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Solid State Fermentation of Orange Pomace for Bioethanol Production

U. Musa[1], I. A. Mohammed[1], S. M. Munnir[1], M.U.Garba[1], A.F. Abdulhamid[2] & E.P Uduak [1]

[1]Chemical Engineering Department,
Federal University of Technology, Minna, Nigeria.
[2] Engineering Department,
National Agency for Science and Engineering Infrastructure,
Abuja, Nigeria.

Abstract: This study is aimed at studying the effect of process variables on solid state fermentation of orange pomace for bioethanol production using *Saccharomyces cerevisiae.* The effect of substrate concentrations (100 - 350 g), fermentation period (24 - 72 hours) and inoculum amount (2.0 - 4.5 g) on solid state fermentation of orange pomace for bioethanol production was investigated. Characterization of the resulting bioethanol was carried out to determine its fuel properties (viscosity, flash point, density, refractive index, specific gravity, pH and boiling point). Experimental results revealed increase in the process variables (substrate concentration, fermentation period and inoculum amount) led to a corresponding increase in bioethanol yield until an optimum condition was reached (substrate loading of 200 g, pH of 4.5, fermentation temperature of 35°C, inoculum amount of 3 g and fermentation period of 72 hours) after which a decline in yield was observed. The maximum ethanol yield of 32.32 % v/v was obtained at these condition. Characterization of the bioethanol sample showed that the ethanol has satisfactory fuel properties that establishes its suitability as an alternative renewable fuel that can be blended with gasoline.

***KEY WORDS*:** Bioethanol, biomass, fermentation, fuel, orange pomace, solid state

1.0 Introduction

Alternative sustainable energy derived from biomass are presently considered as promising and attractive energy source when compared to fossil derived fuels. Cellulosic bioethanol obtained from biomass fermentation is a renewable

and environmentally friendly alternative fuel to petroleum gasoline [1]. It is presently the most commonly used liquid biofuel. Bioethanol has negligible contribution to global warming in comparison to petroleum gasoline [2]. Bioethanol is produced via microbial fermentation and distillation of the ethanoic wash from fermented biomass-extracted sugars. It can be used as a liquid fuel in automobile engines, either wholly or blended with petroleum gasoline [3]. Brazil and USA are the two major producers of ethanol, these two countries accounts for 62 % of the world production. First generation feedstocks (starch and sugar) are mainly used for this bioethanol production in these parts of the world [4]. The use of first generation feedstock is unfit for bioethanol production because starch and sugar feedstocks are basis for human and animal nutrition hence there will be problems on ethical concerns and favourable economics. It is based on this fact that second generation feedstocks (non-food feedstock's) are used for bioethanol. Second generation feedstocks consist of locally available and abundant agricultural waste [5]. Lignocellulose biomass is considered as second generation feedstocks. It is an ideal feedstock for biofuel production because it does not compete with food resources, reduces carbon dioxide emission by about 75% in comparison to fossil derived fuels [6].

Fruit pomaces are viable raw materials for bioethanol synthesis. Pomaces differs significantly from wood (hardwood or softwood). Woody materials are known to be naturally harsh and require thorough pretreatment before fermentation. Pomaces contains very high amount of easily accessible fermentable sugar content. These characteristics make pomaces suitable for all varieties of fermentation media [5].

Solid state fermentation (SSF) is an attractive technology for producing higher yield of bioethanol as compared to submerged liquid fermentation. In this process the microorganisms strive well due to the enabling environment similar to its natural habitat thereby resulting into higher metabolic activities [7]. Solid-state fermentation involves the process of microbial growth and product formation on solid particles in the absence (or near absence) of water; however, the substrate is known to contain sufficient moisture to permit microbial growth and metabolism [8]. Solid state fermentation results into higher bioethanol yields and better product characteristics in comparison with submerged fermentation which is characterized by the cultivation of the microorganisms in a liquid medium. Another great advantage of solid state fermentation over submerged fermentation is the lower capital and operating costs due to the utilization of low cost agricultural and agro-industrial wastes as substrates. The low water volume used in solid state fermentation process has also a large impact on the economy of the process mainly because of the smaller fermenter-size, the more reduced the downstream processing, stirring and sterilization costs [9 – 10]. In solid state fermentation the

microorganisms grow on a moist solid with little or no free water, although the capillary water may be present. Solid state fermentation process is best used for fungi and microorganisms requiring less moisture content; hence this process can also be used for fermentation process involving organisms (bacteria) requiring high water activity [11]. Different researches have reported the solid state fermentation of different fruit pomaces; banana peels [12], sweet potatoes [13], carob pods [14], grape and sugar beet pomaces [15], rice bran [6] for bioethanol production. There are little or no documented literatures on the production of bioethanol from orange pomace.

Orange peel waste (OPW) is the solid residue of orange juice production. Orange peel is an excellent example of a wasted resource. It consists of peels, membranes, cores, juice sacs and seeds which are rich source of pectin, appreciable quantity of cellulose, and soluble sugars. Orange peels is usually available in large quantity as it constitute over 50% of the processed fruits. It can be easily fermented to produce produces ethanol at a temperature between 25 and 35°C [16]. Its commercial uses are limited and its disposal is of great concern from the environmental point of view. The aim of this work is to study the effect of process variables on the solid state fermentation of orange pomace for bioethanol production and also the characterization of the bioethanol to determine its relevant fuel properties.

2.0 Materials and Method

The orange pomace was obtained from Minna, washed in order to remove dirt and sand. Sodium hydroxide, yeast (*saccharomyces cerevisiae*), glucose and peptone were all of analytical grade.

2.1 Test for sugar content (brix) in the orange pomace

This was done with the aid of a hand held refractometer, to ensure that the glucose content in the substrate will be suitable or appropriate for saccharification and fermentation. The pomace collected was pulverized into pulp with the aid of a blender; this was pressed to extort the juice from the pulp. The lens of the refractometer was then cleaned with a cotton wool to guarantee a clean lens surface, after which little drops of the juice was added to the refractometer and it was closed. The sugar content was recorded from the micro-gauge as soon as a sharp colour was observed.

2.2 Pretreatment of the orange pomace

All the glassware were washed and autoclave for 1hr at a temperature of 121°C for sterilization. To a 500 ml conical flask, 150 g of the pulverized pomace was weighed. 30 ml of 4.0% sodium hydroxide buffer in the ratio (5:1) was used for pretreatment for 2 hr to make cellulose more accessible for enzyme activity [17].

2.3 Preparation of the culture media

The yeast (*saccharomyces cerevisiae*) was used for fermentation of the substrate sugar was cultivated for 2 days (48 hrs) before commencing the experiment. 20 g and 10 g of glucose and peptone respectively were diluted in 1L of

distilled water and untainted for 20 minutes at a temperature of 121℃ to produce 100 ml of glucose-yeast-peptone (GYP) medium in a conical flask. 5 ml suspension of the yeast strain (*Saccharomyces cerevisaie*) was introduced in to the prepared culture media. This was incubated at room temperature on a rotary shaker at a speed of 200 rpm for 48hr (2days) before injection into fermentation medium [17].

2.4 Solid State Fermentation

The prepared yeast (*Saccharomyces cerevisiae*) was introduced into the pretreated samples in the conical flasks and covered with foil paper. The mixer was charged into an incubator and allowed to ferment for different fermentation period between 24 and 72 hours and at a constant temperature of 35°C. The resulting ethanol liquor was boiled off via a distillation column apparatus for an hour at 79.5℃. There after the yield of ethanol was deduced by calculating the specific gravity of the ethanol obtained and the resulting value is used to deduce the ethanol concentration.

3.0 Result and Discussion

3.1 Orange Pomace Analysis

The sugar brix was determined with the aid of a refractometer and it was recorded as. The Fehling solution test for reducing sugar was carried out on the substrate. The colour of the substrate changed from bright yellow to red, this indicated the presence of reducing sugar in the sample.

3.2 Effect of Process Variables on Bioethanol Yield.

Effect of fermentation period on bioethanol yield.

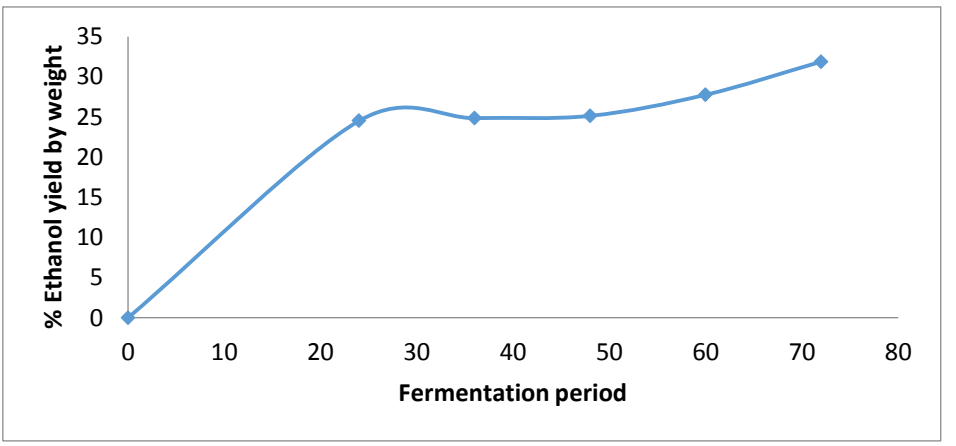

Fig. 1 Effect of fermentation period on ethanol yield

The effect of fermentation period on ethanol yield was carried out between the fermentation period of 24 to 72 hours, at a constant temperature (35 °C), constant substrate loading (150 g), constant yeast strain or concentration of yeast (3.0g). From Figure 3.1, it was observed that there was a corresponding increase in the percentage yield of the bioethanol yield as the fermentation period increased from 24 to 72 hours. Optimum yield of 31.87 % (w/w) of

bioethanol was obtained at a fermentation period of 72 hours (3 days). The increase in bioethanol yield with time was attributed the appreciable contact between the enzyme and the hydrolyzed sugar. This result was in accordance with the result of Kanokphorn *et al*. [18] who reported the fermentation of leaf waste for bioethanol production.

Effect of substrate loading on bioethanol yield

The effect of substrate loading (100 to 350 g) on ethanol yield was carried out at a constant temperature of 35 ^{o}C, optimum fermentation period of 72 hours and constant inoculum amount (3 g).

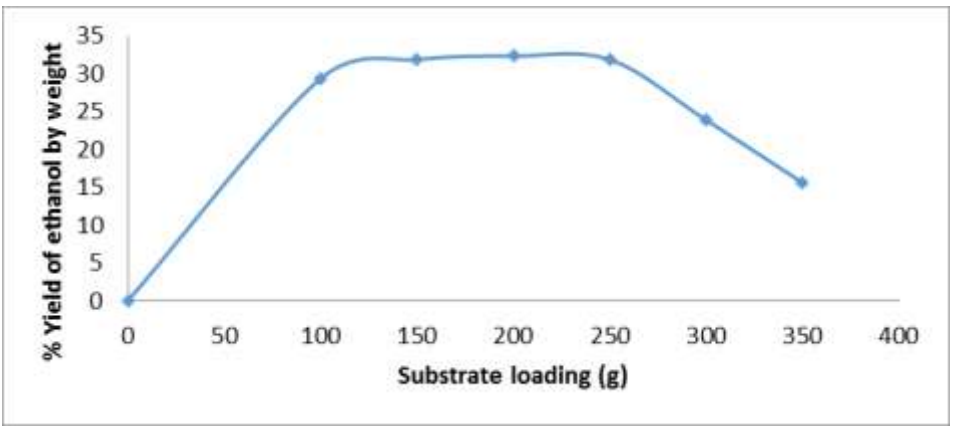

Fig. 2 Effect of substrate loading on bioethanol yield

From on Figure 2 it was observed that bioethanol yield increased significantly as the substrate loading increased from 0 to 100 g. However as the substrate loading increased from 100 – 250g the bioethanol yield was gradual until a maximum yield of 32.32 % (w/w). Subsequently the yield decreased drastically as the substrate loading increased beyond 250 g. Higher substrate loading prevents the ethanol fermentation because the yeast cannot sufficiently act on all the substrate since the inoculum amount is constant (3 g). Another reason for the decrease in ethanol yield is the accumulation of high concentration of ethanol and by products which changes the broth pH [19].

Effect of inoculum amount on bioethanol yield

The effect of inoculum amount (2.0 to 4.5 g) on ethanol yield was carried out at a constant temperature of 35 ^{o}C, constant substrate amount of 150 g, constant pH of 4.5 and constant fermentation period of 72 hours.

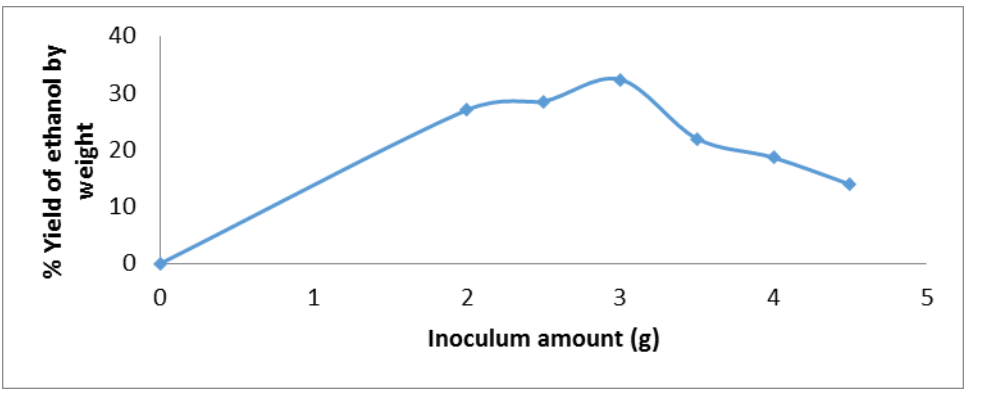

Fig. 3 Effect of inoculum amount on ethanol yield

The percentage yield of bioethanol increased as the inoculum amount increased from 0 to 3 g. there was a decrease in the percentage of bioethanol produced as the inoculum amount increased from 3.5 to 4.5 g. The optimum yield of 32.32 % (w/w) bioethanol was obtained when 3.0 g of inoculum was used. The result is in conformity to the study of Neelakandan and Usharani [20].

3.3 Bioethanol Characterization

The appearance of the ethanol sample after filtration and distillation was clear and colourless. The boiling point of the ethanol sample obtained from this study was 79.20^{o}C which is slightly higher than the boiling point of standard ethanol (78.5^{o}C). The higher value obtained in this work may be due to the presence of impurities in the ethanol sample produced.

Table 1 Properties of Bioethanol Produced

Property	***Standard ethanol**	**Orange Pomace ethanol sample**
Appearance	Clears colourless	Clear colourless
Boiling point (^{o}C)	78.50	79.20
Density (g/cm$^{3)}$	0.789	0.795
Specific gravity	0.789	0.795
Viscosity (cP)	1.20	1.25
Solubility	Miscible	Miscible
Flammability	Flammable	Flammable
Refractive index	1.360	1.334
Flash point (^{o}C)	12.8	12
pH	7.0	6.94

*(Source: Walker, [20])

The density of the sample was 0.795 g/cm^{3} which shows close proximity to the density of the standard ethanol sample. Viscosity is the resistance of a fluid to flow or the property of fluid that resists the force tending to cause the fluid to flow. The viscosity of the ethanol sample was determined to be 1.25 cP. The solubility is a direct measurement of

hydrophobicity or the tendency of water to exclude a substance from solution. Solubility is the maximum concentration which an aqueous solution can tolerate before the onset of phase separation. The ethanol sample was completely miscible with water. This result was in agreement with reported literature [20]. The bioethanol sample produced burns with blue flame when ignited. The flash point of 12 ^{o}C obtained in this study shows close proximity 12.8^{o}C reported for standard ethanol. The pH of the bioethanol sample was 6.94 indicating that the bioethanol sample is neutral and this corresponds with the pH of standard ethanol.

4.0 Conclusion

The study has attempted and succeeded in reporting for the very first time the potential of a typical Nigerian orange pomaces for bioethanol production via solid state fermentation. Optimum yield of 32.32 % (w/w) was obtained at a temperature of 35 ^{o}C, pH of 4.5, substrate loading of 150 g, inoculum amount of 3 g and fermentation period 72 hours. Properties of bioethanol produced were satisfactorily in agreement with standard specification.

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