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Research article

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LAND REMEDIATION AND RECLAMATION TECHNIQUES THROUGH THE BIODEGRADATION OF WASTE PAPERS

通过废纸生物降解的土地修复和复垦技术

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Abstract

Waste materials are one of the major components of solid organic waste that pollutes land. Inappropriate disposal of municipal waste (solid) not only affects land but is also a source of danger to inhabitants. The management of residue from incineration is an integral part of waste disposal/management systems. This research provides a collection and bio-treatment technologies for various types of waste paper such as office paper and newspaper as feedstock using two microorganisms: *Serratia* and *Rhizopus* to convert them to reducing sugars. The percentages of reducing sugar present in the samples before, during and after hydrolysis were determined using the dinitrosalicylic (DNS) acid test method. The effect of temperature on the substrate was analyzed at 37, 40, and 45°C. This research shows that waste paper can be converted into useful materials.

Keywords: Soil Pollution, Remediation, Biodegradation, Waste Paper, Fermentable Sugar

摘要 废弃物是污染土地的固体有机废弃物的主要成分之一。城市废物(固体)的不当处置不仅会影响土地, 而且还会对居民造成危险。焚烧残留物的管理是废物处置/管理系统的一个组成部分。本研究为以沙雷氏菌和根霉属两种微生物为原料的办公用纸、报纸等各类废纸提供收集和生物处理技术, 将其转化为还原糖。使用二硝基水杨酸(域名系统)酸测试方法测定水解之前、期间和之后样品中存在的还原糖的百分比。在 37、40 和 45° C 下分析温度对基板的影响。 这项研究表明, 废纸可以转化为有用

的材料。

关键词：土壤污染、修复、生物降解、废纸、发酵糖

I. INTRODUCTION

Researchers reported that about 95% of MSW (municipal solid waste) is disposed of untreated in landfills and open areas, causing several problems to humans and the environment, which can lead to shorter lifespans of land dwellers [1–2]. The environmental pollution is highest in the overcrowded industrial areas of the developed countries [3]. There is major concern over the effects of land/soil/water pollution over the past three decades. Not only activity of man, but also natural occurrences in the environment cause pollution [4–7]. Inappropriate disposal of MSW is one of the major causes of land/soil/water contamination [4, 8]. The major environmental catastrophe of our world today is land pollution [9]. Apart from waste papers, hydrocarbon also pollutes the environment, which can be remediated using microorganisms [10–13].

The deposition of excess quantities of SW (solid waste) on land and the urgent need for clean energy are topical national issues especially in Nigeria [13]. Managing waste has helped reduce/limit and control pollution in the environment by recycling it into useful materials [14]. This waste occupies appreciable parts of land and contributes extensively to environmental pollution [15].

Waste, when not properly managed, turns into a serious environmental challenge. Solid waste materials, when properly treated and recycled, can be very useful in society [13, 16-19]. However, more awareness must be about waste paper products and recycling to prevent them from being dumped or burned (incineration and land filling), which increases the rate of environmental pollution [20]. The incineration of waste papers results in many ailments that can lead to death, such as neurobehavioral disorders [4, 21-23], asthma [24–28], discomforts such as dizziness and headaches [29], irritation of the sense organs, and affects the lung [30, 31] in the form of respiratory diseases [32, 33]. While some persons see waste as a risk to public health and land, some people see it as a mere aesthetic inconvenience, and others see it as an excellent source of generating income.

The organic materials present in waste materials are exposed to both chemical and biological treatments to break down the cellulose component to fermentable sugars. The use of inorganic acids is involved in the chemical procedures such as sulfuric [34] and hydrochloric acid [35] in hydrolyzing the cellulose composition. Apart from the fact that the use of acid hydrolysis is expensive, it is also not environmentally acceptable, acidic solution resulting from the reaction is not reusable on other cellulolytic waste materials [36, 37]. A more affordable method for decomposing SW material is the use of a multi-component enzyme system such as Cellulase [38].

Materials with lignocellulose such as leaves or other parts of plants are very rich in hemicellulose, cellulose and lignin present as biomass in paper [39]. The (primary) component of plant cell walls that is made up of glucose long chains which are linked by (b-1,4)-glucosidic bonds is known as cellulose. It is reported that about 61% of cellulose and 16% of hemicellulose are present in newspaper, making it an excellent source of sugars [40]. The decomposition of sugar into simpler substances by yeast to produce bio-ethanol is known as fermentable sugars, this is an alternative use to fossil fuels, which are more environmentally friendly, the burning of fuel leads to the emission of greenhouse gases, which is a cause of global warming [41, 42].

The literature reveals waste papers, kitchens, and agricultural wastes as the major components of organic solid waste materials that contribute to solid waste generation [4, 42, 43].

The need to look for an alternative method to dispose solid waste in order to safe land and our environment cannot be over emphasized. The use of waste paper as biomass and Cellulase introduction to convert them to useful materials is therefore required since it is environmentally friendly and affordable [18, 44].

Serratia is a facultative anaerobic, Gram-negative, rod-shaped bacterium related to *Yersiniaceae*. They are 1–5 μm long and non-spore-forming microorganisms [45]. It was named after the Italian physicist Serafino Serrati within the Gamma proteo-bacteria. The only *Serratia* species recognized in the eighth edition of Bergey's Manual was *Serratia marcescens* [46]. The *Serratia* species are ubiquitous and can be found anywhere (including humans) [47].

Rhizopus is a saprophytic fungus, which lives on plants and specialized parasites. They are also found in many organic substances such as bread, leather, mature fruits [48]. *Rhizopus* reproduces by vegetative, asexual and sexual means. It is found in most parts of the world and under varied ecological conditions. About 8–10 known species of *Rhizopus* have been identified.

This research work describes the bioconversion of SW papers into fermentable or reducing sugar using the cellulose of two microorganisms, *Serratia* and *Rhizopus*, at various temperatures.

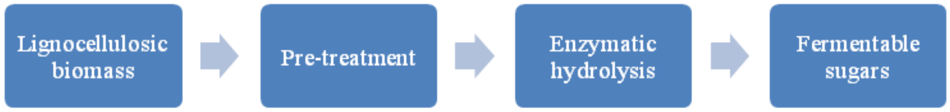

Figure 1. Bio-conversion process

II. Methodology

A. Sources of Raw Materials

The paper materials (biomass) used for this investigation were newspapers (NP) and office papers (OP). The newspapers and office papers were collected for the library and instructor offices of Covenant University, Ota, Nigeria.

B. Microorganism

Serratia and *Rhizopus* clinical isolates were obtained from the Department of Applied Biology, Biotechnology, Covenant University, Ota, Ogun State, Nigeria.

C. Preparation of Cellulase

Dextrose Agar (SDA) slants were used for the two isolates. They were sub-cultured on dextrose agar plate (sterile) and incubated at 27°C five days. The isolates were adjusted to 0.5 McFarland for the conversion process [49–51].

D. De-Inking and Alkaline Pre-Treatment

Sorted used paper was cut into 2 cm x 2 cm and soaked in 5% Sodium hypochlorite for a day (24 h). The paper was washed to neutralize it and was later oven dried at 105°C for another 24 h using Genlab Oven-18L/GLS. The paper samples were soaked in 50 ml of NaOH (2.0 M), alkali-treated substrate was filtered with muslin cloth, washed with distilled water until the neutralization point was reached. Excess water was removed using muslin cloth. De-inking is necessary since the ink particles hinder effective hydrolysis, high ash content embedded in it with inorganic fillers such as calcium carbonate and clay which was added to enhance the printing qualities [16, 17]. Only the paper with ink was deinked.

E. Inoculation of Samples

Samples were steeped in 4% sodium hypochlorite for two days; this was rinsed with water until it reached pH of 7. The temperatures used were selected because of the optimum operating conditions for the two microorganisms. Treated substrates were inoculated with 1-ml microorganism, and the solution was incubated for 7 days at the selected range of 37, 40, and 45°C. Samples were withdrawn daily and analyzed for reducing sugar using the DNS acid method.

F. Proximate Analysis

The proximate analysis was performed using the standard Association of Analytical Chemists [AOAC] method 2000 [51, 52]. The anthrone method was used for the determination of the carbohydrate composition. The concentrations of the sugars were determined by the DNS method, which is popularly known as the dinitrosalicylic acid method [16].

With the anthrone method [16], the glucose calibration curve was plotted, and calculations were done with Microsoft Excel 2018 with a linear equation:

$$y = 1.323x - 0.0819 \quad (1)$$

where y - concentration in mg/ml;
x – absorbance.

G. Tests for Reducing Sugar

The DNS acid test determined fermentable/reducing sugar [16].

III. Results and Discussion

Table 1 reveals the proximate compositions of the paper samples used. OP has the highest moisture content percentage of 7.55%. This can be due to the locations, where the paper was picked from - offices and the university library, where air conditions are always on. The crude ash composition was found to be higher in the office paper with 2.17%; the crude ash composition/content indicated minerals and sand in the sample. The crude ash content also informs the contamination of the samples with the soil. Ash is higher in newspaper sample due to the materials used in the production.

Table 1.
Proximate analysis of waste samples

Analysis	Newspaper (NP)	Office paper (OP)
Moisture content (%)	5.89	7.55
Ash content (%)	9.42	5.50
Crude fiber content (%)	0.61	2.17

From Table 2, the carbohydrate composition of the substrate using the anthrone method was recorded and office paper has the highest concentration of carbohydrate (45.325 g/L). The anthrone method is a popular method used for the analysis of carbohydrate content and cellulose content for lignocellulosic material, its implementation is widely accepted even on an industrial scale.

Table 2.
Concentration of carbohydrate in waste sample concentration using anthrone method

Samples	Absorbance (Abs.)	Concentration (g/L)
Newspaper sample (NP)	30.320	40.032
Office paper sample (OP)	34.320	45.325

For the analysis of fermentable sugar content in the substrate, dinitrosalicylic (DNS) test method was used as an alternative to high-performance liquid chromatography (HPLC) and the Grohmann method for analysis of total reducing sugar as used in [53] using the concentrated sulfuric acid method with modifications in the time of hydrolysis of paper sample. Table 3 shows the sugar content in all samples and it was observed that the office paper had a higher concentration of 1.270 g/L.

Table 3.
Reducing the sugar content in waste paper samples

Waste Paper Samples	Absorbance	Concentration (g/L)
Newspaper (NP)	0.028	0.507
Office Paper (OP)	0.084	1.270

Figures 2 to 7 show the 3D plots for the effect of hydrolysis duration (days) on the concentration of reducing sugar for OP and NP with *Serratia* and *Rhizopus* at 37, 40, and 45°C, respectively.

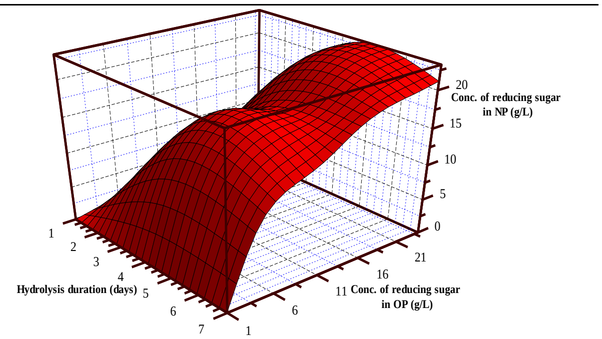

Figure 2. Effect of hydrolysis duration (days) on concentration of reducing sugar for OP and NP with *Serratia* at 37°C

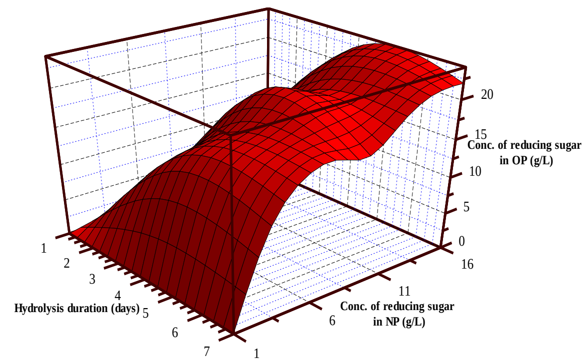

Figure 3. Hydrolysis duration (days) on concentration of reducing sugar for OP and NP with *Serratia* at temperature 40°C

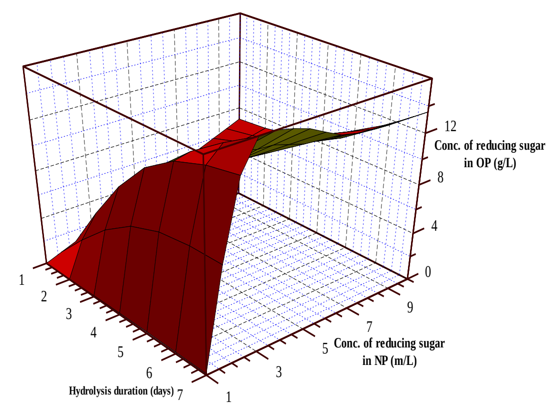

Figure 4. Hydrolysis duration (days) on concentration of reducing sugar for OP and NP with *Serratia* at 45°C

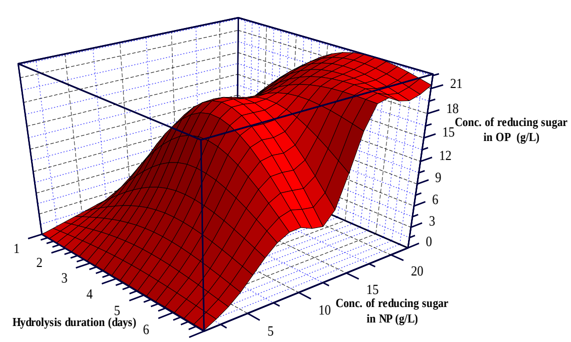

Figure 5. Hydrolysis duration (days) on concentration of reducing sugar for OP and NP with *Rhizopus* at temperature 37°C

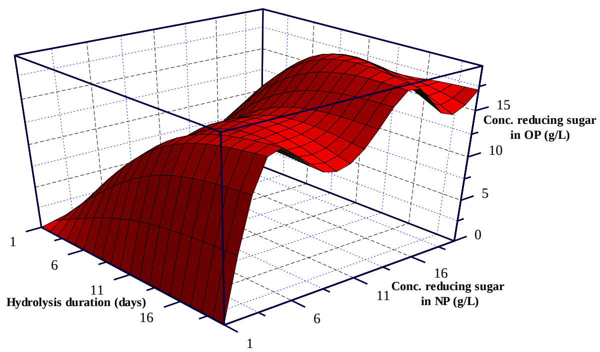

Figure 6. Hydrolysis duration (days) on concentration of reducing sugar for OP and NP with *Rhizopus* at 40°C

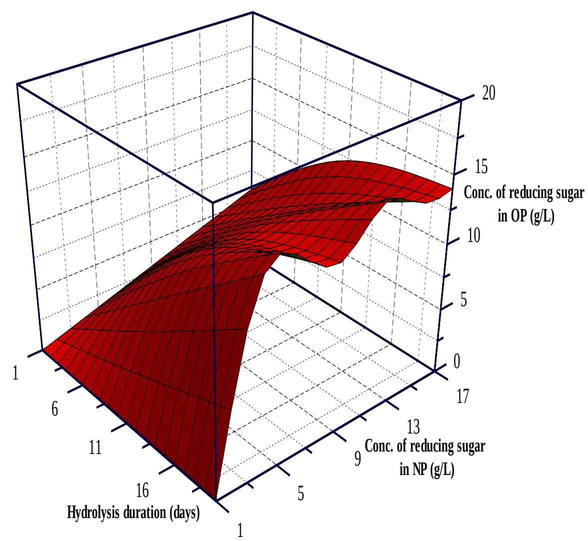

Figure 7. Hydrolysis duration (days) on concentration of reducing sugar for OP and NP with *Rhizopus* at 45°C

These figures show similar trend in the conversion of waste papers to reducing sugar at various temperatures with an increase in the production of reducing sugar with respect to time. After enzymatic hydrolysis for 7 days (1 week) at various 37, 40, 45°C, the concentration of fermentable sugar was measured daily to observe the trend. The general trend of the concentration of the reducing sugar was on the increase from the first day to the seventh day as shown in figures 2 to 9, although slight distortions occurred on certain days, which could be due to reduced microbial activities with the aqueous solution of the mixtures. We observed that *Serratia* and *Rhizopus* after a week at 37°C had the highest effect on the office paper, which could be due to the high susceptibility of it to yield high concentration of sugar after the increase in time to 48 h of alkaline pretreatment with NaOH compared to the analysis carried out by [13] where the pretreatment was for 24 h.

At 37°C, the two enzymes produced high yield of reducing sugar concentrations; observed from figure 8 and 9 with a concentration of 21.991 g/L for *Serratia* with newspaper and 21.391 mg/ml for *Rhizopus* with office waste paper. At temperature 40°C, highest yield of reducing sugar was obtained on the 6th day using *Serratia* while *Rhizopus* had 19.089 mg/ml reducing sugar on the 5th day. Low yield of reducing sugar was recorded for both microorganisms at temperature 45°C, this might be due to the optimum operating temperature of the microorganism. The comparative analysis in Figures 8 and 9 presents the effect of varying hydrolysis temperature on the yield of the reducing sugar.

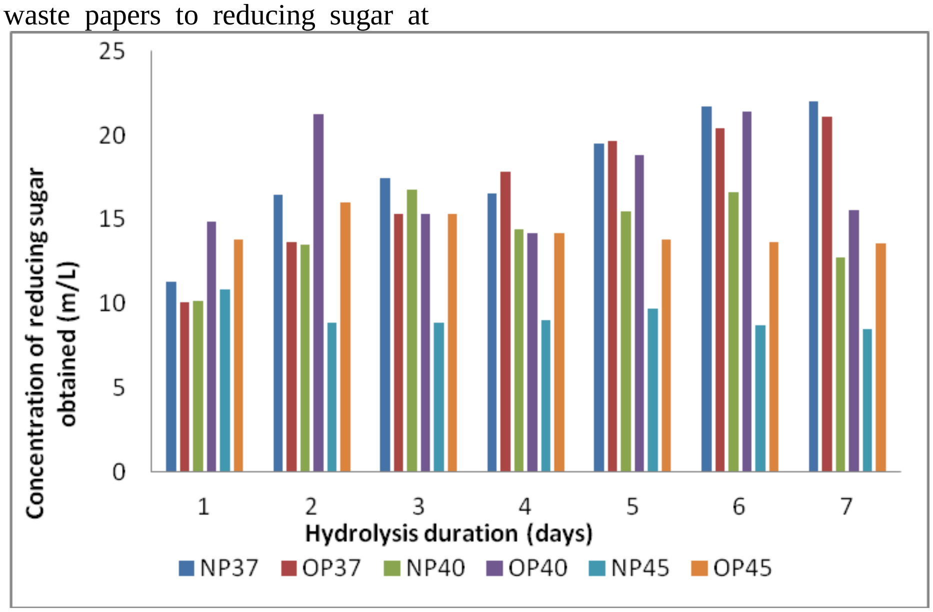

Figure 8. Various temperature effect on the concentration of reducing sugar obtained using *Serratia*

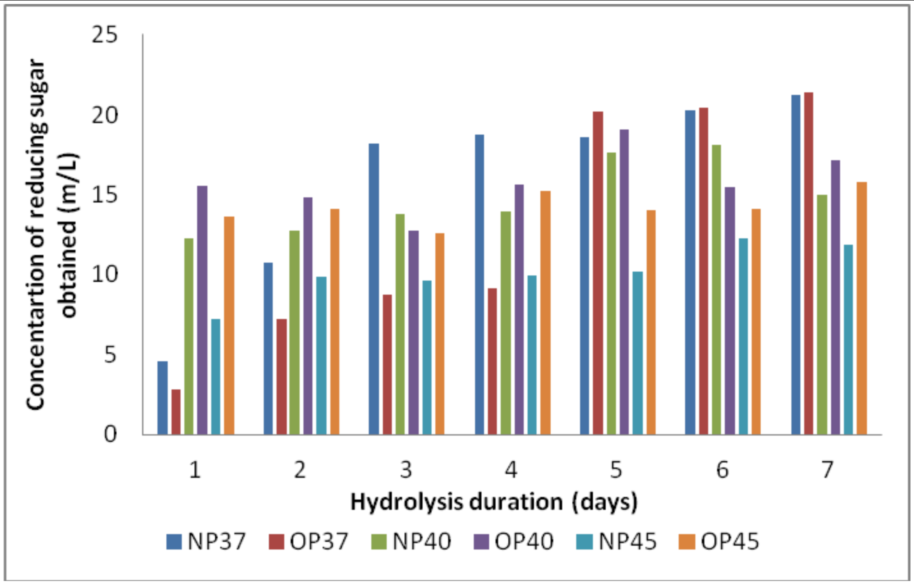

Figure 9. Various temperatures effect on the concentration of reducing sugar obtained using *Rhizopus*
Notes: NP – newspaper at 37, 40, 47°C; OP – office paper at 37, 40, 47°C

The yield of reducing sugars decreased as the temperature increased from 37°C to 40°C. Microorganisms have a particular optimum temperature whereby they function optimally. If the temperature increases from optimum, their function either decreases or they become inactive, which may lead to death in most cases.

IV. Conclusions

The two microorganisms used (*Serratia* and *Rhizopus)* can degrade waste papers into reducing sugars. The bacterial metabolite *Serratia* produces the highest concentration of reducing sugar on the newspaper sample (21.991 g/L) that has the highest susceptibility rate among the other waste paper samples. 37°C is a better saccharification temperature than 40°C, although both fall between the optimum operating temperatures. The enzymatic hydrolysis and alkaline pretreatment of waste paper is of major importance since it could be an alternative solution for solid waste management and production of reducing sugar. The produced reducing sugar can also be fermented to ethanol. Waste papers can therefore be recycled and used in the production of glucose syrups for industrial and commercial purposes. The bioconversion process is environmentally friendly as no harmful products are obtained during the process and low energy is consumed during the process.

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