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Comparative Analysis of the Microbial Production of Ethanol Using Agricultural Waste

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Abstract: *This study investigates the potentials of yam peels, potato peels, cassava peels and plantain peels in microbial production of ethanol. The wastes were subjected to microbiological and chemical analysis. The microbiological analysis was done using pour plate isolation method according to American Society for Testing and Materials (ASTM) Standards. The isolated microorganisms were identified based on their cultural, morphological and biochemical characteristics. Ethanol production was assayed using appropriate chemical method. Our experimental result showed that the total viable bacterial counts was highest in cassava with a count of* $2.2\times10^4\,cfu/g$ *while lowest bacterial counts was observed in potato, with a count of* $0.9\times10^4\,cfu/g$. *Total viable fungal counts were highest in cassava with a count of* $0.9\times10^4\,cfu/g$ *and lowest in potato, with a count of* $0.4\times10^4\,cfu/g$. *Bacterial isolates identified includes bacillus sp., Corynebacterium sp., Pseudomonas sp., Micrococcus sp. and Lactobacillus sp. Fungal isolated includes Penicillium sp., Aspergillus niger., Sachaccharomyces sp. The highest occurring bacterial isolate were bacillus sp. and Corynebacterium sp. with percentage occurrence of 50% each. Lactobacillus sp. and Pseudomonas sp. were the least occurring bacterial isolates with 25% distribution. The highest occurring fungal isolate was Aspergillus niger (75%) while the least was Penicillium sp (25%). The amount of reducing sugar recovered from the samples ranged from 11.29-46.00 mg/ml. In ethanol yield, potato substrate had more amount of ethanol (2.50 ml) while plantain substrate has the least amount of ethanol (0.98 ml). Alcohol number ranged from 1.0 – 3.0, with potato being the highest while plantain the lowest. This study revealed that important industrial materials can be produce using agricultural wastes by microorganisms.*

Keywords: *Microorganisms, Agricultural Wastes, Ethanol, Bacterial Isolates, Fungal Isolates*

INTRODUCTION

The escalating demand for renewable energy sources has fostered interest in biofuel production. Traditional ethanol production heavily relies on food crops like corn and sugarcane, but there is growing enthusiasm for using agricultural waste as a feedstock. Agricultural waste, encompassing crop residues, stalks, husks, and straw, is widely available globally. Experts emphasize the significance of utilizing these waste materials, as it not only reduces environmental pollution but also offers a cost-effective feedstock for ethanol production (Sadh *et al.,* 2018; He *et al.,* 2019). Several studies have been conducted to explore the potential of various microbial strains in fermenting agricultural waste and converting it into ethanol (Mitchel *et al.,* 2020; Patel *et al.,* 2021; Johnson *et al.,* 2023). Experts have also identified bacterial and yeast species as viable microorganisms for ethanol production from agricultural waste (Cherubini, 2010; Munasinghe and Khanal, 2010; Pan *et al.,* 2021). Among bacterial strains, Zymomonas mobilis and Escherichia coli have gained recognition due to their efficient utilization of sugars derived from agricultural waste, leading to high ethanol productivity (Smith *et al.,* 2022). Yeast species such as Saccharomyces cerevisiae and Kluyveromyces marxianus have also been extensively studied for their ethanol production capabilities (Zhang *et al.,* 2019). Agricultural waste contains complex polymers like cellulose and hemicellulose, which require pre-treatment to convert them into fermentable sugars. Experts concur that employing pre-treatment methods is crucial to enhance the accessibility of these polysaccharides (Mosier *et al.,* 2005; Goh *et al.,* 2010; Mussatto *et al.,* 2010; Arora *et al.,* 2019). Noteworthy pre-treatment techniques, including physical, chemical, and biological methods, have been utilized to break down complex structures. Steam explosion, acid hydrolysis, and enzymatic hydrolysis are commonly employed pre-treatment techniques (Lee *et al.,* 2019). Fermentation, a pivotal step in ethanol production, involves the conversion of fermentable sugars derived from agricultural waste into ethanol by selected microorganisms. Experts stress the importance of optimizing fermentation conditions, such as pH, temperature, and oxygen availability, to achieve higher ethanol yields (Kazi *et al.,* 2010). Furthermore, immobilized cell systems and co-cultures have been explored to enhance ethanol production efficiency (Okorondu *et al.,* 2009; Braide and Nwaoguikpe, 2011; Wang *et al.,* 2021). The industrial processes for bioethanol production using mainly grain, tuber and root starches were carried out by (Tasic *et al.,* 2009), cassava bagasse was carried out by (Amenaghawon *et al.,* 2013), pineapple by (Duhan *et al.,* 2013), sugarcane molasses by (Dias *et al.,* 2013 and Behera *et al.,* 2012) as well as sweet potato by (Oyeleke *et al.,* 2012). Among various starchy materials available throughout the world, it is instructive to note that corn, wheat, sweet sorghum, sweet potato and cassava have been successfully utilized for the commercial production of bioethanol (Oyeleke *et al.,* 2012). Braide *et al.,* (2018) investigated the capability of using local strains of Zymomonas mobilis and Saccharomyces cerevisiae to produce bioethanol from cassava, yam and potato peels. The three substrates were subjected to a pretreatment process using acid and enzyme hydrolysis to remove lignin. The ethanolic fermentation was prepared using Z. mobilis and S. cerevisiae. It has also been shown that root crops have greater potential than corn grains as ethanol sources, if economical harvesting and processing techniques are fully developed (Thatoi *et al.,* 2014). Starch is a complex carbohydrate which needs conversion into simpler sugars before being converted into ethanol. While the microbial production of ethanol from agricultural waste holds promises, experts acknowledge several challenges that need to be addressed. Efficient breakdown of recalcitrant lignocellulosic materials, development of robust microorganisms, and optimization of fermentation processes are ongoing research areas (Sarkar *et al.,* 2012; Adegboye *et al.,* 2021). Additionally, economic viability and scalability pose critical considerations for successful industrial implementation (Chen *et al.,* 2020). The selection of microorganisms for industrial bioethanol production depends upon their ability to utilize a wide range of substrates, being resistance against various inhibitory products, and tolerance to high sugar and ethanol concentrations (Hans *et al.,* 2019). Experts agreed that microbial production of ethanol from agricultural waste offers a sustainable and eco-friendly approach to biofuel generation (Kazi *et al.,* 2010; Liu and Hu, 2010). The indication is that, utilizing abundant agricultural waste reduces waste accumulation (Watanabe *et al.,* 2010). Advances in understanding microbial fermentation, pre-treatment techniques, and optimization strategies provide a solid foundation for future progress. Continued research and development efforts are essential to overcome existing challenges and fully unlock the potential of microbial ethanol production from agricultural waste which this study seeks to address. Thus, the aim of this present study is to investigate the potentials of microbial production of ethanol using agricultural waste such as yam peels, potato peels, cassava peels and plantain peels.

MATERIALS AND METHODS

2.1 Materials

The yam peels, potato peels, cassava peels and plantain peels used as raw materials for this study were obtained from a residence in Uselu, New Lagos Road area of Benin City, Edo State, Nigeria. The raw materials were washed, peeled and sun dried to remove dirt from it. The chemicals and reagents used are: Concentrated sulfuric acid, distilled water, sodium hydroxide, diethyl ether, freshly isolated and industrially made yeasts. The chemicals and reagents were of analytical grade and were procured from scientific shops. The apparatus and equipment used include: measuring cylinders, refractometer, beakers, funnels, whatzman filter paper (could be ashless or any other), retort stands, volumetric flasks (or other collector bottles), distillation sets, 250ml conical flasks, round bottomed flasks, heating source (heating mantles, plates or other sources), water bath, thermometers, weighing balance, specific gravity (SG) bottle.

2.2 Methods

A. Microbiological Analysis

One gram (1g) of each sample (yam peels, potato peels, cassava peels and plantain peels) was weighed and aseptically introduced into 9ml of sterile distilled water and properly shaken before a 10 - fold serial dilution, up to 10^{-3}, was performed. This was carried out to obtain fungal isolates using the pour plate method described by Akerele (1990). The media used for the analysis were nutrient agar and potato dextrose agar. For the nutrient agar, the medium was prepared from commercially available dehydrated powder. In the preparation, 28g of nutrient powder was dissolved in 1 litre of distilled water in a conical flask covered with cotton wool and aluminum foil paper. This was stirred and autoclaved at 121^0C for 15 minutes and then cooled to 50^0C, before pouring into petri dishes. For the potato dextrose agar, the medium was used for isolation of fungi from samples and for the preparation of pure cultures. In the preparation, 39g of potato dextrose agar powder was dissolved in the same conditions as above. Streptomycin (0.1 w/v%) was added to the medium to prevent the growth of bacteria. This analysis was carried out according to *ASTM E1757 Standard* practice for *preparation* of biomass for compositional *analysis and* ASTM E1690-08(2021) Standard Test Method for Determination of Ethanol Extractives in Biomass.

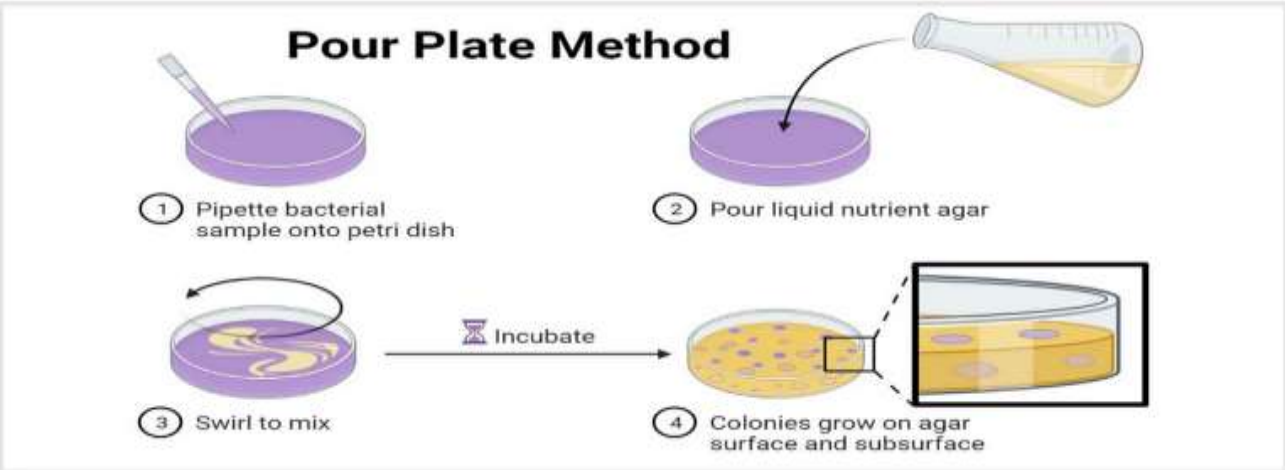

Fig. 1. Pour Plate Method

B. Determination of Total Viable Count

Pour plate method was used for microbial enumeration. Aliquot of 1ml from 10^{-2} dilution was pipetted into sterile petri dish and labeled as such. 20ml of prepared agar was dispensed into the various petri plates. The nutrient agar plates were incubated at 37^{0C} for 24 hours while the potato dextrose agar was kept at room temperature for 48 to 72 hours. The colonies were counted to obtain total viable count.

C. Identification of Bacterial Isolates

The bacterial isolates were characterized and identified based on their cultural characteristics and biochemical reaction which includes gram reaction, motility test, oxidase test, catalase test, coagulase test, indole test, urease test, citrate test and carbohydrate fermentation test.

D. Identification of Fungi Isolates

The fungi isolates were examined macroscopically using their cultural characteristics and microscopically using lactophenol blue.

RESULTS AND DISCUSSION

Our experimental result showed that the total viable bacterial counts was highest in cassava with a count of $2.2\times10^4\,cfu/g$ while the lowest bacterial counts was observed in potato, with a count of $0.9\times10^4\,cfu/g$. Total viable fungal counts were highest in cassava with a count of $0.9\times10^4\,cfu/g$ and lowest in potato, with a count of $0.4\times10^4\,cfu/g$ as presented in Table-1.

Table-1 Total viable bacterial and fungal counts in samples

Samples	Bacterial counts $\left(\times10^4\,cfu/g\right)$	Fungal counts $\left(\times10^4\,cfu/g\right)$
Plantain	1.1	0.5
Cassava	2.2	0.9
Potato	0.9	0.4
Yam	1.3	0.6

Table-2 summarizes the cultural, morphological and biochemical characteristics of bacterial isolates. The bacterial isolates identified includes *Bacillus sp., Corynebacterium sp., Pseudomonas sp., Micrococcus sp.* and *Lactobacillus sp. while* fungal isolates include *Penicillium sp., Aspergillus niger.,* and *Sachaccharomyces sp.*

Table-2 Cultural, morphological and biochemical characterization of bacterial isolates

Characteristics	B_1	B_2	B_3	B_4	B_5
Elevation	Low convex	convex	Low convex	convex	Low convex
Margin	Entire	Entire	Entire	Entire	Entire
Colour	Cream	Cream	Light green	Yellow	Cream
Shape	Circular	Circular	Circular	Circular	Circular
Mortility	+	+	+	+	+
Gram staining	+	+	-	+	+
Cell type	Rod	Short Rod	Rod	Cocci	Rod
Cell arrangement	Chain	Cluster	Single	Single	Chain
Spore stain	+	-	-	-	+
Catalase	-	+	-	+	+
Urease	-	-	+	+	-
Oxidase	+	-	+	-	+
Coagulase	-	-	-	-	-
Indole	-	-	-	-	-
Citrate	-	+	+	+	+
H_2S	-	-	-	-	-
Glucose	+	+	+	+	+
Lactose	-	+	-	-	+
Isolates	*Bacillus sp*	*Corynebacterium sp*	*Pseudomonas sp*	*Micrococcus sp*	*Lactobacillus sp*

Table 3 depict the cultural and morphological characteristics of fungal isolates.

Table-3 Cultural, morphological characterization of fugal isolates

Cultural	Green flat colony with white periphery	Black fluffy colony with reverse side yellow	Medium creamy with convex elevation and entire margin
Morphological			
Nature of hyphae	Septate	Non- Septate	Pseudohyphae
Colour of spore	Green	Brownish	Colourless
Type of spore	Conidiophore	Conidiophore	Chlamydosphore
Appearance of special structure	Brush-like conidia	Foot cells	Budding
Fungal isolates	*Penicillium sp*	*Aspergillus niger.,*	*Sachaccharomyces sp*

Occurrence of the microbial isolates is presented in Table 4. This regime shows that the highest occurring bacterial isolates were *Bacillus sp* and *Corynebacterium sp* (50% each) while *Lactobacillus sp* and *Pseudomonas sp* (25%). The highest occurring fungal isolate was *Aspergillus niger* (75%) while the least was *Penicillium sp* (25%).

Table-4 Distribution of microbial isolates

Microorganisms (Number of occurrence)	Plantain	Cassava	Potato	Yam
Bacteria				
Bacillus sp	-	-	+	+
Corynebacterium sp	-	-	+	+
Lactobacillus sp	-	+	-	-
Pseudomonas sp	-	+	-	-
Fungi				
Aspergillus niger.	-	+	+	+
Penicillium sp	-	-	-	+
Sachaccharomyces sp	-	+	+	-

Key: + means presence of organism - means absence of organism

Table-5 shows the ethanol production of the various agricultural wastes examined as well as the other chemical parameters.

Table-5 Chemical parameters in samples

Samples	Parameters		
	Reducing sugar (mg/ml)	Ethanol yield (ml)	Alcohol number determination (ml)
Potato	46.00	2.50	3.00
Cassava	35.16	1.85	2.5
Yam	16.16	1.10	1.2
Plantain	11.29	0.98	1.0

It was observed from the above result in Table-5 that potato substrate had more amount of ethanol (2.50ml) followed by Cassava (1.85ml) and Yam (1.10ml) while Plantain substrate has the least amount of ethanol (0.98). It is worthy of note from our analysis that the proximate composition of the agronomic waste used in this study encouraged bacterial proliferation more than fungi. The agronomic waste represents potential nutrient source for the proliferation of bacterial species. Bacterial contamination of these wastes may have originated from air flora, utensils for cutting or due to the poor sanitary level in the environment. The fungi isolates recovered in this work has been reported to be active in fermentation of agronomic wastes to useful industrial products. *Aspergillus niger* has been used in recent years to ferment agronomic waste such as cassava, pineapple and sugar cane to citric acid which has wide applications in the pharmaceutical and other industries. The most frequently fungi were *Aspergillus niger* which occurred in cassava, potato and yam peels, this was followed by *Sachaccharomyces sp* which was found in cassava and potato peels and *Penicillium sp* which was only found in yam peels. The amount of reducing sugar recovered from the samples ranged from 11.29 – 46.00 mg/ml, with potato being the highest substrate that yielded reducing sugar while plantain was the lowest substrate. In the ethanol yield, potato substrate had more amount of ethanol (2.50ml) followed by Cassava (1.85ml) and Yam (1.10ml) while Plantain substrate has the least amount of ethanol (0.98). Alcohol number ranged from 1.0 – 3.00, with potato being the highest while plantain was the lowest. These results share similarity to that obtained by Wang et al., (2011) and Braide et. al., (2018). It is also in correlation with the findings of Akponah and Akpomie (2011) which gave ethanol yield after fermentation of yam peels hydrolysed using amylolytic fungi, enzyme and acid as 1.68, 0.56 and 2.7 (%v/w) respectively. Ethanol yield from potato peels were given as 4.02%v/w (amylolytic fungi hydrolysate), 1.94% v/w (enzyme hydrolysate) and 9.38% v/w (acid hydrolysate). Fermentation of the respective cassava peel hydrolysates resulted in 10.5%v/w, 4.07% v/w and 17.52% v/w ethanol. In terms of substrate yield, Akponah and Akpomie (2011) showed that highest ethanol production was observed from cassava root peels, followed by potato peels while yam peels yielded the least ethanol concentration compared to our result where potato substrate had more amount of ethanol (2.50ml) followed by Cassava (1.85ml) and Yam

(1.10ml) while Plantain substrate has the least amount of ethanol (0.98). The implication of this is that both potato peels and cassava peels have the potential to produce highest ethanol yield.

CONTRIBUTION TO KNOWLEDGE

The potentials of agricultural wastes such as yam peels, potato peels, cassava peels and plantain peels in microbial production of ethanol has successfully been investigated. This study showed that ethanol can be produced from agricultural wastes like yam peels, potato peels, cassava peels and plantain peels.

CONCLUSION

This study has shown that ethanol production from agricultural waste material is a good alternative. Cleaner and greener production of the compounds like ethanol is important for sustainable growth. These environmental friendly processes can be made more economical by optimizing the process parameters and finding more effective techniques for conversion of these agricultural wastes and other low-cost raw materials into usable product.

Furthermore, it was found from this study that agronomic waste such as cassava, potato, plantain and yam peels are highly contaminated by microorganisms and that these organisms can use the waste as potential substrate for production of industrially important products such as ethanol, by fermentation process. It is therefore recommended that agricultural waste should be harnesses in the production of useful substances by selecting appropriate bacterial and fungal species as this eco-friendly process will not only help to remove wastes from the environment, but also convert this waste to industrially important products.

CONFLICT OF INTEREST

There is no conflict of interest for this research work.

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