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Effect of Lignocellulose Waste-Biomass Pretreatment of the Peels of *Musa acuminata* (Banana) and *Ananas comosus* (Pineapple) on the Production of Bioethanol via Solid-State Fermentation

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Abstract

The use of fruits both domestically and industrially generates waste from their discarded peels which may lead to environmental pollution and contamination. This work aims at evaluating the effects of chemical pre-treatment of biomass on the physicochemical properties and the effects of process parameters on the production of ethanol via solid-state fermentation. The biomass was dried, ground and subjected to chemical pretreatment using H_2SO_4 and NaOH. pH, total Titratable acidity (TTA), total soluble sugar (TSS), specific gravity (SG), and total reducing sugars (TRS) were carried out before and after the pretreatment of biomass. Acid hydrolysis and neutralisation of the biomass were then carried out before solid-state fermentation. A central composite design (CCD) was used to evaluate the effects of process parameters; fermentation time (X_1), pH (X_2), and biomass load (X_3) on the physicochemical and functional properties of the bioethanol produced. Before pretreatment, the TRS and TSS of pineapple and banana peel biomass were observed to be 16.67 & 5.36% and 8 & 5%Bx respectively. For acid pretreatment, pineapple peel biomass had higher TRS and TSS ranging from 20.69% -

30.0% and 10% - 21%Bx compared to banana peel biomass with TRS and TSS ranging from 14.39% - 21.8% and 9.9% - 14.9%Bx respectively. Whereas, for alkaline pretreatment, pineapple biomass had TRS and TSS ranging from 13.71% - 19.35% and 10% - 17.5%Bx while banana biomass had higher yields of TSS ranging from 10% - 16.5%Bx and TRS ranging from 10.79% - 19.98%. The optimum fermentation conditions for Banana Peel Biomass were fermentation time (48.88 h), pH (6.11), and biomass load (21.96 g/ml); with ethanol conc., density, TRS, and TSS of 2.56%, 1.025 g/ml, 13.93% and 2.59%Bx respectively from Banana biomass pretreated with acid. While for Pineapple Peel Biomass, the optimum conditions were; fermentation time (34.51 h), pH (4.82), and biomass load (24.0 g/ml) with ethanol conc., density, TRS and TSS of 2.42%, 1.015 g/ml, 37.72% and 5.61%Bx respectively from Pineapple biomass pretreated with acid. This study showed that chemical pretreatment has a positive impact on the physicochemical properties of fruit biomass with acid pretreatment being the most effective in the release of sugars and production of bioethanol.

Subject Areas

Analytical Chemistry, Biological Chemistry, Chemical Engineering & Technology, Environmental Chemistry

Keywords

Lignocellulose Biomass, Peels, Musa Acuminate, Ananas Comosus, Pretreatment, Acid, Alkaline, Solid-State Fermentation, Bioethanol

1. Introduction

The bio-refinery sector is faced with the challenge of substrate availability as the first-generation substrate (1G substrate) for ethanol fermentation (sugar and starch-containing substrates) is used for both food and feed [1]. The second-generation substrates (2G substrate) or lignocellulose biomasses usually neglected and discarded as waste could be a plausible solution to augment food and feed sources as well as a substrate for the bio-refinery, thereby contributing to environmental healthiness [2].

However, the complex architectural arrangement of the second-generation substrates kneading the constitutive cellulosic micro fibrils of lignin and hemicellulose forms a lignin carbohydrate complex which is unavailable on the one hand for enzyme action, and so, limits the access of the other constituents due to the low porosity and high crystallinity. These micro fibrils are bundled together to form cellulose fibers, making cellulose an ultrastructure [3]. These complications and the heterogeneous structure of the LCB thereby require more complex chemical processes to extract and make available the still useful components of the second-generation substrates. It is, therefore, necessary to develop novel and efficient methods to sustainably extract and make available the useful components of the second-generation substrates to contribute toward solving the problem of insufficient first-generation substrates for bioethanol production and environmental depletion by waste dumping [4].

This work aims at evaluating the effects of chemical pre-treatment of biomass (pineapple and banana peels) on the physicochemical properties and the effects of process parameters on the production of ethanol via solid-state fermentation. Pineapple and banana are widely consumed tropical fruits with a greater portion of the fruits accumulated as waste. Banana is the second largest produced fruit accounting for 16% of the total fruit products worldwide and the peel which forms a part of the non-edible portion, (accounting for approximately 35% of the whole fruit weight), is discarded as waste [5]. This waste represents an alternative feedstock for the production of value added products such as ethanol as it has no competition with food and feed and is widely distributed, available, and inexpensive [6]. This study aims at evaluating the effect chemical pretreatment of waste Lignocellulose biomass on the production of bioethanol from *Musa acuminata* (Banana) and *Ananas comosus* (Pineapple) fruit peels via Solid-State Fermentation.

2. Methodology

2.1. Material Collection, Preparation and Pretreatment

Banana and pineapple peels were washed with clean water and separately cut into smaller sizes and dried at an oven temperature of 90˚C ± 20˚C for 24 hours. This was followed by crushing into fine particles and sieving to ease the pretreatment phase. The powdered samples, banana and pineapple respectively were weighed using a scale balance and subjected to pretreatment with 2.2% diluted sulphuric acid (H_2SO_4) in a corked glass container. The same procedure was also followed to pretreat samples with 2.2% sodium hydroxide (NaOH). Samples mixed with acid or alkaline were autoclaved at a temperature of 121˚C and a pressure of 2.5 Pa for 15 minutes. Fermentation of the pretreated samples was done by a solid-state fermentation process using yeast (Saccharomyces cerevisiae) through semi-simultaneous saccharification and fermentation [7].

2.2. Separation and Characterization of the Bioethanol

The separation of the ethanol from the fermentation broth was done by simple fractional distillation according to the method [8]. The separated ethanol was characterized as presented in Table 1.

2.3. Experimental Design

In this study, a Central composite design (Box and Wilson Designs) was used in the optimization of bioethanol production. The process parameters; Fermentation time, X_1 (24 - 72), pH, X_2 (5 - 7) and Biomass load, X_3 (15 - 25) were chosen as the independent variables (experimental factors) to assess their effect on the physicochemical and functional properties (responses; TSS, TRS, Ethanol

Table 1. Analytical methods.

Property	Analytical method
Total Titratable Acidity	Titratable acidity was determined by titrating a known amount of aqueous extract of the sample against an alkali solution of known normality using phenolphthalein as an indicator [9]. It is expressed as an equivalence of any organic acids, e.g. citric acid, or malic acid.
pH	The pH was determined using an automatic pH meter by dipping the hydrogen electrode into the sample at room temperature [9].
Total soluble sugars and Specific gravity	Total soluble sugars (TSS) were measured in Brix using a hand refractometer [10].
Total reducing sugars	The total reducing sugars were determined using Lane and Eynon's method which is based on the principle of reduction of Fehling's solution by reducing sugars.
Ethanol concentration	The concentration of ethanol present in the bioethanol (ethanol and water) was determined by volumetric analysis using acidified potassium permanganate [11].
Density	Density was doing using the volumetric method [12]. An empty 50 ml conical flask was weighed and the weight was recorded. The weighed conical was filled with 30 ml of the sample (ethanol) and the weight of both the flask and ethanol was recorded. The density of ethanol was then calculated using the following formula: Density (g/mL) = mass/volume

Concentration, Ethanol Yield and Density) of bioethanol. The CCD is advantageous in that it provides high-quality predictions over the entire design space and also has an embedded fractional design part in it. The coded and real experimental matrix for solid-state fermentation for the bioethanol production was as presented in **Table 2**.

The general quadratic model equation with three factors and interactions

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3$$
$$+\beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + E$$

Analysis of variance (ANOVA) was used to evaluate the effect of the different experimental factors on the responses. The interaction between the experimental factors on the response variable was examined using model equations what was generated for each response variable. Fisher's F-test was used to check the statistical significance of the model whereas the coefficient of determination, R^2 expressed the polynomial model's fit quality. A confidence level of 95% significance was used in this study. From the effect of the three factors, the respective contour plots were plotted for both levels. Furthermore, the optimum conditions were obtained from the regression analysis carried out and also from the focal parameters in the contour plots.

2.4. Data Processing Procedure

All obtained data were analyzed using Microsoft excel 2016 to come out with the mean and standard deviation and displayed using histograms. Experiments were carried out in duplicates and data obtained was subjected to analysis of variance (ANOVA) and Duncan test to assess the effects of different factors on the response and the differences between means respectively using STATGRAPHICS

Table 2. Coded and real experimental matrix for solid-state fermentation for production of bioethanol.

Runs	Fermentation time (X_1)/hrs.	pH (X_2)/na	Biomass load (X_3)/g/mL	X_1	X_2	X_3
1	1	1	−1	72	7	15
2	0	0	−1.6818	48	6	11.5911
3	−1	−1	1	24	5	25
4	−1	1	−1	24	7	15
5	1	1	1	72	7	25
6	0	0	0	48	6	20
7	0	−1.6818	0	48	4.31821	20
8	−1	−1	−1	24	5	15
9	0	0	1.68179	48	6	28.409
10	1.68179	0	0	88.363	6	20
11	1	−1	−1	72	5	15
12	0	0	0	48	6	20
13	−1.6818	0	0	7.63704	6	20
14	0	1.68179	0	48	7.68179	20
15	1	−1	1	72	5	25
16	−1	1	1	24	7	25

centurion version XVII.II. The P-value ≤ 0.05 was used for significant effect or difference, R2-value > 70% and/or standard error <10%, was used for model validation. The model equations of each of the responses variable, Y_x, were generated (Equations (1), (4), (7), (10), (12), (14)) and the effect of the interactions between the experimental factors, X_y, (Equations (2), (5), (6), (8), (9), (11), (13) and (15)) on the response variable were evaluated and their surface response plotted generated with the help of sigma plot software

3. Results and Discussion

3.1. Physico-Chemical Composition of Pineapple and Banana Peel Powder

The results of the physicochemical properties of the used waste biomass are as presented in Table 3 below. The pH of the pineapple peel powder obtained was 4.21 ± 0.01, which is higher than the pH of pineapple peel powder (3.47 - 3.84) obtained by [13] and that of banana peel powder was 5.79 ± 0.02. The high pH observed is a result of the maturity stage of the fruit peels (full ripen) used. [13] reported the initial pH of unripe and ripe pineapple peels before fermentation to be 3.6 and 3.9 respectively. Hence, the pH of the fruit peels increases with an increase in maturity. The total titratable acidity (TTA) was 0.402 ± 0.21 and 0.192 ± 0.07 for banana and pineapple peel powder respectively. Unlike 0.16 for pineapple peel extract but the same as the 0.19 for pineapple core extract reported

Table 3. Physicochemical composition of pineapple and banana peel powder.

Biomass \ Property	pH	TTA	TRS/%	TSS/%Brix	SG/Wort
Pineapple peel powder	4.21 ± 0.01^{a,b}	0.192 ± 0.07^{a}	16.67 ± 0.14^{b}	8.00 ± 0.00^{a,b}	1.040 ± 0.00^{a}
Banana peel powder	5.79 ± 0.02^{a,b}	0.402 ± 0.21^{a}	5.36 ± 0.28^{b}	5.00 ± 0.00^{a,b}	1.030 ± 0.00^{a}

Column scores are values of mean ± standard deviation of two trial values and those having the same superscript (a and b) are not significantly different at $p < 0.05$.

by [14]. This is because the pineapple peel used in this experiment contained some fleshy core of the fruit. The TTA values observed for both pineapple and banana peel powder are low because TTA decreases with ripening or advancement in maturity as organic acids responsible for acidity are being used during respiration resulting in a decrease in acidity.

The TRS and TSS obtained for banana and pineapple peel powder were 5.36 ± 0.28 and 16.67% ± 0.14%, and 5 and 8%Brix respectively. Unlike TRS for pineapple peel biomass which is two times higher than that (7.5%) reported by Chalchisa and Dereje, the TRS for banana peel biomass was lower and the TSS for both biomasses as well was lower than the 9.8%Brix reported for the TSS of pineapple peel extract [15]. The higher amounts of sugar in the pineapple peel powder could be a result of the breakdown of starch into sugars or polysaccharides in the cell wall being hydrolysed by the degradation of organic acids into simple sugars [16]. The SG was 1.040 and 1.030 for pineapple peel powder and banana peel powder respectively which is indicative of the soluble solids present in the biomasses.

3.2. Effect of Acid and Alkaline Pretreatment on the Physicochemical Properties of Pineapple and Banana Peel Biomass

Both acid (2.2% H_2SO_4) and alkaline (2.2% NaOH) pretreatment had an impact on the physicochemical properties (TTA, TSS, TRS, SG and pH) of pineapple and banana peel waste biomass are presented in Table 4 and Table 5 below.

3.2.1. Effect of Acid Pretreatment on the Physicochemical Properties of Pineapple and Banana Peel Biomass

The effect of acid pretreatment on the physicochemical properties of pineapple and banana peel biomass is as presented in Table 4.

1) pH

The pH of both pineapple and banana peel biomasses pretreated with dilute acid (2.2% H_2SO_4) increased with an increase in biomass. There were no statistically significant differences in the pH between acid pretreated samples of pineapple peels (PPAC) and banana peels (BPAC) at a confidence level of 95.0%. But there were statistically significant differences (a decrease) between the pH of both biomasses before and after pretreatment at a confidence level of 95.0%. The low pH values observed after acid pretreatment is as a result of the formation of

Table 4. Effect of acid pretreatment on the physico-chemical properties of pineapple and banana peel biomass.

Property Biomass	Biomass load/g/ml	pH	TTA	TRS/%	TSS/%Brix	SG/Wort
Pineapple peel powder	12	1.51 ± 0.0141^{a}	3.23 ± 0.0707^{a}	20.69 ± 0.0707	10.00 ± 0.0707^{b,c,d}	1.045 ± 0.0041
	15	1.52 ± 0.0109^{a}	7.26 ± 0.0707^{a}	24.00 ± 0.1768	14.00 ± 0.0707^{c,d,e}	1.060 ± 0.0041
	20	1.54 ± 0.0126^{a}	8.32 ± 0.1414^{a}	24.30 ± 0.1414	16.50 ± 0.1414^{d,e,f}	1.071 ± 0.0046
	25	1.59 ± 0.0556^{a}	6.08 ± 0.0707^{a}	27.91 ± 0.0707	17.00 ± 0.0707^{e,f}	1.076 ± 0.0063
	28	1.66 ± 0.0424^{a}	6.40 ± 0.0000^{a}	30.00 ± 0.2828	21.00 ± 0.0000^{f}	1.095 ± 0.0063
Banana peel powder	12	1.39 ± 0.0283^{a}	7.97 ± 0.1414^{a}	14.39 ± 0.0000	9.90 ± 0.1414^{b}	1.045 ± 0.0026
	15	1.56 ± 0.0989^{a}	7.57 ± 0.1414^{a}	19.44 ± 0.7778	10.00 ± 0.1414^{b,c}	1.043 ± 0.0042
	20	1.58 ± 0.7993^{a}	8.71 ± 0.0000^{a}	19.62 ± 0.2828	11.00 ± 0.0000^{b,c}	1.044 ± 0.0029
	25	1.63 ± 0.1992^{a}	6.93 ± 0.0707^{a}	21.80 ± 0.3535	13.50 ± 0.0707^{b}	1.065 ± 0.0025
	28	1.76 ± 0.0141^{a}	7.37 ± 0.0000^{a}	16.60 ± 0.5657	14.90 ± 0.0000^{b}	1.065 ± 0.0036

Column scores are values of mean ± standard deviation of two trial values and those having the same superscript are not significantly different at $p < 0.05$.

organic acids during dilute acid hydrolysis. The pH was also observed to increase with an increase in biomass load and this is because organic acids formed during acid pretreatment reduce with an increase in biomass concentration. This is in line with the report of [17] who carried out acid hydrolysis on pineapple leaves at an acid concentration of 4% - 12%(v/v) and solid-to-liquid ratio of 10% - 20% (w/v) and reported the lowest concentration of formic acid and acetic acid respectively at 12% acid concentration (6.06 and 5.80 g/L) and at 20% solid-to-liquid ratio (1.62 and 3.28 g/L).

2) Total Titratable Acidity (TTA)

Both pineapple and banana peel biomasses exhibited a decrease in TTA with an increase in biomass load except for the increase in TTA observed at 28 g/ml biomass of banana peel biomass. There was no statistically significant difference between the TTA of acid pretreated samples of pineapple peels (PPAC) and banana peels (BPAC) at a confidence level of 95.0%. However, there was statistically significant difference between the TTA content of both biomasses before and after acid pretreatment at a confidence level of 95.0%. Unlike pH, TTA decreases with an increase in biomass load. This is because an increase in pH leads to a decrease in TTA. [17] who carried out acid hydrolysis on pineapple leaves at an acid concentration of 4% - 12% (v/v) and the solid-to-liquid ratio of 10% - 20% (w/v) reported the lowest concentration of formic acid and acetic acid (6.06 and 5.80 g/L) respectively at 12% acid concentration and (1.62 and 3.28 g/L) at 20% solid-to-liquid ratio. Hence, the reduction in the formation of organic acids with an increase in biomass load led to a decrease in TTA values.

3) Total Soluble Solids (TSS)

Both pineapple and banana peel biomasses showed an increase in TSS with an increase in biomass load. Both biomasses had the lowest TSS higher than initial total soluble solids of 5% and 8%Brix for banana and pineapple peel powder respectively. Hence, acid pretreatment led to a significant increase in the TSS of both biomasses.

Statistically significant differences were observed between the TSS of pineapple and banana peel biomasses at a confidence level of 95.0%. The increase observed in the TSS of both biomasses after acid pretreatment is a result of the effective removal of hemicelluloses [18]. Dilute acid pretreatment solubilizes hemicellulose into monomers [19] and also degrades polymers such as proteins and polysaccharides in the cell wall into monomers hence increasing the total soluble solids.

4) Specific Gravity (SG)

The specific gravity (SG) was observed to range from 1.045 to 1.095 and 1.045 to 1.065 for PPAC and BPAC respectively after dilute-acid pretreatment and this is in line with the specific gravity of date fruit must reported at 1.070sp.gr before aerobic fermentation [19]. The specific gravity increased with an increase in biomass load for both pineapple and banana peel biomass samples. The increase in specific gravity after the pretreatment of biomass is a result of an increase in total soluble solids which increased the density of the biomass. Hence, the high SG level in pineapple biomass compared to banana biomass is due to the high concentration of soluble solids in pineapple biomass.

5) Total Reducing Sugars

For pineapple peel biomass, the TRS was observed to range from 20.69 to 30%, while for banana peel biomass, it was observed to range from 14.39 to 21.80% after acid pretreatment. The lowest TRS concentration after pretreatment was 14.39% and 20.69% which was higher than the 5.36% and 16.67% recorded before pretreatment for banana and pineapple peel biomass respectively. The TRS increased with an increase in biomass concentration for both biomasses except for the decrease that was observed at a biomass load of 28g/ml for banana peel biomass.

A statistically significant difference was observed between the concentration of TRS in pineapple and banana peel biomasses after acid pretreatment at a confidence level of 95.0%. Pineapple peel biomass had high TRS than banana peel biomass. The increase observed in the concentration of TRS for both biomasses after acid pretreatment was because, dilute acid pretreatment hydrolyses hemicellulose to sugars with high yields, increases the cellulosic surface area, changes the structure of lignin as well as breaks the lignin-hemicellulose shield in agricultural residues [20]. There is also a high conversion efficiency of xylan to xylose during acid pretreatment. Dilute sulfuric acid in particular, increases the solubility of hemicellulose and makes cellulose more readily available from the lignocellulose biomass [18].

3.2.2. Effect of Alkaline Pretreatment on the Physico-Chemical Properties of Pineapple and Banana Peel Biomass

The effect of alkaline pretreatment on the physicochemical properties of pineapple and banana peel biomass is as presented in **Table 5**.

Table 5. Effect of alkaline pretreatment on the physico-chemical properties of pineapple and banana peel biomass.

Property / Biomass	Biomass load/g/ml	pH	TTA	TRS/%	TSS/%Brix	SG/Wort
Pineapple peel powder	12	11.35 ± 0.07^{a}	0.128 ± 0.00^{a}	13.71 ± 0.28^{a}	10.00 ± 0.00^{a}	1.045 ± 0.00^{a}
	15	9.72 ± 0.85^{b}	0.128 ± 0.00^{a}	15.74 ± 0.21^{b}	11.00 ± 0.00^{a}	1.051 ± 0.00^{a}
	20	8.57 ± 0.64^{c}	0.128 ± 0.00^{a}	15.88 ± 0.00^{b}	14.50 ± 0.01^{b}	1.067 ± 0.01^{a}
	25	8.26 ± 0.54^{c}	0.192 ± 0.00^{a}	16.99 ± 0.21^{c}	18.10 ± 0.00^{c}	1.081 ± 0.00^{a}
	28	7.48 ± 0.03^{d}	1.088 ± 0.14^{b}	19.35 ± 0.28^{d}	17.50 ± 0.02^{d}	1.080 ± 0.02^{a}
Banana peel powder	12	13.01 ± 0.01^{e}	0.188 ± 0.03^{a}	12.13 ± 0.14^{e}	10.00 ± 0.01^{a}	1.045 ± 0.01^{a}
	15	11.85 ± 0.38^{a}	0.268 ± 0.00^{a}	14.49 ± 0.00^{f}	10.50 ± 0.00^{a}	1.046 ± 0.01^{a}
	20	10.02 ± 0.63^{a}	0.268 ± 0.00^{a}	16.48 ± 0.42^{c}	13.50 ± 0.01^{e}	1.058 ± 0.01^{a}
	25	8.65 ± 0.22^{c}	1.206 ± 0.00^{b}	19.98 ± 0.53^{d}	14.00 ± 0.00^{b}	1.063 ± 0.00^{a}
	28	8.43 ± 0.04^{c}	0.2345 ± 0.07^{a}	10.79 ± 0.85^{f}	16.50 ± 0.03^{f}	1.070 ± 0.03^{a}

Column scores are values of mean ± standard deviation of two trial values and those having the same superscript are not significantly different at $p < 0.05$.

1) pH

The pH of pineapple peel banana peel biomasses pretreated with alkaline (2.2% NaOH) PPAL and BPAL respectively, decreased with an increase in biomass load. The pH of both biomasses after alkaline pretreatment was more basic compared to the extremely acidic pH observed in acid pretreatment. The basic pH is an indication that there were little or no organic acids formed during alkali pretreatment biomass.

2) Total Titratable Acidity (TTA)

Unlike in acid pre-treated biomass, the TTA in alkaline pre-treated pineapple and banana peel biomasses (PPAL and BPAL respectively) were low in values. It was observed to range from 0.128 to 1.088 in PPAL and from 0.188 to 1.206 in BPAL. The TTA increased with an increase in both biomasses except for the decrease observed at the biomass of 28 g/ml in BPAL.

3) Total Soluble Solids (TSS)

Like in the acid pre-treated biomass, the TSS in alkaline pre-treated biomass was observed to increase with an increase in biomass load with values ranging from 10.0 to 18.1% and 10 to 16.5% for PPAL and BPAL respectively. For pineapple biomass, the TSS obtained after alkaline pre-treatment was lower than that obtained after acid pre-treatment at the same biomass load for both biomasses except at 25 g/ml where the TSS of PPAL (18.1%) was higher than that of PPAC (17.0%). Banana peel biomass on the other hand had higher TSS for BPAL than BPAC at the same biomass load.

The lower concentration of TSS in pineapple samples after alkaline pre-treatment compared to acid pre-treatment is because, during alkaline pre-treatment, lignin and hemicellulose are reduced while cellulose remains the same. Banana biomass samples, on the other hand, had a high concentration of TSS after alkaline pre-treatment compared to acid pre-treatment. Therefore, alkaline pre-treatment is more efficient in degrading the polymers in the banana peel cell wall into simple monomers as a result of the reduction in the degree of polymerisation during alkaline pre-treatment. Alkali pre-treatment changes the lignocellulosic structure due to cellulose swelling, reduction in crystallinity and degree of polymerisation. Hence, it increased the internal surface area and removed acetyl groups and uronic acids in hemicelluloses [21]. This also gives room for effective and efficient hydrolysis of the hemicelluloses and celluloses into simple and fermentable sugars.

4) Specific Gravity (SG)

Like the TSS, SG followed the same trend. The specific gravity (SG) was observed to range from 1.045 to 1.081 and 1.045 to 1.070 for PPAL and BPAL respectively after dilute-acid pretreatment. The specific gravity increased with the increase in biomass load for both pineapple and banana peel biomass samples except for the decrease observed at biomass load of 28 g/ml for PPAL. For pineapple biomass, the SG obtained after alkaline pre-treatment was lower than that obtained after acid pre-treatment at the same biomass load except at 25 g/ml where the SG of PPAL (1.081) was higher than that of PPAC (1.076%). Banana peel biomass on the other hand had higher SG for BPAL than BPAC at the same biomass load except at a biomass load of 25 g/ml.

Banana peel biomass exhibited high specific gravity after alkaline pretreatment than acid pretreatment implying that there are more dissolved solids in alkaline pretreated banana peel biomass.

5) Total Reducing Sugars (TRS)

For pineapple peel biomass, the TRS was observed to range from 13.71 to 19.55%, while for banana peel biomass, it was observed to range from 10.79 to 19.98% after alkaline pretreatment. This was however lower than the sugar yields obtained under acid pretreatment for both biomasses. The lowest TRS concentration after pretreatment was 13.71% for PPAL which was lower than the initial total reducing sugar content of 16.67% recorded for pineapple peel powder before pretreatment and 10.79% for BPAL which was higher than the 5.36% recorded before pretreatment for banana peel powder. The TRS increased with an increase in biomass concentration for both biomasses except for the decrease that was observed at a biomass load of 28 g/ml for banana peel biomass.

The decrease observed in the total reducing sugar content of banana peel at a biomass load of 28 g/ml could be a result of little or no availability of free water which could have significantly affected the effectiveness of pre-treatment [22].

3.3. Effects of Process Parameters on the Physico-Chemical and Functional Properties of Bio-Ethanol Production via Solid-State Fermentation of Acid Pretreated Pineapple Biomass (PPAC)

A central composite design was used to study the effect of process parameters on the production of bioethanol from acid pre-treated pineapple peel biomass. The process variables evaluated were; Fermentation Time (X_1), pH (X_2) and Biomass Load (X_3). Using the CCD, an experimental matrix for the process factors was generated and the responses were obtained as presented in **Table 6**.

Statgraphics Centurion XVII.II was used for analysis of data and model equations were used to describe the obtained results.

3.3.1. Total Soluble Solid (TSS)

From the analysis of variance (ANOVA) and regression analysis, process factors; fermentation time (X_1), pH (X_2) and biomass load (X_3) showed no statistically significant effect with p-values > 0.05 on the TSS of pineapple biomass pretreated with dilute acid during fermentation. The optimized process conditions for TSS during bioethanol production from pineapple peel pretreated with acid were 7.0, 4.0 and 26.69 for fermentation time, pH, and biomass load respectively for optimum TSS change (10.15). This is in line with the total decrease value of total soluble solids (% Brix) of 10.9 % Brix observed during bioethanol production from sugarcane molasses by instant dry yeast (effect of pretreatment and fermentation temperature) at a temperature of 32˚C [23].

The highest TSS depletion rate was observed to be within the first 24 hrs of

Table 6. Effects of process parameters on the physico-chemical and functional properties of bioethanol produced from acid pre-treated pineapple peel biomass.

	Factors			Responses			
Runs	Fermentation time/hrs	Initial pH	Biomass load/g/ml	TSS/%Brix	TRS/%	Ethanol Conc./%	Density/g/ml
	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4
1	72	7	15	3.50	28.38	2.44	1.021
2	48	6	12	1.80	23.13	2.42	0.968
3	24	5	25	7.40	33.72	2.22	1.011
4	24	7	15	2.80	14.91	2.56	1.005
5	72	7	25	4.70	26.29	2.47	0.994
6	48	6	20	3.50	24.62	2.58	1.005
7	48	4	20	4.00	27.42	2.61	1.001
8	24	5	15	3.30	21.70	2.00	1.029
9	48	6	28	5.00	30.48	2.53	0.989
10	88	6	20	5.50	21.33	2.61	1.011
11	72	5	15	3.70	25.15	2.53	0.954
12	48	6	20	3.70	25.26	2.67	1.019
13	08	6	20	3.50	6.59	2.44	1.009
14	48	8	20	3.50	22.36	2.56	0.989
15	72	5	25	2.50	30.00	2.67	1.004
16	24	7	25	4.50	14.66	2.50	1.019

fermentation. This is probably due to an increase in alcohol leading to a decrease in viable cells [24]. The high concentration of TSS left after fermentation was probably because most of the soluble solids present in the biomass are non-fermentable sugars and so could not be consumed by yeast (*Saccharomyces cerevisiae*) during fermentation [24].

3.3.2. TRS

Unlike TSS, process parameters had statistically significant effects on the TRS of pineapple peel pretreated with acid during bioethanol fermentation as indicated on the pareto diagram in **Figure 1**. The linear effect of fermentation time: pH and squared fermentation time both had a significant negative and positive effect respectively. The following model equation was used to describe the TRS as a function of fermentation time (X_1), pH(X_2) and biomass load (X_3).

$$\begin{aligned} Y_2 &= 13.3575 + 0.109182X_1 - 2.21358X_2 + 1.47829X_3 \\ &\quad -0.00585888X_1^2 + 0.132135X_1X_2 - 0.00938542X_1X_3 \\ &\quad + 0.266496X_2^2 - 0.48025X_2X_3 + 0.0563619X_3^2 \end{aligned} \tag{1}$$

This model equation is valid because $R^2 = 92.7978\%$ which is greater than 80% with a standard error of 3.70921 which is <10%.

It was observed that the TRS content of the fermented broth greatly decreased during fermentation. This is because reducing sugars are consumed by yeast cells and converted to ethanol under anaerobic conditions during fermentation. Reducing sugars, especially glucose, are converted to ethanol through the metabolic pathway of EMP (Embden Mayerhoff Parnas) under anaerobic conditions [23].

3.3.3. Effect of Fermentation Time on the TRS of PPAC

Doubling the fermentation time had a negative significant impact on the TRS of PPAC fermented broth at a p-value of 0.0139. The following model equation was used to examine the effect of doubling fermentation time on TRS.

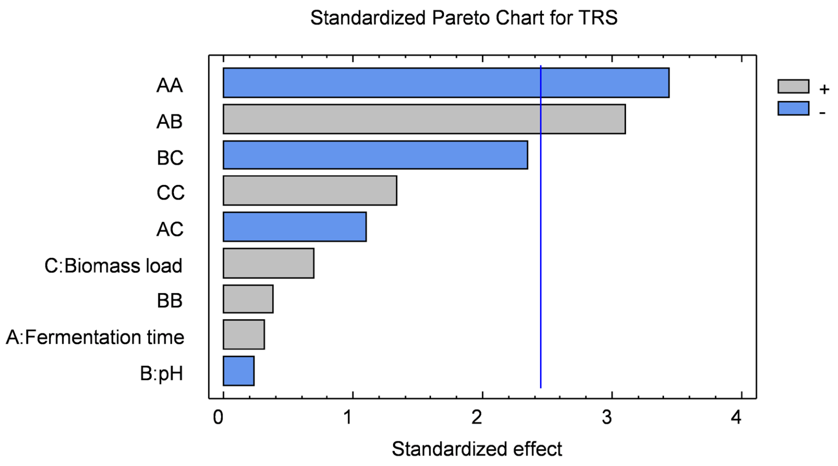


Figure 1. Pareto diagram showing the effect of processing parameters on the TRS in PPAC fermented broth.

$$Y_2 = 13.3575 + 0.109182X_1 - 0.00585888X_1^2 \quad (2)$$

Doubling fermentation time had a negative significance on total reducing sugars probably due to high utilization of reducing sugars during the first few hours (4 - 6 hours as seen in **Figure 1**) of fermentation where yeast cells are multiplying and still very viable and also alcohol is at its lowest. Sugar utilization progressively reduces as fermentation proceeds after the 8th hour due to the reduction in viable yeast cells as a result of an increase in ethanol concentration during fermentation [24]. High ethanol concentrations inhibit cell growth and viability, limiting fermentation productivity [25].

3.3.4. Effect of Fermentation Time and pH on the TRS of PPAC

The interaction of fermentation time and pH (X_1X_2) had a positive significant influence on the PPAC TRS with a p-value of 0.0211. Evaluating the interaction effect of fermentation time and pH (X_1X_2) at constant biomass load gave the following equation.

$$\begin{aligned} Y_2 = {} & 13.3575 + 0.109182X_1 - 2.21358X_2 - 0.00585888X_1^2 \\ & + 0.132135X_1X_2 + 0.266496X_2^2 \end{aligned} \quad (3)$$

Figure 2(a) and **Figure 2(b)** is a response surface plot showing the interaction between two independent variables (fermentation time and pH) and their effects on the response variable (TRS). It was observed that reducing sugar utilisation increased with increasing fermentation time and increasing pH. [26] reported that low pH value of 4.0 or 3.5 significantly reduced yeast growth and increased the residual sugar level in the fermentation broths after studying effects of initial pH value of the medium on the alcoholic fermentation performance of *Saccharomyces cerevisiae* cells immobilized on nipa leaf sheath pieces.

3.3.5. Ethanol Concentration

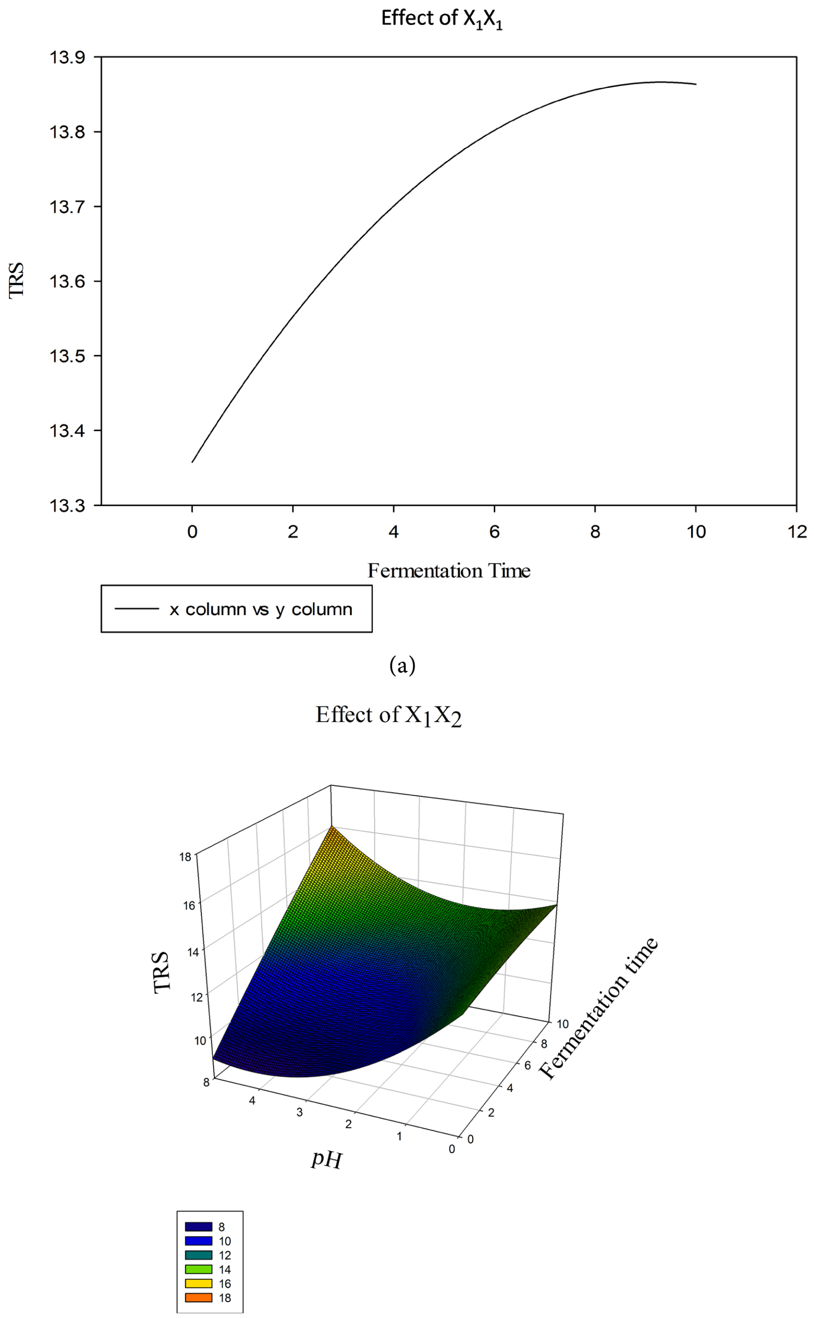
The pareto diagram in **Figure 3** shows the effect of processing parameters on the ethanol concentration of PPAC. The fermentation time and the linear interaction of fermentation time: pH both had a significant negative and positive effect on ethanol concentration respectively. The following equation was generated for PPAC ethanol concentration from the analysis of data obtained using Statgraphics Centurion XVII.II

$$\begin{aligned} Y_3 = {} & -3.00714 + 0.0480431X_1 + 0.747829X_2 \\ & + 0.199208X_2 - 0.0000991333X_1^2 - 0.00588542X_1X_2 \\ & + 0.0000104167X_1X_3 - 0.0201837X_2^2 \\ & - 0.00975X_2X_3 - 0.00333736X_3^2 \end{aligned} \quad (4)$$

This model equation has $R^2 = 82.9334$ % which is greater than 80%, hence it is valid with a standard error of 0.115741 which is <10%.

1) Effect of fermentation time on the ethanol concentration of PPAC

The fermentation time (X_1) had a significant effect on the ethanol concentration of PPAC with a p-value of 0.0119. As fermentation time (X_1) increases, ethanol concentration also increases, this shows that fermentation time affects



(a)

(b)

Figure 2. (a) Effect of fermentation time on TRS; (b) Effect of fermentation time and pH on TRS.

the ethanol concentration positively. This is in line with a report on an increase observed in bioethanol production from microalgae by increasing the fermentation time while keeping biomass and yeast volume constant [27]. Keeping other factors constant and observing the effect of fermentation time only yielded the following equation

$$Y_3 = -3.00737 + 0.0474546X_1 - 0.000097219X_1^2 \tag{5}$$

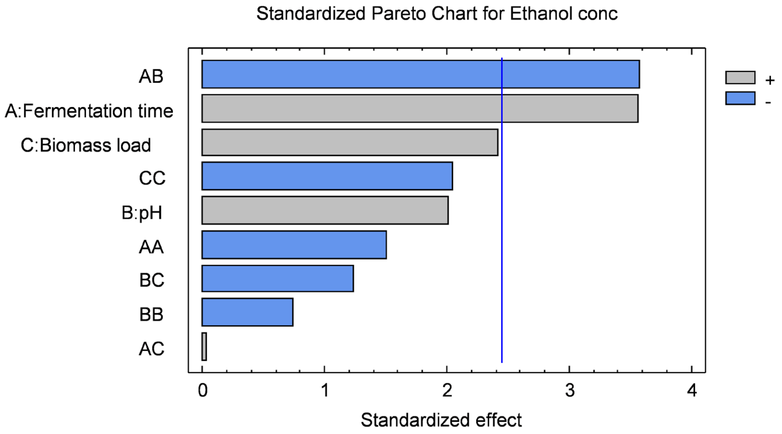


Figure 3. Pareto diagram showing the effect of processing parameters on the ethanol concentration of PPAC.

2) Effect of fermentation time and pH on the ethanol concentration of PPAC

Figure 4(a) and Figure 4(b) presents the effects of fermentation time and pH on the ethanol concentration of PPAC. Increasing the fermentation time (X_1) and pH (X_2) led to a reduction in ethanol concentration of PPAC with a p-value of 0.0117. Hence, the increase in the interaction of fermentation time and pH (X_1X_2) has a negative significant impact on the ethanol concentration of PPAC. Keeping other process factors constant and observing the interaction of fermentation time and pH gave the following equation.

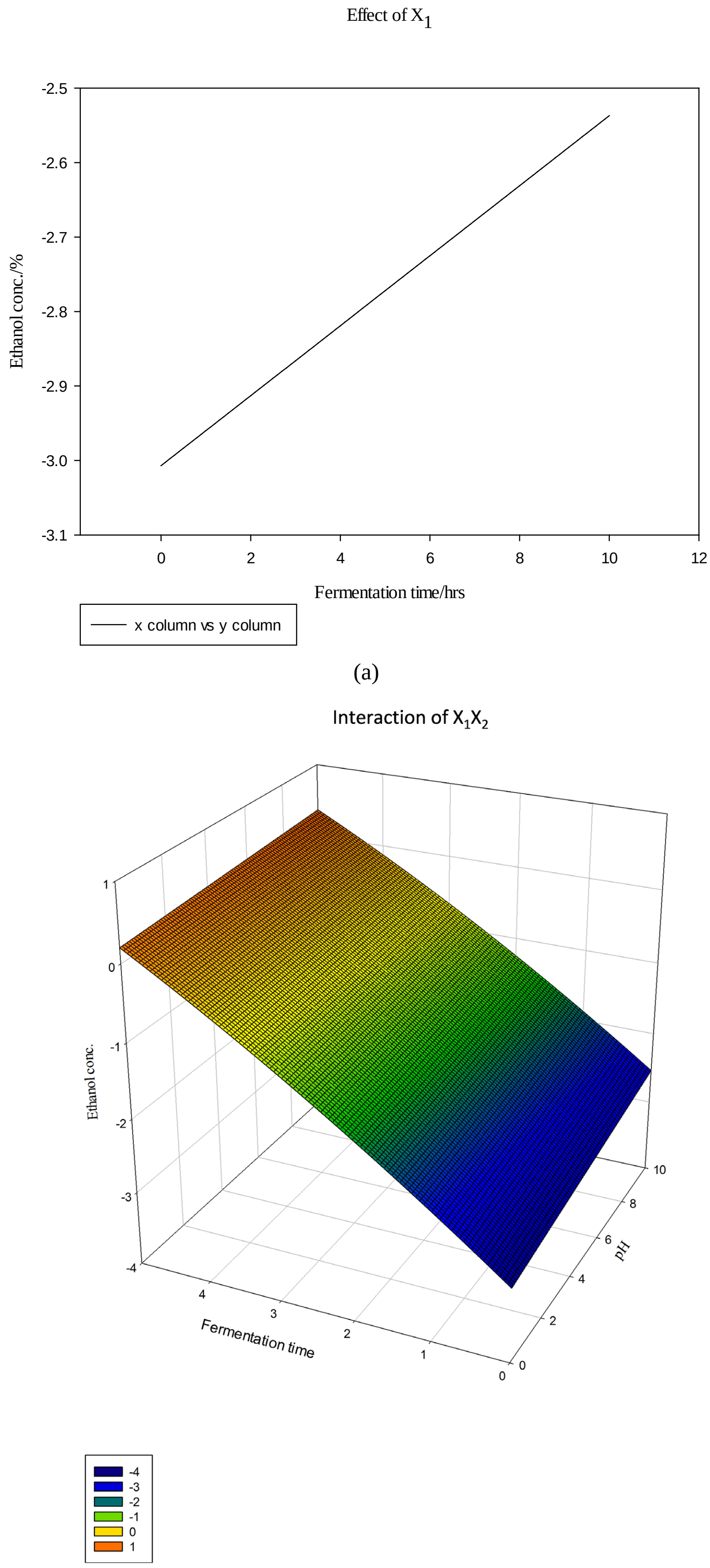
$$\begin{aligned} Y_3 = &-3.00737 + 0.0474546X_1 + 0.746433X_2 \\ &- 0.000097219X_1^2 - 0.00578462X_1X_2 \\ &- 0.0205535X_2^2 \end{aligned} \tag{6}$$

Increase in fermentation time increases ethanol concentration due to conversion of fermentable sugars in ethanol by yeast cells. However, increase in pH affects the effectiveness of yeast cells (*Saccharomyces cerevisiae*) as they are efficient in mild acid conditions. Hence, increasing both fermentation time and pH will have a negative effect on the ethanol concentration.

The optimized process conditions were 88.0, 4.0 and 24.34 respectively for fermentation time, pH, and biomass load for PPAC optimal ethanol concentration of 2.99%. Accordingly, [28] reported the optimum conditions giving the maximum calculated bioethanol production of 30.7 g/L with a bioethanol yield of 42% as follows: pH 5, 25% initial molasses concentration, 35˚C, 116 rpm, and 60 hours after carrying out a response surface optimization of bioethanol production from sugarcane molasses by Pichia veronae Strain HSC-22.

3) Density of bioethanol from acid pretreated pineapple peel

From the analysis using STATGRAPHICS, process parameters had no statistically significant effect on the density of bioethanol produced from acid pretreated pineapple peel biomass.



(a)

(b)

Figure 4. Effect of fermentation time (a), and fermentation time: pH; (b) on the ethanol concentration of PPAC.

3.4. Effects of Process Parameters on the Physico-Chemical and Functional Properties of Bio-Ethanol Production via Solid-State Fermentation of Alkaline Pretreated Pineapple Biomass (PPAL)

A central composite design was used to study the effect of process parameters on the production of bioethanol from alkaline pre-treated pineapple peel biomass. The process variables evaluated were; Fermentation Time (X_1), pH (X_2) and Biomass Load (X_3). Using the CCD, an experimental matrix for the process factors was generated and the responses were obtained as presented in Table 7.

3.4.1. Effect of Process Parameters on the TSS of PPAL Fermented Broth for Bioethanol

From the analysis using STATGRAPHICS, the following model equation was generated for the TSS in PPAL fermented broth for ethanol production.

$$\begin{aligned} Y_1 = &-3.29547 - 0.0485109X_1 + 2.45615X_2 - 0.34087X_3 \\ &+ 0.00151494X_1^2 - 0.0151042X_1X_2 + 0.00114583X_1X_3 \\ &- 0.359259X_2^2 + 0.1325X_2X_3 - 0.00819318X_3^2 \end{aligned} \quad (7)$$

Regression analysis for TSS during the fermentation process of pineapple peel

Table 7. Effects of process parameters on the physico-chemical and functional properties of bioethanol produced from alkaline pre-treated pineapple peel biomass.

	Factors			Responses			
Runs	Fermentation time/h	Initial pH	Biomass load/g/ml	TSS/%Brix	TRS/%	Ethanol Conc./%	Density/g/ml
	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4
1	72	7	15	1.50	19.71	2.64	1.012
2	48	6	12	0.50	18.08	2.67	1.020
3	24	5	25	1.50	14.46	2.58	1.016
4	24	7	15	0.50	14.10	2.61	1.035
5	72	7	25	5.20	19.67	2.67	1.009
6	48	6	20	2.80	18.36	2.44	0.996
7	48	4	20	0.50	12.78	2.25	0.989
8	24	5	15	1.00	51.41	2.33	0.997
9	48	6	28	2.50	10.95	2.53	1.008
10	88	6	20	5.00	19.45	2.42	1.003
11	72	5	15	3.30	62.18	2.64	1.006
12	48	6	20	2.00	08.75	2.67	0.958
13	08	6	20	4.00	33.68	2.67	1.005
14	48	8	20	1.50	22.64	2.67	1.001
15	72	5	25	4.50	11.33	2.50	0.995
16	24	7	25	3.80	24.64	2.56	1.014

biomass pretreated with alkaline (PPAL) is valid. The model equation has R^2 = 92.7361% which is greater than 70% and a standard error of 0.693358 which is <10%.

From the Pareto diagram in **Figure 5**, the doubling effect of fermentation time (X_1X_1) and the interaction effect of pH and biomass load (X_2X_3) had a positive significant effect on the consumption of TSS during fermentation of PPAL while all other interactions and process factors did not show any significant effect. pH (X_2) and the combined effect of fermentation time and biomass load (X_1X_2) showed a positive non-significant impact on the TSS consumption whereas, the doubling effect of pH (X_2X_2) and biomass load (X_3X_3), the combined impact of fermentation time and pH (X_1X_2), biomass load (X_3) and fermentation time (X_1) had a negative non-significant effect.

1) Effect of Fermentation time on the TSS during fermentation of PPAL biomass

From **Figure 6**, it was observed that increasing the fermentation time (X_1) led to an increase in the usage of total soluble solids during the fermentation of alkaline pretreated pineapple peel biomass with a p-value of 0.0068, which implies a significant effect. Hence, there was a decrease in TSS with an increase in fermentation. A decrease was reported in the TSS of tomato, red chili, bottle gourd and carrot juice from 2.9 - 2.1, 1.1 - 0.4, 4.2 - 3.1 and 6.9 - 0.20˚Bx after a fermentation period of 80 hrs [29]. Therefore, keeping other factors constant and observing fermentation time only generated the following equation.

$$Y_1 = -3.29547 - 0.0485109X_1 + 0.00151494X_1^2 \tag{8}$$

2) Effect of pH and biomass load on the TSS consumption during fermentation of PPAL biomass

The interaction of pH and biomass load led to an increase in TSS consumption with a p-value of 0.0355, hence a significant effect as shown in **Figure 7**. Keeping other process factors constant and observing the interaction of pH and biomass load gave the following equation.

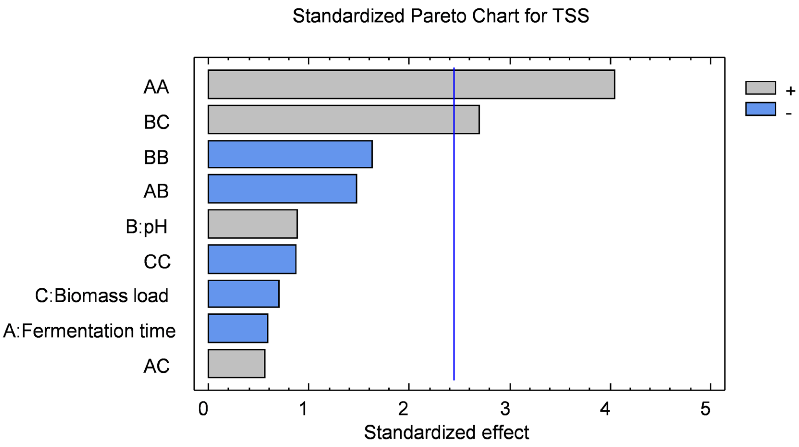


Figure 5. Pareto diagram showing the effect of processing parameters on the TSS in PPAL fermented broth.

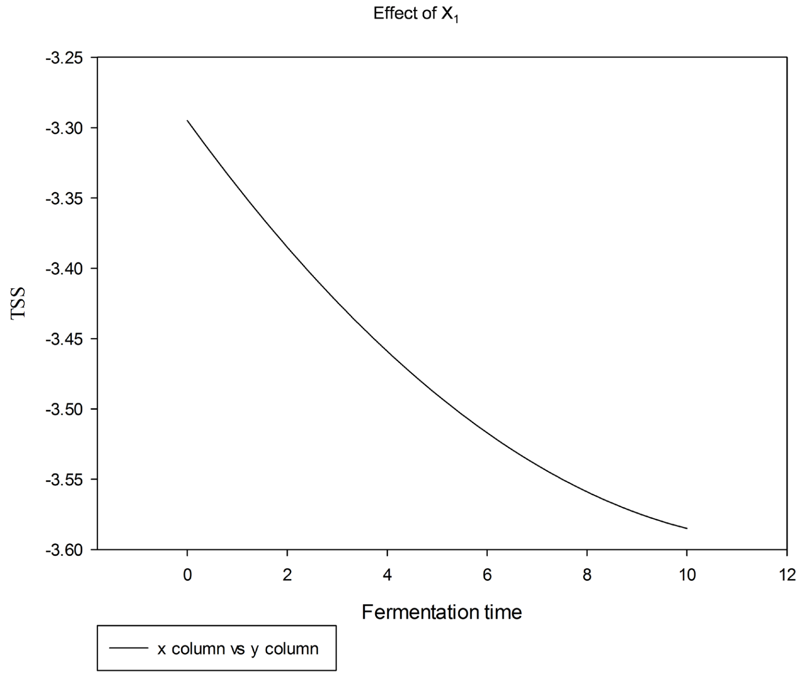


Figure 6. Effect of fermentation time on the TSS of PPAL.

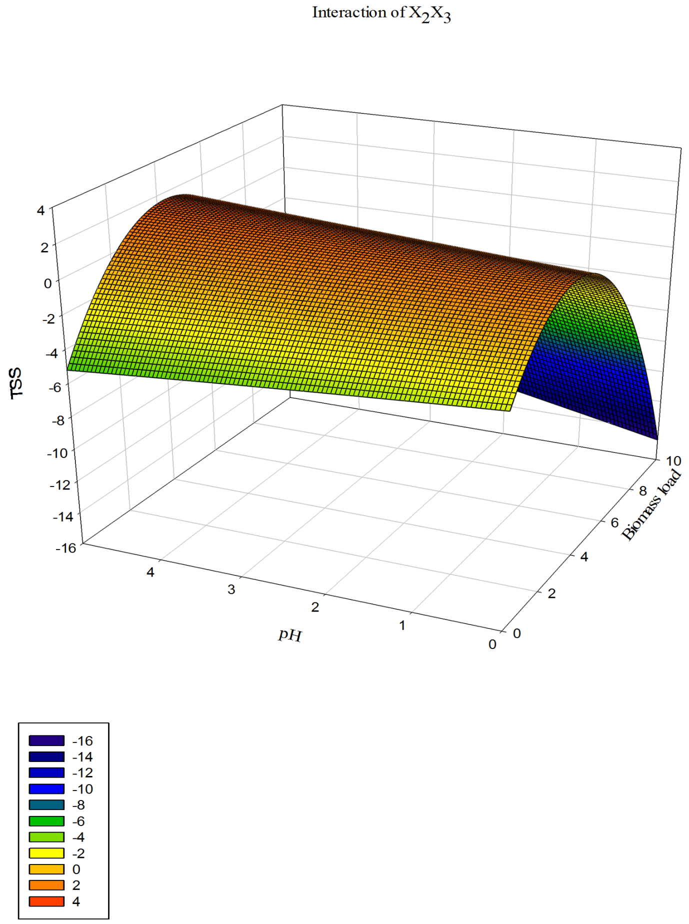


Figure 7. Interaction of pH and biomass load.

$$\begin{aligned}Y_1 &= -3.29547 + 2.45615X_2 - 0.34087X_3 \\ &\quad -0.359259X_2^2 + 0.1325X_2X_3 - 0.00819318X_3^2\end{aligned} \tag{9}$$

The optimized process conditions for TSS during bioethanol production from PPAL were 88.0 hrs, 5.57 and 28.0 g/ml for fermentation time, pH, and biomass load respectively for optimum TSS consumption (7.02% Brix).

3.4.2. Effect of Process Parameters on the TRS of PPAL Fermented Broth for Bioethanol

From the analysis of data using STATGRAPHICS, the following equation was generated for the TRS in PPAL fermented broth for ethanol production.

$$\begin{aligned}Y_2 &= 353.912 - 0.187209X_1 - 45.3902X_2 - 16.9829X_3 \\ &\quad + 0.00908939X_1^2 - 0.0364583X_1X_2 - 0.0255X_1X_3 \\ &\quad - 0.490465X_2^2 + 2.4575X_2X_3 + 0.0527415X_3^2\end{aligned} \tag{10}$$

This model equation is valid with an R^2 = 72.4124 % which is greater than 70% and a standard error of 12.2773 which is >10%.

From the Pareto diagram in Figure 8, the combined interaction of (X_2X_3) had a positive significant impact on the TRS in PPAL fermented broth while all the other interactions and factors had non-significant effects. The square effects of fermentation time (X_1X_1) and biomass load (X_3X_3) had a positive non-significant influence while the effects of (X_3), (X_2), (X_1), and the combined interaction of (X_1X_3), (X_1X_2), and the doubling effects of (X_2X_2), on the other hand, had a negative non-significant effect.

1) Effect of pH and biomass load on the TRS of PPAL fermented broth

From Figure 9 it was observed that increasing both pH (X_2) and biomass load (X_3) led to a positive change in the TRS in PPAL. The interaction effect of pH and biomass load (X_2X_3) had a p-value of 0.0299, hence having a significant impact on the TRS.

$$\begin{aligned}Y_2 &= 353.912 - 45.3902X_2 - 16.9829X_3 - 0.490465X_2^2 \\ &\quad + 2.4575X_2X_3 + 0.0527415X_3^2\end{aligned} \tag{11}$$

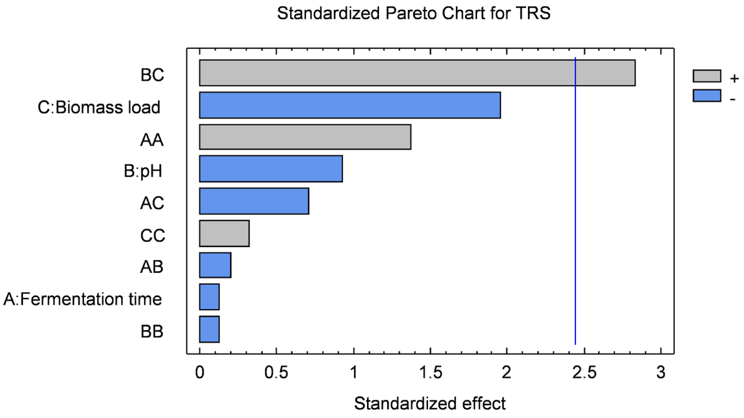


Figure 8. Pareto diagram showing the effect of processing parameters on the TRS in PPAL fermented broth.

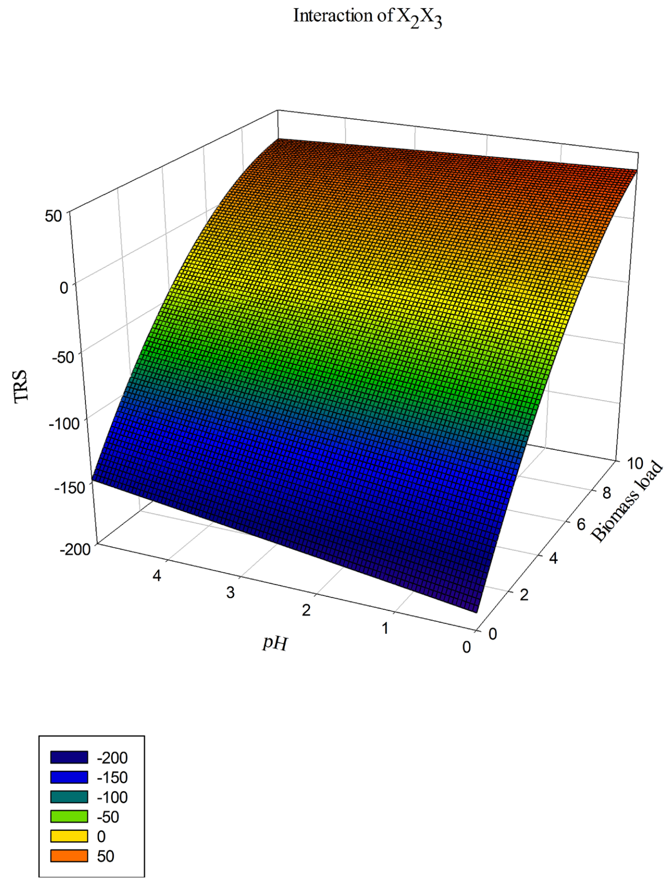


Figure 9. Interaction of pH and biomass load on TRS.

Increasing the pH will affect the effectiveness of yeast (*Saccharomyces cerevisiae*) which functions properly in mild acid conditions. Substrate concentration primarily affects uptake rates and thereby product rate kinetics. High substrate concentration negatively affects ethanol productivity (sugar uptake) due to the repression of glycolytic enzymes [30]. Hence, the interaction of pH and biomass load led to an increase in the uptake of sugars.

2) Effect of process parameters on PPAL Ethanol concentration

From the analysis using STATGRAPHICS, process parameters had no statistically significant effect on the ethanol concentration of bioethanol produced from alkaline pretreated pineapple peel biomass.

3.4.3. Effect of Process Parameters on PPAL Ethanol Density

From the analysis using STATGRAPHICS, the following equation was generated for the density of PPAL bioethanol.

$$\begin{aligned} Y_4 = {} & 1.3401 - 0.000872752X_1 - 0.0604035X_2 - 0.0175674X_2 \\ & + 0.0000155979X_2 - 0.0000833333X_1X_2 - 0.0000125X_1X_3 \\ & + 0.00740646X_2^2 - 0.0008X_2X_3 + 0.000560771X_3^2 \end{aligned} \tag{12}$$

This model equation is valid because R^2 = 71.4298 % which is greater than 70% with a standard error of 0.0139997 which < 10%.

From the Pareto diagram in **Figure 10**, the doubling effect of biomass load (X_3X_3) had a positive significant effect on ethanol density of PPAL while other process factors and interactions had no significant effect. The doubling effects of fermentation time (X_1X_1) and pH (X_2X_2) had a positive non-significant effect while all process factors (fermentation time, pH, and biomass load) and their interactions had a negative non-significant effect on the ethanol density of PPAL.

1) Effect of biomass load on PPAL ethanol density

The standard density of ethanol is 0.789 g/L. Doubling the biomass load of the sample will lead to an increase in ethanol concentration (**Figure 11**) in the water-ethanol mixture thereby adjusting the ethanol density towards its standard. Maintaining fermentation time and pH while doubling the effect biomass load gave the following model equation.

$$Y_4 = 1.3156 - 0.020684X_3 + 0.000632077X_3^2 \tag{13}$$

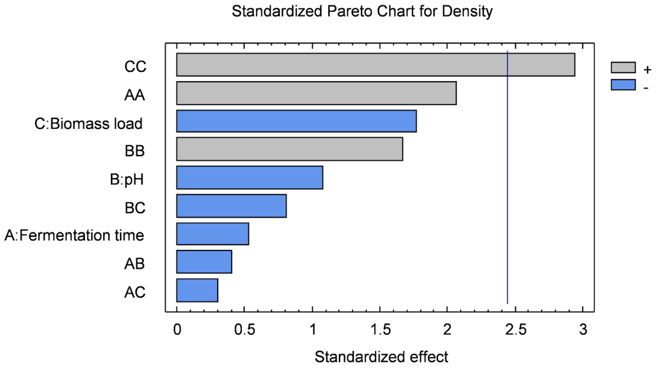


Figure 10. Pareto diagram showing the effect of processing parameters on the Density of PPAL ethanol.

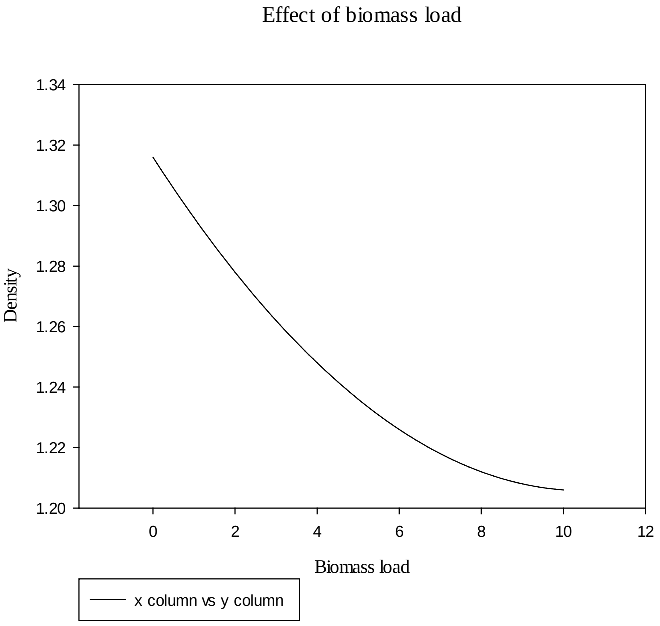


Figure 11. Effect of biomass load on the density of PPAL ethanol.

3.5. Effects of Process Parameters on the Physicochemical and Functional Properties of Bio-Ethanol Production via Solid-State Fermentation of Acid (BPAC) and Alkaline (BPAL) Pretreated Banana Biomass

A central composite design was used to study the effect of process parameters on the production of bioethanol from acid and alkaline pre-treated banana peel biomass. The process variables evaluated were; Fermentation Time (X_1), pH (X_2) and Biomass Load (X_3). Using the CCD, an experimental matrix for the process factors was generated and the responses were obtained as presented in Table 8 and Table 9 for the acid and alkaline pre-treated banana peel biomass respectively.

From the analysis of variance (ANOVA) using STATGRAPHICS, process parameters which were fermentation time, pH, and biomass load showed no statistically significant effects on the physicochemical and functional properties of acid pretreated banana peel biomass. Like the acid pretreated banana peel, the process parameters and their interactions for the alkaline pretreated banana peel biomass had no statistical significant effect on the physicochemical properties on the production of the bioethanol.

Table 8. Effects of process parameters on the physicochemical and functional properties of bioethanol produced from acid pre-treated banana peel biomass.

Runs	Factors			Responses			
	Fermentation time/h	Initial pH	Biomass load/g/ml	TSS/%Brix	TRS/%	Ethanol Conc./%	Density/g/ml
	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4
1	72	7	15	2.00	08.47	2.64	1.010
2	48	6	12	1.00	11.70	2.61	0.949
3	24	5	25	2.50	09.22	2.61	0.995
4	24	7	15	1.50	02.47	2.58	0.972
5	72	7	25	4.00	08.36	2.22	0.985
6	48	6	20	2.20	13.62	2.56	1.010
7	48	4	20	1.20	16.92	2.61	1.012
8	24	5	15	1.80	10.38	2.47	1.032
9	48	6	28	1.50	16.25	2.72	0.981
10	88	6	20	1.50	15.51	2.67	1.008
11	72	5	15	0.50	14.12	2.31	0.948
12	48	6	20	1.00	11.25	2.61	1.064
13	08	6	20	1.50	08.10	2.67	1.005
14	48	8	20	5.50	15.54	2.44	0.988
15	72	5	25	2.00	09.35	2.67	1.010
16	24	7	25	2.50	10.61	2.61	1.005

Table 9. Effects of process parameters on the physico-chemical and functional properties of bioethanol produced from alkaline pre-treated banana peel biomass.

	Factors			Responses			
Runs	**Fermentation time/h**	**Initial pH**	**Biomass load/g/ml**	**TSS/%Brix**	**TRS/%**	**Ethanol Conc./%**	**Density/ g/ml**
	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4
1	72	7	15	0.50	07.51	2.67	1.002
2	48	6	12	0.90	10.24	2.61	1.074
3	24	5	25	0.60	04.85	2.47	1.021
4	24	7	15	1.50	05.95	2.47	1.065
5	72	7	25	2.00	13.65	2.61	1.025
6	48	6	20	2.00	09.20	2.61	0.986
7	48	4	20	0.00	10.89	2.61	0.976
8	24	5	15	0.00	05.04	2.42	0.966
9	48	6	28	0.50	11.38	2.40	1.027
10	88	6	20	0.50	07.88	2.67	0.995
11	72	5	15	1.00	01.29	2.56	1.062
12	48	6	20	1.00	07.94	2.33	0.900
13	08	6	20	0.00	07.34	2.53	1.000
14	48	8	20	2.50	07.98	2.50	1.014
15	72	5	25	1.50	11.10	2.50	0.985
16	24	7	25	2.00	07.97	2.28	1.010

Like acid pretreated banana peel, process parameters and their interactions for the production of bioethanol from alkaline pretreated banana peel biomass had no statistically significant effects on the physicochemical properties of the ethanol.

1) Effect of process parameters on the density of BPAL bioethanol

From the analysis using STATGRAPHICS, the following equation was generated for the density of BPAL bioethanol

$$\begin{aligned} Y_4 = {} & 1.89308 + 0.00128826X_1 - 0.114154X_2 - 0.0645732X_3 \\ & + 0.0000338209X_1^2 - 0.000565323X_1X_2 - 0.0000564667X_1X_3 \\ & + 0.012971X_2^2 - 0.0002728X_2X_3 + 0.00167314X_3^2 \end{aligned} \tag{14}$$

This model equation has $R^2 = 65.134$ % which is less than 70% implying that the regression analysis for the Density of Banana peel biomass pretreated with acid (BPAL) is not valid. Standard error = 0.042866 which is <10%.

From the Pareto diagram in Figure 12, the doubling effect of biomass load (X_3X_3) had a positive significant effect on ethanol density of BPAL while other process factors and interactions had no significant effect. The doubling effects of fermentation time (X_1X_1) and pH (X_2X_2) had a positive non-significant effect while all process factors (fermentation time, pH, and biomass load) and their interactions had a negative non-significant effect on the ethanol density of BPAL.

i) Effect of biomass load on the density of BPAL ethanol

The standard density of ethanol is 0.789 g/L. Increasing the biomass load of the sample will lead to an increase in substrate concentration (Figure 13) thereby increasing ethanol concentration in the water-ethanol mixture hence adjusting the ethanol density towards its standard. Maintaining fermentation time and pH while doubling the effect biomass load gave the following model equation.

$$Y_4 = 1.89308 - 0.0645732X_3 + 0.00167314X_3^2 \quad (15)$$

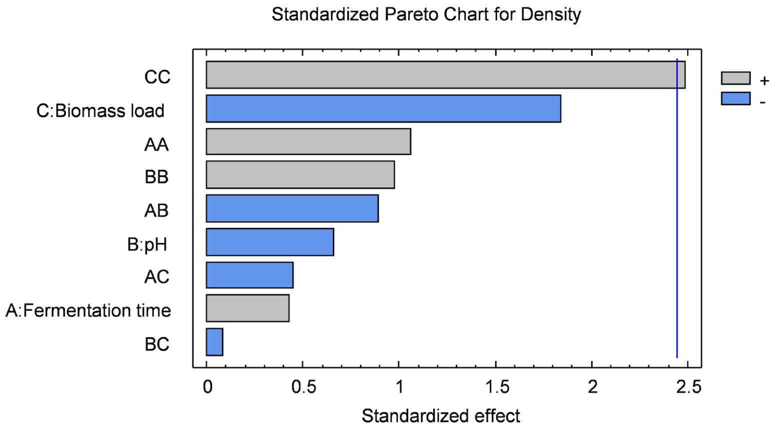


Figure 12. Pareto diagram showing the effect of processing parameters on the Density of BPAL ethanol.

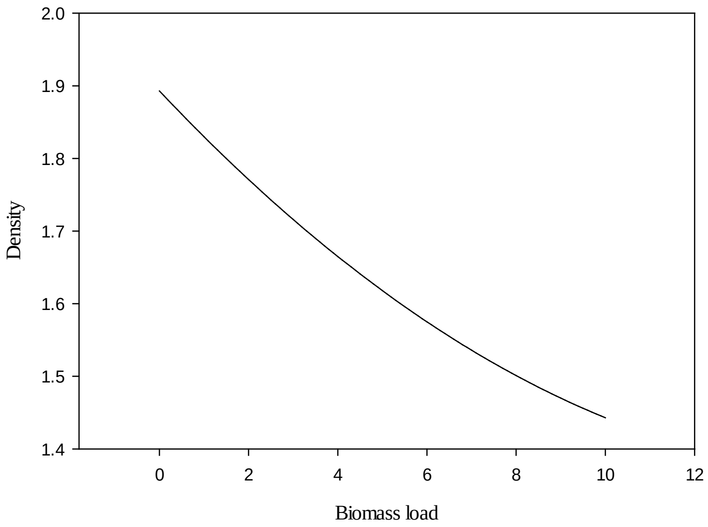


Figure 13. Effect of biomass load on the density of BPAL ethanol.

4. Conclusion

The results of this research showed that chemical pretreatment (dilute acid and alkali pretreatment) had a significant effect on the physicochemical properties of banana and pineapple peel lignocellulose biomasses. Both chemical pretreatment methods significantly affected the reducing or fermentable sugars of both biomasses but dilute acid (2.2% sulfuric acid) pretreatment was the most effective as both biomasses recorded the highest reducing sugars after acid pretreatment. Process parameters (Fermentation time, pH and biomass load) generally affected the physicochemical and functional properties of food-grade ethanol produced through solid-state fermentation. The optimized process conditions (fermentation time, pH and biomass load) for bioethanol production from BPAC were; 48.88 h, 6.11, and 21.96 g/ml respectively with 2.56%, 1.025 g/ml, 13.93% and 2.59%Bx for ethanol concentration, density, TRS and TSS respectively. While for BPAL, the optimized conditions were 77.05 h, 7.0, 27.99 g/ml for fermentation time, pH and biomass load respectively yielding 2.56%, 1.048 g/ml, 16.57% and 1.30%Bx for ethanol conc., density, TRS and TSS respectively. PPAC had 34.51 h, 4.82, and 24.0 g/ml for fermentation time, pH and biomass load respectively giving 2.42%, 1.015g/ml, 37.72% and 5.61%Bx for ethanol conc., density, TRS and TSS respectively. While, PPAL on the other hand had 7.17 h, 7.27, and 27.99 g/ml for fermentation time, pH and biomass load respectively giving 2.73%, 1.059 g/ml, 51.81% and 5.50%Bx for ethanol conc., density, TRS and TSS respectively. Acid pretreatment was observed to be the most effective for both biomasses with higher yields of TRS (14.39% to 21.8% and 20.69% to 30.0%) for banana and pineapple peel respectively.

Conflicts of Interest

The authors declare no conflicts of interest.

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