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Original Research Article

Bioethanol Production from Pineapple and Cassava Peels Using Fungal Isolates as Inoculants

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ABSTRACT

Globally, the production of biofuels as an alternative energy source from renewable raw materials to complement energy needs has become of utmost importance. The goal of this study was to compare bioethanol production from pineapple and white cassava peels utilizing inoculants such as *Neurospora crassa, Aspergillus oryzae* and *Saccharomyces cerevisiae*. The cassava and pineapple peels were obtained from the white cassava and abacaxi pineapple, respectively. The fungal isolates: *Neurospora crassa, Aspergillus* oryzae, and *Saccharomyces cerevisiae* were isolated from burnt wood, steamed rice, and fresh palm wine (from oil palm) at 37ºC. Sabouraud Dextrose Agar (SDA) at pH 5.6 was used to grow the fungal isolates, and morphological observations were used to confirm the isolates. The substrates were fermented with different inoculant combinations, distilled on days 4 and 8, and the physicochemical parameters were determined. The results showed that *Aspergillus oryzae* and *Saccharomyces cerevisiae* in combination gave the highest bioethanol yield of 48.67±5.7 ml with the pineapple peels at day 8; whereas *Aspergillus oryzae and Neurospora crassa* in combination gave the highest bioethanol yield of 38.33±2.03 ml with the cassava peels at day 4. This observation was statistically significant (p<0.05). The findings led to the conclusion that pineapple peels have a higher bioethanol yield than cassava peels. The inoculants utilized in this research work indicate the best prospects for bioethanol production from abacaxi pineapple and white cassava.

***Keywords*:** Bioethanol, fermentation, pineapple peels, cassava peels, inoculants, fungi.

Introduction

Energy plays an important role in our daily lives and is a critical component of any country's socioeconomic development. In a variety of methods, the plentiful energy around us can be stored, converted, and increased for our benefit. Energy production has been a source of concern for both scholars and governments.[1] These sources of energy (solar, wind, biofuel, water, biomass and geothermal) can be categorized into three groups: fossils, renewable, and nuclear (fissile). Currently, fuels and chemicals are majorly obtained from unsustainable mineral resources, petroleum, natural gas, which leads to environmental pollution, greenhouse gas emissions, and problems with energy security. The renewable energy sources include biomass, hydro, and solar (both thermal and photovoltaic), geothermal, and marine energy sources.[1] Globally, there is a rising awareness of renewable energy generation, owing to the high price of fossil oil and the looming energy crisis.[2] A variety of factors are responsible for this dwindle in fuel sources and these include excessive consumption of fossil fuels, population boom and industrial advancement in many countries.[3] The consequences of this ever-increasing demand for fuel have led to the generation of high levels of pollution and greenhouse gases which have been on a rapid increase in the atmosphere for a few decades now.[3] It is thus of utmost economic and ecological importance to optimize the production of biofuels as an alternative energy source from renewable raw materials to complement energy needs.[4] Bioethanol is a good substitute to motor fuel.[5] This is because, bioethanol can be blended with gasoline and its usage causes low emission of greenhouse gases.[6] Hence, it is considered the most reliable biofuel for the future.[7]

Biofuel is expected to be one of the dominating renewable energy sources in the transport sector in the nearest future.[8,9] Notably, bioethanol is a type of ethanol produced from biomass and agricultural wastes through enzyme hydrolysis and fermentation process.[10,11,12] However, bioethanol yield depends majorly on the type of microorganism(s) utilized; this is of utmost importance for fermentation processes and industries.[13,14] *Saccharomyces cerevisiae* is a specie of yeasts (unicellular fungus) that has been used in fermentation since ancient times.[15] Palm wine contains about 13% of sucrose just after being tapped, and yeast spores especially *S. cerevisiae* which is a major yeast found in palm wine from reports, infects the juice and ferments the sugars.[16] *Neurospora crassa* is a type of fungus that has been used in genetic, cellular and biochemical research.[17] It is a saprotroph and is found on burned vegetation or trees after forest fires.[18] *N. crassa* has been employed in the fermentation of glucose to ethanol in the presence of oxygen.[19] *Aspergillus oryzae* is a filamentous fungus used to ferment soy beans, saccharify rice and other grains in making beverages and spirits particularly in East Asia.[20] It can contaminate carbon-rich and starchy foods such as bread, beans, rice as well as various trees and plants resulting in food spoilage. *A. oryzae* is one of the most potent fungi which having attributes of a generally regarded safe micro-organism (GRAS) as confirmed by the FDA.[21] The fungus is able to secrete good amounts of several degrading enzymes giving it the ability to degrade proteins and various starches into amino acids and sugars respectively. These attributes make *A. oryzae* essential in fermentation.[22] Also, there are speculations that smaller petroleum reserves might be depleted in no distant time from now. Thus, there is enormous energy security concern.[23] In view of this, there is a shift of

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focus towards other renewable sources for energy production. A suitable candidate for a renewable source of energy is bioethanol which is the subject of this study. This study was designed to carry out a comparative study of bioethanol production from pineapple and cassava peels using *Neurospora crassa*, *Aspergillus oryzae*, and *Saccharomyces cerevisiae*, singly and in combinations, as inoculants.

Materials and Methods

Sample collection

The peels of *Manihot esculenta* and *Ananas comosus* were obtained from the whole white cassava and abacaxi pineapple respectively, all purchased at Marian market, in Calabar Municipality (latitude 4º57'6.12''N, longitude 8º19'19.19''E and altitude 11 meters), Cross River State in February, 2019. The voucher specimens has been deposited at the School of Preliminary Studies, Federal College of Dental Technology and Therapy, Trans-Ekulu, Enugu State., under the numbers SPS/22/004 and SPS/22/005. The *M. esculenta* and *A. comosus* peels were washed with clean water, sorted to remove debris, and allowed to drain. The peels were dried at 37ºC for one month to remove moisture. The dried peels were homogenized using a manual blender. The dried samples were stored in sealed plastic bags at 37ºC before use.

Source of fungal isolates

S. cerevisiae strains were isolated from fresh palm wine,[24] *Aspergillus oryzae* strains were isolated from steamed rice,[25] and *Neurospora crassa* strains were isolated from burnt tree from a burnt bush.[26] *Aspergillus oryzae*, *Saccaharomyces cerevisiae* and *Neurospora crassa* were grown in complete Sabourad dextrose agar (SDA) growth media which contained (sucrose – 2%, Casein hydrolysate – 1 g, yeast extract – 2.5 g, $MgSO_4.7H_2O$ – 5 g, NaCl – 103.2 g).

Identification of isolates (inoculum)

A block of Sabouraud Dextrose Agar (SDA) (approximately 0.00065 square meter) was cut from the agar plate using a sterile scalpel blade and placed on a glass slide on another Petri dish containing moist blotting paper. A small portion of the colony was picked up from an inoculated plate using a spud and inoculated on the four sides of the block a little below the surface. A cover slit was carefully placed over the agar block and gently pressed for adhesion. The lid was placed on the plate carrying the inoculated block and it was incubated at room temperature for 48 h. After 48 h, the mycelia growth and spore produced were observed. This was done by removing the slit on the block and placing it on a clean slide carrying a few drops of lactophenol cotton blue. The slide was examined under the microscope with a low power of 20x objective to locate the isolate and 40x objective was used to confirm the presence of fungal structures for identification. The fungal structures were stained deep blue against a clear pale blue background. This was done for the three fungal strains used in this research.

Morphological observations

After three days of incubation on Sabourad dextrose agar (SDA) incorporated with chloramphenicol; macroscopically, isolates were observed for the following features: if suspected colonies of *A. oryzae* had properly grown fungi mycelium with characteristic white and fluffy strands becoming black later similar to salt and pepper appearance covering the outside of the steamed rice; for *S. cerevisiae*, colonies had uniquely earthy smells. Other characteristics were colour ranging from cream white to colourless, shape was oval, round shapes occurring singly and colonies of fungal strains possessed morphological features of *S. cerevisiae*; and the colonies suspected to be *N. crassa* fungi had the orange colour of the vegetative spores, characterized by a hyphal growth which elongates at the tip. It also had repeated apical budding, which formed chains of proconidia having a resemblance of beads on a string. The colonies of fungi inoculated on solid media had morphological features of *N. crassa.*

Fermentation of substrates

The inoculants, 10 ml of each were added to all the substrate samples respectively. The single strains were inoculated with 10 ml of each infusion, two strains had 5 ml of each infusion and three strains had 3.5 ml of each infusion. The inoculated substrates in the fermenting vessels were kept tightly capped at room temperature for 4 and 8 days. The samples were in triplicates, making a total of 88 fermenting vessels for fermenting days 4 and 8. At the end of days 4 and 8 incubation periods, samples in each group were ready for distillation. The fermented brew was distilled using a simple distillation process. The brew was heated in a flask to a temperature of 80^{o}C. The pure liquid; the distillate (bioethanol) was collected in a flask. The distillation process was repeated until the entire fermentation brew was exhausted. The distillates (bioethanol) were stored in sample bottles, well-labeled until ready for analysis.

Determination of some physicochemical properties of the bioethanol

The volume of the bioethanol collected was determined using a measuring cylinder and expressed as a quantity of bioethanol produced in millimeters (ml).

The concentration of ethanol was determined using a UV 5800PC spectrophotometer, at a wavelength of 340 nm. The ethanol concentration (%v/v) was calculated by extrapolation of the standard ethanol absorbance versus concentration curve. The standard curve was determined by adopting the methods of Oyeleke and Jubril.[27]

The viscosities of the bioethanol produced was determined using a digital viscometer expressed in m Pa.S at 20ºC.

For density, an empty 50 ml of pycnometer made of borosilicate was weighed. The pycnometer was filled with the sample (bioethanol). Any excess was wiped off the sides and the weights of each bioethanol were recorded. The density was calculated using the formula:

$$\text{mass (g)} = \text{mass of pycnometer} + \text{sample} - \text{mass of empty pycnometer}$$

$$density\ \left(\frac{g}{ml}\right) = mass/volume$$

For specific gravity, distilled water was filled into the pycnometer and the weight was recorded. The specific gravity was calculated using the formula:

$$\text{specific gravity} = \text{density of ethanol}/\text{density of water}$$

Statistical analysis

The data generated were subjected to the analysis of variance (ANOVA) using SPSS version 22.0. Differences between means were subjected to post-hoc analysis at $p<0.05$.

Results and Discussion

Using suitable fermentation-causing organisms, it is possible to obtain an ethanol yield of up to 90 – 97% of the theoretical value in a fermentation medium.[28] The fermentation-causing organism of interest were carefully chosen concerning their ability to withstand stress during the fermentation process. The fermentative efficiency of *A. oryzae*, *N. crassa*, and *S. cerevisiae*, singly and in combinations concerning ethanol production was accessed in this study. The use of fruit peels in bioethanol production from an economic point of view is highly recommendable as they are readily available both commercially and domestically. Pineapple and cassava peels have high contents of carbohydrates and are suitable to be utilized as substrates to drive fermentation processes including the production of bioethanol since this process requires sugary starch, and fibrous materials.[29] The bioethanol produced using pineapple substrates in fermentation day 4 produced its highest volume having a mean value of 38.33 ± 2.03 ml in samples inoculated with A. oryzae + *N. crassa* strains and the lowest yield mean value was 24.67± 2.85 ml in samples inoculated with *S. cerevisiae* strain. The fermentation day 8 produced a significantly high volume having a mean value of 48.67 ± 5.7 ml in samples inoculated with *A. oryzae* + *S. cerevisiae* strains, and lowest volume mean value was 34.33 ± 1.76 ml inoculated with *A. oryzae* + *S. cerevisiae* + *N. crassa* strains. The control produced low volumes of bioethanol having mean

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values of 21.00 ± 0.00 ml and 21.00 ±0.00 ml for fermentation days 4 and 8 respectively as shown in figure 1. The bioethanol produced using cassava substrates in fermentation day 4 produced its highest volume having a mean value of 32.00 ± 2.08 ml in samples inoculated with *N. crassa* strain and the lowest yield mean value was 24.33 ± 2.85 ml in samples inoculated with *S. cerevisiae* + *N. crassa* strains. Similarly, fermentation day 8 produced a high volume having a mean value of 27.00 ± 2.65 ml in samples inoculated with *N. crassa* strain and its lowest volume and the mean value was 18.00 ± 1.53 ml in samples inoculated with *S. cerevisiae* strain. The control produced low volumes of bioethanol having mean values of 21.00 ± 0.00 ml and 21.00 ± 0.00 ml for fermentation days 4 and 8 respectively as shown in figure 2. In comparison, the bioethanol produced in fermentation day 4 using pineapple substrates in samples inoculated with *A. oryzae* + *N. crassa* strains, had a higher volume (38.33 ± 2.03 ml) than the cassava substrates (32.00 ± 2.08 ml). From the results, the volume of ethanol produced by the different substrates differed significantly (p<0.05) and there was also an observed significant (p<0.05) difference for fermentation-causing organisms. It was observed that the bioethanol yield from the pineapple peels was better than the cassava peels, although the volume varied from one fermentation-causing organism to the other (whether singly or in combination). From the pineapple peels, bioethanol yield seemed to increase with an increase with fermentation time, but the reverse was observed for the cassava peels. Mohammed et al.,[30], report that pineapple peels have one of the greatest potentials for high bioethanol yield and attribute this potential to the high content of free reducing sugars in its peels. This increase in ethanol yield could be since the substrate can readily be hydrolyzed to sugar by the amylolytic activity of the fermentation-causing organisms, and subsequent conversion of sugar to ethanol by fermentation in the medium. Thus, there is the available nutrient for fermentation-causing organisms for long periods of the fermentation process. The observed decrease in ethanol yield with an increase in fermentation time with cassava peels could be attributed to the exhaustion of substrates in the medium after about four (4) days of fermentation. This observation is in agreement with the findings of Shilpa et al.,[31] and Zainal et al.,[32] who carried out a similar study using cassava peels as they observed that the optimum yield for ethanol was on the fourth day. *A. oryzae* and *S. cerevisae* in combinations gave the highest bioethanol yield with pineapple peels and this was observed to be on day 8 whereas, *A. oryzae* and *N. crassa* in combinations gave the highest bioethanol yields with cassava peels and this was observed to be on day 4. As single organisms, *A. oryzae*, or *N. crassa* had better bioethanol yields than *S. cerevisae* with both pineapple and cassava peels. Chibuzor et al.,[33] reported that *S. cerevisiae* had low bioethanol yields from cassava peels also. However, it was also evident from this study that even in the absence of a fermentative organism, the water control recorded a considerable amount of bioethanol yields from both pineapple and cassava peels on both days 4 and 8, and this process of ethanol production in the presence of hydrochloric acid (HCl) is often referred to as "acid hydrolysis". For pineapple, the densities of the bioethanol produced for fermentation day 4 inoculated with *S. cerevisiae* strain had the highest density with an average value of 1.23 ± 0.10 g/ml and the bioethanol with the lowest density had an average value of 0.71 ± 0.01 g/ml in samples inoculated with *A. oryzae* + *N. crassa* strains. The bioethanol inoculated with *S. cerevisiae* + *N. crassa* strains had the highest density, having an average value of 0.99 ± 0.01 g/ml on fermentation day 8 and the lowest density average value was 0.86 ± 0.12 g/ml, inoculated with *N. crassa* strain. The densities of the control for fermentation days 4 and 8 had average values of 0.94 ± 0.00 g/ml and 0.96 ± 0.00 g/ml respectively (Figure 3). For cassava, the densities of the bioethanol produced for fermentation day 4, inoculated with *N. crassa* strain had the highest density with an average value of 0.94 ± 0.10 g/ml and the bioethanol with the lowest density of 0.58 ± 0.04 g/ml inoculated with *A. oryzae* + *N. crassa* strains. The bioethanol inoculated with *N. crassa* strain had the highest density (0.98 ± 0.01 g/ml) on fermentation day 8 and the lowest density was 0.89 ± 0.05 g/ml, inoculated with *A. oryzae* strain. The densities of the control for fermentation days 4 and 8 had average values of 0.05 ± 0.00 g/ml and

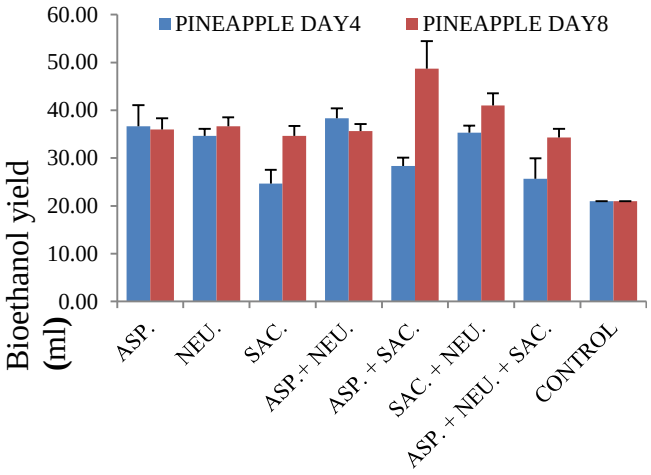

Figure 1: Bioethanol yield (volume) of pineapple substrate using different inoculants on fermentation days 4 and 8. ASP- *Aspergillus oryzae;* NEU- *Neurospora crassa*; SAC- *Saccharomyces cerevisiae*. The mean differences were considered significant at p<0.05.

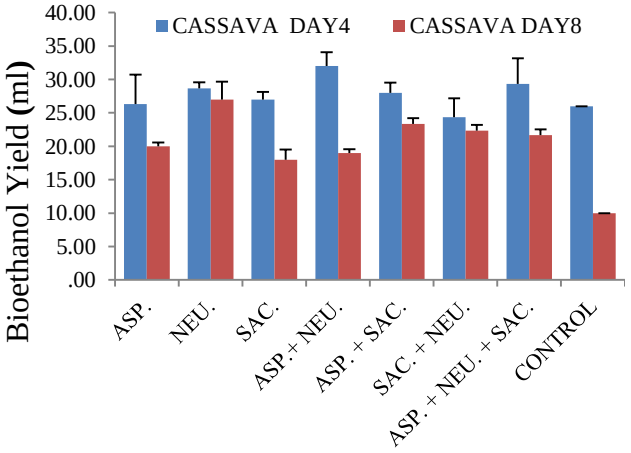

Figure 2: Bioethanol yield (volume) of cassava substrate using different inoculants on fermentation days 4 and 8. ASP- *Aspergillus oryzae;* NEU- *Neurospora crassa*; SAC- *Saccharomyces cerevisiae*. The mean differences were considered significant at $p < 0.05$.

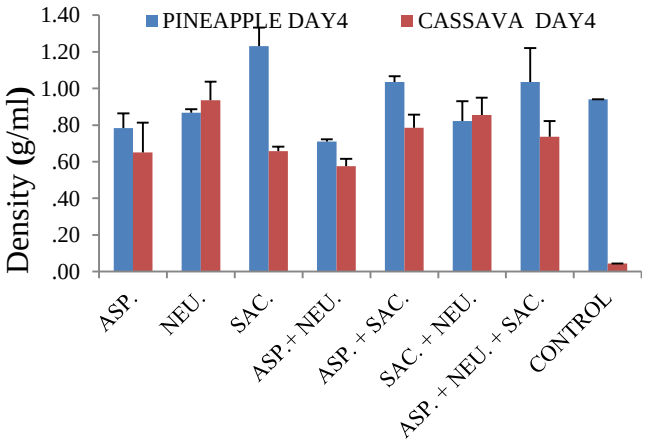

Figure 3: Bioethanol densities of pineapple and cassava substrates using different inoculants on fermentation day 4. ASP- *Aspergillus oryzae;* NEU- *Neurospora crassa*; SAC- *Saccharomyces cerevisiae.* The mean differences were considered significant at $p < 0.05$.

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0.12 ± 0.00 g/ml respectively (Figure 4). During fermentation, the density of the medium decreases as sugars are converted to alcohol.[34] It was thus not a surprising observation from this study that, the lower the ethanol yield, the higher the density of the medium; and the higher the ethanol yield, the lower the density of the medium. From this study, pineapple substrates on day 4 inoculated with *A. oryzae* + *N. crassa* with average densities of 0.71 ± 0.01 g/ml and cassava substrates on day 4 inoculated with *A. oryzae* + *S. cerevisiae*, and *A. oryzae* + *N. crassa*, *A. oryzae* +*S. cerevisiae* had average densities (0.78 ± 0.07 and 0.74 ± 0.08g/ml). These average densities approached the bioethanol standard density which is 0.78 g/ml. Specific gravity is the ratio of the density of a substance to the density of water[35] and it indicates how much sugar has turned into alcohol.[34] The specific gravity of ethanol has been reported to be less than one (<1) having a value of 0.79, indicating it is less dense than water.[36] On fermentation day 4, the highest value for specific gravities of bioethanol produced using pineapple substrates inoculated with *S. cerevisiae* strain was 1.28 ± 0.11, and the lowest value of 0.74 ± 0.01, was obtained when *A. oryzae* + *N. crassa* strains were used as inoculants, while on fermentation day 8, the highest value for specific gravities of bioethanol produced inoculated with *S. cerevisiae* + *N. crassa* strains was 1.03 ± 0.02, and the lowest value of 0.90 ± 0.12, was obtained when *N. crassa* strain was used as inoculant. The specific gravities of the control for fermentation days 4 and 8 had mean values of 0.98 ± 0.00 and 1.00 ± 0.00 respectively (Figure 5). On fermentation day 4, the highest value for specific gravities of bioethanol produced using cassava substrates inoculated with *N. crassa* strain was 0.97 ± 0.11, and the lowest value of 0.60 ± 0.04, was obtained when *A. oryzae* + *N. crassa* strains were used as inoculants, while on fermentation day 8, the highest value for specific gravities of bioethanol produced inoculated with *N. crassa* strain was 1.02 ± 0.00, and the lowest value of 0.93 ± 0.05, was obtained when *A. oryzae* strain was used as inoculant. The specific gravities of the control for fermentation days 4 and 8 had mean values of 0.05 ± 0.00 and 0.13 ± 0.00 respectively (Figure 6). It was recorded that the specific gravities of the bioethanol obtained from this study was close to the standard value using pineapple substrates on days 4 and 8, inoculated with *A.oryzae* + *N.crassa*; while specific gravities for cassava substrates < 1 for days 4 and 8 were inoculated with *A.oryzae* + *S.cerevisiae* and *A. oryzae* + *N.crassa* + *S.cerevisiae*. The knowledge of the specific gravity of fluids being blended is important because it influences the torque and horsepower of motor engines or machines. The large difference in specific gravities between bioethanol and fossil fuels may cause separation, which may cause engine damage and failure. A stable blend of biofuel and petrol or diesel is possible only when the density of the two is close.[37]

A high ethanol concentration in the fermentation broth has several advantages such as increased fermentor throughput, reduced processing costs, reduced energy cost per liter of ethanol, and reduced risk of bacterial contamination.[38] Ethanol concentration may be utilized as an indicator of the quality of ethanol produced and/or the efficiency of the processes and methods utilized in the production of ethanol.[38] On fermentation day 4, the highest concentration of bioethanol produced using pineapple substrates inoculated with *S. cerevisiae* strain was 0.84 ± 0.08% (v/v), and the lowest concentration of 0.07 ± 0.00% (v/v), was obtained when *A. oryzae* + *N. crassa* strains were used as inoculants, while on fermentation day 8, the highest concentration of bioethanol produced inoculated with *S. cerevisiae* strain was 0.27 ± 0.21% (v/v), and the lowest concentration of 0.07 ± 0.00% (v/v), was obtained when *A. oryzae* strain was used as inoculant. The concentrations for the controls of fermentation days 4 and 8 were 0.08 ± 0.00% (v/v) and 0.14 ± 0.00 % (v/v) respectively (Figure 7). On fermentation day 4, the highest concentration of bioethanol produced using cassava substrates inoculated with *S. cerevisiae* + *N. crassa* strains was 0.26 ± 0.19% (v/v), and the lowest concentration of 0.06 ± 0.02% (v/v), was obtained when *S. cerevisiae* strain was used as inoculant, while on fermentation day 8, the highest concentration of bioethanol produced inoculated with *A. oryzae*, *A. oryzae* +*N.crassa*, *A.oryzae* + *S. cerevisiae*, *S. cerevisiae* + *N. crassa* and *A. oryzae*+ *N. crassa* + *S. cerevisiae* strains respectively was 0.07 ± 0.01% (v/v), and the lowest concentration of 0.06 ± 0.00% (v/v), was obtained when *N. crassa* strain was used as inoculant.

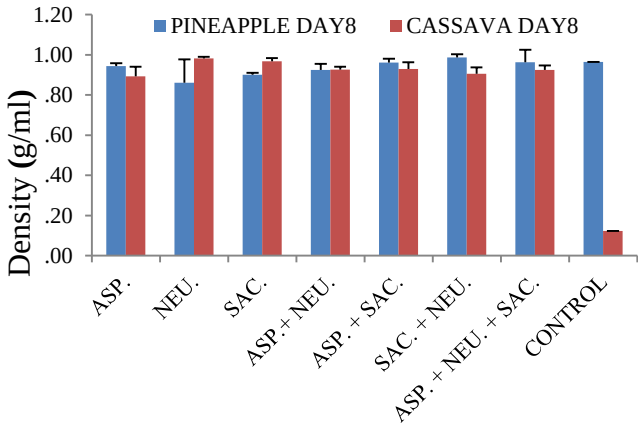

Figure 4: Bioethanol densities of pineapple and cassava substrates using different inoculants on fermentation day 8. ASP- *Aspergillus oryzae;* NEU- *Neurospora crassa*; SAC- *Saccharomyces cerevisiae.* The mean differences were considered significant at $p<0.05$.

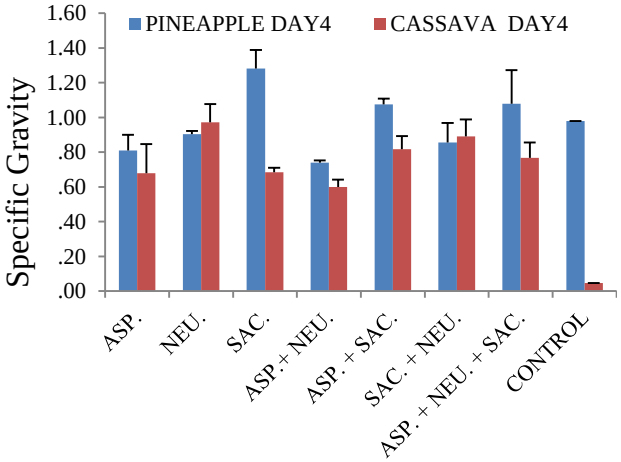

Figure 5: Bioethanol specific gravities of pineapple and cassava substrate using different inoculants on fermentation day 4. ASP- *Aspergillus oryzae;* NEU- *Neurospora crassa*; SAC- *Saccharomyces cerevisiae.* The mean differences were considered significant at $p<0.05$.

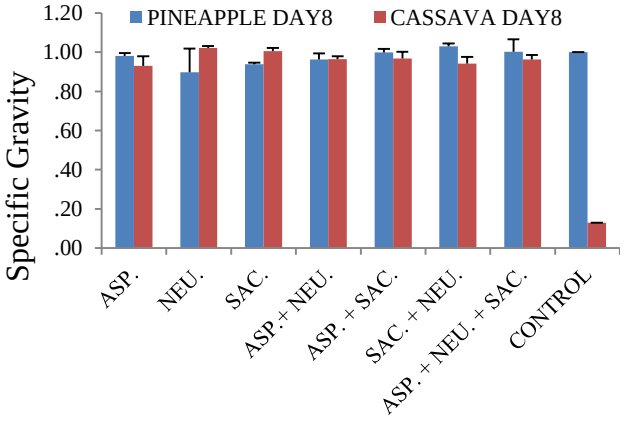

Figure 6: Bioethanol specific gravities of pineapple and cassava substrates using different inoculants on fermentation day 8. ASP- *Aspergillus oryzae;* NEU- *Neurospora crassa*; SAC- *Saccharomyces cerevisiae.* The mean differences were considered significant at $p<0.05$.

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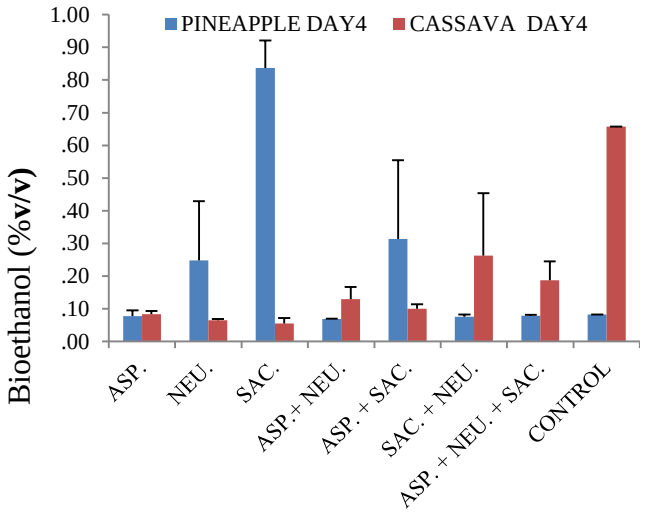

Figure 7: Bioethanol concentrations of pineapple and cassava substrates using different inoculants on fermentation day 4. ASP- *Aspergillus oryzae;* NEU- *Neurospora crassa*; SAC- *Saccharomyces cerevisiae.* The mean differences were considered significant at $p<0.05$.

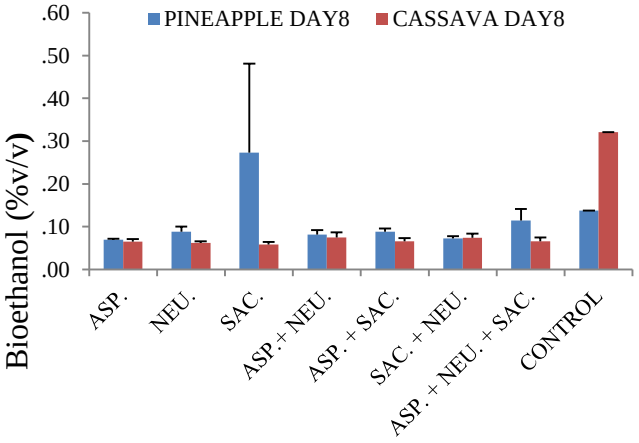

Figure 8: Bioethanol concentrations of pineapple and cassava substrates using different inoculants on fermentation day 8. ASP- *Aspergillus oryzae;* NEU- *Neurospora crassa*; SAC- *Saccharomyces cerevisiae.* The mean differences were considered significant at $p < 0.05$.

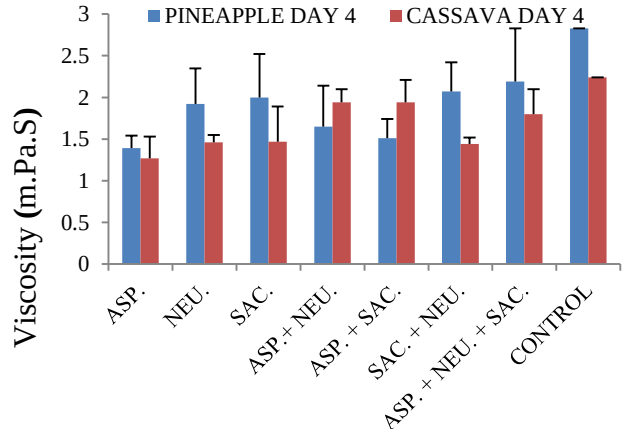

Figure 9: Bioethanol viscosities of pineapple and cassava substrates using different inoculants on fermentation day 4. ASP- *Aspergillus oryzae;* NEU- *Neurospora crassa*; SAC- *Saccharomyces cerevisiae.* The mean differences were considered significant at $p<0.05$.

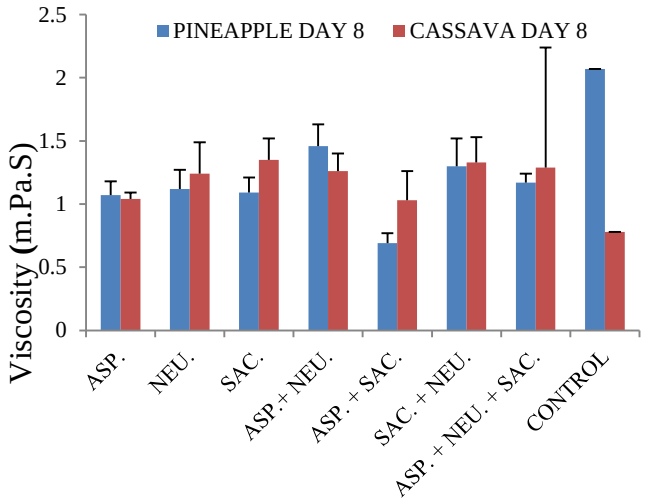

Figure 10: Bioethanol viscosities of pineapple and cassava substrates using different inoculants on fermentation day 8. ASP- *Aspergillus oryzae;* NEU- *Neurospora crassa*; SAC- *Saccharomyces cerevisiae.* The mean differences were considered significant at $p<0.05$.

The concentrations for the control of fermentation days 4 and 8 were 0.66 ± 0.00% (v/v) and 0.32 ± 0.00% (v/v) respectively (Figure 8). From the results with pineapple peels, the highest observed ethanol concentration during the fermentation process was on day 4 with *S. cerevisiae* being the fermentation-causing organism. However, for cassava peels, the highest observed ethanol concentration was also on day 4 with the combination of *S. cerevisiae* and *N. crassa* being the fermentation-causing organisms. Amidst the several advantages of a high ethanol concentration in the fermentation broth, it is considered one of the major stresses or factors responsible for decreased ethanol production. At high concentrations, ethanol can cause changes in the lipid bilayer of cell membranes by making them hyperpolarized thereby increasing membrane fluidity and consequently decreasing membrane integrity.[39] The increase in permeability of cell membranes to small molecules and ions will cause perturbation of cell homeostasis which in turn will impact negatively on several metabolic pathways.[40] Thus, ethanologenic organisms must be ethanol tolerant if a high ethanol yield is desirable. It was observed that, of the three fermentation-causing organisms of choice, the least ethanol yield was recorded in *S. cerevisiae* and it may be that these species of *S. cerevisiae* as used in this study were affected by ethanol concentration in the fermentation medium. High viscosity syrup is among the key factors affecting ethanol fermentation efficiency, viscosity reduction will be one of the necessary factors for large-scale industrial production of ethanol.[41]

On fermentation day 4, the highest viscosity of bioethanol produced using pineapple substrates inoculated with *A. oryzae* + *N. crassa* + *S. cerevisiae* strains was 2.19 ± 0.64 m.Pa.S, and the lowest viscosity of 1.39 ± 0.15 m.Pa.S, was obtained when *A. oryzae* strain was used as inoculant, while on fermentation day 8, the highest viscosity of bioethanol produced inoculated with *A. oryzae* +*N.crassa* strains was 1.46 ± 0.17 m.Pa.S, and the lowest viscosity of 0.69 ± 0.08 m.Pa.S, was obtained when *A. oryzae* + *S. cerevisiae* strains was used as inoculants. The viscosities for the control of fermentation day 4 and 8 were 2.83 ± 0.00 m.Pa.S and 2.07 ± 0.00 m.Pa.S respectively (Figure 9). On fermentation day 4, the highest viscosity of bioethanol produced using cassava substrates inoculated with *A. oryzae* + *S. cerevisiae* strains was 1.94 ± 0.27 m.Pa.S, and the lowest viscosity of 1.27 ± 0.26 m.Pa.S, was obtained when *A. oryzae* strain was used as inoculant, while on fermentation day 8, the highest viscosity of bioethanol produced inoculated with *S. cerevisiae* strain was 1.35 ± 0.17 m.Pa.S, and the lowest viscosity of 1.03 ± 0.23 m.Pa.S, was obtained when *A. oryzae* + *S. cerevisiae* strains was used as inoculants. The viscosities for the control of fermentation day 4 and 8 were 2.24 ± 0.00 m.Pa.S and 0.78 ± 0.00 m.Pa.S respectively (Figure 10). From the results, a significant ($P<0.05$) viscosity reduction was observed with an increase in

fermentation time for both pineapple and cassava peels with all fermentation-causing organisms.

Conclusion

The results of this investigation suggested that *A. oryzae*, both alone and in conjunction with other inoculants, might be the best combination for turning pineapple peels into bioethanol. According to the study, selecting pineapple peels on day 4 may also affect how much ethanol is produced at its best, improving yield with just slight changes in the physicochemical variables.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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