the influence of Z is negligible compared to X and Y. The interactions XY and YZ increase the yield while XZ negatively influence the response. Quadratic effect of (Y) is the highest followed by (X) while quadratic effect of (Z) is the lowest. The interactions involving Z are negligibles compared to interaction XY.

$$\text{Phenolic compound (mg/100 g)} = 79.72 + 207.59 \times X + 481.74 \times Y - 6.14 \times Z - 22.52 \times X^2 + 35.01 \times X \times Y - 2.37 \times X \times Z - 231.01 \times Y^2 + 5.39 \times Y \times Z + 0.06 \times Z^2 \quad (17)$$

3.2.4 *Optimizing area*

The optimal area represents a surface that reveals the highest value of responses. Thus, from Figure 3 (a) there are two optimal areas; in the left and right with the value of 35%. The highest cellulose content is obtained for the time of 5 h and the NaOH concentration ranged between 2 M to 2.3 M. Meanwhile, Figure 3 (b) presents one optimal area beginning from 800 mg/100 g going through the right side. This optimum is obtained for time varying between 3.7 h and 5 h and NaOH concentration ranged from 1.3 M to 2 M.

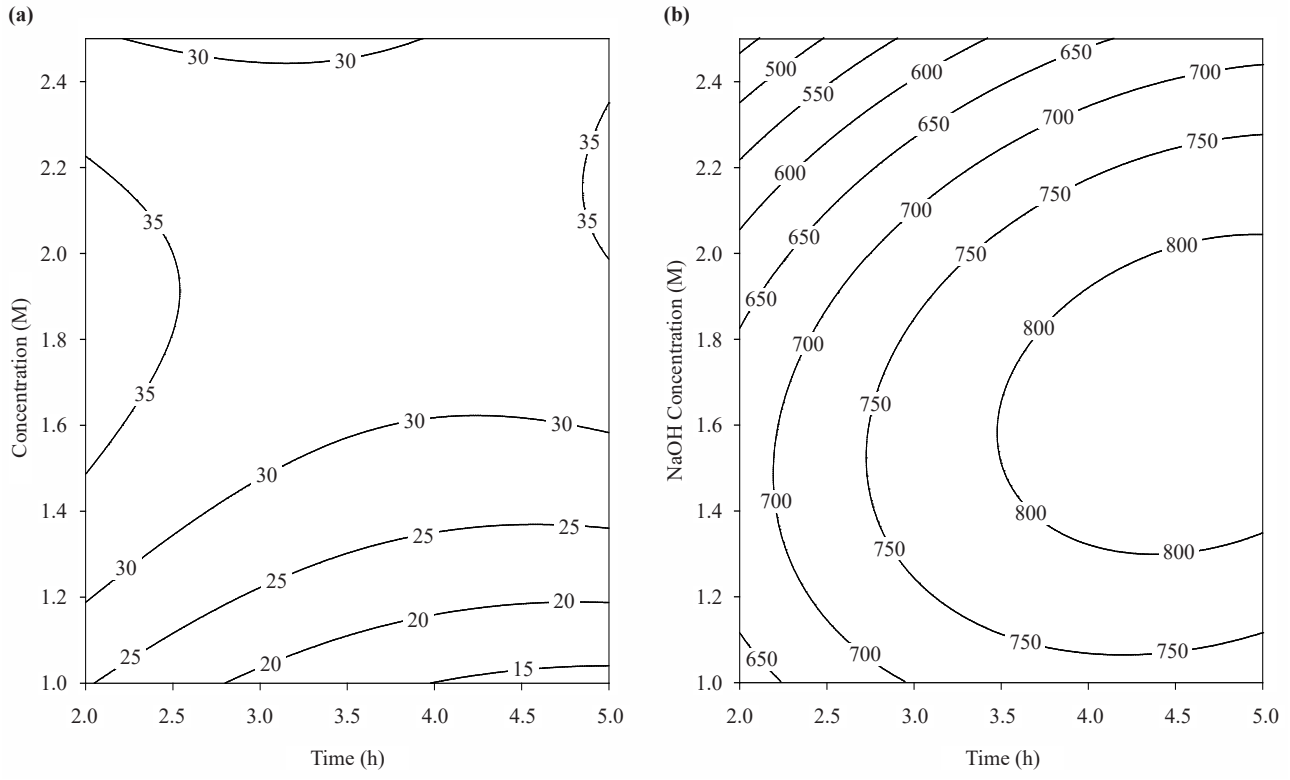

Figure 3. Iso-curve response of cellulose content (a) and phenolic compound content (b)

3.3 *Acid pretreatment optimization*

The objective in this part is the determination of the best condition to obtain the highest amount of sugar-free from cattle manure previously pretreated with sodium hydroxide (NaOH). This optimum is obtained from the acid pretreatment response matrix, model and iso-curve response.

3.3.1 *Response matrix*

The results obtained after the experiment are presented in Table 5 revealing that sugar-free content ranges from 41.54% to 46.16%. This variation indicates that the physico-chemical parameters influence the sugar content and may justify the importance of finding optimal conditions using experimental design.

Table 5. Response matrix of acid pretreatment

Experiments	Acid Concentration (M)	Time (min)	Temperature (°C)	Sugar-free (%)
1	2.25	5.50	177.50	43.73
2	0.50	1.00	140.00	41.94
3	4.00	1.00	140.00	41.54
4	0.50	10.00	140.00	45.83

Table 5. (cont.)

Experiments	Acid Concentration (M)	Time (min)	Temperature (°C)	Sugar-free (%)
5	4.00	10.00	140.00	46.16
6	0.50	1.00	215.00	43.10
7	4.00	1.00	215.00	44.03
8	0.50	10.00	215.00	43.37
9	2.25	5.50	177.50	43.63
10	4.00	10.00	215.00	45.57
11	0.50	5.50	177.50	43.39
12	4.00	5.50	177.50	44.66
13	2.25	1.00	177.50	42.91
14	2.25	10.00	177.50	45.11
15	2.25	5.50	140.00	43.86
16	2.25	5.50	215.00	44.56
17	2.25	5.50	177.50	43.61

3.3.2 *Validation of model, modeling and optimal area*

3.3.2.1 *Validation of model, modeling*

The validation indexes are shown in Table 6 and as explained in paragraph 3.2.2, it is evident from Table 6 that the model is validated and this model is illustrated by equation (18). This equation reveals that acid concentration (X) and temperature (Z) negatively influence sugar content while time (Y) positively influences sugar content. The quadratic effects of X and Y negatively affect sugar content while quadratic effect of Z positively affect response. The interaction XY and XZ positively influence sugar content while interaction YZ negatively influences studied response.

Table 6. Condition to validate the models

Validation Index	Values
R^2	0.97
Adjusted R^2	0.94
AAD	0.02
Bfl	1.00
Afl	1.04

$$\text{Sugar-free (\%)} = 42.7258 - 0.9771 \times X + 1.1077 \times Y - 0.0242 \times Z - 0.0071 \times X^2 + 0.0317 \times X \times Y$$

$$+ 0.0061 \times X \times Z - 0.0018 \times Y^2 - 0.0050 \times Y \times Z + 0.0001 \times Z^2 \quad (18)$$

3.3.2.2 *Optimizing*

Figure 4 illustrates the iso-curve response of sugar-free content. This figure reveals that the optimum area reaches 45.5% for concentration between 3.5 M and 4 M and time ranged from 9 min to 10 min.

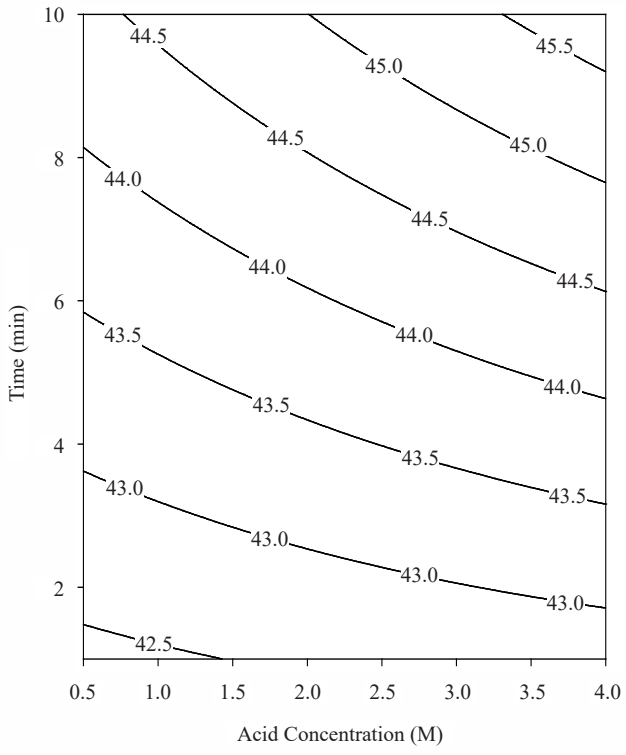

Figure 4. Iso-curve response of sugar free content

3.4 *Hydrolysis optimization*

This part allows to display of the hydrolysis optimum condition from cattle manure previously pretreated with NaOH and H_2SO_4. The best alkaline pretreatment is found in the following conditions: The ratio of 23.50 from cattle manure mass/NaOH solution is heated with NaOH concentration ranging between 2 M to 2.3 M during 5 hours. The alkaline pretreated cattle manure passes through the acid pretreatment which occurs at a sulfuric acid concentration between 3.5 M-4 M, time ranging from 9 min to 10 min with a temperature of 177 °C. The hydrolysis optimum conditions are assessed from cattle manure previously pretreated with sodium hydroxide (NaOH) and sulfuric acid (H_2SO_4). This optimum is obtained from the acid hydrolysis response matrix, model and iso-curve response.

3.4.1 *Response matrix*

Table 7 presents the response matrix from the centered composite design and indicates that experiments 5 and 8 show the highest value of sugar yield while experiments 1 and 6 present the lowest value. Thus, sugar yield varies from 62.63% to 80.73% with a variation of 18.1% indicating that time and H_2SO_4 concentration influence response.

Table 7. Hydrolysis response matrix

N°	Coded Matrix		Test Matrix		
	X	Y	H_2SO_4 concentration (M)	Time (hour)	Sugar yield (%)
1	-1	-1	2.00	0.50	62.63
2	+1	+1	3.00	2.00	71.41
3	0	0	2.50	1.25	67.66
4	0	0	2.50	1.25	66.54
5	+α	0	3.20	1.25	79.15
6	-1	+1	2.00	2.00	64.45
7	0	+α	2.50	2.31	65.57
8	+1	-1	3.00	0.50	80.73
9	0	-α	2.50	0.18	68.67
10	-α	0	1.75	1.25	66.47
11	0	0	2.50	1.25	66.54

X_1 : H_2SO_4 Concentration; X_2 : Time

3.4.2 *Validation of model and modeling*

The condition to validate the model is present in section 3.2.2 and accordingly, it is evident from Table 8 that the model of sugar yield with respect to time and H_2SO_4 concentration is validated. This model is presented by Equation (19) showing that taking individually, H_2SO_4 concentration (X) and time (Y) decreases sugar-free yield. The interaction XY, the quadratic effect of X and Y positively influences sugar-free yield.

Table 8. Validation of model

Validation Index	Values
R^2	0.97
Adjusted R^2	0.95
AAD	0.10
Bfl	1.01
Afl	1.11

$$\text{Sugar-free (\%)} = 196.552 - 96.308 \times X - 34.337 \times Y + 18.050 \times X^2 + 10.9067 \times X \times Y + 3.062 \times Y^2 \quad (19)$$

3.4.2.1 *Optimizing*

Figure 5 presents the iso-curve response of sugar-free from acid hydrolysis. The amount of sugar at this step determines the quantities of bioethanol at the fermentation step. This Figure reveals that the maximum value is 78% of sugar is obtained for time ranging from 1.9 h and 2.0 h and the sulfuric acid concentration between 2.9 M and 3.0 M. From the kinetic hydrolysis model results presented in Table 9, the sugar yield can reach 100% if the acid hydrolysis reaction occurs at 210 °C. And at this temperature, the reaction time can be reduced to less than 1 hour.

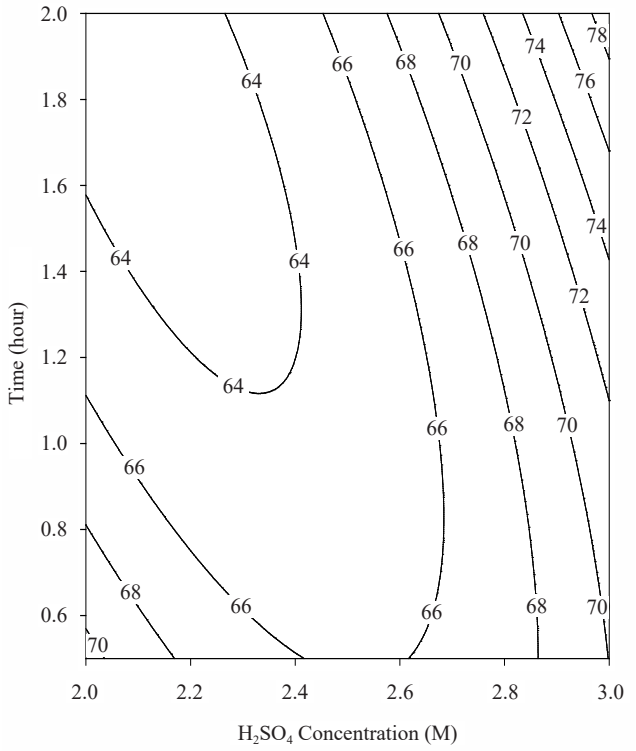

Figure 5. Iso-curve response sugar-free in function of time and acid concentration

3.5 *Bioethanol potential from cattle manure*

Table 9. Kinetic Model Cs = 1.5%

T (°C)	K_1	K_2	tmax (s)	CA (%)	CB (%)
120	8.3901E-06	0.00018942	17,218.0304	24.8271134	1.21047791
150	0.00022132	0.00152296	1,481.83671	22.8290948	4.11600655
170	0.00150021	0.00515333	337.803512	20.9918714	6.00812864
190	0.00850086	0.01555656	85.6492239	19.3025026	7.69749736
210	0.04124628	0.04254181	23.8716448	17.7490897	9.25091029

k_1 : Rate constant in glucose production (s^{-1}); k_2 : Rate constant in the breakdown of glucose (s^{-1}); CA : Cellulose content (%); CB : Glucose content (%); tmax : Maximum time

The bioethanol potential has been estimated from cellulose amount per year and sulfuric acid hydrolysis results. By applying equations (6) and (15) the amount of cellulose per year is estimated at 593,271 t/year and the amount of bioethanol potential is estimated at 461,433,000 L/year. The optimum hydrolysis result reveals sulfuric acid concentration between 2.9 M and 3.0 M. The kinetic hydrolysis model based on sulfuric acid concentration is presented in Table 9 showing that the maximum time of hydrolysis and the cellulose decrease when the temperature increases while the quantity of glucose follows the evolution of the temperature. Hence, temperature plays a very important role during the hydrolysis of cellulose. The increase in temperature accelerates the reaction and reduces hydrolysis time. The decrease in cellulose content is due to the conversion of cellulose into glucose, in the same way, the increase in sugar content. The results from the kinetic hydrolysis, compared to the experiments from the acid hydrolysis optimization model point out that, the sugar yield at the acid hydrolysis step can be improved if the reaction occurs at 210 °C and at the same time the residence time can be reduced.

4. Conclusion

In this study, where the goal was the valorization of cattle manure for bioethanol production, we found that the absorption bands from FTIR of investigated manure show the presence of O-H alcohol, C-H bond in macromolecules, C = O carbonyl, KCl, $CaCl_2$, PO_4^{3-} and CO_3^{2-} revealing that it is rich in cellulose, hemicellulose and lignin. The presence of these macromolecules in cattle manure is well explained by SEM which points out the porous structure justified by the presence of lignin on the surface of the cellulose and the heterogeneous particle size illustrating the varying proportion of cellulose, hemicellulose and lignin. The pretreated cattle manure pores are wider compared to unpretreated. The optimum conditions to produce bioethanol from cattle manure are obtained when alkaline/acid pretreatments, and acid hydrolysis, are successively applied before fermentation. This optimum is reached when alkaline pretreatment occurs for 5 hours with NaOH concentration ranging between 2 M to 2.3 M for a cattle manure mass/solution ratio of 23.50 while, the acid pretreatment occurs at acid concentration between 3.5 M-4 M, with time ranged from 9 min to 10 min with a temperature of 177 °C meanwhile, acid hydrolysis occurs at sulfuric acid concentration between 2.9 and 3M for the time ranging from 1.90 hours and 2 hours. These conditions lead to giving the total sugar of 78% to proceed fermentation step. From the amount of cattle manure generated per year, the amount of cellulose per year is estimated at 593,271 t/year and the amount of bioethanol potential is estimated at 461,433,000 L/year. The kinetic hydrolysis model based on sulfuric acid concentration shows that the maximum time of 4 hours for the temperature of 120 °C and when the temperature increases the maximum time decreases at the same time the quantity of glucose follows the evolution of the temperature. Hence, temperature plays a very important role during the hydrolysis of cellulose.

Conflict of interest

The authors declare no competing financial interest.

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