

## Bioconversion of Waste Foolsap and Newspaper to Fermentable Sugar

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### ABSTRACT

The aim of this project work was to evaluate the effect of bacteria – *Serratia* in the enzymatic hydrolysis of Foolsap [FS] and Newspaper [NP] into fermentable or reducing sugars. The effect of temperature and hydrolysis time (number of days) on the extent of concentration of reducing sugar yield were assessed and quantified using Dinitrosalicylic acid test method (DNS). A proximate analysis was carried out on the substrate before hydrolysis. Alkaline pre-treatment using sodium hydroxide and deinking process for the removal of paper with ink for higher susceptibility of the substrate before the enzymatic hydrolysis were carried out. The temperature effect on the waste sample were analysed at 37°C, 40°C and 45°C for 7 days, it was observed that at 37°C, saccharification was higher than at 40°C and 45°C. The general observation was in that *Serratia* had a very good effect on the waste paper samples. A 3-D surface plot revealed that the yield of sugar increased along with the hydrolysis time (number of days), with Foolsap having the highest yield of about 21mg/ml. The comparative analysis shows that the highest yield was obtained at the temperature of 37°C, for both substrates used.

**Keywords:** *Serratia*, waste paper, enzymatic hydrolysis, fermentable sugars, microorganisms

### INTRODUCTION

Waste materials such as paper, agricultural biomass and cooking oil can be very useful when properly recycled [1-4]. However, paper products are presently recycled on a limited basis. Most waste papers are usually dumped or burnt, thus increasing the rate of environmental pollution [5]. Therefore, the use of enzymes as a catalyst in the bioconversion of waste paper materials to fermentable sugars is an important step that needs to be considered in the producing sugars using an environmental-friendly process [6]. Crude oil constitutes another material that pollutes environment. This can be remediated using microorganisms [7-10].

Cellulose is a naturally abundant material that can be derived from the sources that are sustainable and renewable, biodegradable, carbon

neutral and are environmentally safe [11]. It can be obtained from bacteria [12], cotton [13], sisal [14], tunicates [15], wood pulps [16] and ramine [17]. Cellulose, lignin and hemicellulose are essential components of lignocellulosic biomass which include different parts of plants that serve as paper materials, such as woods and leaves [18]. Hemicellulose, a primary component of xylose is responsible for the interlink bonds present in cellulose molecules [19]. Paper and material structural strength is due to the presence of lignin, possessing a high degree of different interconnected molecular structure. Shleser [20] reports that newspaper contains nearly 61% of cellulose and 16% hemicellulose; therefore, these materials can serve as an appropriate feed stock for the production of sugars.

Recycling of waste paper has led to reduction in the cellulose fiber length, having lower quality

compared to the paper originally manufactured from pulps [21]. It is therefore advised that waste paper should be promoted as bioenergy resource due to the high expense required in recycling papers and the high cellulosic content of the recycled papers.

Waste paper would contribute greatly to solid waste if they are not recycled. When this waste is not properly managed it can turn into a serious environmental problem. An alternative means by which solid wastes are treated include incineration [22], land filling [23], waste organic materials are exposed to biochemical and chemical methods in order to aid the release of fermentable sugars, this occurs while the cellulose component of waste materials is broken down. The chemical procedure involves the use of inorganic acids such as sulphuric [24] and hydrochloric acids [25] in hydrolysing the cellulolytic content. This is an expensive method and it is not environmentally friendly, the resulting acid solution is difficult to recycle for further use on other cellulolytic waste materials available [26]. Other methods of degrading the waste cellulose material is the use of cellulase which is a multi-component enzyme system [21].

## MATERIALS AND METHODS

The paper materials used for this investigation were foolscap papers and newspaper. These materials were obtained from the Covenant University environs. Microorganism *Serratia* was sourced from Applied Biology and Biotechnology Unit Laboratory of Covenant University, Ota, Ogun State.

Preparation of cellulase done using the method [6, 27-30]. Deinking and Alkaline Pre-treatment were carried out using the method [6].

The method of [31-33] was used to analyse the percentage of moisture, ash, crude fibre composition, the carbohydrate content was determined using the Anthrone method.

The waste papers with the sizes 2 cm by 2 cm were physically sorted and treated. 5 g of each paper substrate were soaked in 100 ml of 2.0 M NaOH (1%) solution in a flask and kept for the duration of 24 hours. Deinking was carried out on papers with ink. The paper substrate were soaked in 4% sodium hypochlorite for 48 h, and then washed with water until neutralized (pH of 7) and left to dry overnight at 105°C in the oven.

Enzymes, as generally known, are specific in action and are active in operation under specific

conditions, which are popularly known as optimum conditions. General optimum conditions for such type of bacteria are within the range of 35° to 50°C; hence, the analysis was focused within the range. For this work, 37°C, 40°C and 45°C were used. The pre-treated paper substrates were mixed with 1ml *Serratia* solution and incubated for 1 week at a temperature range of 37°C to 45°C. The samples were withdrawn daily and analysed using the DNS acid method for reducing sugars.

Test for Reducing Sugar. The test for reducing sugar was carried out using the DNS acid test. The absorbance was recorded for each sample that was collected.

The standard glucose solution was prepared by the dissolution of 100 mg of glucose in 100 ml of distilled water. The working standard solution was prepared by pipetting out 10 ml of stock standard solution into a flask and diluting it to 100 ml with distilled water.

0 to 1.0 ml of the working standard solution were collected in separate test tubes. The volume of each test tube was made up to 3 ml using distilled water. 1.0ml of distilled water was used as blank. The absorbance of each sample was read following the DNS method below.

1% NaOH was prepared by dissolving 1 g of NaOH in 100 ml of distilled water, 1 g of Dinitrosalicylic acid, 200 mg crystalline phenol and 50 mg of sodium sulphite were dissolved in 100 ml of 1% NaOH. 40% of potassium sodium tartrate was prepared by dissolving 40 g of potassium sodium tartrate in 100ml of distilled water.

The supernatant of the entire sample was collected using a pipette. 1 ml of each supernatant was collected and the volume was equalized to 3 ml using distilled water. 1 ml of Dinitrosalicylic acid solution was added to each test tube containing the samples. The contents of the test tubes were boiled in a water bath for 10 minutes. 1 ml of 40% potassium sodium tartrate was added to each test tube while they were warm. The tubes were allowed to cool and the absorbance was read at 575 nm.

## RESULTS AND DISCUSSION

Table 1 shows the proximate analyses carried out on the two samples of paper used. It was revealed that Newspaper had the highest composition in three (3) out of four (4) tests carried out under the proximate analysis on the raw substrate.

The higher moisture content might probably be due to where the papers were picked from – Library where air conditioning operates for about 14 hours daily. The crude ash content was found to be higher in the foolscap sample with 9.76%; the crude ash content indicates the presence of mineral. The carbohydrate content of the paper samples using the Anthrone method was recorded with Newspaper having the highest concentration of carbohydrate of 40.03 mg/ml. The result obtained proved that the proximate values have no effect on the yield of fermentable sugar produced, since Foolscap produced more.

Figures 1-3 show the yield of reducing sugar in both waste paper samples hydrolysed for one week (7 days). It was observed that foolscap had the highest concentration of sugar, followed by Newspaper irrespective of the total carbohydrate composition present in the substrate used. The reducing sugar yield is dependent on days of hydrolysis. The more the days, the greater sugar yield obtained.

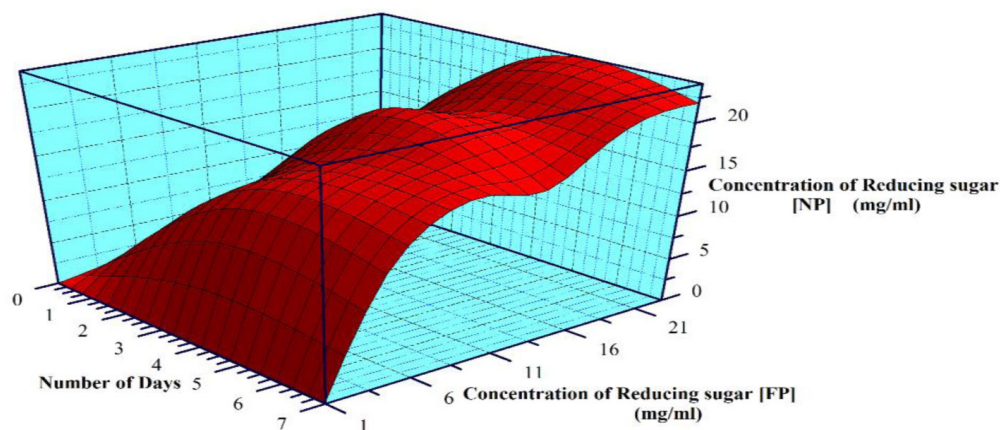
Figures 4 and 5 show the effects of different temperature on the yield of the reducing sugar (comparative analysis). After 7 days (1 week) of enzymatic hydrolysis at a temperature of 37°C, the concentration of reducing sugar was measured daily to observe the trend of increase or decrease and the results were recorded. The general trend of the concentration of the fermentable sugar was on the increase from the first day to the seventh day for both FS and NP, this is a similar observation to the work carried out by [34] on the biodegradation of five paper samples using *Trichoderma viride* as cellulase. The results indicated that the effect of *Serratia* on the waste paper samples after a week at 37°C, had the highest effect on the

**Table 1.** Proximate analyses on waste paper samples

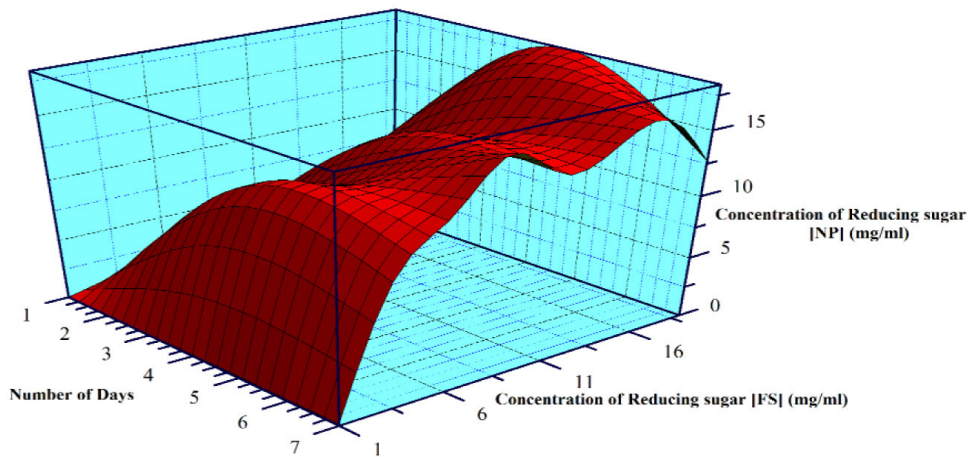
Specifications	Value
pH ( $\pm 0.01$ )	7.99
EC ( $\mu\text{S}/\text{cm}$ ) ( $\pm 1$ ) <sup>1</sup>	52200
TDS (mg/L) ( $\pm 0.1$ ) <sup>2</sup>	36540.0
T. Hardness (mgCaCO <sub>3</sub> /L) ( $\pm 0.01$ ) <sup>3</sup>	6142.00
C. Hardness (mg CaCO <sub>3</sub> /L) ( $\pm 0.01$ ) <sup>4</sup>	1018.50
M. Hardness (mgCaCO <sub>3</sub> /L) ( $\pm 0.01$ ) <sup>5</sup>	5123.50
T. Alkalinity (mgCaCO <sub>3</sub> /L) ( $\pm 0.01$ ) <sup>6</sup>	263.30
HCO <sub>3</sub> <sup>-</sup> Alkalinity (mgCaCO <sub>3</sub> /L) ( $\pm 0.01$ )	263.30
Turbidity (NTU) ( $\pm 0.01$ )	0.20
L. Index ( $\pm 0.01$ ) <sup>7</sup>	1.31
Ca <sup>2+</sup> (mg/L) ( $\pm 0.01$ )	407.40
Mg <sup>2+</sup> (mg/L) ( $\pm 0.01$ )	1228.66
Na <sup>+</sup> (mg/L) ( $\pm 0.1$ )	7800.0
K <sup>+</sup> (mg/L) ( $\pm 0.01$ )	437.5
Silica (mg/L) ( $\pm 0.01$ )	0.00
Cl <sup>-</sup> (mg/L) ( $\pm 0.01$ )	14704.92
SO <sub>4</sub> <sup>-2</sup> (mg/L) ( $\pm 0.1$ )	2618.8
NO <sub>3</sub> (mg/L) ( $\pm 0.01$ )	0.00
F (mg/L) ( $\pm 0.001$ )	2.79
PO <sub>4</sub> <sup>-3</sup> (mg/L) ( $\pm 0.001$ )	0.001

foolscap substrate samples which could be due to the high susceptibility to yield high concentration of sugar.

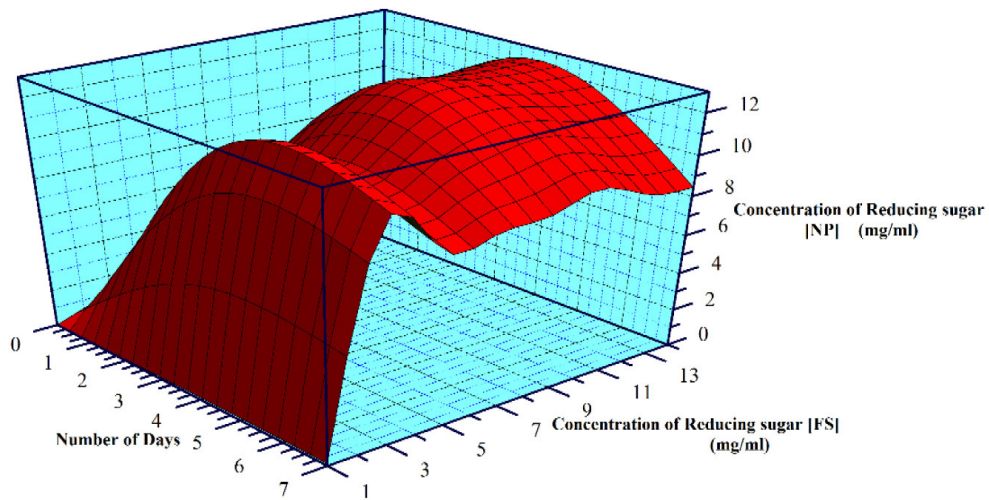
Reducing sugar was also produced at 40°C but not as much as at 37°C; however, lesser yield was recorded for the highest temperature, i.e. 47°C. Thus, we can deduce that *Serratia* operates best at the temperature between 37° and 40°C, since higher yield was recorded at these two temperatures. Although bacterial enzymes such *Serratia* used for this work have an optimum range of temperature that aid its microbial activities (37–45°C), this is seen from the work



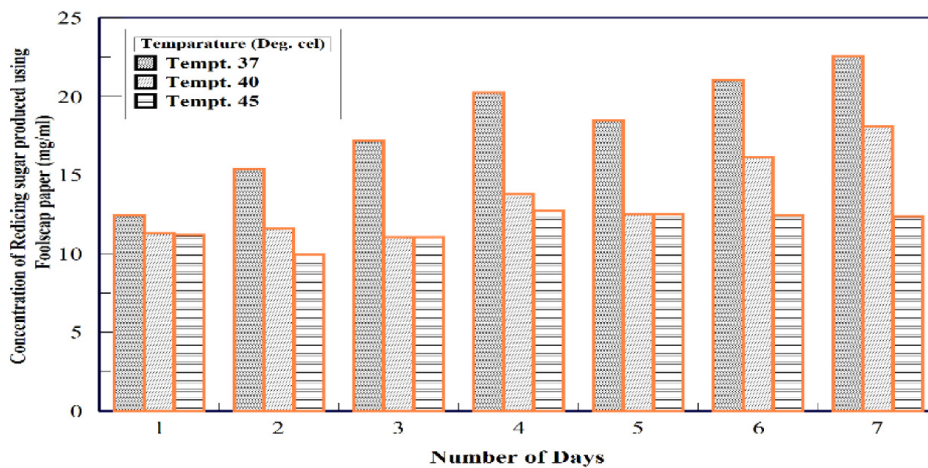
**Figure 1.** Effect of hydrolysis time (number of days) on the concentration of reducing sugar obtained from Foolscap [FP] and Newspaper [NP] using *Serratia* at temperature 37°C



**Figure 2.** Effect of hydrolysis time (number of days) on the concentration of reducing sugar obtained from Foolscap [FP] and Newspaper [NP] using *Serratia* at 40°C



**Figure 3.** Effect of hydrolysis time (number of days) on the concentration of reducing sugar obtained from Foolscap [FP] and Newspaper [NP] using *Serratia* at 45°C



**Figure 4.** Effect of different temperature on the yield of Reducing sugar using Foolscap paper as substrate

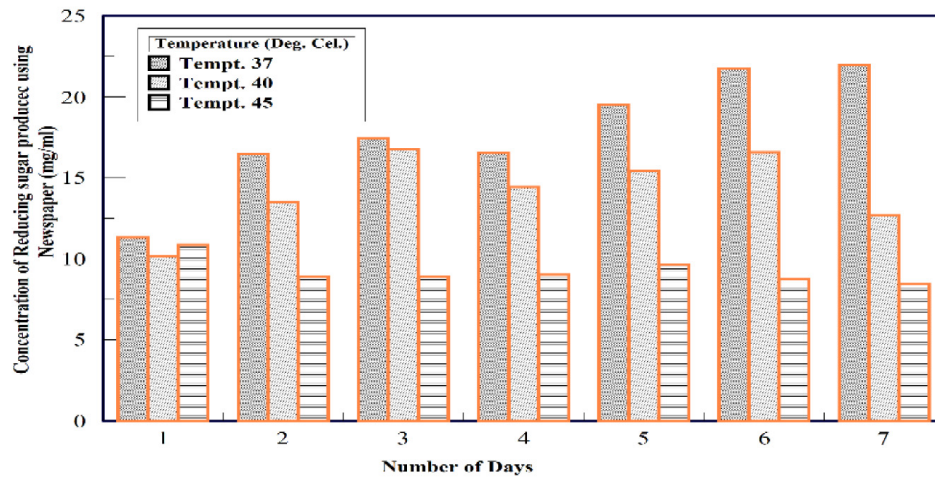


Figure 5. Effect of different temperature on the yeild of Reducing sugar using Newspaper as substrate

of [35] on the effect of high growth temperature on *Serratia marcescens*; therefore, the trend being studied is not a situation of a temperature increase above the optimum, but rather that 37°C is a more suitable temperature for high saccharification than 40°C and 45°C.

However, the comparative analysis of the temperature and the metabolites shows that the 37°C is a more optimum temperature in the production of reducing sugar from the waste paper substrates and also that *Serratia* have the highest effects in the bioconversion on the paper samples used.

## CONCLUSION

*Serratia* have the ability to degrade papers into fermentable sugars. The temperature range of 37°C to 45°C is an optimum temperature range for the bacterial actions on the substrate (waste paper samples); also, the temperature 37°C is a better temperature for saccharification than 40°C and 45°C. The enzymatic hydrolysis of waste paper is of major interest, seeing it could be an alternative solution for the solid waste material treatment and corresponding glucose and more so, ethanol production. The waste papers that cannot be recycled can be utilized in the production of glucose syrups of very high purity level in glucose for industrial and commercial satisfaction. The bioconversion process is “environmentally friendly” because no harmful side products are obtained at any time during the process, also the demand for energy during the process is low.

## Acknowledgements

The authors appreciate the sponsorship of Covenant University.

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