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(RESEARCH ARTICLE)

Production and characterization of bioethanol from *Solanum lycopersicum* stalk hydrolysates by the simultaneous saccharification and fermentation using *Zymomonas mobilis* and *Saccharomyces cerevisae*

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Abstract

The limited oil reserves, pollution concerns, global warming has led to an increased financial support for sustainable and environmental friendly sources of energy. In the last decades there is an increasing interest in the development of the bioethanol production from lignocellulosic residues, which do not compete directly with food. However, the low efficient conversion of cellulosic biomass to biofuels hinders its success hence the need to constantly explore new, cheap and readily available raw materials. In this work, the capability of typed strains of *Z. mobilis, S. cerevisiae* as well as the co culture of *Z. mobilis* and *S. cerevisiae* (1:1) to produce ethanol by saccharification and fermentation process from tomato stalk was investigated and compared. The results shows the optimal ethanol yield of 4.00, 6.70 and 10.97% (v/v) was obtained from *S. cerevisiae, Z. mobilis* and the co culture of *S. cerevisiae and Z. mobilis* after 6 days of fermentation. The combination of *S. cerevisiae* and *Z. mobilis* yielded the highest ethanol when compared with the yields obtained when the organisms were used separately since the combination of the bacteria and yeast undergoes EMP and ED pathways which converts sugar into alcohol. The identity of the produced ethanol was confirmed by its density and IR spectrum. The results of this study suggest that agro waste should no longer be discarded but converted to useful products like bioethanol since it contains fermentable sugars.

Keywords: Bioethanol; Bioprocesses; *S. cerevisiae*; *Z. mobilis*

Abbreviations*:* ED - Entner – Doudoroff, EMP - Embden-Meyerhof-Parnas

1. Introduction

The world today is constantly being faced with the problem of oil production *viz-a-viz* matching the rapid industrialization triggering ever-increasing demand of fuels especially bioethanol, biodiesel, biohydrogen among others [1]. These recent challenge has challenge the world to pay special concern to greenhouse gas emissions and energy security for a sustained economic development. For a reduced dependence on oil from fossil reserves, use of biofuels such as bioethanol from abundantly available lignocellulosic biomass is of great interest nowadays in an effort to meet the 10% mandate binding target for biofuels from renewable sources in the transport for all European member states by 2020 [2].

Biofuels may be classified under the categories of first or second generation biofuels [3]. First generation biofuels are generally made from carbohydrates, lipids and oils or agro-industrial wastes using conventional technologies. Second generation biofuels are generally derived from lignocellulosic biomass including cellulosic plant biomass such as the

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stalks, stems, wood among others. Nigeria is amongst the rapidly expanding large economy, facing a formidable challenge to meet its energy needs to support its growing population and this seems to be highly difficult target to meet. Thus, it is a must for countries like Nigeria to invest in renewable energy researches. The readily available source are plant biomass which is readily abundant and renewable source of energy-rich carbohydrates which can be efficiently converted by microbes into biofuels of which, bioethanol is widely produced on an industrial scale today [4].

Solanum lycopersicum is a perennial grass about 1-3 meters high with a weak stem that often sprawl over the ground. The stalks are rich in cellulose and hemicelluloses and hence could be exploited for bioethanol production [5]. Irrespective of the type of lignocellulosic biomass, the improvement in ethanol production technology from inexpensive lignocellulosic feedstock like wood biomass, agricultural and forestry residues, biodegradable fraction of industrial and municipal wastes, the basic structural composition of lignocellulosic biomass consists of cellulose, hemicellulose and lignin. Since lignocellulosic materials are very complex, not one pretreatment method can apply for all the materials. Taherzadeh and Karimi [6] have established several methods generally classified as physical, physico-chemical, chemical and biological pretreatment and elaborating reviewed these methods. One of the most commonly used pretreatment methods is steam explosion, with the addition of H_2SO_4 which removes most of the hemicellulose, followed by enzymatic hydrolysis to convert cellulose to glucose [7-8]. The release of hexose and pentose sugars during pretreatment and enzymatic hydrolysis is often accompanied by liberation of compounds such as furans, weak organic acids and phenolics compounds [9] that inhibits growth, ethanol yield and productivity of fermenting microorganisms like *Zymomonas mobilis, Saccharomyces cerevisiae* among others [10-12]. This enzymatic hydrolysis can be performed simultaneously with the co-fermentation of glucose and xylose in a process referred to as simultaneous saccharification and co-fermentation. Simultaneous saccharification and co-fermentation although inexpensive offers several advantages which include continuous removal of end-products of enzymatic hydrolysis that inhibit cellulases or β-glucosidases[13] and higher ethanol productivity and yield than separate hydrolysis and fermentation [14-15]. The simultaneous saccharification and co-fermentation concept is one of the interesting process options and the potential of such process for the biological conversion of the lignocellulosic raw material of *Solanum lycopersicum* using *Zymomonas mobilis* and *Saccharomyces cerevisiae* to produce bioethanol to the best of our knowledge has not been reported previously hence the aim of this study.

2. Material and methods

2.1. Sample collection and authentication

Fresh stalks of *Solanum lycopersicum* plant used as cellulosic substrates were collected locally at Dundaye village of Wamakko Local Government Area of Sokoto State, North-Western Nigeria in the month of June, 2017. They were identified and authenticated at the Bayero University, Kano herbarium where an Accession Number: BUKHAN 0367 was issued. They were cut into small pieces, sun dried for 4 days and later dried in a hot air oven at 70^{o}C for 6 hours. They were grounded into fine powder using a milling machine (Electric grain milling machine). The powdered sample was stored at room temperature in an air tight glass container prior to its use.

Figure 1 *Solanum lycopersicum* stalk (a) before and (b) after collection

2.2. Isolation and identification of the hydrolyzing *Aspergillus niger*

Aspergillus niger were isolated from maize grains using direct plating technique. 20 maize grains were picked randomly and surface sterilized by soaking for 1 minute in sodium hypochlorite (2.5%), and rinsed with sterile distilled water. The grains were blotted with sterile filter paper and plated on potato dextrose agar containing 7.5% sodium chloride and 1.0 g of streptomycin sulphate in 1 litre of media [16]. The plates were incubated at 25 ^{o}C and monitored for fungal growth daily for seven days. The resulting cultures were identified based on cultural and morphological characteristics using taxonomic keys [17-18]. Target moulds were sub-cultured to obtain pure single spore cultures. A small amount of the growth colonies were taken and smeared on a glass slide, covered with a slip and heated slightly to remove air bubbles before being viewed under microscope. The organism identified was compared with the standard structure of *Aspergillus niger* as described by Robert and Ellen [19].

2.3. Fermenting microorganisms and culture conditions

The procedure described by Sneha*l et al*. [20] was adopted with slight modification. Procured *Zymomonas mobilis*AX101 strain reported to produce cellulases were maintained on potato dextrose agar (PDA) medium and were revived once in a month on PDA slants at 30 ± 2°C for 7 days and were stored at 4 °C. Procured *Sachromycescerevisiae*TMB3400 was maintained on malt extract-glucose-yeast extract-peptone (MGYP) medium with the composition 0.3 g% malt extract, 1.0 g% glucose, 0.3 g% yeast extract, 0.5 g% peptone and 2.0 g% agar. The pH was maintained within the 6.4 - 6.8 range. The biomass was obtained by cultivating the yeast cells in MGYP broth medium and 1 x 10^7 cells were used for inoculation into fermentation media. During the fermentation process biomass obtained was deflocculated by washing 2-3 times with sterile normal saline (0.9% NaCl). The enzyme broth was filtered using coarse filter paper and the filtrate obtained was centrifuged at 10,000 rpm for 15 min. The crude enzyme extract obtained was analyzed for various enzyme activities. Cellulolytic enzymes obtained are used for carrying out saccharification and saccharified hydrolysate utilized for ethanol production.

2.4. Simultaneous saccharification and fermentation

The fermentation was carried out along with saccharification, as described by Kroumov *et al*. [21] and Oghgren *et al.* [22]**.** Eighteen 250 cm^3 of conical flasks sorted into 3 groups with 3 conical flasks per group with each containing 10 g of the hydrolyzed tomato stem samples was set up. 0.125 g $MgSO_4.7H_2O$, 0.50 g $(NH_4)_2SO_4$, 0.50 g KH_2PO_4, and 0.516 g of $FeSO_4.7H_2O$ were added as nutrient. The flasks were covered with cotton wool, wrapped in aluminum foil, autoclaved for 15 min at 121 °C, and allowed to cool to room temperature. The harvested *A. niger* spore suspension co-cultivated with equal amount of the fermentative organisms were aseptically inoculated into each flask and incubated at 30 °C according to the following protocol;

Group A: inoculated with *Sachromyces cerevisae*

Group B: inoculated with *Zymomonas mobilis*

Group C: inoculated with *Sachromyces cerevisae* and *Zymomonas mobilis*

One flask was removed from each group every twenty four hour incubation period for a period of six days. The obtained fermented broth from each conical flask in each group was bulked together ready for fractional distillation.

2.5. Fractional distillation

The fermented broth was transferred into a round-bottom flask fixed to a distillation column with a running tap water through the column. A conical flask was fixed to the other end of the distillation column to collect the distillate. A heating mantle with the temperature adjusted to 78.3 °C was used to heat the round-bottomed flask containing the fermented broth for each group. The distillate collected was measured using measuring cylinder [23]. The percentage bioethanol yield was calculated according to the expression proposed by Gunasekaran and Kamini [24].

$$\text{Bioethanol Yield (\%)} = \frac{\text{Volume of Bioethanol Produced}}{\text{Volume of sample Used}}$$

2.6. Qualitative test for ethanol

The 2 cm^3 of acetone was added to a test tube containing 4 drops of the fractionated bioethanol. 2 drops of chromic acid were then added. The test tube was fitted with a tight cork. The mixture was shaken vigorously [25-26].

2.7. Density determination

The procedure described by Emtron [27] was employed. The volume of the bioethanol distillate from each fermentative organism with reference to the original mass of the sample utilized was employed to determine the density of the produced bioethanol according to the expression

$$\text{Density } (g/cm^3) = \frac{\text{Mass of Sample (g)}}{\text{Volume of ethanol produced } (cm^3)}$$

2.8. Infrared spectroscopy

The produced bioethanol distillate was subjected to IR spectroscopy to establish the presence or otherwise of important functional groups to confirm or otherwise if ethanol was actually produced [28].

3. Results and discussion

3.1. Percentage yield

The percentage yield of bioethanol produced as well as the volume of the bioethanol produced using *S. cerevisiae* and *Z. mobilis* as fermentative organisms as well as the co-fermentation using *S. cerevisiae* and *Z. mobilis* is represented in Tables 1 and Figure 1.

Table 1 Percentage yield of the produced bioethanol

Fermentative organism	Percentage yield
S. cerevisiae	4.00
Z. mobilis	6.70
S. cerevisiae + Z. mobilis	10.97

Table 1 reveals the percentage yield of bioethanol produced from the fermentative microorganisms in the order *S. cerevisiae + Z. mobilis* > *Z. mobilis* > *S. cerevisiae* which is in agreement with the findings of Oyeleke and Jibrin [23].

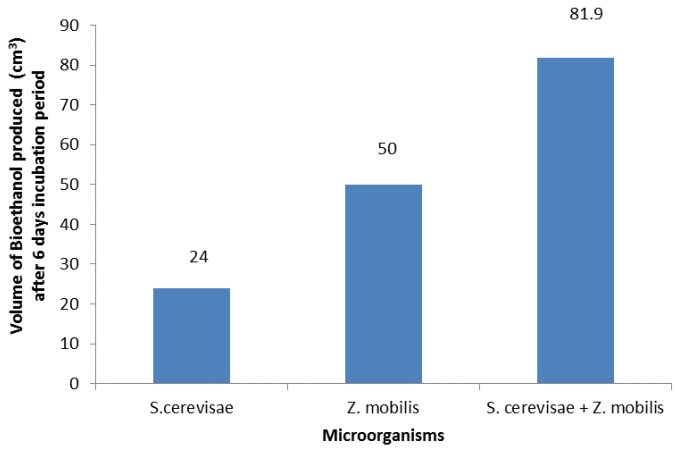

Figure 2 Volume of bioethanol produced from *Solanumlycopersicum*stalk

The volume of bioethanol produced from tomato stalk when hydrolyzed with *Aspergillus niger* and fermented with *Zymomonas mobilis* and *Saccharomyces cerevisiae* is in the order *S. cerevisiae + Z. mobilis* > *Z. mobilis* > *S.* cerevisiae. This is a confirmation that *Z. mobilis* possesses pyruvate decarboxylase and alcohol dehydrogenase as reported by Gunasekaran and Chandra [29] unlike *S. cerevisiae* and that these enzymes facilitate ethanol production by ensuring the continuous fermentation of pentose sugars which are normally found in hemicelluloses biomass. The volume of ethanol

produced from *Z. mobilis* inoculated hydrolysate is much lower than that reported by Oyeleke and Jibrin [23] on guinea corn and millet husk.

3.2. Qualitative test for ethanol

The change in colour of the mixture forming a blue-green precipitates within few second of adding drops of chromic acid confirms the presence of ethanol.

3.3. Density determination

The density of the produced bioethanol using *S. cerevisiae* and *Z. mobilis* as fermentative organisms as well as the co-fermentation using *S. cerevisiae* and *Z. mobilis* is represented in Table 2.

Table 2 Density of produced bioethanol

Organisms	Density (g/cm^3)
S. cerevisiae	0.908
Z. mobils	0.875
S. cerevisiae + Z. mobilis	0.869

The results reveals and further confirms that *S. cerevisiae* produces the least volume of bioethanol while that produced when *S. cerevisiae + Z. mobilis*w as used to co-ferment the tomato stalk produces the largest volume of bioethanol since the same quantity of biomass was employed within the fermentative days of the study. The produced bioethanol is within the acceptable density of 0.8033 g/cm^3 for the standard ethanol.

3.4. Infrared spectroscopy confirmation of the produced bioethanol

The IR spectrum of the produced bioethanol is shown in Figure 3. The IR spectrum reveals the presence of the following functional groups with frequencies: C-O (1043.7 and 1084.7 cm^{-1}), C-H (2903.6 and 2981.9 cm^{-1}) and O-H (3347.1 cm^{-1}) which is a confirmation of the broad and intense O-H peak in the 3650-3200 cm^{-1} region while that of the C-O stretch is seem in the region of 1300-1000 cm^{-1} and C-H stretch observed in the (2800-3000 cm^{-1}) region.

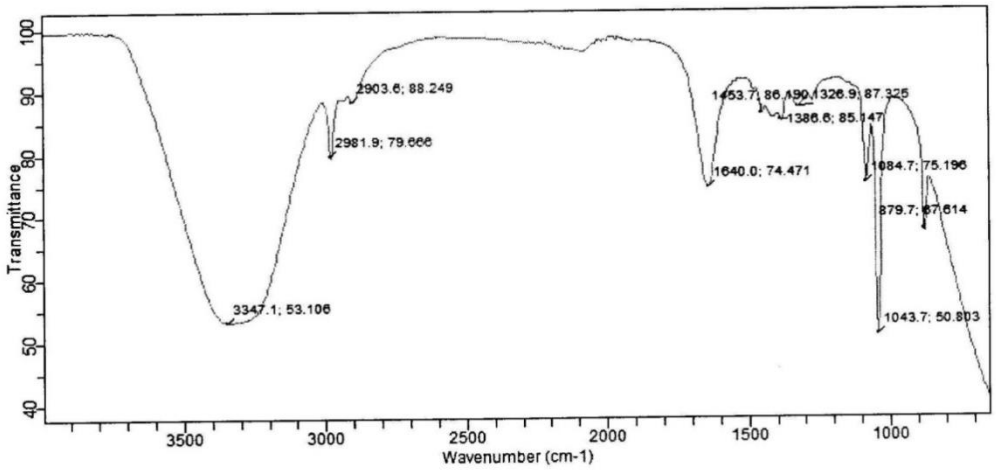

Figure 3 Infrared spectrum of bioethanol produced from the *Solanumlycopersicum*stalk

4. Conclusion

Tomato (*Solanum lycopersicum*) is a major food crop in Nigeria used in virtually all food preparations. The stalk of this crop has majorly no beneficial application but its stalk known for the accumulation of cellulosic biomass waste in agricultural practices. This study shows that *Solanum lycopersicum* stalks could serve as novel material for the production of ethanol since the result of the fermentation using *S. cerevisiae* and *Z. mobilis* individually as well as the co-culture fermentation using the combination of *S. cerevisiae and Z. mobilis* to produce ethanol is very encouraging and an attractive alternative technology for the production of biofuels specifically bioethanol. There is still need thou to develop

a more efficient and economic pretreatment process and a hyper-cellulase producing strains of microorganisms for the improved saccharification capable of utilizing both pentose and hexose sugars which in turn would increase ethanol production.

Compliance with ethical standards

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Disclosure of conflict of interest

We the Authors of this research work have no conflict of interest and state that we are solely responsible for all the contents and writings presented in this research work.

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