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Bioethanol Production from Elephant Grass (*Pennisetum purpureum*)

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Abstract

The problem of global warming has been undeniably accepted worldwide. One of the causes of this situation is the increase in the greenhouse gases. This study was carried out to produce bio-ethanol from elephant grass (*Pennisetun purpureum*). The grass was sun dried, milled and pretreated by steam explosion method at 160°C. Seven sets of 350 g of the sample was mixed with 350 ml of water boiled at 100°C and left to stand for 24 hours, after which it was sieved. Sets of 25 ml extract was decanted into reactor bottles and autoclaved at 121°C, at 15 psi for 15 mins. Five milliliter (5 ml) of *Saccharomyces cerevisiae* of 0.98 optical density and 5 ml of *Aspergillus* spp., was then introduced into the reactors. The reactor bottles were corked properly, sealed with aluminum foil and rocked at room temperature for 6 days. Samples were pooled and taken for distillation at 24 hours intervals. Ethanol yield increased from day one to sixth day, which had ethanol yield of 9 ml from 25ml of plant extract while the control had 0.5 ml ethanol yield on day 6. The pH of the medium decreased from 6.50 to 6.00 for the reactor bottle containing microbial inoculants and the control had a pH of 5.53 to 5.85 while the temperature of the reactor bottle containing the microbial inoculants increased from 25°C to 30°C and the control had a temperature of 24°C and 28°C. The optimized condition for ethanol production was established at pH 6.0 and temperature 30°C. Under these conditions, an ethanol yield of 9 ml was obtained from 25 ml of plant extract. The control experimental setup showed low ethanol yield of 0.5 ml of 25 ml of plant extract.

Keywords: Elephant grass, bioethanol, reactor, *Sacharromyces cerevisiae*

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Introduction

Bioethanol is an alcohol produced by fermenting the sugar components of renewable plant biomass (Keith, 2009). It is made mostly from sugar and starch crops such as sugar cane and corn among others. Bioethanol feedstock can contain either sucrose, starch or a lignocellulosic material (Macedo et al., 2008). The chemical properties of lignocelluloses components make them of enormous biotechnological values for the production of affordable fuel ethanol. Also, it is less expensive than starch and sugar crops and is also renewable and available in large quantities (Aiyejagbara, 2015). Cellulose is the major polymeric component of plant material and is the most abundant polysaccharide on earth (Liming and Xueling, 2004). It is one of the three major components of all plant cell walls with two other components - hemicelluloses and

lignin. The digestive system of man and most other animals (except ruminants) do not contain the necessary enzymes (cellulase) for hydrolyzing β – glycosidic linkages. However, cellulases are found in ruminants, various insect, fungi, bacteria and algae (Lee, 1992).

Cellulose exists in a number of crystalline and amorphous topologies. Its insolubility and heterogeneity make native cellulose a recalcitrant substrate for enzymatic hydrolysis. Microorganisms meet this challenge with the aid of a multi-enzymatic system. Specific enzymes act in synergy to elicit effective hydrolysis (Lee and Fan, 1982). Many fungi are capable of producing extra-cellular enzymes that can degrade cellulose. They are *Trichoderma viride, Aspergillus niger* and *Aspergillus flavus*. Bacteria such as *Clostridium thermacellum* can also produce cellulases (Lee, 1992).

Currently, serious attention has been diverted from non-renewable fossil fuels towards renewable, bio-degradable and non-polluting bio-fuels. This has ignited tremendous research in the field of bio-energy crops all over the world such as Brazil, the United States, European Union (EU), Asia, Australia and Africa where there has been a shift of dependency on fossil fuels to bio-fuels due to inflating oil prices. The global biofuel production tripled during 2000 to 2007, although it accounted for less than 3% of global transportation fuel supply (Coyle, 2007 and Mohanty et al., 2013). Due to the non-renewable nature of fossil fuels and various environmental problems associated with their exploration, production and use, a lot of efforts were made to develop renewable and environmentally friendly bio-energies (Ogbonna et al., 2001; Zhang et al., 2003).

Among the various bio-energies, fuel ethanol is already commercially produced in many countries where it is used as octane enhancer, blended with gasoline in various ratios to produce gasohol or used directly in specially designed ethanol engines. Gasohol containing less than 10% ethanol (E10) can be used in most engines without modifications. A major advantage of bio-ethanol is that the feedstock (agricultural materials) is varied, renewable and can be produced in many places. Most African countries have large areas of fertile land that is not currently used for production of food crops (World Bank Report, 2013). The utilization of energy crops as a source of renewable fuels is a concept with great relevance to current ecological and economic issues at both national and global scales. Crops that are currently being adapted for bio-energy are limited because of their potential role as food for human or animal or animal consumption (Mohanty et al., 2013). Energy derived from renewable substrates possesses a number of advantages over fossil derived energy. These include environmental friendliness and profitability (Tran et al., 2011).

Owing to depleting reserves and competing industrial needs of petrochemical feedstocks, there is global emphasis in ethanol production by microbial fermentation process. Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology (Ali et al., 2014). At present, the problem of global warming has been undeniably accepted worldwide. The cause of this situation is the increase in the greenhouse gases, therefore the present study was undertaken to utilize lignocellulosic biomass (elephant grass – *Pennisetum purpureum*) for bioethanol production. The choice of elephant grass as feedstock was made because it is widely distributed in Africa and production of bioethanol from it, if achieved, would be sustainable.

Materials and Method

Sample Collection/Preparation

Plate1a: Elephant grass

Plate1b: Elephant grass

Plate 2: Sun dried grass

Plate 3: Dry milled grass

Biomass Pre-treatment by steam explosion.

The biomass was treated with high pressure saturated steam by autoclaving at 121^{o}C, 15psi for 15 mins followed by a sudden increase in temperature to 160^{o}C for 5 mins. The pressure was suddenly reduced, leading to explosive decompression of the material and subsequent increase in enzyme accessibility.

Extraction/ Sterilization

Dry milled grass (350 g) was homogenized with 350 ml of 100°C boiled water and allowed to stand for 24 hrs. The liquid was then extracted from the grass using clean muslin cloth and the extract was heat sterilized.

Microorganisms

Aspergillus spp. and *Saccharomyces cerevisiae* were isolated from soil and palm wine, respectively, and maintained on Potato Dextrose agar.

Media Preparation

Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth were prepared according to manufacturer's instruction.

Development of inoculants

Aspergillus spp. and yeast inoculum **(***Saccharomyces cerevisae*) were developed and scaled up according to the methods of Ogbonna et. al. (2013).

Setting up of fermentation

The fermentative production of bioethanol was carried out by simultaneous saccharification and fermentation with shaking. The pre-treated substrates were used for all the experiments. Substrate (25 ml), decanted into each bioreactor bottle, was autoclaved at 121^{o}C/15psi for 15 mins. Fermentation process was carried out according to Ali et. al. (2014).

Simultaneous saccharification and fermentative production of bioethanol by Saccharomyces cerevisiae and Aspergillus spp.

Co-culture of yeast (*Saccharomyces cerevisiae*) and *Aspergillus* spp. was employed. *Saccharomyces cerevisiae* culture (5 ml, 0.87 optical density) and 5 ml of 72 hr old *Aspergillus* spp. were added into 25 ml crude plant extract, sealed with aluminum foil, placed on a shaker at room temperature for 6 days. Twenty four-hour samples were withdrawn from the culture broth and taken for the estimation of bioethanol produced according to the methods of Ogbonna and Okoli (2010).

Physicochemical analysis

Temperature determination

The temperature range was monitored by inserting a Unicon® thermometer.

pH range

The pH was determined using a labtech digital pH meter (Photic 20®).

Results and Discussion

The production of ethanol from elephant grass by the efficient lignocellulolytic enzyme system of *S. cerevisae* and *Aspergillus* spp. is essential for the bioconversion of elephant grass to economic important product (ethanol). The optimization of the bioconversion of *Pennisetum purpureum* took into consideration the pH, temperature and the carbon-rich grass growth substrate. The ethanol yield for the various days increased from Day 0 which had no ethanol yield to Day 6 with ethanol yield of 9 ml from 25 ml plant extract (Fig. 2). The control had no ethanol yield on Day 0 and 0.5 ml on Day 6.

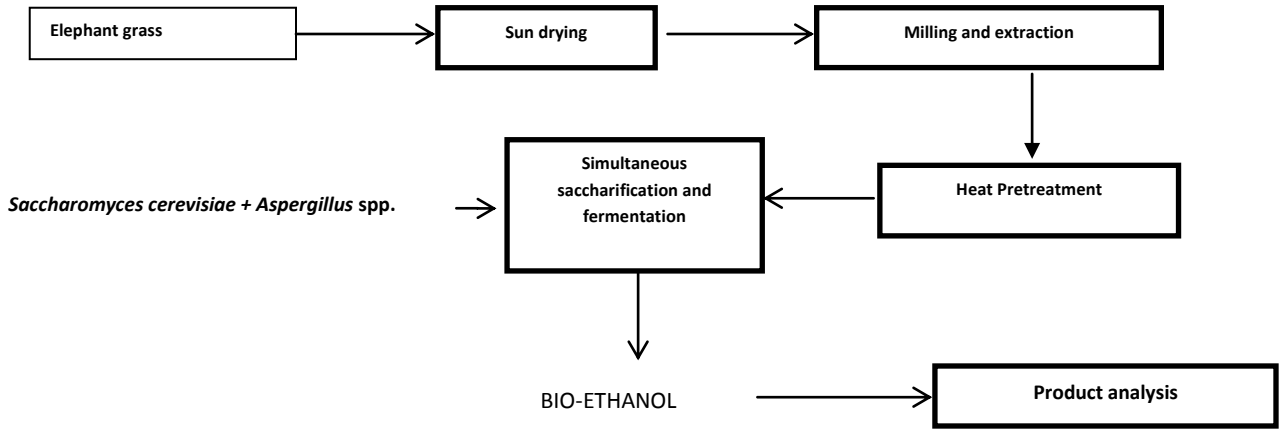

Fig 1: Schematics for production of ethanol from *Pennisetum purpureum*.

A maximum temperature of 30°C was achieved between Day 5 and 6 as shown in Fig. 3. The temperature for optimum ethanol yield 30°C at the sixth day while the control also showed an increase in temperature ranging from 25°C to 28°C. The quantity of ethanol produced was maximum at pH 6 (Fig. 4). This is due to the fact that *Aspergillus* spp. and *Saccharomyces cerevisiae* function best in an environment with pH 6 (Berg, 2007).

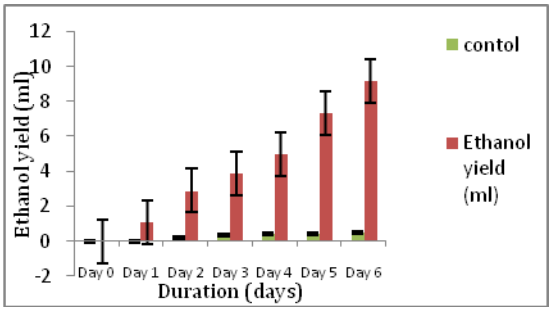

Fig.2. Ethanol yield from *Pennisetum*

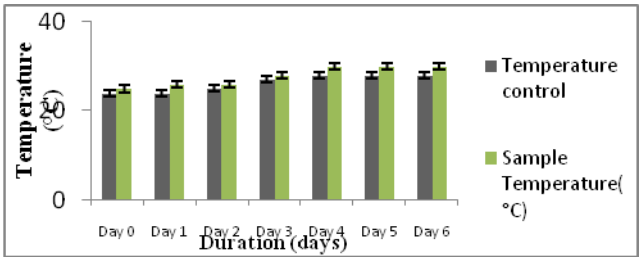

Fig.3 Temperature range for ethanol production

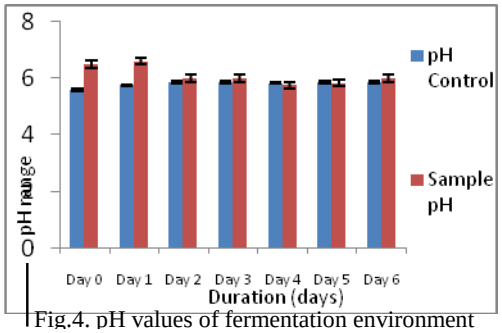

Fig.4. pH values of fermentation environment

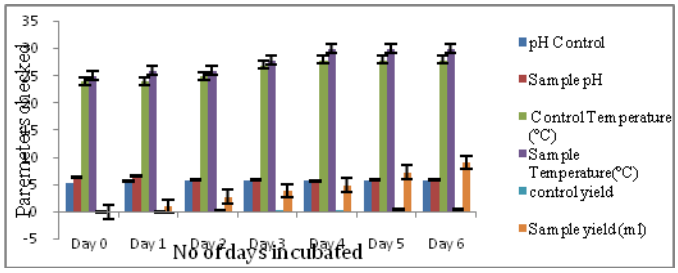

Fig.5 Physicochemical indices during the fermentation process

Researchers had experimented on various raw materials and different fermentation methods for bioethanol production; but recently, attention has become focused on lignocellulosic materials for bioethanol production. Cellulosic substrates were used by Arthe et al. (2008) for bioethanol production through microbial extracellular enzymatic hydrolysis and fermentation with an ethanol yield of 8.9 g/l. In another report, enzymatically pre-treated agricultural residues were inoculated with

different fungal cultures by Seema et al. (2007) for ethanol production. In our study, cheap and abundant plant materials were used for bioethanol production. The results obtained were similar to those of Arthe et al. (2008) and Seema et al. (2007). Cellulolytic activity and ethanol yields were low in the flasks where substrate alone was available (control reactors).

Elephant grass (*Pennisetum purpureum*) is, therefore, a suitable raw material for the production of bioethanol, using *Saccharomyces cerevisae* and *Aspergillus* spp. as substrate fermenter. It is recommended that Elephant grass which is an abundant, renewable and cheap source of feedstock be used for industrial production of ethanol.

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