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# Determination of optimum conditions for maximum bioethanol yield from the Nigerian sweet potato (*Ipomoea Batatas* (L.) *Lam*)

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# Abstract

Bioethanol is an important biofuel produced from biomass and is generally regarded as the future of fuels. This research was carried out for the main purpose of determining the optimum process conditions required for maximum bioethanol yield from the pulp of local sweet potato. The variety of potato used in this research is the Nigerian sweet potato (*Ipomoea Batatas* (L.) *Lam*) containing high amount of starch and the experiment was carried out with the use of Baker's Yeast (*Saccharomyces cerevisae*) as the biocatalyst. In this study, the effect of acid concentration for hydrolysis, pH and fermentation period was examined. The sweet potato pulp was hydrolyzed using dilute sulphuric acid solutions of 0.5M, 1.5M and 2.5M and heated for 30 minutes at 100 °C yielding 0.9% (v/v) reducing sugar for fermentation. The hydrolysates were then subjected to fermentation at different pH values for different fermentation periods of 2, 3.5 and 5 days respectively. The pH values ranged from 4.5 to 6.5, an increment of 1.0 was used during the examination of pH with 1.0 M sodium hydroxide solution (NaOH) used for adjustment. The rate of bioethanol production was seen to increase as the pH and fermentation periods increased with the exception of the fermenting cultures of pH of 6.5. The maximum bioethanol yield of 13.40 ml, equivalent to 3.350% (v/v), was gotten from the hydrolysate obtained using the dilute acid of concentration 0.5M and left to ferment for 5 days at a pH of 5.5.

Keywords: Sweet potato; Bioethanol; Biofuel; Fermentation; Hydrolysis; Saccharomyces cerevisae

# 1. Introduction

Global energy demands for everyday use in industry, transportation, power generation, heating and other aspects of human life is on a steady rise [1]. Every country needs energy to keep up with the pace of rapid development going on in other parts of the world. This is also not unconnected to the rise in the world's population [2]. Thankfully, fuels from fossil sources have been there for humans and contributed immensely to economic and infrastructural development of many nations especially since the turn of the twentieth century [3]. The United States Department of Energy, DOE, defines fossil fuels as fuels that are obtained from non-renewable sources which accumulated in the earth crust as a result of decomposition of plants and animals buried in the ground long ago [DOE Portal, https:// www. energy. gov/ science- innovation/ energy

sources/fossil#:~:text=Fossil%20energy%20sources%2C%@)including%20oil,buried%20by%20layers%20of%20ro ck, Last accessed 04/07/2022]. Examples of fossil fuels are crude oil, natural gas and coal. As it stands today, crude oil account for more than 37% of the world's energy needs [4]. The National Academy of Sciences also attributes 81% of the energy used in the United States of America to be from crude oil, coal and natural gas [National Geographic Portal: https://education.nationalgeographic.org/resource/fossil-fuels/, Last accessed 04/07/2022]. Between the three, coal is largest domestically produced energy source in the US and is used extensively to produce electricity in the country.

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Despite the impact of fossil fuels on humans and world development in general, they are not without issues. One demerit of fossil fuels is their finite nature [5]. There are serious concerns about rapid depletion of fossil sources due to the increasing worldwide demand for energy [3]. Fossil sources are not sustainable and would ultimately be used up. According to researchers, crude oil, a major fossil fuel, would experience a massive decline in annual production by 2050 [6]. There are cases where millions of dollars have been spent searching for energy sources such as oil prospecting but to no avail. The unsustainable nature of fossil fuels is the reason for high cost of production which ultimately results in the hike of global energy prices [7]. Another major demerit with the use of fossil fuels is the role they play in environmental pollution. Fossil fuels often lead to the emission of Greenhouse Gases (GHG) due to incomplete combustion in engines [8]. These gases cause global warming which successive governments across the globe have taken a stand against [9]. They aid in the depletion of the ozone layer causing direct rays of sunlight to reach us. These and many more are the reasons why top companies around the world are trying to leave petroleum and other fossil-based feedstock behind for plant-based options (biomass) [10]. There hasn't been a time in history when the clamour for alternative sources of energy has been more pronounced [3]. This is why most modern day research is now concentrated on renewable production of biofuels and biochemicals.

One of the most prominent biofuels is bioethanol. Bioethanol is a renewable source of energy and a worthy substitute for petrol in motor engines. It has been in use in vehicle engines since 1925 [2]. It is a form of fuel produced from fermented lignocellulosic biomass [5]. It is considered clean because it is biodegradable, has little or no toxicity and poses no significant threat to the environment [11]. In addition, bioethanol offers less corrosion tendencies, more energy efficiency, good blending ability with diesel fuel and gasoline for internal combustion engines [12]. Bioethanol has an environmental advantage over other fossil fuels in that it reduces the emission of pollutants from about 80-90 % to around 40-50% [8]. During combustion, this fuel splits to carbon dioxide (CO<sub>2</sub>) and water.

The production of bioethanol follows three paths. The first step is usually the hydrolysis of the feedstock to breakdown the plant polymers (that is lignocellulosic and hemicellulosic polymers) into simple sugars ready for fermentation with the most important simple sugar monomers being glucose and xylose [10]. This step requires critical care because its sugar yield determines the overall yield of bioethanol. Hydrolysis of biomass can be done through the use of acid (Acid hydrolysis) and the use of enzymes (Enzymatic hydrolysis) [13]. The latter often occurs after pretreatment of the lignocellulosic biomass. The second step in bioethanol production is the conversion of the sugar monomers into bioethanol through the fermentation process using microorganisms [9]. Common yeast (*Saccharomyces cerevisae*) is commonly used because it can be easily cultured even in large scale processes and it has a short germination time [12]. Other microorganisms used in fermentation *Zymomonas mobilis, Pichia stipites, Scheffersomyces shehatae, Aspergillus niger, Trichoderma harzianum*, etc. The third and final step is usually the bioethanol separation and purification phase with often involves distillation [2]. In summary the production of bioethanol follows the equation:

## $C_6H_{12}O_6$ (Glucose) $\rightarrow 2CO_2$ (carbon dioxide) + 2ATP (Energy) + Bioethanol [2].

Countries currently using bioethanol in large quantities include Brazil and the US [2]. Just recently, the Nigerian National Petroleum Company (NNPC) Ltd, the regulatory body in the petroleum and allied sector, established the Renewable Energy Division (RED) to position the country amongst the top players in bioethanol production and usage [Business Day Portal: https://www.businessday.ng/amp/energy/oilandgas/article/nnpcs-clean-energy-plan-opens-opportunity-for-investors/, Last accessed: 05/07/2022]. Bioethanol is one of the most promising fossil fuel alternatives for the future and if its production is optimized, it could replace them whilst ensuring environmental sustainability [12].

Plants that generally have starch and cellulosic components have the potential to produce bioethanol [9]. Grains from starchy foods and wastes from starch processing plants could also be used for its production [10]. Amongst the commonly used materials for bioethanol production are potato, wood, maize, straw, sweat sorghum, barley, wheat, sugarcane, sugar beet, and cassava. Derman et al [14] used palm fruit bunches as the starting material for bioethanol production. Onwuakor et al [5] used risk husk. Agro-wastes also do well in bioethanol production. According to Otoikhian and Amune [8], sugarcane bagasse, yam peels, cassava peels, potato peels and maize wastes are common agro-wastes used in bioethanol production. In Brazil, bioethanol is produced using sugarcane [13] while Europe and US produces her bioethanol mostly from maize and other starchy grains [15].

The Nigerian sweet potato (*Ipomoea Batatas (L.) Lam*) is a staple food used vastly in the country. It is current ranked fifth, coming after rice, wheat, maize and cassava, on level of importance amongst developing nations [16]. Not just in developing countries, potatoes generally place second in the commonly used food in the world [2]. Being a root and tuber crop, sweet potatoes have high contents of starch, pectin and vitamins with starch accounting for about 30 – 85 g per 100 g of the tuber crop [17]. They also do not need complex pretreatment for bioethanol production due to their starchy nature [2]. Sweet potatoes in Nigeria often get wasted due to the poor processing facilities and technological

knowhow. These wastes can be put to use in bioethanol production [13]. This would increase economic activity, bring wealth to farmers and make Nigeria attain one of its sustainable development goals (SDGs).

The production of bioethanol from sweet potatoes peels have been evaluated by various researchers in the past. However, the study of bioethanol production from the plant's tuber is an area many researchers haven't gotten into. This research therefore seeks to discover the optimum process conditions for the production of bioethanol from the tubers of the Nigerian sweet potato. The effects of acid concentration for hydrolysis, pH and fermentation time are evaluated herein. The ultimate aim is to establish the Nigerian sweet potato as a worthy raw material for bioethanol production thereby increasing local content and environmental sustainability.

# 2. Material and methods

# 2.1. Material Used

The potato tubers (Freshly harvested potato roots 10 to 12 months old) were obtained from Ihiagwa Market in Ihiagwa, Owerri, Nigeria. The biocatalyst used in this study, dry commercial Baker's Yeast (*Saccharomyces cerevisiae*) was obtained from a local chemical store in Owerri, Nigeria. Other reagents such as high grade Sulphuric acid (98 wt %), Distilled water, sodium hydroxide (NaOH), the fermentation minerals (Yeast extract, Peptone, malt extract) as well as the apparatus used were obtained from the Chemical Engineering Laboratory, Federal University of Technology, Owerri, Nigeria.

# 2.2. Material Preparation

## 2.2.1. Preparation of sweet potato samples

The sweet potato was prepared for hydrolysis by the following method. The tubers were weighed, peeled and cut into chips. The chips were soaked in water for 30 minutes and subsequently taken to a local mill for grinding. The grounded potato was then processed according to the process described in Azad et al [15]. It was pressed in a sieve and the water was drained. The pressing continued until the pulp was obtained and taken to the laboratory for analyses. 8.70kg of potato yielded 3ml of potato pulp.

## 2.2.2. Preparation of sulphuric acids (concentrations of 0.5M, 1.5M and 2.5M)

Calculations involving the amounts of water and sulphuric acid needed to get the desired concentrations were carried out beforehand. These calculations informed the mix ratio done in the laboratory.

To obtain 0.5M sulphuric acid, 500 ml of distilled water was initially measured using a measuring cylinder and poured into a round bottom flask. Approximately 27.2ml of sulphuric acid (98% w/w) was measured and added to 500ml of distilled water. The mixture was diluted with distilled water up to the 1000ml mark and gently stirred. Nine fractions of 100ml were drawn out and kept.

Same procedure was followed to obtain acids of concentrations 1.5M and 2.5M. But in these cases, 81.5ml and 135.7ml of sulphuric acids (98% w/w) were used respectively.

## 2.2.3. Preparation of the biocatalyst (Saccharomyces cerevisae)

The biocatalyst was first activated using the method described in Nadir et al [10]. 100ml of distilled water was heated to  $40^{\circ}$ C and 0.5% (w/w) of Dry Baker's Yeast (*Saccharomyces cerevisae*) added to the distilled water. The mixture was left to cool for 10 minutes.

The inoculum was prepared according to the method described in Azad et al [15]. The broth culture used was modified Yeast-Malt-Peptone-Dextrose (YMPD) medium but potato marsh was used in place of dextrose as carbon source. 10g of mash potato was added to an Erlenmeyer flask containing 1000ml of distilled water. 3g of yeast extract, 5g of peptone and 3g of malt extracts were subsequently added. The activated biocatalyst (*Saccharomyces cerevisae*) was then added to flask and stirred. The Erlenmeyer flask containing the inoculum was autoclaved at 121°C at 15psi for 15 minutes. The pH of the whole system was adjusted to 6.00 followed by incubation for 24 hours at 30°C with a shaking 150 rpm to allow for proper yeast cell propagation.

## 2.3. Acid hydrolysis of the sweet potato pulp

In preparation for fermentation, holes were bored into the top of the reagent bottles to serve as vents for produced gases. Pipes were passed through these holes to collect the effluent gases into a bowl of water. 100ml of the modified YMPD culture medium (containing the biocatalyst) was asceptically inoculated into the hydrolysates and stirred. The pH was adjusted to the desired points using concentrated NaOH (sodium hydroxide). The reagent bottles were corked and left to ferment at room temperature (35°C) for the designated periods (2, 3.5 and 5 days). The bottles were shaken daily and the pH monitored on daily basis to ensure the pH remains within the designated range.

# 2.4. Bioethanol collection through distillation

After fermentation, batch-wise distillation was carried out for each of the 27 reagent bottles. The distillation unit was set up by placing a distilling flask on a hot plate for heating and cool water to run through a condenser for collection of the effluent bioethanol gas. This was done to eject the ethanol from the mixture of ethanol, water and other impurities. 400ml of each fermented sweet potato hydrolysate was transferred to a distillation flask and heated to 90°C to ensure all produced bioethanol leaves the system. A thermometer was fixed in the setup to measure and maintain constant temperature throughout the distillation process. The thermometer reading was constantly observed.

## 2.5. Experimental runs

The experiment was carried out a total number of 27 times following the same procedure to find the relationship between variables: acid concentrations (0.5M, 1.5M and 2.5M), pH (4.5, 5.5 and 6.5) and fermentation periods of 2, 3.5 and 5 days.

# 2.6. Assessment of bioethanol quality

Upon distillation, the produced bioethanol samples were taken and assessed for quality using the following properties – colour, odour and boiling point. The colour of the produced bioethanol was determined by sight just as the odour was determined by smell. The boiling was determined by heating the solution in a corked glass flask with the probe of an electronic thermometer inserted for taking readings. The peak temperature after which there was no further rise was taken as the boiling point.

## 2.7. Determination of percentage bioethanol yield

Upon certification, the percentage yield from each experimental run was determined using the equation provided in Onwuakor et al [5]. The ratio of the volume of the produced bioethanol to the volume of fermented sweet potato hydrolysate (i.e 400ml) is multiplied by 100. This is given mathematically as:

Percentage Yield = 
$$\frac{\text{volume of produced bioethanol}}{400} \times 100$$
.....1.

# 3. Results

The results obtained upon completion of the 27 experimental runs are tabulated below:

Experimental run	рН	Acid Concentration used (mol/dm3)	Fermentation period (days)	Obtained bioethanol volume (ml)	Bioethanol Yield (%)
1	4.50	0.50	2.00	10.40	2.600
2	5.50	0.50	2.00	11.30	2.825
3	6.50	0.50	2.00	9.80	2.450
4	4.50	0.50	3.50	11.20	2.800
5	5.50	0.50	3.50	11.40	2.850
6	6.50	0.50	3.50	10.10	2.525
7	4.50	0.50	5.00	12.10	3.025
8	5.50	0.50	5.00	13.40	3.350
9	6.50	0.50	5.00	8.50	2.125
10	4.50	1.50	2.00	8.80	2.200
11	5.50	1.50	2.00	10.20	2.500
12	6.50	1.50	2.00	9.10	2.275
13	4.50	1.50	3.50	9.90	2.475
14	5.50	1.50	3.50	10.40	2.600
15	6.50	1.50	3.50	10.10	2.525
16	4.50	1.50	5.00	10.50	2.625
17	5.50	1.50	5.00	10.90	2.725
18	6.50	1.50	5.00	9.40	2.350
19	4.50	2.50	2.00	7.20	1.800
20	5.50	2.50	2.00	7.90	1.975
21	6.50	2.50	2.00	6.40	1.600
22	4.50	2.50	3.50	8.30	2.075
23	5.50	2.50	3.50	8.90	2.225
24	6.50	2.50	3.50	6.20	1.550
25	4.50	2.50	5.00	8.90	2.225
26	5.50	2.50	5.00	9.70	2.425
27	6.50	2.50	5.00	7.20	1.800

**Table 1** Experimental runs, pH, period of fermentation, volume of bioethanol obtained and bioethanol percentage yield(%)

Further breaking down the obtained results according to the concentration of the acids used in hydrolysis, we obtained the following:

pН	Fermentation period (days)	Bioethanol volume (ml)	Bioethanol Yield (%)
4.5	2	10.40	2.600
4.5	3.5	11.20	2.800
4.5	5	12.10	3.025
5.5	2	11.30	2.825
5.5	3.5	11.40	2.850
5.5	5	13.40	3.350
6.5	2	9.80	2.450
6.5	3.5	10.10	2.525
6.5	5	8.50	2.125

Table 2 Obtained volume and percentage yield of bioethanol at constant concentration of hydrolyzing acid (0.5M)

Table 3 Obtained volume and percentage yield of bioethanol at constant concentration of hydrolyzing acid (1.5M)

рН	Fermentation period (days)	Bioethanol volume (ml)	Bioethanol Yield (%)
4.5	2	8.80	2.200
4.5	3.5	9.90	2.475
4.5	5	10.50	2.625
5.5	2	10.20	2.500
5.5	3.5	10.40	2.600
5.5	5	10.90	2.725
6.5	2	9.10	2.275
6.5	3.5	10.10	2.525
6.5	5	9.40	2.350

Table 4 Obtained volume and percentage yield of bioethanol at constant concentration of hydrolyzing acid (2.5M)

рН	Fermentation period (days)	Bioethanol volume (ml)	Bioethanol Yield (%)
4.5	2	7.20	1.800
4.5	3.5	8.30	2.075
4.5	5	8.90	2.225
5.5	2	7.90	1.975
5.5	3.5	8.90	2.225
5.5	5	9.70	2.425
6.5	2	6.40	1.600
6.5	3.5	6.20	1.550
6.5	5	7.20	1.800

# 4. Discussion

In this work, the pulp of the Nigerian sweet potato (*Ipomoea Batatas (L.) Lam*) was used to produce bioethanol through fermentation. Since the substrate has a high starch content, acid hydrolysis was used to break down and convert the starch into usable glucose for the fermentation process. Starch is the form in which food is majorly stored in plants. Baker's Yeast (*Saccharomyces cerevisae*), first activated in lukewarm water and grown in a nutrient medium, was used in the fermentation as the biocatalyst. The nutrient medium used for this study is the modified Yeast-Malt-Peptone-Dextrose (YMPD). The modification was due to the use of sweet potato marsh instead of dextrose as a carbon source. The pH of the whole system was corrected using sodium hydroxide (NaOH) to maintain a constant pH of 4.5, 5.5 and 6.5 throughout the duration of fermentation.

The fermenting system for the production of bioethanol was kept stable in the laboratory without disturbance throughout the fermentation period with constant checks on the pH of each fermenting system. As fermentation took place, it was observed that the formation of bioethanol began just after 24 hours and was evidenced by appearance of bubbles in the bowl of water which where the collector pipes from the top of the reagent bottle were sent to. Prior to this period, the microorganisms can be said to be in lag phase where they are adapting to their new environment [18]. The gas evolved upon fermentation is carbon dioxide gas ( $CO_2$ ) as confirmed by the equation of the fermentation reaction. This was also observed by Onwuakor et al [5] who produced bioethanol from rice husk using *Saccharomyces cerevisae*.

The production of bioethanol came with an associated drop in the pH of the fermenting cultures. From 4.5, 5.5 and 6.5, the pH of the various fermenting systems were observed to reach 4.0 and 4.1. To keep the system consistent with the desired pH for analysis, 1.0M NaOH (sodium hydroxide) was used for adjustment with the aid of a pH meter. On attainment of the desired pH, the system was then closed at allowed to ferment.

# 4.1. Assessment of bioethanol quality

The bioethanol obtained in the various fermentation batch experiments and upon distillation had clear, see-through colors just like those produced from petroleum feedstock. The smell was like that of wine while their boiling points ranged between 77 – 84 °C. While it can be said that the presence of impurities may be responsible for this variation, the boiling points are within the range obtained by Ademuiluyi and Mepba [19] thus confirming that the produced solutions were indeed bioethanol. The presence of impurities could be due to the evaporation and collection of substances other than bioethanol during the distillation process. Since the solutions were heated up to 90°C for complete vaporization, it is possible that some substances with higher boiling points might have migrated alongside bioethanol albeit in minute or negligible amounts. This then calls for further purification of produced bioethanol which by integrating another separation stage.

## 4.2. Effects of acid concentration, Ph and fermentation period on bioethanol yield

From the results in table 1, it can be seen that concentration of the acid used in hydrolysis, pH, and fermentation period had an effect on the volume of bioethanol produced. This goes to show they are among the most important parameters in the production of this important biofuel. A change in one or more of them would alter the system and lead to a rise or a fall in the rate of bioethanol production. Thus any industrial-scale process must incorporate these three parameters in a way that would ensure optimum production of the bioethanol.

## 4.2.1. Combined Effects of pH and Fermentation Period on Bioethanol Yield at Constant Acid Concentration of 0.5m

Figure 1. shows the production volume of bioethanol at different pH of the fermenting system and at different fermentation periods for the hydrolysates obtained from the acid concentration of 0.5M. At constant pH, the volume of bioethanol produced is seen increasing as the fermentation period increased from 2 to 5 days. This can be said to result from the log phase since the biocatalyst is now adapted to the new environment and has started reproduction. The increase in bioethanol production with corresponding increase in fermentation period was also observed by Ogunsuyi [20]. However, the rate of bioethanol production initially increased from 4.5 to 5.5 and reduced when the pH reached 6.5.

Hence from figure 1, the maximum yield of bioethanol for sweet potato hydrolysates from the 0.5M acid (13.40 ml representing a yield of 3.350%), was achieved on the 5th day at a pH of 5.5.





#### 4.2.2. Combined Effects of pH and Fermentation Period on Bioethanol Yield at Constant Acid Concentration of 1.5m

The interaction between the pH of fermenting system, fermentation period and volume of bioethanol produced at a constant acid concentration of 1.5M can be seen from figure 2. The rate of production of bioethanol increased progressively as the fermentation period increased from 2 to 5 days with the exception of the fermenting system with pH of 6.5 at the 5th day which dropped slightly. The rate of bioethanol production at each fermentation period saw a consistent pattern throughout the three fermentation periods. Bioethanol production increased from pH values increased from 4.5 to 5.5 but began dropping at pH value of 6.5.

Hence from figure 2, the maximum yield of bioethanol for sweet potato hydrolysates from the 1.5M acid (10.90 ml representing a yield of 2.725%), was also achieved on the 5th day at a pH of 5.5.



Figure 2 Combined effects of pH and fermentation period at constant hydrolyzing acid concentration of 1.5M

## 4.2.3. Combined Effects of pH and Fermentation Period on Bioethanol Yield at Constant Acid Concentration of 2.5m

Figure 3 shows that the volume of bioethanol production increased with a corresponding increase in fermentation period for the fermenting systems with a pH of 4.5 and 5.5. Conversely, the volume of bioethanol production for the fermenting systems with pH of 6.5 decreased from with an increase in fermentation period and increased by the last day of analysis (5th day). The rate of bioethanol production at each fermentation period is consistent with that of the hydrolysates from the 1.5M acid. The production of bioethanol production increased as pH values increases from 4.5 to 5.5 but began dropping at pH value of 6.5.

Hence from figure 3, the maximum yield of bioethanol for sweet potato hydrolysates from the 1.5M acid (9.70 ml representing a yield of 2.425%), was achieved on the 5th day and also at a pH of 5.5.





#### 4.3. Optimum Production Conditions

The general optimum yield of bioethanol corresponds to the highest volume recorded throughout the experiment. The process conditions for this optimum value are a combination of the singular optimum conditions discussed earlier.

Table 4 The optimum production conditions for bioethanol p	production from sweet potato
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Process conditions	Optimal value
Acid concentration	0.5M
рН	5.5
Fermentation period	5 days
Bioethanol volume	13.40 ml
Bioethanol Yield	3.350%

Table 4. Shows the optimum production conditions of bioethanol production from sweet potato. The optimum fermentation period is 5 days at a constant pH of 5.5 and with hydrolysates produced using an acid concentration of 0.5M. This is similar to the findings of Ude and Oluka [6] who got the optimum pH for bioethanol production at 5.7 further validating the findings of Tsunatu et al [21] who discovered that that the closer the pH of the fermenting system is to 7.00, the lower would be the bioethanol yield. Ojewunmi et al [18] also obtained 0.5M as the optimum acid concentration for bioethanol production from sweet potato peels. They discovered that acids with higher concentration were not able to produce fermentable sugars at higher temperatures thus validating this finding.

# 5. Conclusion

The aim of the project was to uncover the optimal process conditions for the production of bioethanol from potato. The bioethanol production was studied using the Nigerian variety of sweet potato (*Ipomoea Batatas* (L.) Lam) containing a high starch content of about 65-80% (w/w). This came to be by varying 3 independent variables or conditions (acid concentration, pH and time). *Saccharomyces cerevisiae* was used as a biocatalyst in the fermentation of the hydrolysates. The optimal values of concentration, pH and time were obtained at 0.5M, 5.5 and 5 days respectively. Maximum ethanol yield was gotten as 13.40ml corresponding to 3.350% v/v of substrate. This work has been able to establish that starch from potato pulp can be a viable source of bioethanol if the optimal process conditions can be used during production.

# **Compliance with ethical standards**

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## Disclosure of conflict of interest

The authors declare that they have no conflict of interests.

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