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Microbial Mediated Production of Bio-ethanol from the Rhizome of *Imperata cylindrica* (L.) P. Beauv. (Spear Grass)

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Authors' contributions

This work was carried out in collaboration among all authors. Author HOS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors VBG, BEO and IOA managed the analyses of the study. Author CJU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The microbial mediated production of bio-ethanol from the rhizome of *Imperata cylindrica* (spear grass) was carried out in this research work. Proximate analysis of the rhizome was done to determine the protein, carbohydrate, moisture, ash, lipid, and fibre contents. Sugar profile analysis was carried out using Gas Chromatography (GC). The sugars obtained from the rhizome include ribose, xylose, arabinose, rhamnose, fructose, glucose, maltose, lactose, and sucrose. Ethanol yield increased simultaneously from day 5 to day 20 for all reactors in six different experimental design i.e, *Saccharomyces cerevisiae* alone; *Serratia marcescens* alone, *Aspergillus flavus* + *Saccharomyces cerevisiae*; *Apergillus flavus* + *Saccharomyces cerevisiae* + *Serratia marcescens; Aspergillus flavus* + *Serratia marcescens* including the control (containing no microorganism). The highest ethanol yield was achieved at pH 7 and temperature range between 28^0C to 38^0C. The

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reducing sugar decreased from 0.37 to 0.03 g/mol as the period of incubation increased while cell density increased from 0.1 to 0.68 nm as the period of incubation increased. Reactor containing co-cultures of *Saccharomyces cerevisiae, Serratia marcescens* and *Aspergillus flavus* in combination produced the highest ethanol yield at day 20 while the reactor containing only *Serratia* sp produced the lowest ethanol yield. This study has demonstrated that the use of *Saccharomyces cerevisiae, Serratia marcescens* and *Aspergillus flavus* co-cultures is more effective in bio-ethanol production using *I. cylindrica* (*spear* grass) compared to individual cultures.

Keywords: Imperata cylindrical; bio-ethanol yield; Saccharomyces cerevisiae; Serratia marcescens; Aspergillus flavus.

1. INTRODUCTION

Biofuels have been identified as a promising renewable alternative to fossil fuels. Biofuel can be employed in contemporary infrastructures and even combined with fossil fuels to reduce emission and provide domestically produced energy. Many arguments have been put forward for the use of biofuels including that it is more sustainable and can provide answer to the worlds future energy need in the face of dwindling oil resources. Furthermore, the advantages of biofuel production outweigh those of the fossil fuel from the environmental standpoint.

Bio-ethanol is one biofuel from plant material with promising prospect due to the abundance of biomass materials that can be exploited for its production and the availability of technology to make its production simple, scale able and commercially profitability. The production of ethanol from plant materials is a source of energy that is renewable with no net addition of CO_2 to the environment since it is produced from plants and recycled into plants through photosynthesis, making bio-ethanol an environmentally friendly and beneficial energy source. It can be said that the only liquid transportation fuel that contributes little or nothing to green house gas effect is bio-ethanol [1].

The concern across the world about climate change and the need to lessen the concentrations of green-house-gases has necessitated the demand for the use of bio-ethanol over fossil fuel [2]. Sources of biomass such as crops, agricultural waste, perennial grass and household waste have been explored by scientists all over the world as potential viable feed stock for energy production. However using consumable food crops such as rice, cassava, wheat, corn, sugarcane, and soya beans for the production of bio-ethanol has raised major concerns about the sustainability of biofuel production technology [3].

There is the need for an increase in the production of bio-ethanol due to the growth in its demand as an alternative source of renewable biomass energy. New feedstock for bio-ethanol is constantly being sought, as well as suitable microbial processes for its production. Studies have been carried out using different raw materials and different procedures for bio-ethanol production, but presently, it has been noticed that lignocellulosic plant materials are the focus for bio-ethanol production [4-8]. An invasive, rhizomatous (with underground horizontal stems that can penetrate soil for long distances) of the aggressive plant, *Imperata cylindrica* (Spear grass), that could become a threat to other plant species found around it and underground grown food crops (i.e. like yam, cassava etc.) was chosen for the production of bio-ethanol in this study. This research thus aimed to access the potentials of the rhizome of *Imperata cylindrica* (Spear Grass) for microbial mediated bio-ethanol production.

2. MATERIALS AND METHODS

2.1 Sample Collection

Rhizomes of *Imperata cylindrica* (spear grass) were collected from the farm from Agbarho town of Delta state. The plant *I. cylindrica* was identified and authenticated by Prof. Bosa E. Okoli of the Biofuel Centre, University of Port-Harcourt. The plant vouchers of the whole plant and rhizomes were deposited in the Department of Plant Science and Biotechnology Herbarium, University of Port Harcourt.

Pure cultures of the yeast *Saccharomyces cerevisiae*, the mould *Aspergillus flavus* and the bacterium *Serratia marcenses* used for this study, were obtained from Microbiology Major Laboratory, Faculty of Science, University of Port Harcourt.

2.2 Sample Preparation / Pretreatment

The plant rhizomes were properly washed with clean water, sun dried to remove all moisture and hence concentrate the sugar for 3 days. The dried sample was milled into a powdered form for easy extraction and pretreatment. Exactly 5000 ml of water boiled to 100^0C was introduced into 500 g of the milled rhizome and left to stand for 24 hours, after which a muslin cloth was used to obtain the aqueous extract.

2.3 Proximate Analysis of the Rhizome of *Imperata cylindrica* (Spear Grass)

The crude protein content was determined using the Kjeldahl method described by Sluiter et al. [9]. Carbohydrate was determined by Cleg Anthrone Method as described by Sluiter et al. [9] Moisture determination was by air oven method. Lipid determination was by the soxhlet extraction method. Ash determination was by the furnace method as described by sluiter et al. [10]. The crude fibre value was gotten by difference between the whole and the sum of the other constituents.

2.4 Scale-up of Inoculums

A loopful each of *Aspergillus flavus and Saccharomyces cerevisiae* was inoculated into different tubes containing 14ml Sabouraud dextrose broth and incubated at 25^0C for 12 h. *Serratia marcescens* was inoculated into 10 ml of nutrient broth and incubated at 25^0C for 12 hours.

2.5 Saccharification of Spear Grass Rhizome Extract

Six flasks containing 200 ml of spear grass rhizome extracts were each inoculated with 14 ml of the 12 hours old culture of *Aspergillus flavus* and incubated at 25^oC for 12 hours for hydrolysis to take place.

2.6 Fermentation Set-up

Fermentation experiments were carried out as described by Ali et al. [11]. The fermentative production of bio-ethanol was done using batch fermentation with shaking method. The pre-treated plant substrate was used for all the experiments. Two hundred (200) ml of the substrate was introduced into bottles and autoclaved at 121^oC at 15 psi for 15minute. Exactly 14 ml of *Aspergillus flavus*, *Saccharomyces cerevisiae* and *Serratia marcescens* were each transferred into 500ml flasks containing 200 ml solution of the rhizome extract in the experimental set-up.

The seven fermentation set-ups were

A. Control (200 ml of extract in 500 ml conical flask without microorganisms)
B. 200 ml of pretreated extract + *Saccharomyces cerevisiae* = (Sacc)
C. 200 ml of pretreated extract + *Aspergillus flavus* + *S. cerevisiae* = (Asp + Sacc)
D. 200 ml of pretreated extract + *S. cerevisiaee* + *S. marcescens* = (Sacc + S)
E. 200 ml of pretreated extract + *Serratia marcescens* = (S)
F. 200 ml of pretreated extract + *A. flavus* + *S. cerevisiae* + *S. marcescens* = (Asp + Sacc + S)
G. 200 ml of pretreated extract + *A. flavus* + *S. marcescens* = (Asp + S)

The flasks were all incubated at room temperature (28^0C) for 20 days. All the experiments were performed in duplicates and aseptically. Throughout the fermentation process, 25 ml each of sample were taken every 5 day intervals to estimate of bio-ethanol yield. Samples were also taken to determine the temperature, pH, cell density and for sugar analysis.

2.7 Ethanol Concentration

The ethanol concentration was determined using a refractometer after distillation as described in Ademiluyi and Mepba [12].

2.8 Sugar Analysis

The Sugar Profile was determined by Gas Chromatography (GC: HP 6890 powered with HP ChemStation with Rev.A 09.01 [1206] Software).

2.9 Reducing Sugar Determination

Reducing sugar was determined using dinitrosalicylic acid as described by Miller et al. [13].

2.10 Cell Density

Exactly 1 ml of sample treatment each was examined at 690 nm using un-inoculated fermentation medium as blank. This was done for the various sampling days i.e. day 1, 5 10, 15 and 20.

2.11 Statistical Analysis

Statistical Package for the Social Sciences (SPSSv21) was used for the Analysis of Variance (ANOVA) among the various treatments.

3. RESULTS

3.1 Proximate Composition of the Rhizome - *Imperata cylindrica* (Spear Grass)

The proximate composition of the rhizome of *Imperata cylindrica* is shown in Table 1. The moisture content was highest at 62.85% and ash the least at 1.55%.

3.2 Sugar Profile of the Rhizome of *I. cylindrica* (Spear Grass)

The result in Fig. 1 shows the different types of sugar found in the rhizome using Gas chromatography (GC). The sugar present includes ribose, xylose, arabinose, rhamnose, fructose, glucose, maltose, lactose and sucrose. The result showed that glucose and sucrose had the highest value.

3.3 Bio-ethanol Yield

The result of bio-ethanol yield as presented in Fig. 2 revealed that at day 1 ethanol yield was zero (0) for all set-ups, from day 5, ethanol production increased for all the set-ups and maximum ethanol was produced at day 20. Set-up F had the highest ethanol yield of 11.4%, followed by C, and G with both 9.8%. Control had the lowest ethanol yield.

Table 1. Proximate composition of the rhizome of *Imperata cylindrical*

Nutrient	Quantity (%)
Protein	6.13
Carbohydrate	7.25
Moisture	62.85
Ash	1.55
Lipid	7.5
Fibre	14. 73

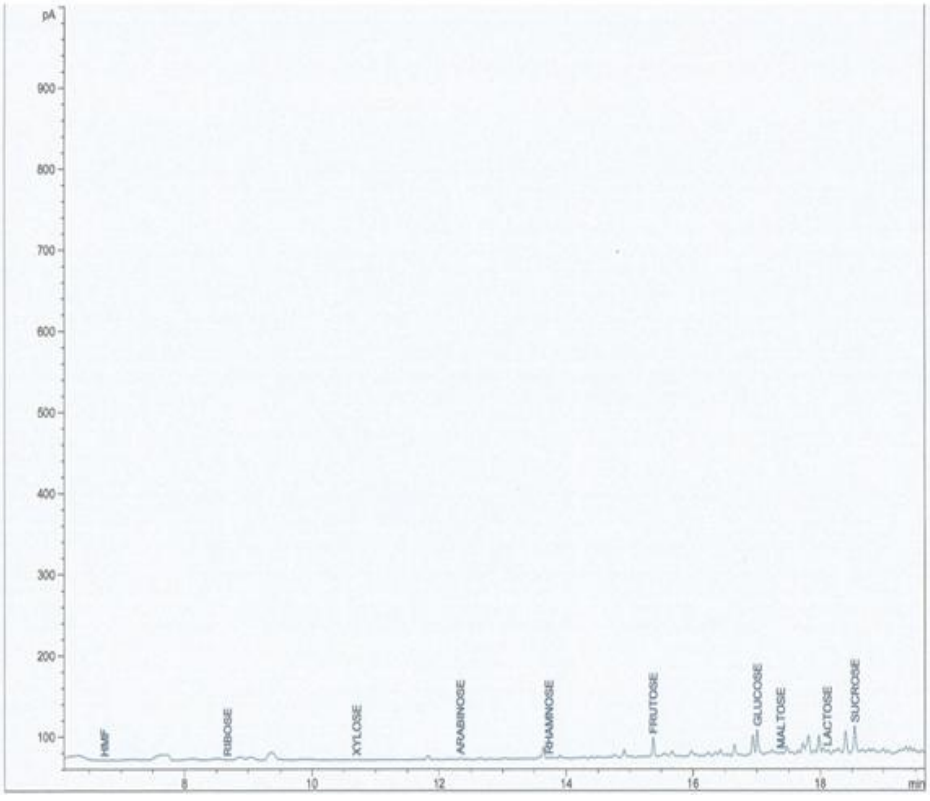

Fig. 1. GC chromatogram showing the different sugars presents in the rhizome of *Imperata cylindrica* (spear grass)

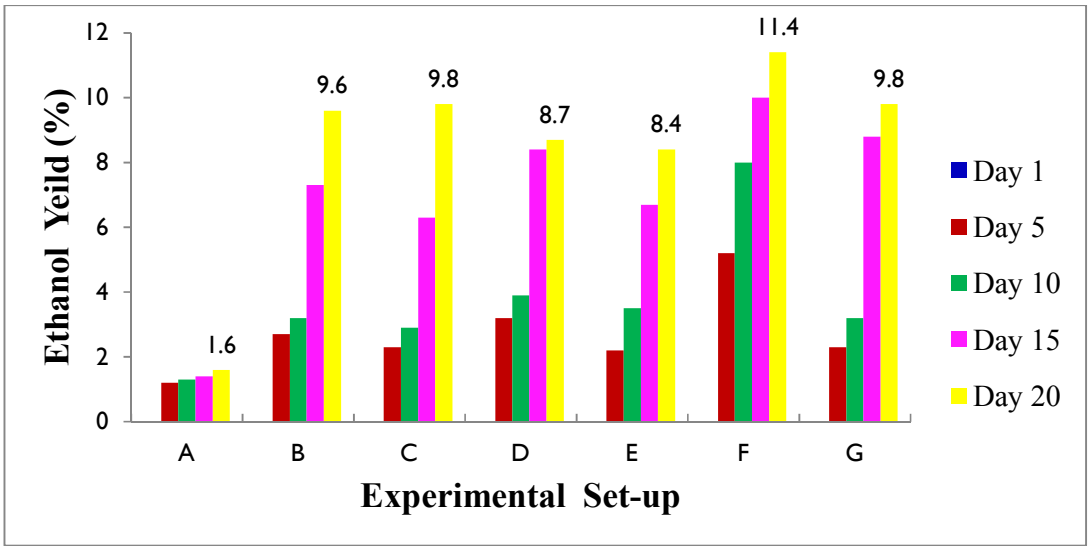

Fig. 2. Ethanol yield obtained from the rhizome extract of *Imperata cylindrical,* where A= Control; B = Extract + *Serratia marcescen;* C = Extract + *Aspergillus flavus* + *S. cerevisiae*; D = Extract + *S. cerevisiae* + *S. marcescen;* E = Extract + *Saccharomyces cerevisiae;* F = Extract + *A. flavus* + *S. cerevisiae* + *S. marcescens* G = Extract + *A. flavus* + *S. marcescen*

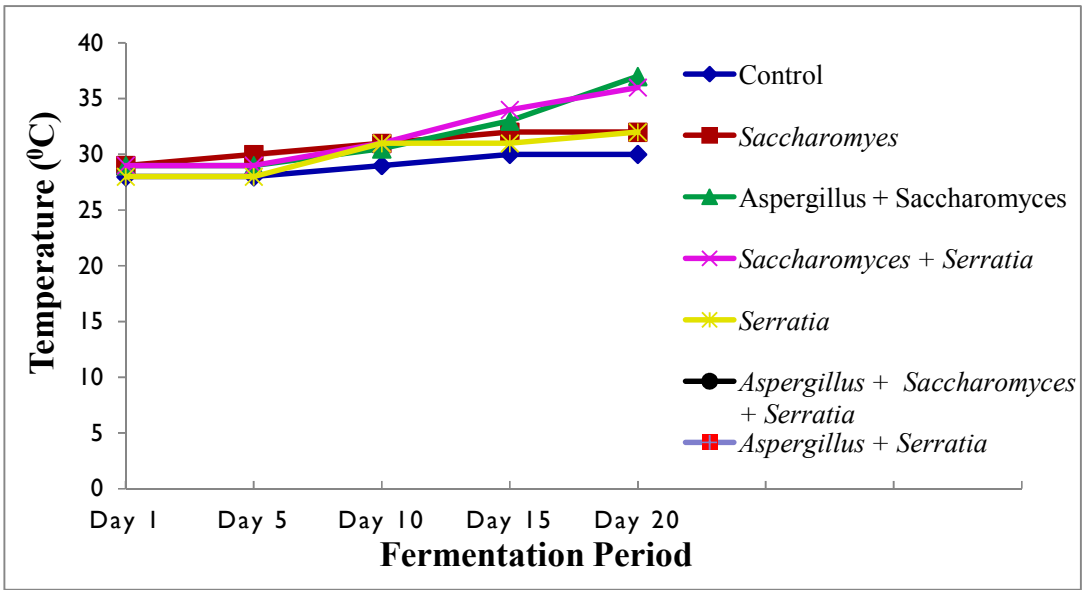

Fig. 3. Temperature variation during the production of bio-ethanol from the rhizome extract of *Imperata cylindrical*

3.4 Effect of Temperature on Bio-ethanol Production

The different temperature ranges for the various sample treatments are as presented in Fig. 3. The temperature for all set-ups increased as the days increased. The highest temperatures were recorded at day 20 for all the samples. The highest temperature recorded was 38^0C and the lowest was 28^0C.

3.5 Effect of pH on Bio-ethanol Production

Results in Fig. 4 show the effect of pH on bio-ethanol using the rhizome of *Imperata*

cylindrica (Spear grass). The pH value ranged between 4.8 and 7.0. The pH for all the set-ups decreased from day 1 to day 20.

3.6 Effect of Reducing Sugar on Bio-Ethanol Production

Fig. 5 shows the reducing sugar concentration with fermentation time. The chart showed that the sugar present in the rhizome extract of *Imperata cylindrica* reduced simultaneously as the fermentation period increased.

3.7 Effect of Cell Density on Bio-ethanol Production

Results of cell density with fermentation time are shown in Fig. 6. It was observed that cell density increased gradually for all experimental set-ups during the fermentation process.

4. DISCUSSION

The results of proximate analysis of the rhizome of *Imperata cylindrica* (Spear grass) revealed that moisture had the highest with 63%. This was attributed to the rhizomes of *I. cylindrica* being able to absorb enough water from the soil due to their slender shape (1 – 1.5 mm diameter) and roots (up to1.2 m deep in the soil). The fibre was 15%, lipid 8% and 7% carbohydrate. The carbohydrate and sugar contents of this rhizome made it an interesting feedstock for bio-ethanol production.

The results obtained for reducing sugar and ethanol yield revealed that there was a corresponding increase in ethanol yield as reducing sugar decreased in all set-ups for the period of fermentation. This is in congruence with the study of Ali et al. [14] who observed in his set-up that as the ethanol yield increased, the concentration of the reducing sugar decreased. In another study by Itelima et al. [15], results showed that in all the substrates used, the concentration of the reducing sugar decreased gradually as the fermentation period and ethanol yield increased. Ethanol yield was also observed to increase as the period of fermentation increased for all set-ups in the present study, similar to the findings of Itelima et al. [15].

The highest yield of bio-ethanol produced was observed in set-up F (11.4%), followed by C and G (9.8%), which all had the microorganism *Aspergillus flavus* present. The observed high yield in bio-ethanol was as a result of the presence of protease enzyme found in *A. flavus* that helped to break down the peptide bonds and lignocellulosic cell wall of the plant rhizome; so that *S. cerevisiae* and *S. marcescens* can have fast access in converting the starch and sugar contained in the rhizome of *I. cylindrica* to bio-ethanol during fermentation process. Many authors have relied on *Sacchromyces* spp. for the production of bio-ethanol [14,16-18]. Co-cultures of *Aspergillus* and *Saccharomyces* has also been used in the manufacturing of

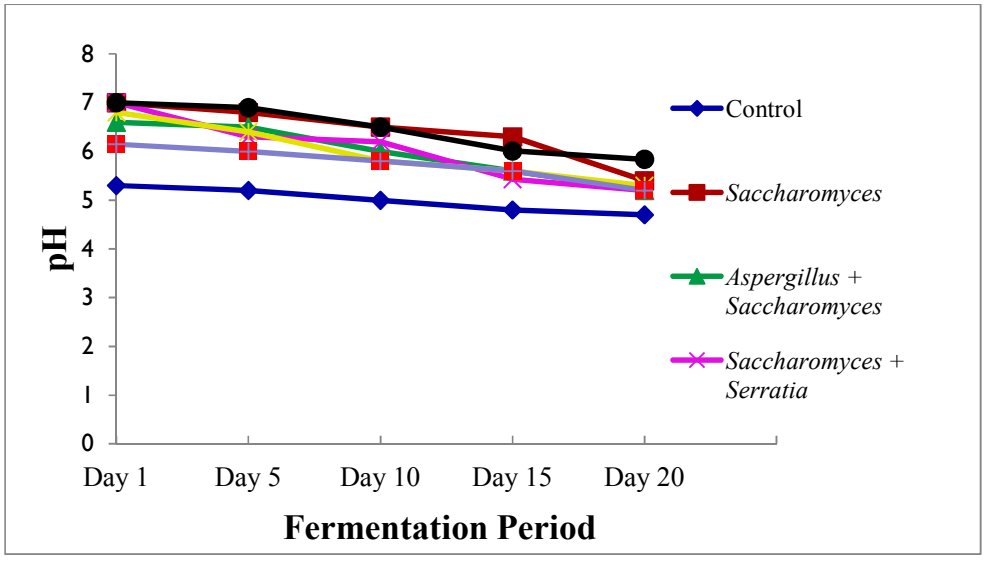

Fig. 4. pH variation during the production of bio-ethanol from the rhizome extract of *Imperata cylindrica*

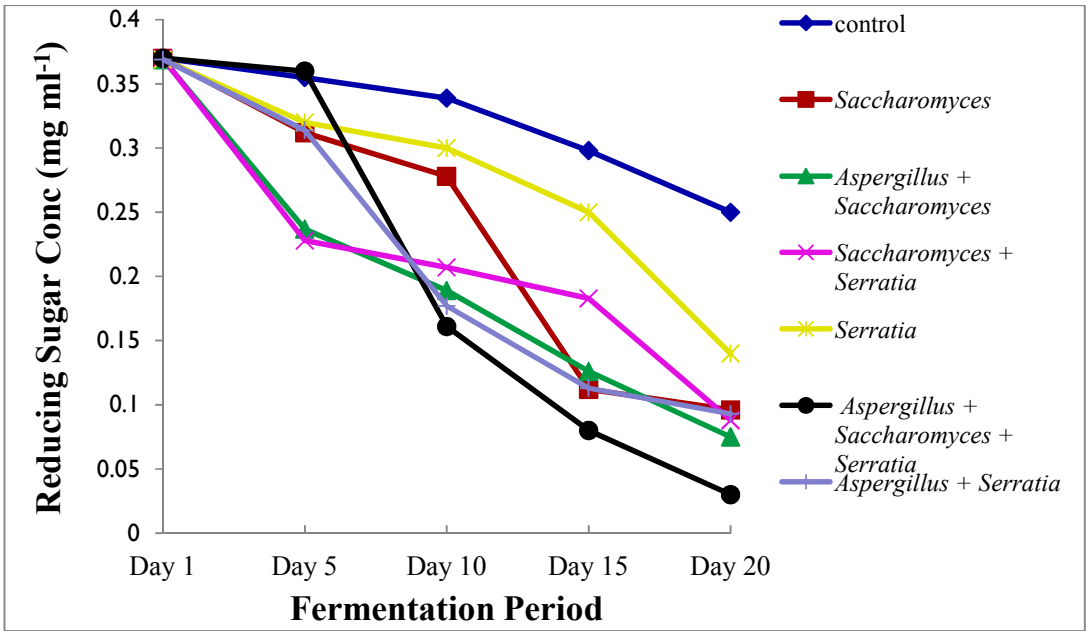

Fig. 5. Reducing sugar concentration with fermentation time

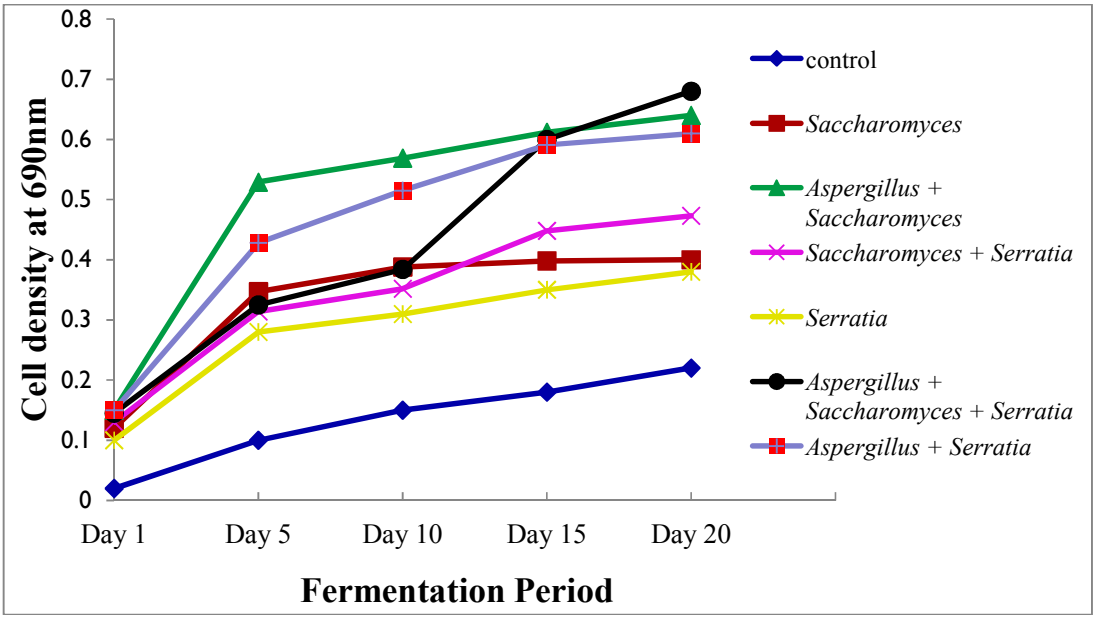

Fig. 6. Cell density with fermentation time

bio-ethanol [19,20]. The results from the statistical analysis showed significant difference ($p<0.05$) in ethanol yield between all the microbial mediated treatments (B-G) and the control, but not between them.

The temperature during the fermentation process ranged from 28°C – 38°C, with set-up F (*A. flavus* + *S. cerevisiae* + *S. marcescens*) having the highest temperature of 38°C, probably due to higher activity rate by the three inoculants during bio-ethanol production. The set-ups having one microorganisms, B = *S. marcescens* alone and E = *S. cerevisiae alone* had the least temperature of 28°C. This result may be due to the low activity when a single organism is involved in the

fermentation process. Temperature increases the rates of enzyme reactions up to the point where the enzymes began to denature [21]. Above that temperature, reaction dropped precipitously as the enzyme got denatured. The results showed that ethanol yields were relatively constant with temperature.

A gradual and continuous decrease in pH was recorded in this study, a clear indication that pH decreases towards acidity and these acids were stored in the fermentation medium all through the fermentation process. This decrease is attributable to the acid producing activity of *S. cerevisiae, A. flavus and S. marcescens* during the fermentation of the sugars present in the plant extract. The results obtained from this study are also similar to the results obtained in the study of Ali et al. [13]. They observed that as ethanol production increased, pH decreased. It is evident therefore that the production of acid and oxidation of sugar in the process of fermentation could have lowered the pH in the reactors.

A progressive reduction of sugar was observed in all experimental set-ups. This decrease was because microorganisms were converting the sugar found in the plant extract (substrate) into bio-ethanol. The control also had reduction in sugar, with little ethanol produced. Set-up F which had the highest yield of bio-ethanol, also showed more reduction of sugar. As more ethanol was produced daily, the sugar present in the medium reduced as well. Set-up E (*S. marcescens*) alone had the least reduction in sugar. This was due to the absence of *A. flavus* enzyme that facilitated the breakdown of lignocellulosic cell wall and absence of *S. cerevisiae* the chief fermenter used mainly to reduce sugars in the production of bio-ethanol.
There was also a corresponding increase in the cell density as the period of fermentation increased. The same observation was reported by Itelima et al. [15]. The increase in cell densities in all the set-ups from day 1 to day 20 of the fermentation periods indicated that the sugar contents of the rhizome were utilized to produce bio-ethanol.

5. CONCLUSION

The rhizome of *Imperata cylindrica* (spear grass) is a suitable raw material for bio-ethanol production with *Saccharomyces cerevisiae, Aspergillus flavus*, and *Serratia marcescens* co-cultures. For environmental sustainability, production of bio-ethanol from none food crops, will definitely reduce the level of fossil fuel usage and grant a friendly environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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