OBAFEMI AWOLOWO UNIVERSITY

STUDIES INTO THE PRODUCTION OF BIOETHANOL FROM CORNCOB USING CRUDE CELLULASE ENZYME AND DILUTE ACID

BY

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This is to certify that AGBONJINMI Omowumi Sarah executed this research work in partial fulfilment of the requirements for the award of Master of Science Degree in Agricultural and Environmental Engineering, Faculty of Technology, Obafemi Awolowo University, Ile-Ife.

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DEDICATION

I dedicate this work to the almighty God and my family.

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All glory, honour and adoration be to the Lord Jesus Christ , the author and finisher of my faith, the creator of the heavens and earth, my all in all, the lifter of my head, my father, the closest friend anyone could ask for.

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ABSTRACT

This study investigated the production of bioethanol from corncob using crude cellulase enzyme and dilute tetraoxosulphate (VI) acid. The conversion of the cellulosic content of corncob to glucose and the fermentation of the hydrolysates to bioethanol was also studied. This is with the view to converting this abundant agricultural waste into value-added product.

The dried corncob was pretreated mechanically by milling with a hammer mill, sieved with a screen of 1mm, then chemically pretreated with sodium hydroxide in concentrations ranging from (1- 2) %v/v. The enzymatic hydrolysis was carried out on a portion of the substrate with the use of crude cellulase enzyme in liquid form which was isolated from corncob-decomposing bacterium (*Bacillus pantothenticus*) by mixing with the pretreated biomass and thoroughly agitated. The acid hydrolysis was done by adding the dilute tetraoxosulphate (VI) acid to the pretreated substrate, then thoroughly agitated and heated. The concentration of the glucose yields obtained during hydrolysis was determined using the Dinitrosalicylic acid (DNS) method. Fermentation of the glucose was done with the use of fermenting organism *Saccharomyces cerevisaie* (Brewers' yeast). Shake culture experiment was carried out on the glucose for the ethanol production by introducing 5 % innoculum size into each glucose sample. Acid dichromate method was used in determining the bioethanol concentration. The study was carried out as a fractional factorial design with temperature A (50, 60, 70 ^{o}C), NaOH concentration B (1, 1.5, 2 %v/v) and H_2SO_4 concentration C (1.5, 2, 2.5 mol/dm^3) as factors that generated twenty experimental runs. Response Surface Methodology (RSM) was used for the analytical and optimization studies using the Central Composite Design (CCD).

Results obtained showed that the factors A, B and C are significant model terms ($p<0.05$). in the production of glucose and ethanol from acid hydrolysis. The highest glucose obtained during acid hydrolysis was 1467.78 g/L at 60 ^oC temperature, 1.5%v/v NaOH concentration and 2.5 mol/dm^3 H_2SO_4 while the lowest glucose yield obtained was 141.43 g/L at 70 ^oC temperature, 1.5%v/v NaOH concentration and 2.00 mol/dm^3 H_2SO_4 concentration. The highest and lowest ethanol yield obtained from fermentation of acid hydrolysates was 45.34 g/L and 30 g/L respectively. The optimum condition for the ethanol production from acid hydrolysates was found to be temperature (69.74 ^oC), NaOH concentration (2.00%v/v) and H_2SO_4 concentration (2.50 mol/dm^3). The highest and lowest glucose obtained during enzymatic hydrolysis was 253.99 g/L and 23.23 g/L respectively while the highest and lowest bioethanol yields were 91.81 g/L and 54.52 g/L. The optimum conditions for the highest bioethanol yields from enzymatic hydrolysates are 1 ^oC temperature, 1.5% v/v NaOH concentration.

It can be concluded from this study that crude cellulase enzyme is more effective and cheap in converting corncob to bioethanol than dilute tetraoxosulphate (VI) acid.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Farm waste materials are classified into two broad groups namely livestock wastes and plant waste materials. Most of these farm wastes are biodegradable and some had already been discovered as suitable materials for biogas production through research (Adelekan and Bamgboye, 2009). However, large quantities of this agricultural residue produced in Nigeria and other developing countries are still underutilized and not properly managed. The current management practice of these wastes is usually that of burning or leaving the wastes on the farm to decompose contributing to land and air pollution. Agricultural by-products such as rice husk, corncob, cornstalk, palm kernel shell and many others are generated in very large quantities in Nigeria and other agrarian countries annually (Onuegbu *et al*., 2012).

Corn is so proficient at supplying essential calories that they are considered the quintessential subsistent crops. However, the success of this starch crop as staple food limits its potential development as renewable energy source For instance, carbohydrate crops such as corn and cassava which has become an important feedstock in biofuel production, may to a large extent have fuelled the current food crises worldwide by direct competition with food supply and arable land (Datar *et al.*, 2007; Srinorakutara *et al*., 2008; Akponah and Akpomie, 2011). It therefore becomes imperative that the searchlight be turned at present to the use of non-food starchy items for the production of bioethanol. Corn is one of the most abundant cereal produced in Africa amongst all the staple food crops grown, as a result, corncob, a renewable resource of lignocellulosic biomass (Caye *et al*., 2008) from agricultural processing; represents about 30% of agro-wastes (Asante 2004; Kumar *et al.*, 2008; Zakpaa, *et al*., 2009). Presently, corncobs are being burnt as fuel in the households of peasant rural farmers, as a result, the gases emitted during burning increases air pollution and other potential health hazards. In some other places, the corncobs are left on the land for a long time to decay, thereby, claiming farmland that could be used for the cultivation of other food crops. The constant dumping of corncobs indiscriminately in the surroundings constitutes nuisance and also pose serious health risks to the human occupants as a result of the environmental pollution. The typical composition of corncob by dry base cellulose is (30-40 % w/w), hemicellulose (35-40 % w/w), and lignin (10-20 %, w/w) (Demirbas 2005; Mosier *et al*., 2005). This unusually high content of cellulose and hemicellulose in corncob justifies it as an attractive feedstock for bioethanol (Chandrakant and Bisaria 1998; Wyman 2007). Cellulose and hemicellulose can be hydrolysed with chemicals and/or enzymes to monomeric sugars, which can be subsequently converted biologically to bioethanol (Caye *et al*., 2008).

Wyman, (1996) defines bioethanol as a versatile transportation fuel and fuel additive that offers excellent performance and reduces air pollution compared to conventional fuels. Its production and use adds little, if any, net release of carbon dioxide to the atmosphere, drastically reducing the potential for global climate changes. Bioethanol, also called ethyl alcohol (C_2H_5OH) was described as a biodegradable, renewable, low in toxicity clear liquid fuel that burns completely in cars without leaving any carbon residue, causes little or no environmental pollution (Jason, 2009; Adelekan, 2010). As a renewable energy source, bioethanol is expected to significantly reduce carbon dioxide (CO_2) emissions when the entire fuel cycle is considered and can also replace diesel fuel in Compression Ignition (CI) engines (Brent and Bailey, 1996). Bioethanol can be added to gasoline to improve its combustion, thereby reducing tailpipe emissions of carbon monoxide, CO and unburned hydrocarbons that form smog; and as a neat or pure fuel, bioethanol has extremely favourable properties that can reduce smog-forming emissions (Wyman, 1996). The global energy usage depends on fossil fuel supply which produces greenhouse gases and pollutants, causing climate change and other environmental problems, (Chien- Lun, 2007) while according to Wyman (1996), the economic and social effects of global climate change would likely pale anything that has occurred in recent times. As pressing as economic issues are, the world is also faced with potentially even greater environmental consequences, if care is not taken with these effects, it might be difficult to reverse. On the other hand, there is growing evidence that global conventional fossil fuel is close to the point where half of the accessible reserves have been depleted; pointing towards the real possibility that production will not be able to sustain the world demand in the near future (Kerr, 2005) and all over the globe, there are several reports on the increase in demand for bioethanol production through the process of fermentation due to the dwindling number of oil reserves resulting in soaring prices of fossil fuels (Brooks, 2008). Bioethanol is being considered as a potential liquid fuel due to the limited amount of natural resources (Masami *et al*., 2007) and in particular, that it is produced from non- food lignocellulosic waste products, such as wood chips and straw, or non- food crops such as willow, could be an environmentally- friendly alternative (Wyman and Goodman, 1993). Therefore, through a sustained research program and an emerging economic competitiveness, the technology for bioethanol production is poised for immediate widespread commercial applications (Wyman, 1996).

Production of bioethanol from maize agro-waste has been attempted with enzymes from different sources for hydrolysis of lignocellulose and with different organisms for fermentation (Zakpaa *et al*., 2009). According to Wijffels and Barbosa, (2010), feedstock for producing bioethanol should come from non - food crops or agricultural wastes and residues which are referred to as second and third generation feedstock that includes lignocellulosic materials (wood straw, grasses), sucrose-based (sugar cane and molasses), starch-based (wheat, corn, barley, sweet sorghum and sweet potato, cassava, yam).