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Bioconversion of waste newspaper into fermentable sugars at different temperatures with different *Aspergillus niger* cellulase concentrations

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ABSTRACT

Used newspaper is a major component of wastepaper that forms part of waste cellulosic materials which exhibits the potential to be developed as a resource of bio-energy. An initial step in utilizing the bio-energy potential of used newspaper is to bioconvert the cellulose compound into fermentable sugars such as glucose. This step can be performed with a hydrolytic enzyme such as cellulase from *A. niger*. Various amounts of used wastepaper have been treated with different concentrations of cellulase from *A. niger* at different incubation temperatures. Optimum degradation was observed at 50^0C with the highest cellulase concentration and during degradation of the highest amount of wastepaper incubated.

1. INTRODUCTION

Used newspaper is a major component of waste paper that is classified as organic waste and which is part of solid waste mainly referred to as municipal solid waste. Like with other organic waste materials, cellulose is a major structural component of newspaper. Cellulose is an abundant and natural material made from renewable and sustainable resources which is biodegradable, carbon neutral with a low environmental health and safety risk [1]. Cellulose can be obtained from many sources such as ramie [2], tunicates [3], bacteria [4], sisal [5], cotton [6] and wood pulps [7]. Waste paper including newspaper, being a cellulose biomass provides a potential resource of raw material for the conversion into fermentable sugars such as glucose which can then be further fermented into renewable bio-chemicals and pharmaceuticals. Several million tons of paper which are produced and used globally results in extremely high amounts of waste paper which continue to be finally part of municipal waste. Recycling of waste paper results in a shortening of the fiber length of cellulose with the quality of the resulting paper produced poorer than paper made from virgin pulps. With the higher cost of producing paper from recycled paper and due to the high cellulosic content, waste paper has the potential to be developed as a resource of bioenergy. If not recycled, waste paper could become part of solid waste which if not correctly managed results in a major environmental problem and concern. An alternative to the traditional ways of treating solid organic waste such as land filling [8] and incineration [9], organic waste materials can be exposed to chemical and biochemical procedures releasing fermentable sugars during degradation of the cellulose content of these waste materials. The chemical procedure is performed by hydrolyzing the cellulose content with inorganic acids such as hydrochloric acid [10] as well as sulfuric acid [11]. This method is relative expensive and not environmental benign and it is also difficult to recycle the acid for use on other waste cellulose materials [12]. The biochemical process of degrading cellulose can be achieved by treating waste cellulose with a multi-component enzyme system known as cellulase. Cellulase can be obtained from bacterial [13] and fungal [14] resources. Although the catalytic action of cellulase has been investigated the mechanism of cellulose degradation by cellulase enzymes is not yet resolved [15]. The inability to completely hydrolyze cellulose by cellulase action is due to the complexity of the cellulase enzyme, features of the cellulose structure, heterogeneous nature of catalysis and different behavior of different cellulose substrates towards various cellulase enzymes [16, 17]. The optimum degradation of cellulose by cellulase enzymes is also sensitive for changes in catalytic properties such as incubation pH and temperature, enzyme concentration as well as the amount of cellulose to be degraded and also the type of cellulose and cellulase used during the incubation [18].

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During this investigation the bioconversion of waste newspaper has been investigated by converting this waste cellulose material with cellulase from *A. niger* into fermentable sugars. The degradation was performed with different masses of used newspaper at different incubation temperatures while treating this waste paper substrate with different *A. niger* cellulase concentrations.

2. MATERIALS AND METHODS

2.1 Waste paper material

Used newspaper was collected for degradation with cellulase enzyme. This paper material was prepared as circular pieces with diameter of 6mm and masses of 0.0138g,0.0306g and 0.0695g were transferred in triplicate to test-tubes.

2.2. Buffer solution

The cellulase catalyzed degradation of used newspaper is classified as a heterogeneous catalysis and the process needs to be performed at a pH-value allowing maximum degradation of waste cellulose materials by the cellulase enzyme. A pH 5.0, tris buffer solution was prepared by dissolving 1.2 g of tris in 2000 ml of distilled water resulting in a 0.005 M buffer solution and the pH adjusted with concentrated hydrochloric acid and potassium hydroxide (2%).

2.3. Cellulase solution

To purify the cellulase enzyme from sugars, crude *A. niger* (0.1 g) cellulase enzyme was weighed and dissolved in 50 ml of the Tris buffer. The cellulase enzyme-buffer solution was mixed with a magnetic stirrer until a homogenous solution was obtained at a cellulase concentration of 2.0 $mg.ml^{-1}$. This enzyme solution was transferred to a dialyses tube and dialyzed against distilled water for a period of 24 hours at room temperature. Aliquots from this cellulase stock solution were taken and mixed with used newspaper to degrade it into glucose.

2.4. Incubation mixture and sampling

Used newspaper was bio-treated with cellulase from *A. niger* in order to degrade the cellulosic component of newspaper into fermentable sugars, mainly glucose. The different masses of used newspaper were separately incubated with 10µL, 20 µL, 40 µL, 60µL, 80µL and 100µl of the enzyme solution. The total incubation volume was 1000µl with each incubation mixture filled with the Tris-buffer to the required volume. All incubations with the different masses of newspaper and treatment with different enzyme volumes were performed at temperatures of 30^0C, 40^0C, 50^0C and 60^0C during the incubation period of 2h.

2.5. Standard glucose solutions and sugar analysis

The concentration of sugars released from used newspaper during degradation was determined from a standard glucose calibration curve by using the DNS method [19]. A standard glucose stock solution at concentration of 20.00 $mg.ml^{-1}$ was prepared. Samples were taken from this stock solution to prepare diluted glucose solutions at concentrations of 0.50 $mg.ml^{-1}$, 2.00 $mg.ml^{-1}$, 4.00 $mg.ml^{-1}$, 6.00 $mg.ml^{-1}$ and 8.00 $mg.ml^{-1}$.After incubated the different used newspaper materials with the cellulase enzyme the enzymatic reactions were terminated by transferring the DNS reagent (1.5 ml) into the various test-tubes and heat it for a period of 10 minutes in a boiling water bath. The resulting colored solutions were read at 520 nm and the sugar content determined from the calibration curve.

3. RESULTS AND DISCUSSION

The development of organic waste materials such as used newspaper as a resource of bioenergy would become more topical as the effect of fossil fuel combustion on climate is experienced. Cellulose the most abundant organic polymer is considered as an almost inexhaustible bio-resource and can be derivatized to yield various useful products [20, 21, 22]. Waste newspaper one of the cellulosic materials consists of almost 61 % cellulose and 16 % hemicellulose as well as a variable amount of other fillers such as ink [23, 24]. The higher cellulosic content in the waste newspaper than other lignocellulosic materials indicated that it is an alternative resource for cellulose related production [25]. Currently the bioconversion of the carbohydrate ingredient of wastepaper to bioethanol has been investigated [26] a process that initially requires the bioconversion of cellulose into fermentable sugars such as glucose. In an effort to produce more effective cellulase enzymes the organisms are grown on waste newspaper [27]. An important component of developing wastepaper including waste newspaper as a renewable energy resource is the bioconversion of these waste materials cellulose's sections into glucose a fermentable sugar. During this investigation the initial saccharification of used newspaper was observed by exposing different masses of waste newspaper at different incubation temperatures to different concentrations of the *A. niger cellulase* enzyme.

When different masses of newspaper were treated with 10ul of the enzyme solution the 50 pieces showed the maximum sugar production at all the incubation temperatures (Figure 1). It was followed by lower sugar formation obtained from the 22 pieces and the lowest sugar concentration was obtained when 10 pieces of waste newspaper were incubated. The highest amount of sugar formation was obtained during incubation at 50^0C for all the masses. Incubation at 60^0C and at the two lower temperatures showed very similar degradation rates for all three masses of newspaper. The amount of sugar produced at 50^0C from the 50 pieces of newspaper was 42% higher than the lowest amount of sugar produced at 30^0C and 40^0C for similar masses. When degrading the 22 pieces the highest degree of saccharification at 50^0C was 44% higher than the amount of sugar produced at 30^0C. The lowest mass of newspaper (0.0138g) showed an increase of 31% sugar formation from the incubation temperature of 30^0C to 50^0C. Maximum sugar formation of 4 $mg.ml^{-1}$ from 50 pieces when incubated at 50^0C was 207% higher than the lowest amount

of 1.3 mg.ml^{-1} obtained from 10 pieces of newspaper degraded at both 30^{0}C and 40^{0}C.

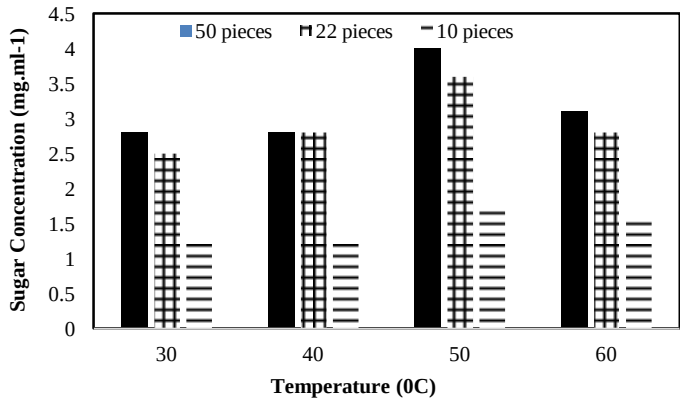

Fig. 1: Bioconversion of different masses of waste newspaper with 10ul of *A. niger cellulase* at different temperatures.

During the bioconversion of the various masses of newspaper with 20ul of the cellulase enzyme solution (Figure 2), maximum bioconversion was obtained with these three masses at an incubation temperature of 50^{0}C. Maximum sugar formation from the 50 pieces at 50^{0}C was 28% higher than the lowest amount obtained during degradation at 40^{0}C. The 22 pieces showed maximum degradation during cellulase catalyzed bioconversion at 50^{0}C and the amount of sugar produced was 75% higher than the lowest amount produced at 30^{0}C. Sugar obtained from the lowest mass of 10 pieces resulted in relative low sugar concentrations between 1.1 to 2.1 mg.ml^{-1}. These concentrations were obtained during exposure to the various temperatures. Maximum sugar production at 4.2 mg.ml^{-1} from the 20 pieces was obtained during incubation at 50^{0}C and was 280 times higher than the lowest sugar formation of 1.1 mg.ml^{-1} obtained when 10 pieces of newspaper was incubated at 60^{0}C.

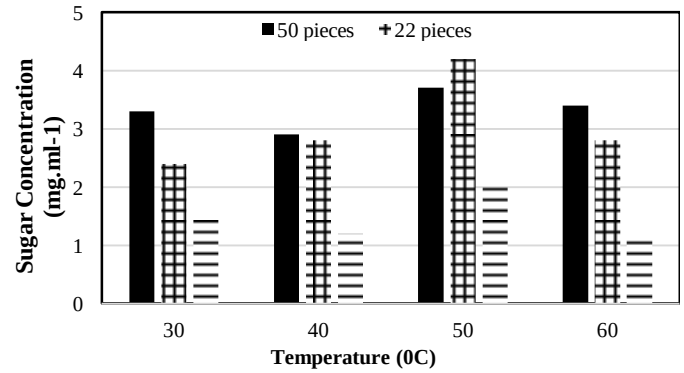

Fig. 2: Bioconversion of different masses of waste newspaper with 20ul of *A. niger cellulase* at different temperatures.

The cellulase catalyzed bioconversion of waste newspaper into fermentable sugars when treating the different masses with 40ul of cellulase enzyme revealed that maximum sugar formation was obtained from both 50 pieces and 22 pieces at the concentration of 4.1 mg.ml^{-1}when incubated at a temperature of 50^{0}C (Figure 3). Sugar formation from the 50 pieces of newspaper at the other temperatures resulted in a decrease of 17% sugar formation at 30^{0}C, 40^{0}C and 60^{0}C. The 22 pieces incubated at 50^{0}C resulted in the same maximum amount of sugar produced at 30^{0}C from the 50 pieces of paper, but it was 58% higher than the lowest concentration of 2.6 mg.ml^{-1} obtained during incubation at 30^{0}C. Bioconversion of the lowest mass of paper (10 pieces) also resulted in the highest sugar formation during incubation at 50^{0}C and lowest sugar production at 40^{0}C. Waste newspaper treated with 60ul of the *A. niger cellulase* showed maximum sugar production when both the 50 pieces and 22 pieces were incubated at 50^{0}C (Figure 4). The concentration of 4.6 mg.ml^{-1} was 28% higher than the concentration of 3.6 mg.ml^{-1} produced when these masses of paper were incubated with the cellulase enzyme at 30^{0}C, 40^{0}C as well as 60^{0}C. The lowest amount of paper (10 pieces) was maximally bioconverted at an incubation temperature of 50^{0}C which resulted in sugar concentration of 2.5 mg.ml^{-1} while the lowest concentration was obtained at 40^{0}C at a concentration of 1.3 mg.ml^{-1}. The maximum sugar concentration of 4.6 mg.ml^{-1} obtained at 50^{0}C when 50 and 22 pieces were degraded was 250% higher than the lowest amount of sugar produced from 10 pieces of newspaper at an incubation temperature of 40^{0}C.

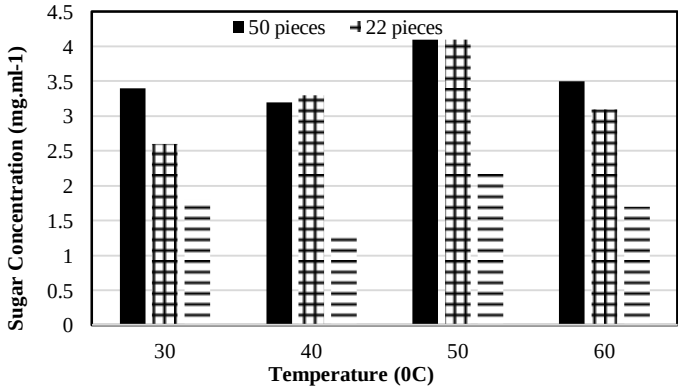

Fig. 3: Bioconversion of different masses of waste newspaper with 40ul of *A. niger cellulase* at different temperatures.

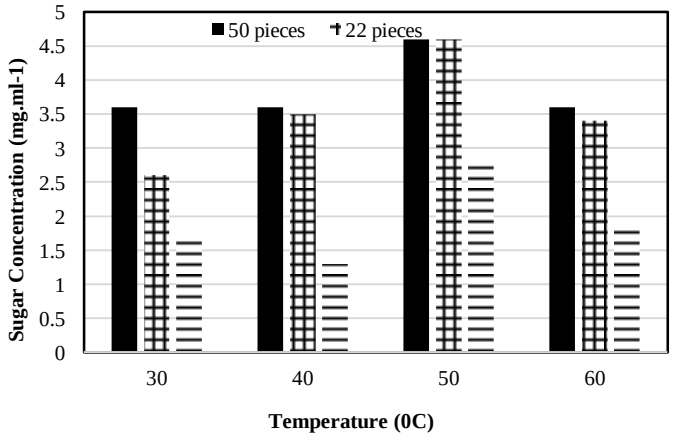

Fig. 4: Bioconversion of different masses of waste newspaper with 60ul of *A. niger* cellulase at different temperatures.

The incubation of 22 pieces and 50 pieces of waste newspaper with 80ul *A. niger* enzyme resulted in the highest amount of sugar production at a concentration of 4.6 mg.ml^{-1} when the incubation was performed at a temperature of 50^{0}C (Figure 5). Degradation of the 50 pieces resulted in a lower concentration of 3.8 mg.ml^{-1} at the other three temperatures of 30^{0}C, 40^{0}C and 60^{0}C. When bioconverting the 22 pieces the lowest sugar concentration was obtained at 30^{0}C at the concentration of 2.6 mg.ml^{-1}. The 10 pieces of newspaper was also maximally degraded at a temperature of 50^{0}C to an extent of 2.4 mg.ml^{-1} that was 100% higher than the lowest concentration of 1.2 mg.ml^{-1} obtained during degradation at 40^{0}C. The maximum sugar formation obtained when exposed to 80ul of enzyme solution was 283% higher at the concentration of 4.6 mg.ml^{-1} than the lowest concentration of 1.2 mg.ml^{-1}.

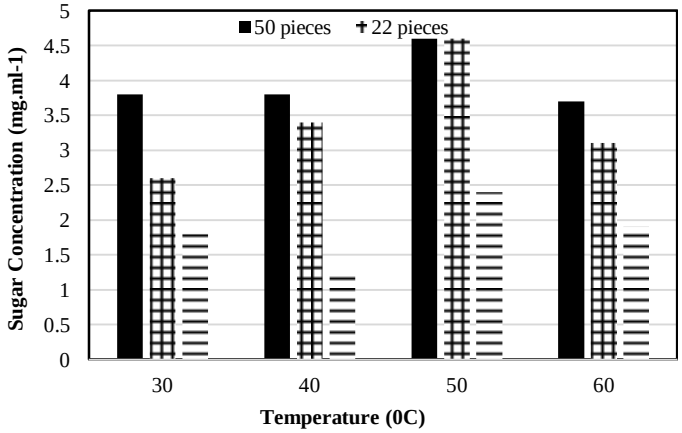

Fig. 5: Bioconversion of different masses of waste newspaper with 80ul of *A. niger cellulase* at different temperatures.

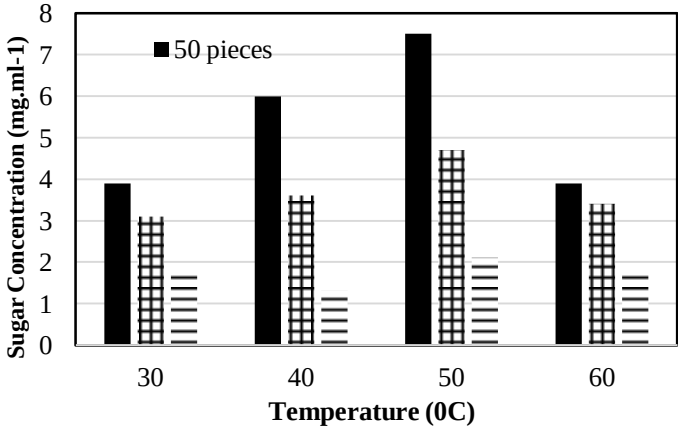

Fig. 6: Bioconversion of different masses of waste newspaper with 110ul of *A. niger cellulase* at different temperatures.

Using 100µl of *A. niger* enzyme solution was the highest enzyme concentration used to bioconvert the various masses of used newspaper at different incubation temperatures (Figure 6). This enzyme solution caused a maximum amount of sugar production at the concentration of 7.5 mg.ml^{-1} when 50 pieces were incubated at the temperature of 50^{0}C. At 30^{0}C and 60^{0}C the lowest concentration of 3.9 mg.ml^{-1} sugar was obtained at 6 mg.ml^{-1} sugar concentration produced when the incubation was performed at 40^{0}C. When 22 pieces of paper were used the maximum degradation was also observed at 50^{0}C at a concentration of 4.7 mg.ml^{-1} that was 52% higher than the lowest concentration of 3.1 mg.ml^{-1} obtained at 30^{0}C. During the incubation of 10 pieces the maximum sugar concentration was obtained at 50^{0}C and the lowest at 40^{0}C. The overall highest concentration of produced sugar during treatment of the newspaper with 100ul of enzyme was obtained from the 50 pieces. When incubated at 50^{0}C the sugar production was 480% higher than the lowest concentration of 1.3 mg.ml^{-1} sugar obtained when 10 pieces was incubated at 40^{0}C**.** The maximum degree of saccharification with all three masses of paper when treated with different enzyme concentrations was observed during incubation at 50^{0}C. Figure 7 reflects the sugar releasing profile from 10 pieces of newspaper when treated with different cellulase concentrations during incubation at 50^{0}C. The lowest amount of sugar production was obtained when 10ul of the enzyme was used for incubation resulting in a sugar concentration of 1.7 mg.ml^{-1}. The highest amount of sugar produced resulted when 60ul of the enzyme was used during the incubation resulting in a sugar concentration of 2.5 mg.ml^{-1}. The sugar formation from the 10 pieces of paper when exposed to 80ul and 100ul was 2.4 mg.ml^{-1} and 2.1 mg.ml^{-1}, respectively. The lowest sugar formation when the highest enzyme concentration was used could be the result of the relative low presence of the newspaper substrate which lower the effective interaction between the cellulase enzyme and the newspaper substrate.

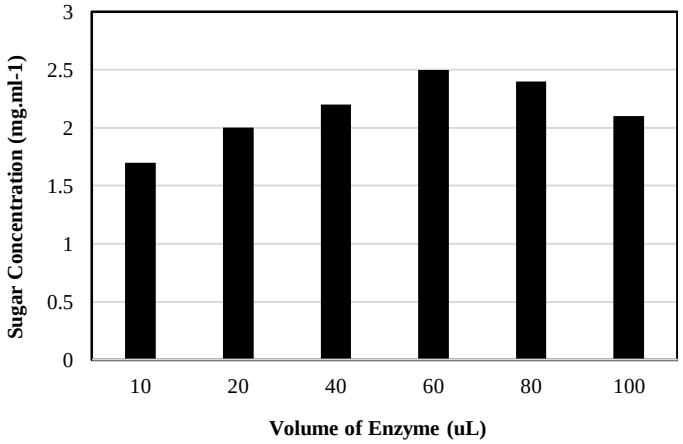

Fig. 7: The relative sugar production from used newspaper when 0.0138g of paper was incubated at 50^{0}C with different enzyme concentrations.

Both incubations with the masses 0.0306g (Figure 8) and 0.0695g (Figure 9) showed an increased tendency of sugar formation when increasing cellulose concentrations were used to degrade the paper material. During the degradation of the 22 pieces a maximum bioconversion was obtained which resulted in a sugar concentration of 4.7 mg.ml^{-1} obtained when treated with 60µl, 80µl and 100µl. This maximum amount of sugar was 30% higher than the lowest concentration of 3.6 mg.ml^{-1}. When

degrading the 50 pieces of newspaper with different enzyme concentrations the resulting sugar concentration also increase from 3.7 $mg.ml^{-1}$ to 7.5 $mg.ml^{-1}$obtained from the 100µlof enzyme solution. This highest amount was 100% higher than the lowest sugar concentration obtained from the 10µl and 20µl enzyme solution.

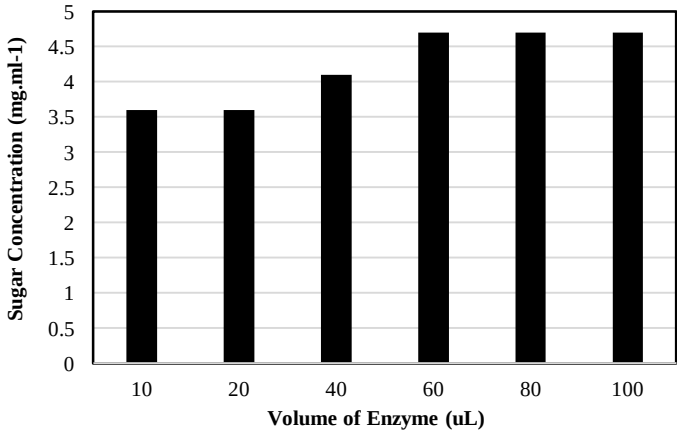

Fig. 8: The relative sugar production from used newspaper when0.0306gof paper was incubated at 50^0C with different enzyme concentrations.

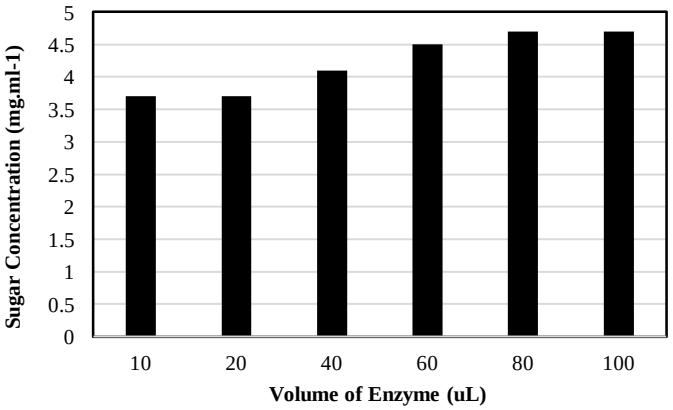

Fig. 9: The relative sugar production from used newspaper when 0.0695g of paper was incubated at 50^0C with different enzyme concentrations.

For quite a while wastepaper has been the research topic for the development of an alternative and renewable energy resource [28]. From this investigation it was shown that newspaper is susceptible for bio-conversion into fermentable sugars, but the process is sensitive for a change in incubation temperature, substrate concentration, enzyme concentration and pH. For maximum bio-conversion of waste newspaper into fermentable sugars the saccharification process should be performed at optimum catalytic conditions.

4. CONCLUSIONS

Cellulase catalyzed bio-conversion of waste paper such as newspaper is a heterogeneous catalytic procedure and the process is more complicated due to the different phases of the cellulase enzyme and newspaper substrate. The bio-conversion profiles of newspaper into sugars are therefore not as ideal as what could be obtained or expected from a homogeneous catalytic process. It can however be concluded that bio-conversion of different masses of newspaper with different concentration of the cellulase enzyme resulted in maximum degradation of newspaper at 50^0C. Increasing cellulase enzyme concentrations also resulted in an increase of the degree of the bio-conversion. Similar increasing sugar concentrations were observed with increasing masses of newspaper degraded. Waste paper is a major component of organic solid waste of which millions of tons are produced annually. Waste newspaper can be as a result of its renewable nature be developed as a resource for the formation of different bio-chemicals and pharmaceuticals which would address the issue of climate change, limit pollution and ensure a clean and healthy environment.

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