

Full Length Research Paper

Production of bioethanol from guinea cornhusk and millet husk

Oyeleke, S. B.* and Jibrin, N. M.

Department of Microbiology, Federal University of Technology, Minna, Niger State, Nigeria.

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The use of guinea corn husk and millet husk (agricultural waste with no appreciable value to industries or competitive use as food) as alternative and cost-effective feed stock for the production of bioethanol was examined. The methods used, included: acid hydrolysis with 2.5 M H₂SO₄, and simultaneous saccharification and fermentation with *Aspergillus niger* and *Zymomonas mobilis* isolated from soil and palm wine, respectively. Ethanol yield from guinea corn husk (26.83 g/l) and millet husk (18.31 g/l) was maximum at 120th h and with ethanol concentrations of 67.7 and 63.8%, respectively. The least ethanol concentration of 30% was obtained with *A. niger* on millet husk. *A. niger* and *Z. mobilis* may be better organisms for ethanol production from Guinea corn husk and millet husk.

Key words: Guinea corn husk, millet husk, hydrolysis, saccharification, fermentation, distillation.

INTRODUCTION

Bioethanol is a renewable energy source produced mainly by the sugar fermentation process; although it can also be synthesized by chemical processes such as reacting ethylene with steam (Anuj et al., 2007). Ethanol fuel blends are widely sold in the United States of America. The most common blend is 10% ethanol and 90% petrol (E10). Vehicle engines require no modification to run on E10 and vehicle warranties are not affected. Only flexible fuel vehicles can run on up to 85% ethanol and 15% petrol blends (E85) (Tanaka, 2006).

The natural energy resources such as fossil fuel petroleum and coal are being utilized at a rapid rate and these resources have been estimated to last only a few years. Therefore, alternative energy sources such as ethanol, methane and hydrogen are being considered. Some biological processes have rendered possible routes for producing ethanol and methane in large quantities. A worldwide interest in the utilization of bioethanol as an energy source has stimulated studies on the cost and efficiency of industrial processes for ethanol production (Tanaka, 2006).

Human activities generate large amounts of waste such as crop residues, solid waste from mines and municipal waste. They may become a nuisance and sources of pollution. It is therefore important to handle them judiciously to avoid health problems, since these wastes may harbour pathogenic microorganisms (Ledward et al., 2003).

Agricultural wastes, including wood, herbaceous plants, crops and forest residues, as well as animal wastes are potentially huge source of energy. In Nigeria, large quantities of these wastes are generated annually and are vastly underutilized. The practice is usually to burn them or leave them to decompose. However, studies have shown that these residues could be processed into liquid fuel such as biogas and bioethanol, or combusted to produce electricity and heat (Soltes, 2000). Ethanol production processes only use energy from renewable sources and there is no net CO₂ emission to the atmosphere, thus making ethanol an environmentally beneficial energy source. In addition, ethanol derived from biomass is the only liquid transportation fuel that does not contribute to the green house gas effect. This reduction of green house gas emission is the main advantage of utilizing biomass conversion into ethanol (Anuj et al., 2007).

Traditionally, ethanol has been produced in batch fermentation with fungal strains such as *Aspergillus niger*, *Mucor mucedo* and *Saccharomyces cerevisiae*, which can not tolerate high concentrations of ethanol. Therefore, improvement programmes are required in order to obtain alcohol-tolerant strains for fermentation (Gunasekaran and Chandra, 2007). *Zymomonas mobilis*, a Gram-negative bacterium, is considered an alternative organism in large scale ethanol production. Its advantages

*Corresponding author. E-mail: droyeleke@yahoo.com.

over yeasts include higher sugar uptake and ethanol yield, lower biomass production and higher ethanol tolerance. The only limitation of *Z. mobilis*, compared to the yeasts, is that its utilizable substrate range is restricted to glucose, fructose and sucrose. This organism can be isolated from palm wine or rotten oranges (Gunasekaran and Chandra, 2007). Several agricultural wastes have been tested for their bioethanol-producing potential. In the present study, the utilization of some agricultural residues (guinea corn husk and millet husk) for the production of bioethanol was evaluated. The objectives of the study were to produce bioethanol from guinea corn husk and millet husk residues through fermentation using *A. niger* and *Z. mobilis*; to compare guinea corn husk and millet husk for their bioethanol producing potential; and to optimize ethanol production from guinea corn and millet husk through hydrolysis.

MATERIALS AND METHODS

Collection and processing of samples

Guinea corn husk and millet husk were collected from waste dumping sites in Minna metropolis. The samples were dried and ground to a powder form using a Waring blender (Binatone).

Isolation and characterization of microorganisms

A. niger was isolated from soil and identified in the microbiology laboratory of the Federal University of Technology Minna, following standard procedures described by Cheesbrough (2003) and Oyeleke and Manga (2008). The isolates were identified by comparing their characteristics with those of known taxa using the schemes of Domsch and Gams (1970).

Z. mobilis was isolated from palm wine using standard solid medium (5 g/l yeast extract, 20 g/l glucose, 20 g/l agar; pH 6.8), as described by Obire (2005). The medium was supplemented with actidione (cycloheximide) to inhibit yeast growth. One ml (1 ml) of palm wine was serially diluted in sterile distilled deionised water and aliquots of the dilutions were aseptically plated onto the medium using the pour plate technique. The agar plates were incubated at 37°C in an anaerobic jar for 24 - 48 h. After incubation, the bacterial colonies that grew on the agar medium were counted using a colony counter and expressed as colony forming units (cfu)/ml of sample. Colonies differing in size, shape and colour were selected from different agar plates and subcultured on standard solid medium by the streak plate technique. The agar plates were and incubated at 37°C in an anaerobic jar for 24 h. The subsequent pure cultures were maintained on agar slant for further characterization and identification. The bacterial isolates were characterized based on colonial morphology, cultural characteristics and biochemical tests as described by Cheesbrough (2003) and Oyeleke and Manga (2008). Biochemical tests that were performed on the bacterial isolates included: Gram staining, catalase test, oxidase test, urease test, motility test, carbohydrate fermentation test, indole test and coagulase test. *Z. mobilis* was identified by comparing the characteristics of the isolates with those of known taxa using Bergey's Manual of Determinative Bacteriology (Holt et al., 1994; Obire, 2005).

Bioethanol production

Methods used for production of bioethanol include hydrolysis, fermentation and fractional distillation.

Hydrolysis

One hundred grams (100 g) of guinea corn husk was weighed into seven 2-l conical flasks, and 1 l of 2 M H₂ SO₄ was added to each conical flask. The flasks were covered with cotton wool, wrapped in aluminum foil, heated for 2 h in a water bath and then autoclaved for 30 min at 121°C. The flasks were allowed to cool, filtered through No. 1 Whatman filter paper and the pH was adjusted to 4.5 with 0.4 M NaOH. The same procedure was repeated for millet husk.

Fermentation

The fermentation was carried out along with saccharification (simultaneous saccharification and fermentation [SSF]), as described by Kroumov et al. (2006) and Oghgren et al. (2006). The flasks containing the hydrolyzed samples were covered with cotton wool, wrapped in aluminium foil, autoclaved for 15 min at 121°C, and allowed to cool at room temperature. *Z. mobilis* and a spore suspension of *A. niger* were aseptically inoculated into each flask and incubated at 30°C. Two flasks of each sample (guinea corn husk and millet husk) were removed after every 24 h, up to 7 days.

Fractional distillation

The fermented broth was dispensed into round-bottom flasks fixed to a distillation column enclosed in running tap water. 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°C was used to heat the round-bottomed flask containing the fermented broth.

Determination of quantity of ethanol produced

The distillate collected over a slow heat at 78°C was measured using a measuring cylinder, and expressed as the quantity of ethanol produced in g/l by multiplying the volume of distillate collected at 78°C by the density of ethanol (0.8033 g/ml). g/l is equivalent to the yield of 100 g of dried substrate (Humphrey and Okafogau, 2007).

Determination of percentage ethanol concentration

A standard ethanol density curve was prepared by taking series of percentage (v/v) ethanol solutions, which were prepared in volumetric flasks, and the weight was measured. The density for each of the prepared ethanol solutions was calculated and a standard curve of density against percentage ethanol was plotted. The percentage ethanol concentration of ethanol produced was obtained by comparing its density with the standard ethanol density curve.

RESULTS

Cultural, morphological and biochemical characteristics of isolates

The organisms used for fermentation were *A. niger* and *Z. mobilis*. *A. niger* showed a black mycelium on the agar medium, it had septate hyphae, long and smooth conidiospores, and long unbranched sporangiospores with a large and round head. *Z. mobilis* was found to be Gram-negative short rod, catalase-positive, oxidase- and urease-negative, motile and hetero-fermentative, producing gas from glucose, fructose and sucrose. Maltose and arabinose were not fermented.

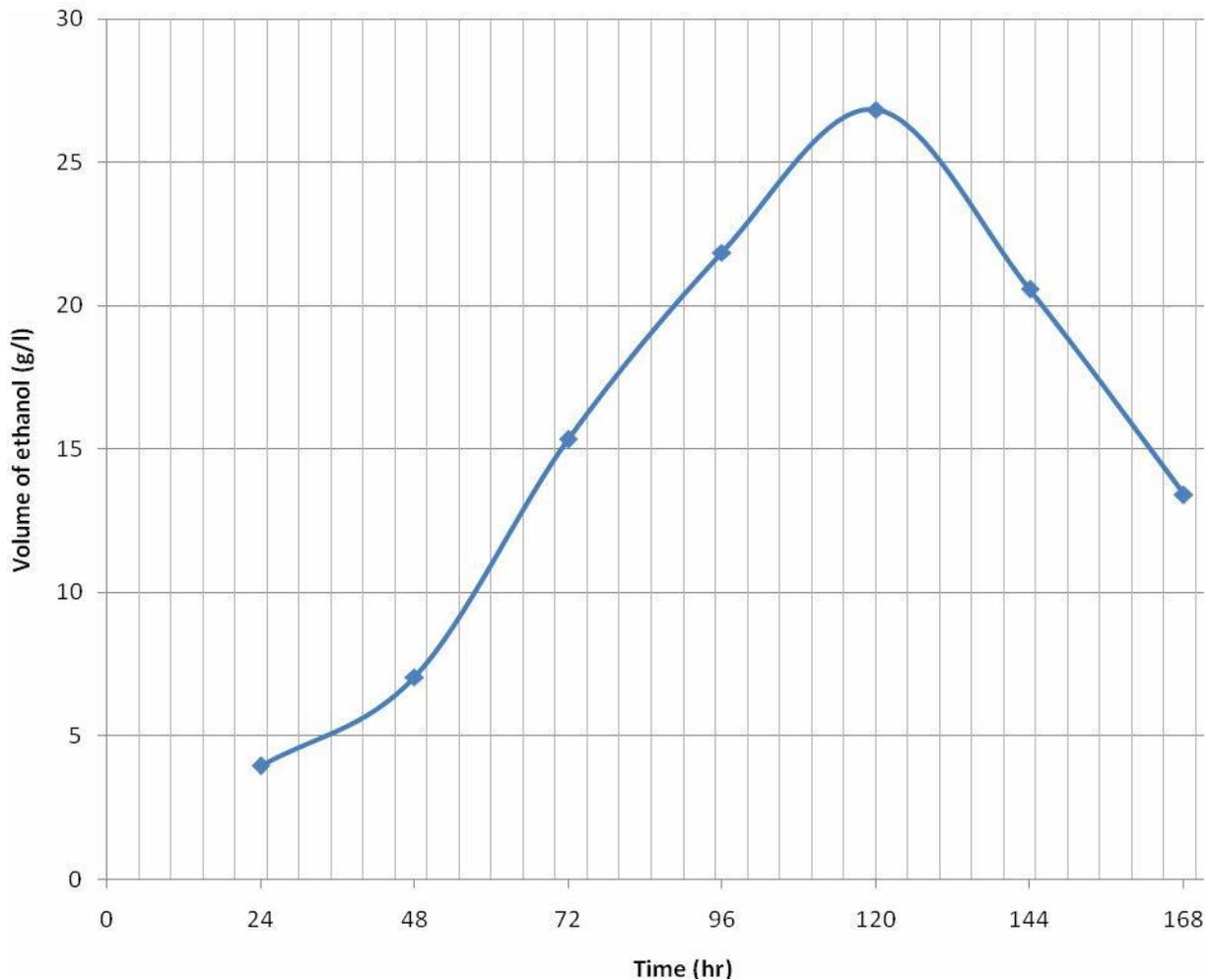


Figure 1. Ethanol produced (g/l) from Guinea corn husk using *A. niger* and *Z. mobilis* simultaneously.

Ethanol produced from guinea corn husk using *A. niger* and *Z. mobilis* simultaneously

Figure 1 shows the volume (g/l) of ethanol produced from guinea corn husk after acid hydrolysis with 2.5 M H₂SO₄ and SSF with both *A. niger* and *Z. mobilis*. The highest volume (26.83 g/l) was produced at 120th h of fermentation, followed by 21.85 g/l at 96th h. The lowest volume (3.94 g/l) was produced at 24th h.

Ethanol produced from millet husk using *A. niger* and *Z. mobilis* simultaneously

Figure 2 shows the volume (g/l) of ethanol produced from millet husk after acid hydrolysis with 2.5 M H₂SO₄, and SSF with both *A. niger* and *Z. mobilis*. The highest volume of ethanol (18.31 g/l) was produced at 120th h of fermentation, followed by 14.94 g/l at 96th h. The lowest volume of ethanol was produced at 24th h.

Ethanol produced from guinea corn husk using *A. niger* and *Z. mobilis* separately

Figure 3 shows the volume of ethanol produced from guinea corn husk after acid hydrolysis with 2.5 M H₂SO₄, and separate fermentation with *A. niger* and *Z. mobilis*. The highest yield of 20.32 g/l was observed at 120th h with *Z. mobilis*. On the other hand, in the case of *A. niger*, the highest yield of 18.23 g/l was observed.

Ethanol produced from millet husk using *A. niger* and *Z. mobilis* separately

Figure 4 shows the volume of ethanol produced from millet husk after acid hydrolysis with 2.5 M H₂SO₄, and separate fermentation with *A. niger* and *Z. mobilis*. The highest yield of 14.10 g/l was observed at 120th h with *Z. mobilis*. In the case of *A. niger*, the highest yield of 10.24 g/l was observed. In both cases, either using guinea corn

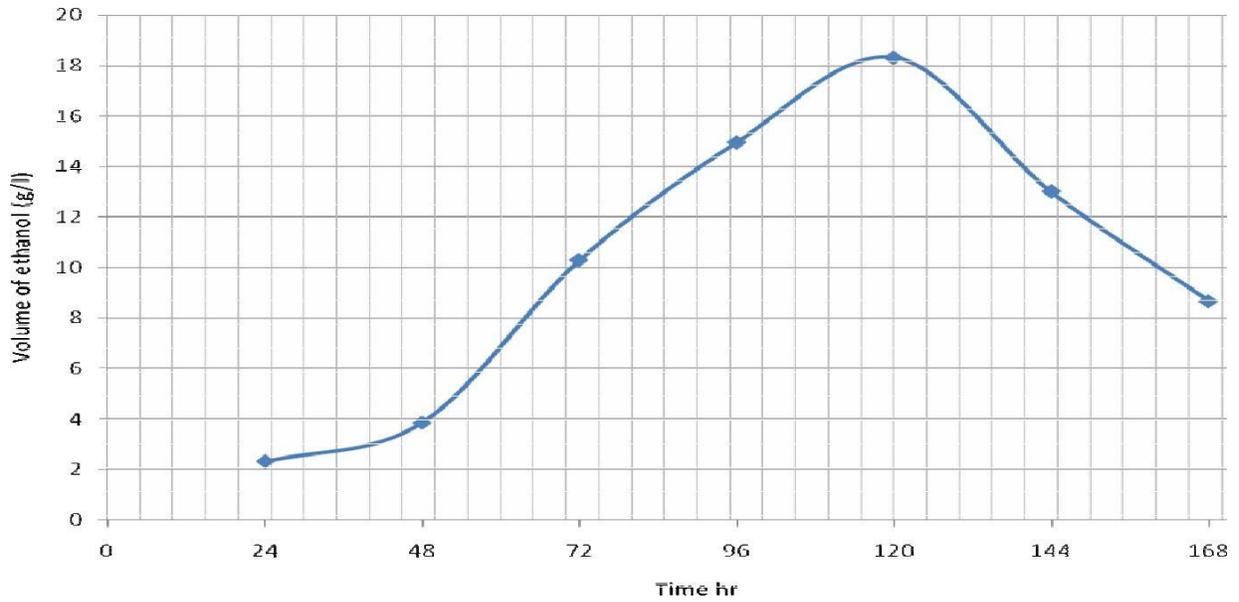


Figure 2. Ethanol produced (g/l) from millet husk using *A. niger* and *Z. mobilis* simultaneously.

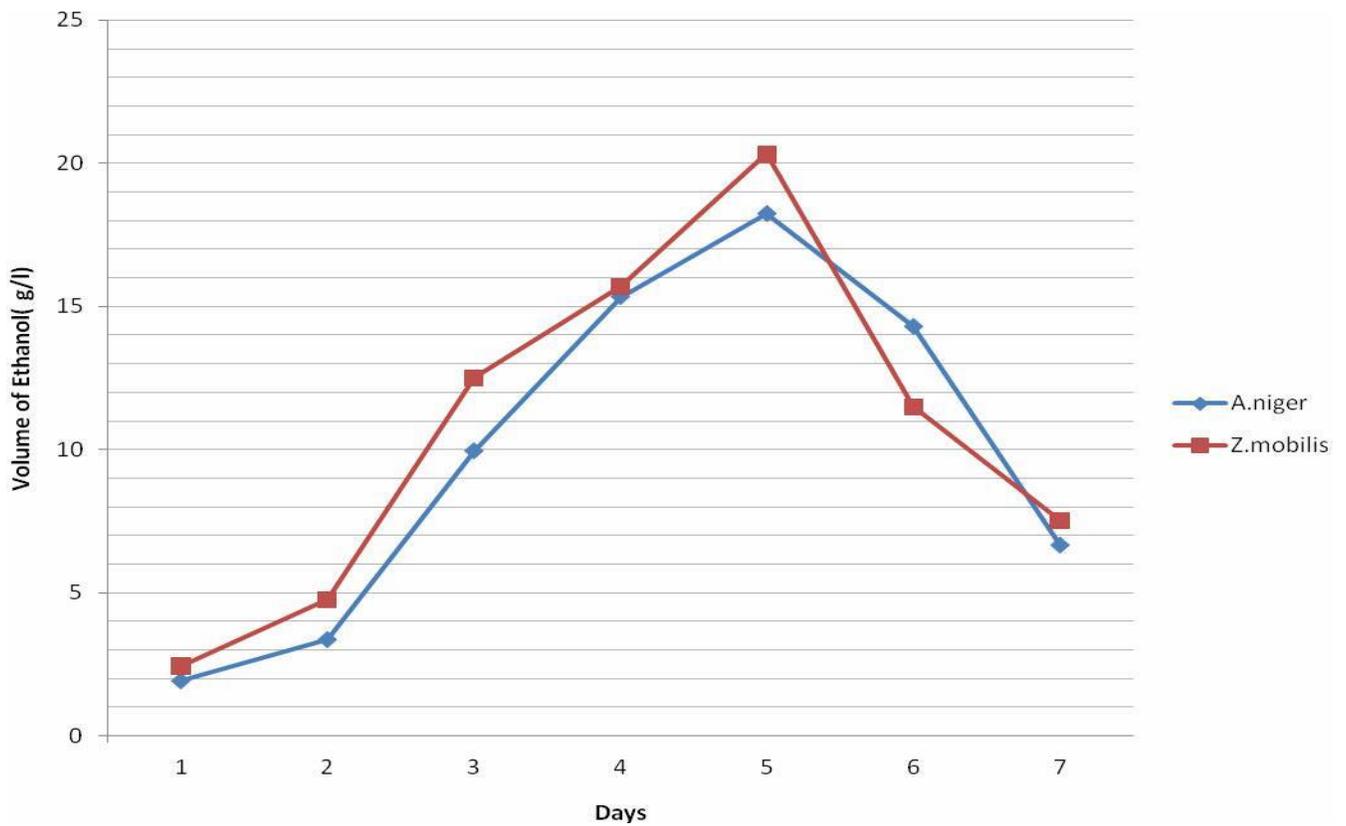


Figure 3. Ethanol produced from Guinea corn husk using *A. niger* and *Z. mobilis* separately.

husk or millet husk, the volume of ethanol produced at each fermentation time examined using *Z. mobilis* was higher compared to that using *A. niger*.

Percentage ethanol concentration

Table 1 shows the percentage ethanol concentration. The

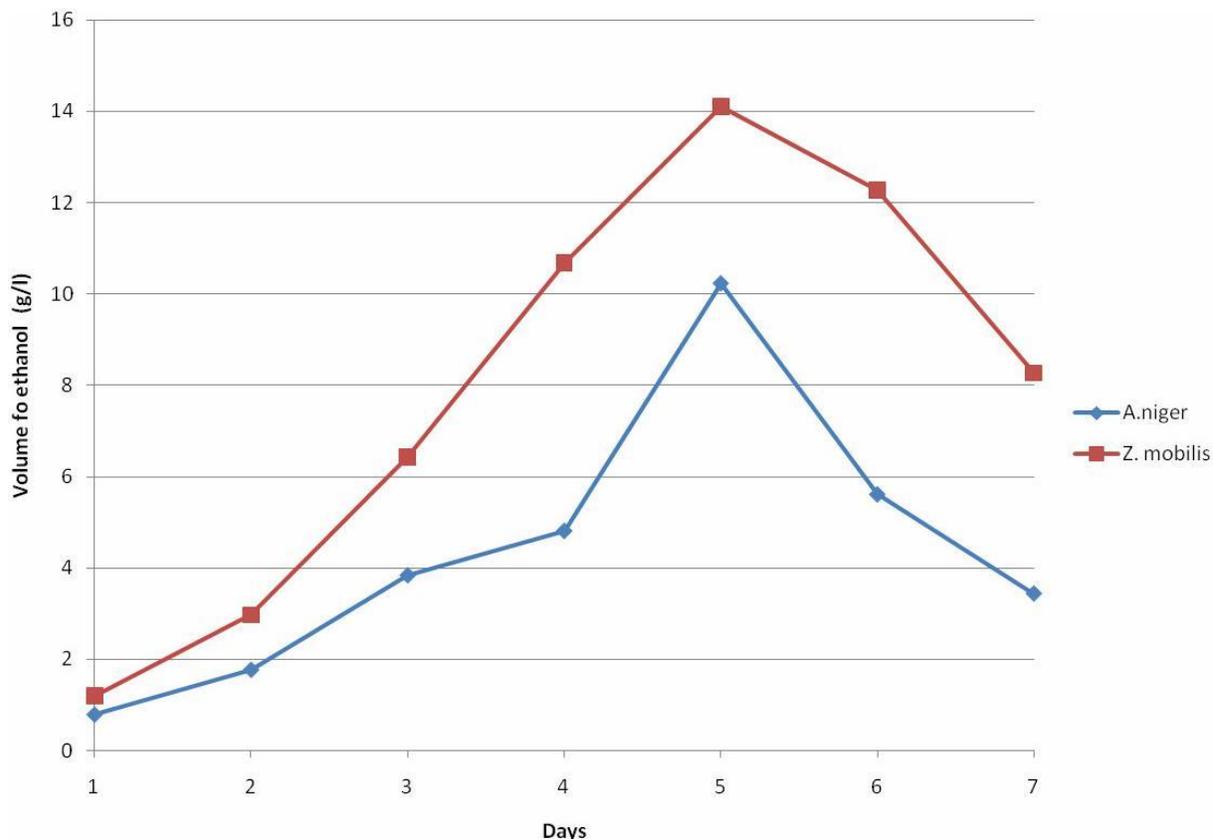


Figure 4. Ethanol produced from millet husk using *A. niger* and *Z. mobilis* separately.

Table 1. Ethanol concentration.

Microorganisms	Density		Ethanol concentration (%)	
	Guinea corn	Millet husk	Guinea corn husk	Millet husk
<i>A. niger</i> + <i>Z. mobilis</i>	0.905	0.907	67.7	63.8
<i>Z. mobilis</i>	0.927	0.916	42.0	49.9
<i>A. niger</i>	0.933	0.943	38.0	30.0

highest concentrations of 67.7 and 63.8% were obtained using both microorganisms simultaneously to ferment guinea corn husk and millet husk, respectively. The lowest concentration (30%) was obtained with *A. niger* on millet husk.

DISCUSSION

Guinea corn husk and millet husk were used to produce ethanol through acid hydrolysis, and SSF with *A. niger* and *Z. mobilis*. In SSF, the two different microorganisms behaved differently, according to their nutrient requirements, but synergistically in the degradation of organic substrate. *A. niger* was capable of producing starch / carbohydrate hydrolases from saccharified guinea corn husk and millet husk. The saccharification products were simultaneously utilized by *Z. mobilis* for ethanol produc-

tion. *Z. mobilis* is able to produce ethanol due to the presence of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH), which are key enzymes in ethanol formation, as reported by Gunasekaran and Chandra (2007). It was also stated by the authors that the ADH of *Z. mobilis* appears to facilitate continuation of fermentation at high concentration of ethanol. The maximum volume of ethanol (27.10 g/l) produced from guinea corn husk and millet husk (18.24 g/l) in this study at the 120th h is in agreement with Agulejika et al. (2005) who also reported maximum ethanol yield at 120th h from fresh fruit (64.01 g/l) and waste fruits (21.14 g/l) using *Z. mobilis*. The higher ethanol yield from fresh fruit was due to higher presence of fructose and glucose in fresh fruits, as stated by Micheal and Rosaline (2000). The maximum volume of ethanol (27.10 g/l) produced from guinea corn husk in this study is in agreement with that (27.7 g/l) reported by

Lekneth et al. (1994) on sweet sorghum. The maximum volume of ethanol produced from guinea corn husk (27.10 g/l) and from millet husk (18.24 g/l) are both lower than the 59 g/l reported by Gunasekran and Chandra (2007) at 120th h from cassava starch hydrolysate. This is due to cassava containing more carbohydrates, which could be fermented to ethanol. The volumes of ethanol generated from guinea corn husk were higher than the volumes generated from millet husk. This could be due to presence of more carbohydrates in guinea corn husk compared to millet husk, which could be fermented to ethanol. The highest ethanol concentration of 67.7% and 63.8% were observed when *A. niger* and *Z. mobilis* were used simultaneously on guinea corn husk and millet husk, respectively. However when both organisms were used separately on the Guinea corn husk and millet husk, *Z. mobilis* generated more ethanol in each case. The percentage concentration of ethanol generated with *Z. mobilis* was also higher than that of *A. niger*. These may be due to presence of alcohol dehydrogenase (ADH) in *Z. mobilis*, which appears to facilitate fermentation at high ethanol concentration, as reported by Gunasekran and Chandra (2007).

The results revealed that ethanol could be produced from agricultural residues, such as guinea corn husk and millet husk, using *Z. mobilis* and *A. niger* as fermenting organisms. Considering the cost-effectiveness, in addition to being a means to control environmental pollution, the use of guinea corn husk and millet husk for ethanol production is concluded as a worthwhile venture.

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