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Comparative Studies on Bioethanol Production from Some Sugar Based Agricultural Wastes

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ABSTRACT: ***The suitability of some sugar-based agricultural wastes (pineapple peels, banana peels, and plantain peels) were examined for bioethanol production. They were subjected to different physico-chemical pretreatments in order to identify the most effective process and optimize the yield of bioethanol. They were further hydrolyzed by cellulase enzymes from Trichoderma ressei micro-organism isolated from the soil. The various hydrolysates obtained were subsequently fermented to bioethanol using co-cultures of Pichia stipitis and Saccharomyces cerevisiae fermentative yeasts. Separate hydrolysis and co-fermentation (SHCF) and simultaneous saccharification and co-fermentation (SSCF) methods were adopted and their bioethanol yields compared. The fermentation results revealed that the maximum bioethanol yields for pineapple peels, banana peels, and plantain peels were 4.94, 3.85, and 4.57 (% w/v wet biomass) respectively at 72 hours fermentation period. SSCF strategy was observed to be more effective as it gave better bioethanol yields in all the considered substrates and was less time consuming. Mixed cultures of Trichoderma ressi, Pichia stipitis and Saccharomyces cerevisiae through SSCF process resulted to a better fermentation yield when compared with previous studies by other workers.***

Keywords: Bioethanol, sugar-based, Trichoderma ressei, Saccharomyces cerevisiae, Pichia stipitis, Separate hydrolysis and co-fermentation (SHCF), simultaneous saccharification and co-fermentation (SSCF).

1. INTRODUCTION

Energy consumption has steadily increased over the years as many countries have become industrialized and the world's population keeps increasing. A liquid transportation biofuel bioethanol now represents a key contributor to the energy profile of most developed countries. Its demand has increased as a result of diminishing crude oil reserves and

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environmental problems associated with the burning fossil fuels (Riungu *et al*., 2014). The burning of fossil fuel at the current rate is likely to create environmental crisis globally. Its use generates carbon dioxide, carbon monoxide, sulphur dioxide, methane and a significant quantity of nitrous oxide. Most of these harmful gases are formed due to incomplete fossil fuel combustion and since bioethanol contains 35% oxygen, it results in a more complete combustion of fuel and reduced tailpipe emissions (Anuj *et al*., 2007). Apart from alleviating the global decline in crude oil production, biofuels play important roles in fuel decarbonisation and act to mitigate damaging impacts of greenhouse gas emissions. Bioethanol is made from sucrose containing feedstocks, starchy materials and lignocellulosic biomass.

Bioethanol is a fermentation derived alcohol that is obtained from carbohydrate materials in contrast to alcohol made synthetically from petroleum sources (Graeme, 2010). It is conventionally produced from sugar and starch containing feedstocks. However, due to their primary food value, these feedstocks are unable to fulfill the worldwide demand for bioethanol production. This study investigated and improved on the feasibility of producing bioethanol from agro-wastes. Fruit peel agro-wastes with little or no commercial value were evaluated for their suitability and cost effectiveness as feedstocks for bioethanol production.
The aim of this research is to produce bioethanol as an environmentally friendly and cost effective energy source from the reduced sugar hydrolysates obtained from the considered fruit peel wastes.

2. MATERIALS AND METHODS

Sample Preparation for Pretreatment

Ripe pineapple, banana and plantain were purchased, washed with distilled water, air-dried and their peels collected. These fresh peel samples were cut to sizes of about 3-5cm in length prior to further treatments.

Physico-Chemical Pretreatment

Chemical hydrolysis and mechanical comminution were carried out simultaneously. Fifteen (15) grams of each substrate was mechanically comminuted in a high speed blender with 100ml of each of the following listed reagents for 5 minutes: (1) H_2O, (2) 1% NaOH, (3) 1% KOH, (4) 1% $Ca(OH)_2$,(5) 1% H_2SO_4 ,(6) 1% HCl and (7) 1% HNO_3. Thereafter, the hydrolysate sugar content of the broth was analyzed using a high precision brix refractometer. In the second step hydrolysis, the broth was incubated for 10 minutes at 120°C after the first step mechanical comminution. The pretreated broth that yielded the highest soluble hydrolysate sugar content was used for enzymatic hydrolysis. The bioethanol yield was optimized by increasing the quantities of substrate and solution to 75g substrate/500ml solution (ratio 3:20).

Measurement of Sugar Level

A high precision refractometer (Grand-index with automatic temperature compensation) was used to analyze the substrate's hydrolysate sugar levels in degree brix (Maroulis *et al*., 2003).

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In a typical measurement, a drop of the sample was put on the glass surface of the refractometer and the sugar level subsequently determined. The results in degree brix were subsequently converted to weight of sugar using:
Weight of hydrolysate sugar (g/L) = °Brix x Specific gravity x 10 (Robert, 2003). (1)

pH Adjustment and sterilization
The pH of the pretreated agro-waste was adjusted to 5.0 in a bowl by adding the required amount of 2.5 M H_2SO_4. Subsequently, samples were sterilized in an autoclave at a temperature of 121°C for 20 minutes and cooled to appropriate temperature before the introduction of microorganisms.

Separate hydrolysis and co-fermentation of hydrolysates
Each of the substrates were inoculated with 25ml *Trichoderma ressi* innoculum and were incubated in a shaking incubator at a rate of agitation of 150 rpm at 45°C for 48 hours. They were then filtered and the soluble hydrolysate sugar yields in the filtrates were measured using a refractometer. Their specific gravities were also measured at this time using a hydrometer. The filtrates were autoclave sterilized and were then subjected to co-fermentation by inoculation with 25ml of *S. cerevisiae* and 25ml of *P. stipitis* inoculums under aseptic condition. They were incubated on a shaker with 150 rpm agitation rate at 30°C for 72hours. The fermented broth samples were filtered and the total sugar and bioethanol contents were determined.
Bioethanol yield was calculated based on alcohol distillate density at 20°C using equations 2 and 3 respectively (Park, 2000; Hadeel *et al*., 2011; Igwe *et al.*, 2012).

$$\text{Ethanol \% (w/v)} = \frac{126.582\ (OSG-FSG)}{OSG} \quad (2)$$

Where OSG is original specific gravity (specific gravity before fermentation), FSG is Final specific gravity (specific gravity after fermentation) and 126.582 is from the Specific gravity of water / Specific gravity of pure ethanol.

$$\text{Specific gravity of sample} = \frac{(X_2 - X_1)}{(X_3 - X_1)} \quad (3)$$

Where: X_1 is weight (g) of empty pycnometer, X_2 is weight (g) of pycnometer + sample and X_3 is weight (g) of pycnometer + water.

After specific gravity values were calculated, the percentage alcohol of each sample was determined using a standard alcohol density table (International Union of Pure & Applied Chemistry, 1985).

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Simultaneous Saccharification and co-fermentation of hydrolysates and fermentation time optimization experiment.
The pretreated substrates were simultaneously inoculated each with 25ml of *Trichoderma ressi*, 25ml of *S. cerevisiae* and 25ml of *P. stipitis* inoculums under aseptic condition. They were incubated on a shaker with 150 rpm agitation rate at 30°C for 72hours.

Ethanol Recovery
Bioethanol was recovered in a rotary evaporator at a temperature of 85^{o}C for 3hrs. Distillates were dried overnight using 3A molecular sieves to absorb water molecules. They were decanted, filtered and redistilled to remove sieve dust and achieve absolute bioethanol.

3. RESULTS AND DISCUSSION

Table 1: Total Hydrolysate Sugars (g/L) Achieved from Substrates after Physico-chemical Pretreatment and Enzymatic Hydrolysis

Substrates	Weight of sugar (g/L) After Physico Chemical Pretreatment	Chemical Reagent Involved	Maximum weight of sugar(g/L) After Enzymatic Hydrolysis	Maximum Hydrometer(OSG)
Pineapple peels	48.9	1% NaOH	84.1 ± 0.082*	1.035 ± 0.0016*
Bananapeels	42.7	1% NaOH	79.4 ± 0.141*	1.031 ± 0.0014*
Plantain peels	61.4	1% NaOH	94.3 ± 0.108*	1.036 ± 0.0022*

*= Standard deviation, OSG = Original Specific Gravity

Data from Table 1 show that enzymatic hydrolysis equally made significant contribution to the hydrolysate sugar weight of substrates though the physico-chemical pretreatment step was more contributory to the total hydrolysate soluble sugar realized. This might be as a result of the inhibitory effects of the sugars released during physico-chemical pretreatment on cellulose activities.

Table 2: Bioethanol yield at 20^{o}C from Hydrometer and Pycnometer Measurements

Substrate	Hydrometer Readings		Pycnometer Readings		Bioethanol yield (%w/v)			
	FSG SHCF	FSG SSCF	SG SHCF	SG SSCF	SHCF Hydro meter	SHCF Pycno meter	SSCF Hydro meter	SSCF Pycno meter

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Pineapple peels	1.010±0.0008*	0.997±0.0014*	0.9950±0.0003*	0.9929±0.0001*	3.06	3.43	4.65	4.94
Banana peels	1.015±0.0016*	1.002±0.0022*	0.9967±0.0004*	0.9944±0.0003*	1.96	2.24	3.56	3.85
Plantain peels	1.015±0.0016*	1.001±0.0008*	0.9958±0.0002*	0.9934±0.0002*	2.57	2.86	4.28	4.57

*=Standard deviation

The bioethanol yields as obtained from the hydrometer and Pycnometer readings are presented in Table 2. Higher bioethanol yields were obtained from the substrates with SSCF process when compared with yields from SHCF process. One of the possible reasons for this is that there was more synergistic action when the three involved fermentative micro-organisms were inoculated at the same time in SSCF process. Another reason might be as a result of the degradation of monomeric sugars obtained during enzymatic hydrolysis in SHCF before yeasts were inoculated to act on them (Graeme, 2010).

Among the tested sugar based substrate peels, ripe pineapple peels gave the best bioethanol yield followed by ripe plantain peels while ripe banana peels gave the least yield.

4. CONCLUSION

This study shows that these sugar based agro-wastes of no commercial value can be utilized in the production of good quality bioethanol with implications for improved waste management, income and efficient energy generation.

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