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TWO-STAGE ACID HYDROLYSIS OF BANANA PEELS FOR ETHANOL PRODUCTION

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Abstract - Two-stage acid hydrolysis of banana peels for ethanol production was studied. The banana peels were separately treated with phosphorous acid (H_3PO_4) and sulphuric acid (H_2SO_4) and later subjected to treatment with the two acids in a two- stage wise configuration to produce glucose. Factors affecting glucose production were investigated. Yeast fermentation of the produced glucose was carried out to obtain ethanol. Process factors for the fermentation process as well as its kinetics and thermodynamics properties were also investigated. High Performance liquid chromatography (HPLC) was used to analyse the ethanol produced. Analysis of the results showed that two–stage acid hydrolysis involving serial application of H_3PO_4 and H_2SO_4 produced the highest glucose content of 2.2662g/l from biomass of 100g/l, acid strength of 4% and temperature of 70^oC for 120minutes. At a pH of 7 for a period of seven (7) days fermentation gave a maximum of 35%v/v ethanol concentration from 50% ethanol yield at 5g/250ml yeast concentration. It was revealed that banana peels, of about 18.8% cellulose content, can effectively be used for ethanol production.

Keywords. Banana peels, Hydrolysis, fermentation, kinetics, Thermodynamics

1. Introduction

Continuous use of fossil fuel has caused global warming and remarkable change on the climate. There is also a rapid increase in the consumption of conventional fossil fuel, which clearly implies a general decrease in energy supply. Mushimiyimana and Tallapragada, (2016) reported that one of the attributes of this century is the production of fuel for automobile engines because of thousands of vehicles and engines running on gasoline and other petroleum fractions. As an alternative to fossil fuel, ethanol has attracted worldwide attention because of its potential usefulness as energy resources in several and various capacities especially as automotive fuel. Ethanol has comparative advantage over conventional petroleum fuels; it possesses higher octane rating and its usage is safer, it gives an improvement in the quality of air due to its cleanliness and burning quality. There are even national policies to blending 20-30% ethanol in gasoline in several countries by 2030 (Jahid et al., 2018). Ethanol can be produced from the conversion of fermentable carbohydrates (Braide et al., 2016).

Lots of agricultural raw materials rich in fermentable carbohydrates have been under examination globally for conversion to sugar and ethanol but the cost of carbohydrate raw materials is comparatively high for production. So, inexpensive feedstock (agricultural waste) such as lignocellulosic biomass (LCB) is being considered for production of ethanol (Arumugam and Manikandan, 2011). Banana (*Musa acuminata*) peels are readily present and full of potentials, yet an underutilized carbohydrate–rich material for glucose yield (Brooks, 2008). Banana being a major cash crop of many regions can generate large amount of waste after harvest. The suitability of banana peels in production of glucose was determined after comparison with some other lignocellulosic wastes (Anhwange et al., 2009). This banana peels can be hydrolyzed to produce glucose for ethanol production.

Hydrolysis is a process where large molecules of carbohydrate are split into parts by the addition of water molecule such that one part gains hydrogen ion (H^+) while the other part gains the remaining hydroxyl ion (OH^-) from the water molecule. Hydrolysis can be performed in the presence of enzymes, organic acids or inorganic acids (Mussatto and Robberto, 2004; Sanchez and Cardona, 2008). Acid hydrolysis can be applied in various ways to improve yield of glucose from banana peels. Acid hydrolysis is dependent on the concentration of the acid solution in use such that the breaking of bonds (β–1, 4–glycosidic bonds) provides the hydrolysis of cellulosic molecules into simple sugar molecules (Salam et al., 2013). Two kinds of acid hydrolysis are applicable; concentrated and dilute hydrolysis (Hendriks and Zeeman 2009). The major advantage of dilute acid hydrolysis is the use of small quantity of acid concentration (about 2–5%) at high temperatures to give an acceptable glucose yield. The produced glucose can be converted to ethanol through fermentation process.

Fermentation is a gradual splitting of large organic molecules of carbohydrates into simpler molecules such as alcohols by micro-organisms (Ababio et al., 2013). A common micro-organism used in fermentation is yeast. It involves a variety of enzymes that bring about the decomposition of starches and sugars to ethanol. Fermentation is a biological way of producing ethanol by allowing micro-organisms to feed on simple sugars produced from the hydrolysis of biomass, such as banana peels. Obtaining a qualitative and quantitative alternative through improved acid hydrolysis of agricultural wastes (such as banana peels) is of great research interest. Due to reduced efficiency and the need to improve on soft acidic hydrolysis, improvement on how to access cellulose and then hydrolyzing it efficiently is a needful challenge (Sanchez and Cardona, 2008). This research work seeks to produce ethanol from glucose produced from acid hydrolysis of banana peels.

2.0. Materials and Method

2.1. Preparation of Banana Peels

Banana peels were washed to remove dirt and dried in an oven at $105^{O}C$ to a constant weight. They were then crushed in a mortar to particulate size and stored in a nylon bag in readiness for characterization and pre-treatment.

2.1.1 Base (Alkaline) Pre-treatment of Ground Banana Peels

50g of ground banana peels was dissolved with 500ml of 4% ($^{W}/_{V}$) NaOH (1N NaOH) in a beaker. It was heated in a water bath at a constant temperature of 50°C for 1 hour. The sample was withdrawn and neutralized with 10%HCl and subsequently with distilled water until pH of 5 was reached. The solution was filtered using sieving net and the residue was dried in an oven at 70°C for 3 hours in readiness for proximate analysis and acid hydrolysis.

2.1.2 Proximate Analysis of Ground Banana Peels before and after Pre-treatment

The proximate analysis was carried out before and after pre-treatment according to the method used by Joshua et al., (2016).

Extractive Content:

The biomass sample of banana peels was dried to a constant weight. The dried sample of about 50g was poured into a thimble and then placed in the Soxhlet extractor. 360ml of water was then placed on the heating mantle. The Soxhlet extraction unit was then coupled to the flask. After the extraction was conducted for 4 hours using ethanol, the supernatant was collected. It was evaporated until a volume of 15.5ml was reached where there was no solvent observed again. It was oven dried at 105°C to completely evaporate moisture from the biomass to a constant weight. The resulting residue gave the extractive content of the biomass.

Lignin Content:

14ml of cold 72% sulphuric acid was added to 1g of the extractive free biomass sample and was stirred. The mixture was allowed to rest for 2 hours before being washed in a filter conical flask and diluted to 3% H_2SO_4. The mixture was then boiled for 4 hours under reflux. The insoluble was allowed to rest,

filtered out, washed and oven dried at 105°C for 2 hours to completely evaporate moisture from the biomass, then cooled and weighed as the lignin content.

Cellulose Content:

Into a 250ml beaker, 2g of the extractive free biomass and 100ml of 17.5% NaOH solution were added and stirred at 30°C for 30 minutes. The content of the beaker was then filtered, washed with 25ml of 9.5% NaOH solution and 20ml distilled water. The residue was washed again with distilled water and 40ml of 10% acetic acid and further with 1 litre distilled water. The residue was finally dried at 105°C for 4hours to a constant weight as the cellulose content.

Hemicellulose Content:

1g of extracted dried biomass was poured into 250ml flask. 150ml of 0.5moldm^{-3} NaOH was added. The mixture was boiled for 4 hours with distilled water. It was filtered after cooling through vacuum filtration and washed until neutral pH. The residue was dried to a constant weight at 105°C in an oven. The difference between the sample weight before and after the treatment is the hemicellulose content $(\%\,{}^{w}/_{w})$ of dry biomass.

Hemicellulose content $(\%\,w/w) =$

$100[(W_B - W_R)/W_B]$ **(1)**

Where W_B is the weight of extracted dried biomass and W_R is the constant weight of residue.

2.1.3. Acid Hydrolysis of Pre-treated Biomass

One-stage acid hydrolysis was performed using 2g of cellulose each separately poured into 20ml of 1%H_2SO_4 and 1%H_3PO_4) contained in two different 250ml beakers. They were respectively placed in isothermal shaker at a temperature of 30°C with an agitation speed of 150 rpm for 2 hours (120 min). At 30 minutes interval, the biomass sample was withdrawn to determine the glucose concentration. The experiment was repeated for temperatures of 40, 50, 60 and 70°C respectively.

Two-stage acid hydrolysis was performed using one different acid for the first 15 minutes of the hydrolysis process and then centrifuged the solution to separate and remove the first acid by decantation. The second acid was added and used to hydrolyze the cellulose resulting from the first-stage hydrolysis for the next round of 15 minutes of the acid hydrolysis. Considering the order of treatment, phosphoric acid was first used, followed by sulphuric acid. After hydrolysis, undissolved particles were separated by centrifugation and the supernatant neutralized with O.1N NaOH and water to a pH of 5. The filtrate was then analysed for glucose concentration and the residue discarded. Glucose was determined by 3, 5 – dinitrosalicyclic (DNS) acid reagent method.

2.1.4. Effect of Process Factors on Glucose Production

Various factors that affected glucose production were examined. They include acid strength, biomass dosage, time and temperature.

1) Effect of Acid Strength on Glucose Produced

Effect of acid strength was studied using 2g biomass in 20ml solution at room temperature for 2 hours using 1%, 2%, 3% and 4% concentrations of different acids (H_2SO_4, H_3PO_4 and H_3PO_4/H_2SO_4 and H_2SO_4/H_3PO_4) to determine the influence of acid strength in hydrolysis.

2) Effect of Biomass Dosage on Glucose Produced

Effect of biomass dosage was studied at room temperature for 2 hours using 0.5g, 1.0g, 1.5g, and 2.0g of pre-treated biomass in 20ml solution for 4% concentration of different acids. Effect of biomass dosage was studied to know the significance of biomass dosage in acid hydrolysis.

3) Effect of Time on Glucose Produced

Effect of time was studied using 2g biomass in 20ml solution at room temperature for 30mins, 60mins, 90mins, and 120 minutes with 4% concentration of different acids. Effect of time was studied to observe the significance of time in acid hydrolysis.

4) Effect of Temperature on Glucose Produced

Effect of temperature was studied using 2g biomass in 20ml solution at 40°C, 50°C, 60°C

and 70°C for 30 minutes with 4% concentration of different acids. Effect of time was studied to determine the significance of time in acid hydrolysis.

2.2. Kinetics of Glucose Production by Acid Hydrolysis

The kinetics of the glucose production was studied using 4% acid concentration of various types of acids by varying temperature from 40°C to 70°C at an interval of 10°C with corresponding time from 30 minutes to 120 minutes. Biomass dosage was maintained at 100g/l each time and corresponding glucose quantities were produced. According to Latinwo and Agarry, (2015), first-order model was proposed for hydrolysis of cellulose to glucose using the reaction scheme: Cellulose → Glucose

The cellulose concentration C is a function of time t.

Mathematically,

$$\mathrm{Ln}\frac{C_0}{C_0-x} = kt \qquad \textbf{(2)}$$

Where C_0 is initial cellulose concentration in g/l, $C_0 - x$ is cellulose concentration after time t in g/l, x is glucose quantity in g/l, k is specific rate content in min^{-1} and t is time taken for hydrolysis in minute.

Equation (2) was used to validate the first – order reaction for the acid hydrolysis.

2.3. Thermodynamics of Glucose Production

The rate constants k obtained from the kinetics of glucose produced was used to evaluate the thermodynamic properties of the acid hydrolysis. As reported by Patil and Madhamshettiwar (2014), Arrhenius equation was used to evaluate activation energy:

$$\mathrm{Ln}k = \frac{-Ea}{RT} + \mathrm{In}\,A \qquad \textbf{(3)}$$

Where k is rate constant, Ea is activation energy, R is universal gas constant (0.008314 $kJmol^{-1}K^{-1}$), T is temperature in Kelvin and A is pre exponential constant.

Equation (3) is used to obtain the activation energy (Ea) which in turn used to evaluate the enthalpy of acid hydrolysis, ($\mathbf{\Delta}H^0$) at various temperatures. Mathematically, $\mathbf{\Delta}H^0$ according to Mukherjee et al (2017) can be expressed as

$$\mathbf{\Delta}H^0 = Ea - RT \qquad (4)$$

Free activation enthalpy ($\Delta H^{\ddagger}$)and entropy of activation($\Delta S^{\ddagger}$) of acid hydrolysis were evaluated from Eyring-Polanyi equation with knowledge of the formula for Gibb's free energy. Mathematically,

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger} \qquad \textbf{(5)}$$

$$\mathrm{In}\left(\frac{K}{T}\right) = -\frac{\Delta H^{\ddagger}}{R}\left(\frac{1}{T}\right) + \left[\mathrm{In}\left(\frac{kb}{h}\right) + \frac{\Delta S^{\ddagger}}{R}\right] \qquad \textbf{(6)}$$

Where $\Delta H^{\ddagger}$activation enthalpy in KJmol-1, Kb is is Boltzmann constant ($1.38065 \times 10^{-23} JK^{-1}$), h is Planck's constant ($\simeq 6.626 \times 10^{-34} JS$) and $\mathbf{\Delta}G^0$ is Gibbs free energy or free activation enthalpy.

Equation (6) $\Delta S^{\ddagger}$ is evaluated, having substituted values for kb and h, at different temperatures. Mathematically,

$$\Delta S^{\ddagger} = R\left[\mathrm{In}\,\frac{K}{T} - \mathrm{In}\,\frac{kb}{h}\right] \qquad \textbf{(7)}$$

Gibb's free energy $\Delta G^{\ddagger}$ was also evaluated at various temperatures.

2.4. Fermentation of Glucose

Fermentation was carried out in a batch reactor containing 250ml of the glucose solution from the best acid hydrolysis and inoculated with 5g baker's yeast (*Saccharomyces cerevisiae)*. Fermentation was anaerobic and tube was fixed through the cap of the fermentor to allow the escape of CO_2 gas produced. The mixture of the glucose solution and yeast was then incubated on a shaker with 300 rpm at 30°C for 7 days maintained at pH of 6. At the end of the fermentation, the fermented liquor was separated, distilled and the yield of ethanol was measured. High Performance Liquid Chromatography (HPLC) analysis was done on the ethanol produced.

2.5. Effect of Process Factors on Fermentation of Glucose to Ethanol

Various factors that affected ethanol production were examined. They include time, yeast concentration and pH.

1) **Effect of Yeast Concentration on Ethanol Yield**

The effect of yeast concentration was studied at pH 6 for 7 days using 2, 3, 5 and 7g of yeast per 250ml glucose solution as the yeast concentrations.

2) **Effect of Glucose Fermentation Time on Ethanol Yield**

The effect of time was carried out at pH 6 using 5g per 250ml glucose solution as yeast concentration for 3, 5, 7 and 9 days.

3) Effect of pH on Fermentation on Ethanol Yield

The effect of pH on fermentation was carried out using 5g per 250ml glucose solution as yeast concentration for 7 days at pH 3, 5, 7 and 9. The pH was adjusted using 10% HCl and 0.1 NaOH.

3. Results and Discussion

3.1 Characterization of Biomass (Banana Peels)

Table 1 shows that pre-treatment of biomass resulted in reduction of moisture, extractives, hemicellulose and lignin contents while it increased cellulose content by 55.5%. It showed cellulose to be highest in content amongst other components. This increase in cellulose content is in agreement with the report of Zhu et al., (2006) who pre-treated palm bunch with NaOH solution and observed 47.56% increase in cellulose. The main reason for pre-treatment of the biomass is to make the lignocellulose structure porous to access its cellulose content which is a direct source of glucose. Pre-treatment brings about increase in reaction rate during hydrolysis.

Table 1 Characterization of Biomass (Banana Peels)

Content	Untreated biomass	Treated biomass	Reduction/Increment (%)
Moisture (%)	4.691	1.325	71.8
Extractives (%)	3.5397	2.5928	26.8
Hemicellullose (%)	6.8247	4.312	36.8
Lignin (%)	5.001	2.5779	48.5
Cellulose (%)	12.0617	18.7556	55.5

3.2 Effects of Process Factors on Hydrolysis of Biomass

Effects of acid strength, biomass dosage, hydrolysis time and temperature were investigated. Figure 1 shows a general increase in the concentration of glucose as the strength of acid increased from 1% to 4%. This is in line with Talebnia et al., (2007) who reported that glucose concentration increased with increase in mild acid concentration. Increase in concentration increases the surface area of the substance and therefore allows for frequent surface contact between reactants necessitating increase in chemical reaction. The result of the reaction is increase in conversion of biomass into glucose.

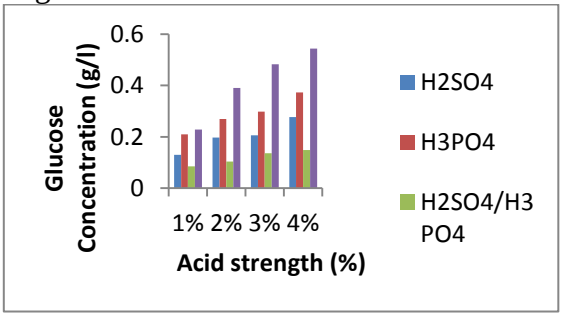

Fig. 1 Effect of acid strength on glucose concentration by acid hydrolysis

In Figure 2, it can be observed that glucose concentration increased as biomass dosage increased. Phosphoric acid produced the highest glucose concentration followed by the combination H_3PO_4/H_2SO_4 and sulphuric acid. The reason for this can be attributed to the phenomenon of ionization where H_2SO_4 ionizes completely in water thereby creating no additional water for hydrolysis. However, H_3PO_4 ionizes partially in water thereby creating additional water molecules for further hydrolysis of the plantain peels to glucose.

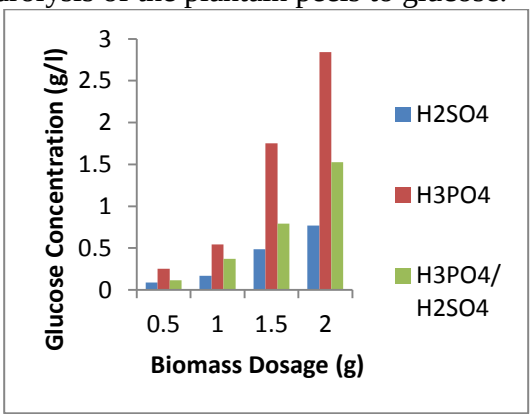

Fig. 2 Effect of biomass dosage on glucose concentration by acid hydrolysis

Figure 3 shows increase in glucose yield with time at various temperatures of 40, 50, 60 and 70°C. This showed that time has a significant effect on the yield of glucose during acid hydrolysis. Consequently, the rate of formation of product increases with time.

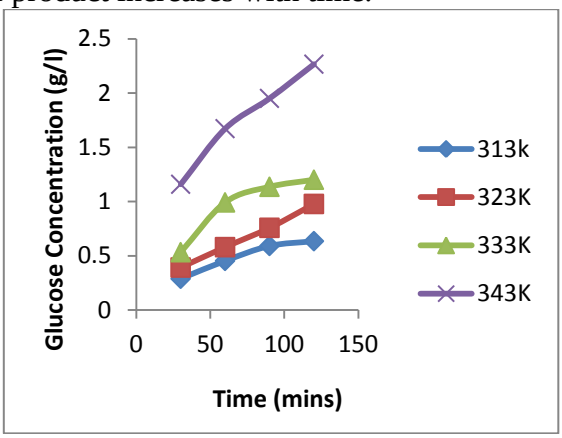

Fig. 3: Effect of time on glucose concentration by acid hydrolysis using 4% H_3PO_4/H_2SO_4

Figure 4 shows that effect of temperature on glucose concentration. Increase in temperature gave increase in glucose concentration. Comparable observations have been made by Ajani et al (2012) and Kupianen et al., (2014) who reported that increase in temperature caused increase in the yield of glucose during hydrolysis. Talebnia et al 2007 reported that Acid hydrolysis of orange peels gave an increased yield with increase in temperature. It was also reported by Megawati et al (2010) that at temperature range 160 - 220°C, total glucose yield increased for acid hydrolysis of rice husk. This could be attributed to the fact that increase in temperature causes agitation and raises the average kinetic energy of the molecules of the biomass. As the molecules move about, they collide and come in contact with one another with increased frequency thereby reacting and consequently increasing in the rate of chemical reaction.

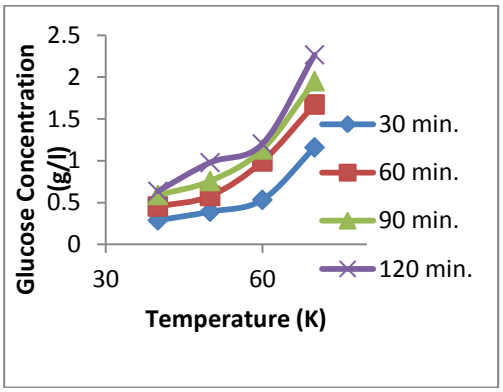

Fig. 4 Effect of temperature on glucose concentration by acid hydrolysis using 4% H_3PO_4-H_2SO_4

3.3 Kinetics on Acid Hydrolysis

A first – order kinetic model for acid hydrolysis was examined. Table 2 shows the rate constants and their corresponding coefficients of correlation for hydrolysis using different acid types. It can be observed that the two – stage acid hydrolysis rate constant was the highest ($10 \times 10^{-5} mins^{-1}$) at the highest temperature of 343k with a corresponding co-efficient of correlation (0.977). As temperature was increased from 313K to 343K, the rate constant (k) increased from 4×10^{-5} $mins^{-1}$ to $10 \times X10^{-5}$ $mins^{-1}$ while the coefficient of correlation (R^2) increased from 0.949 to 0.977. This implied that there was increase in conversion of cellulose to glucose and the chemical reaction proceeded fast as rate constant, K, increased.

Table 2 .Rate constant (K) and Coefficient of Correlation (R^2)

Type of acid	313K		323K		333K		343K	
	$Kx10^{-5}$ (min^{-1})	R^2	$Kx10^{-5}$ (min^{-1})	R^2	$Kx10^{-5}$ (min^{-1})	R^2	$Kx10^{-5}$ (min^{-1})	R^2
H_2SO_4	2	0.986	3	0.970	4	0.955	5	0.991
H_3PO_4	2	0.935	4	0.997	4	0.839	7	0.978
H_3PO_4-H_2SO_4	4	0.949	7	0.997	7	0.866	10	0.977

3.4 Thermodynamics on Acid Hydrolysis

Thermodynamics of acid hydrolysis of banana peels was studied and the evaluated thermodynamic parameters are presented in Table 3. It can be observed from Table 3 that change in enthalpy (ΔH^0) decreased generally with increase in temperature while change in entropy (ΔS^0) and change in Gibb's free energy

(ΔG^0) increased with temperature. The two – stage acid hydrolysis generally had the lowest activation energy, enthalpy and Gibb's free energy but highest in entropy change. According to Latinwo and Agarri, (2015), lower values of activation energy presupposed how easy acid hydrolysis would occur. Activation energy of two – stage acid hydrolysis was 25.6329KJmol^{-1}. The activation energies of acid hydrolysis of banana skin, maize stalks, corn fibre, groundnut shell, sawdust, sunflower seed hull, maize cobs, corn cob, cowpea shells and rice husk gave 37.83, 34.29, 64.35, 78.35, 26.6, 80.34, 76.71, 72.6, 44.37 and 39.60 kJmol^{-1} respectively (Mosier et al., 2002, Megawati et al., 2010 and Ajani et al., 2012). Studies showed that for $\Delta G^0 < O$, reaction is said to be spontaneous while $\mathbf{\Delta}G^0 = 0$, the system is said to be at equilibrium such that no net change occurs. If $\Delta G^0 > 0$, reaction is not spontaneous, hence, the acid hydrolysis is not spontaneous. However, Low value of activation energy (Ea) shows fast rate of reaction while high values of Ea is an indication that the rate of reaction is slow. Also low value of ΔH^0 meant fast reaction rate as high value of ΔH^0 meant slow reaction rate.

Table 3 implied that this acid hydrolysis had a fast rate of reaction. A large negative value of ΔS^0 (which is unfavourable) indicates that the transition state is highly ordered or has a more rigid structure compared to the ground state and reaction rate is slow. Positive values (less negative values) of ΔS^0 (which is favourable) indicates that the transition state is highly disordered compared to the ground state and reaction rate is fast. This acid hydrolysis has less negative values that are approximately equal to zero which corresponds to positive values and so reaction proceeded fast.

Table 3: Thermodynamics Parameters

Parameters (kJ/mol)		**Acid types using 4% acid strength**		
		H_2SO_4	**H_3PO_4**	**H_3PO_4/H_2SO_4**
Activation energy, Ea (kJ/mol)		28.19	34.69	25.63
	$\mathbf{\Delta}H_{313}$	25.59	32.08	23.03
$\mathbf{\Delta}$H(J)	$\mathbf{\Delta}H_{323}$	25.51	32.00	22.95
	$\mathbf{\Delta}H_{333}$	25.43	31.92	22.86
	$\mathbf{\Delta}H_{343}$	25.34	31.84	22.78
	$-\mathbf{\Delta}S_{313}$	0.3353	0.3353	0.3295
$\mathbf{\Delta}$S(J/K)	$-\mathbf{\Delta}S_{323}$	0.3321	0.3298	0.3252
	$-\mathbf{\Delta}S_{333}$	0.3301	0.3301	0.3254
	$-\mathbf{\Delta}S_{343}$	0.3284	0.3256	0.3227
	$\mathbf{\Delta}G_{313}$	130.54	137.03	126.16
$\mathbf{\Delta}$G(kJ/mol)	$\mathbf{\Delta}G_{323}$	132.78	138.53	127.99
	$\mathbf{\Delta}G_{333}$	135.35	141.84	131.22
	$\mathbf{\Delta}G_{343}$	137.98	143.52	133.47

3.5 HPLC Analysis of Ethanol Produced

Table 4 shows the various values attributed to various peaks in the HPLC chromatogram. The peaks are best analyzed using their retention time, height and peak area with corresponding concentrations. It can be observed that peak 3 corresponds to 5.707minutes, 66.371% height and 44.233% peak area to present the highest concentration of 48.7153g in 100ml solution at 254nm wavelength. This is attributed to alcohol (-OH) functional group. This is an indicative of the presence of the alcohol component. This confirmed the presence of ethanol.

Table 4: HPLC Peak Table for Ethanol Produced

Peak	Ret. Time (min)	Conc. (g/100ml)	Height	Area %	Height %
1	3.327	0.13731	653	0.174	0.161
2	4.563	0.16986	788	0.215	0.194
3	5.707	48.7153	269362	44.233	66.371
4	6.139	13.19625	72849	16.739	17.950
5	6.461	16.75392	47553	21.251	11.717
6	8.624	1.95830	4903	2.484	1.208
7	9.450	2.75925	4093	3.500	1.008
8	19.322	8.99008	5645	11.403	1.391
Total			405846	100.000	100.000

Conclusion

Banana peels was found to be rich in cellulose content (18.7556%) which can be effectively production, however, the yield can be influenced by several process factors especially biomass dosage, acid type, acid concentration, time, temperature and fermentation pH.

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