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Effect of ink on the saccharification of waste office paper during the biodegradation with cellulase from *Trichodermaviride* at different temperatures

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Abstract

Waste office paper is a major section of the organic part of solid waste that could be developed as a resource of bioenergy. The saccharification of waste cellulose a structural component of waste paper is an important step during the degradation of waste paper into glucose a fermentable sugar. The sugar yield during biodegradation of waste paper will determine the extent of a bioproduct such as bioethanol production. Waste office paper is covered to various extents with ink and this interaction between ink and paper limits the susceptibility of paper for the hydrolytic action of cellulase. Different masses of ink free waste office paper as well as office paper covered 50% and 100 % with ink were incubated with cellulose from Trichodermaviride at different temperatures. The extent of waste paper biodegradation has decreased with increased coverage of ink. Optimum degradation was concluded for ink free office paper followed by paper covered 50 % with ink with the lowest bioconversion experienced with office paper completely covered with ink. Maximum bioconversion of all three types of waste paper was observed during an incubation temperature of 40^{0}C and mass of 0,12 g per incubation mixture.

Keywords: Waste office paper, cellulase, saccharification, ink.

Introduction

Solid waste production with waste paper as a major component is a universal concern as global volumes of waste is increased annually[1]. Also distressing is the negative environmental effect such as climate change which results from fossil fuel combustion[2,3].To conserve the environment a search for alternative and renewable energy resources is topical with the development of bioenergy resources considered as a possible route to generate clean energy[4,5,6]. The conversion of the organic component of solid waste into fermentable sugars for further bioproduct development is extensively researchedand will be a topical issue for the future[7,8,9].

Office paper is a major component of waste paper with large volumes produced daily[10]. Although being recycled the loss of fiber quality would allow waste paper only to be recycled a number of times where after it becomes part of solid waste. Cellulose a structural component of paper materials can be hydrolyzed by cellulase enzymes into glucose that could be fermented intobioproducts such as bioethanol. Thesaccharification of cellulose is a crucial step in the development of waste paper as a resource of bioenergy. Besides the complexity of the degradation procedure by cellulase, a multi-component enzyme system the susceptibility of the cellulose substrate for cellulase enzyme is also a variable that would influence the extent of sugar production from waste cellulose such as waste paper[11]. The physical interaction between the cellulase enzyme and waste paper is an important prerequisite for the catalytic action of the cellulase enzyme. Many sections of waste office paper is covered with ink that could influence thebinding ability of cellulase to the paper material and thus effect the degradationof the cellulose component of waste paper into fermentable sugars. During this investigation different masses of ink free office paper as well as office paper covered 50 % (one side) and 100 % (both sides) with ink have been treated with cellulase from *Trichodermaviridi.* The incubations were performed at different temperatures in order to conclude the effect of ink on the extent of sugar production from waste office paper.

Material and Methods

Enzyme and substrate: Commercial cellulase from *Trichodermaviride* (0,5g) [Merck, EC3.2.1.4., Onozuka R-10) was prepared in 50 ml, 0,005 MTris buffer, pH 5,0. Waste office paper disks with diameter of 5 mm were prepared as free of ink, 50 % and 100 % covered with ink. The paper disks weighed atmasses of 0.008 g, 0.016 g, 0.024 g, 0.032 g, 0.04g, 0.12 g and 0.2 g were exposed separately to the hydrolytic action of the cellulase enzymes.

Cellulase incubation and sugar determination: All waste office paper treatments with the cellulase enzyme were performed in triplicate. The different masses of paper materials were mixed with the Tris buffer (400 ul) and finally mixed with the enzyme solution (100 ul). The various incubation mixtures were incubated during a period of 2 h at temperatures of 30^{0}C,

40^0C, 50^0C and 60^0C. At the end of the incubation period the sugar concentration of each sample was determined with the DNS-method[12] using glucose as a standard.

Results and Discussion

The development of alternative and renewable energy resources would become more topical as the effects of fossil fuel combustion realizes[13,14]. Also a major concern for densely populated areas is the increased production and non-management of solid waste of which the organic component such as waste paper, domestic waste, and garden waste is a major component[15]. The negative effect of dumped organic waste is documented as mainlygreenhouse gases released from landfills during the anaerobic fermentation of organic cellulose[16]. Waste paper has been identified and investigated as a possible resource for bio-energy development[17] but little information is available on the effect of ink on the cellulaseaction during the saccharification of waste paper.

The incubation of all three types of office paper materials (free of ink, 50 % and 100 % covered with ink) at the different incubation temperatures showed an increase in sugar formation with increasing massesof office paper hydrolyzed. At all temperatures and with all masses the paper free of ink showed the highest degree of sac charification followed by paper 50 % covered with ink while the paper materials completely covered with ink were the least susceptible for degradation by the cellulase enzymes.

A maximum sugar concentration of 2,7mg.ml^{-1} was obtained from 0,12 g office paper covered 100 % with ink when incubated at 40^0C (figure 2). At the same incubation temperature a maximum sugar concentration of 6.0 mg.ml^{-1} was calculated from 0,12 g of paper mass, 50 %covered with ink paper whilst the maximum sugar concentration of 6,5 mg.ml^{-1} was released by the ink free paper at a mass of 0.2 g. The amount of sugar released from the various paper materials at all other incubation temperatures were less than the sugar concentrations produced at 40^0C.

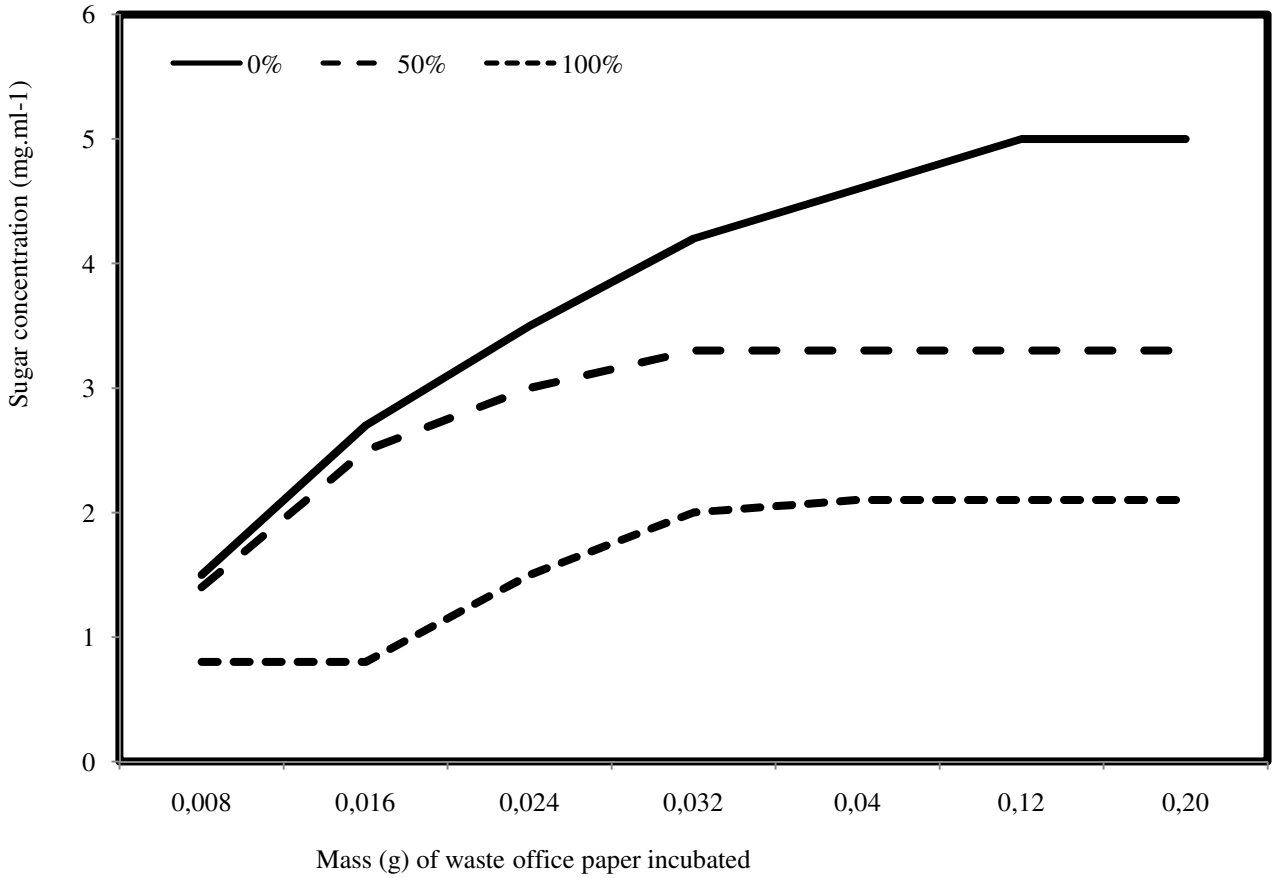

Figure-1
Saccharification of office paper covered with different degrees of ink by cellulase from *Trichodermaviride*at an incubation temperature of 30^0C

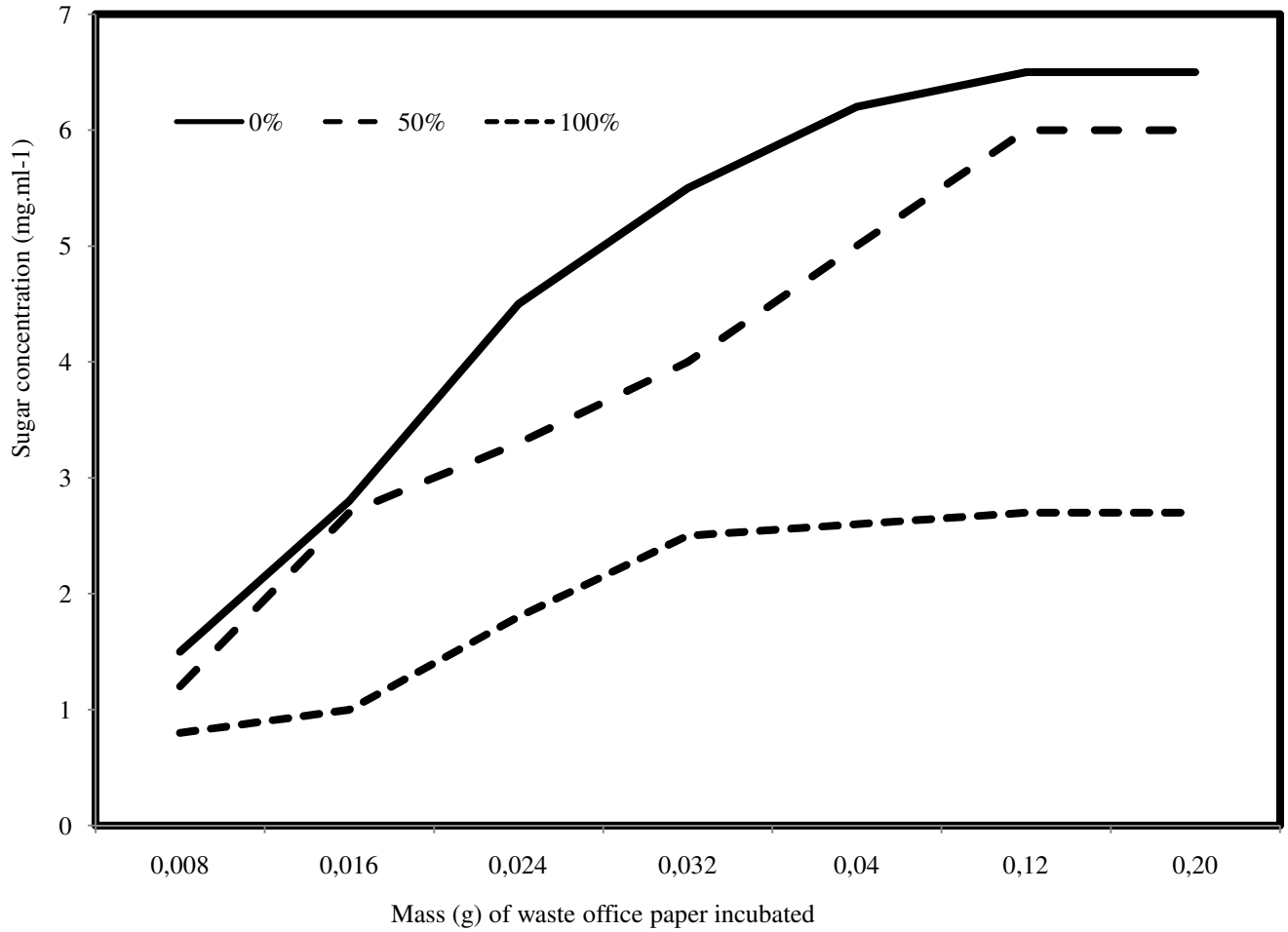

Figure-2
Saccharification of office paper covered with different degrees of ink by cellulase from *Trichodermaviride*at an incubation temperature of 40^0C

The maximum saccharification of office paper which is 100 % covered with ink at an incubation temperature of 40^0C was 22 % higher than the maximum sugar formation from the same paper when incubated at a temperature of 30^0C (figure 1) and 50^0C (figure 3). The maximum conversion of office paper 50 % covered with ink at 40^0C was 40 % higher than the amount of sugar release at 50^0C and 45% higher than the sugar released at 30^0C. Office paper that was 50% covered with ink showed a 66 % increase in sugar formation when incubated at 50^0C relative to the amount of sugar released at 60^0C. When incubated at 60^0C office paper completely covered with ink was not susceptible for cellulase action at all and as a result no sugar was released from this type of paper during the incubation period.

During the saccharification of ink free office paper the maximum conversion was observed at an incubation temperature of 40^0C at a sugar concentration 23 % higher than the yield at 30^0C,and 38% higher than the calculated amount at 50^0C and 50 % more than the amount of sugar released at 60^0 (figure 4). Differences in the amount of sugar formation was not only observed during the saccharification of the paper materials at different incubation temperatures but major differences were also observed in the concentration of sugar formation during the degradation of office paper differently covered with ink while incubated at the same temperature. When incubated at 30^0C the amount of sugar released from ink free office paper was 58 % higher than sugar released from office paper covered 100 % with ink and 34 % higher than the sugar yield from paper 50 % covered with ink. At 50^0C the ink free office paper produced 12 % more sugar than office paper 50 % covered with ink and 48 % more than office paper completely covered with ink. At an incubation temperature of 60^0C no sugar was obtained from the paper totally covered with ink whilst the ink free paper resulted in a sugar concentration of 3,2 $mg.ml^{-1}$ that was 60 % higher than the concentration of sugar released from office paper that was 50 % covered with ink.

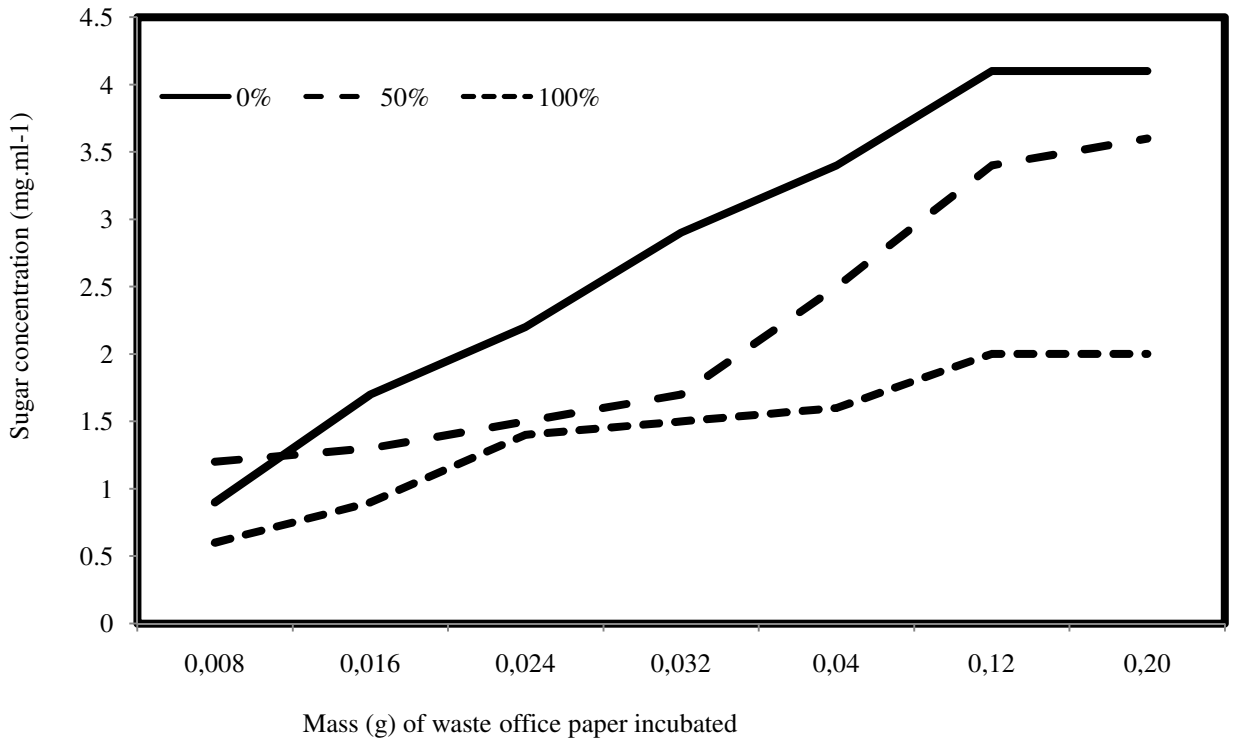

Figure-3
Saccharification of office paper covered with different degrees of ink by cellulase from *Trichodermaviride*at an incubation temperature of 50^{0}C

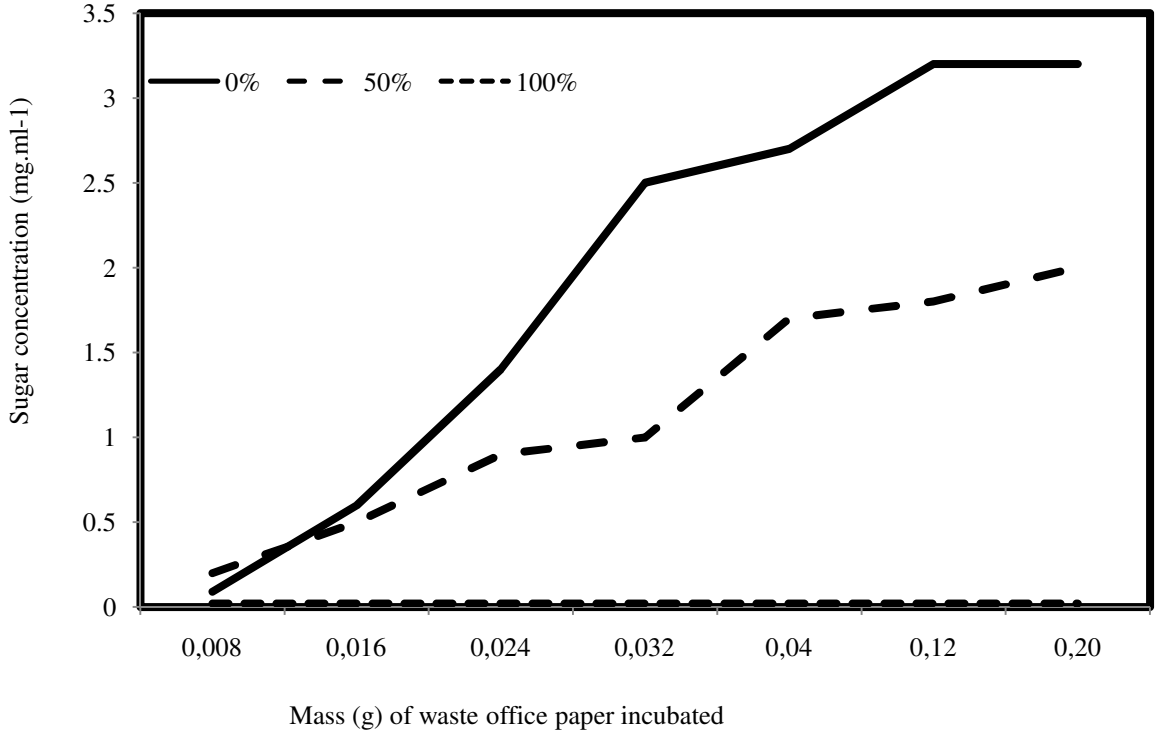

Figure-4
Saccharification of office paper covered with different degrees of ink by cellulase from *Trichodermaviride*at an incubation temperature of 60^{0}C

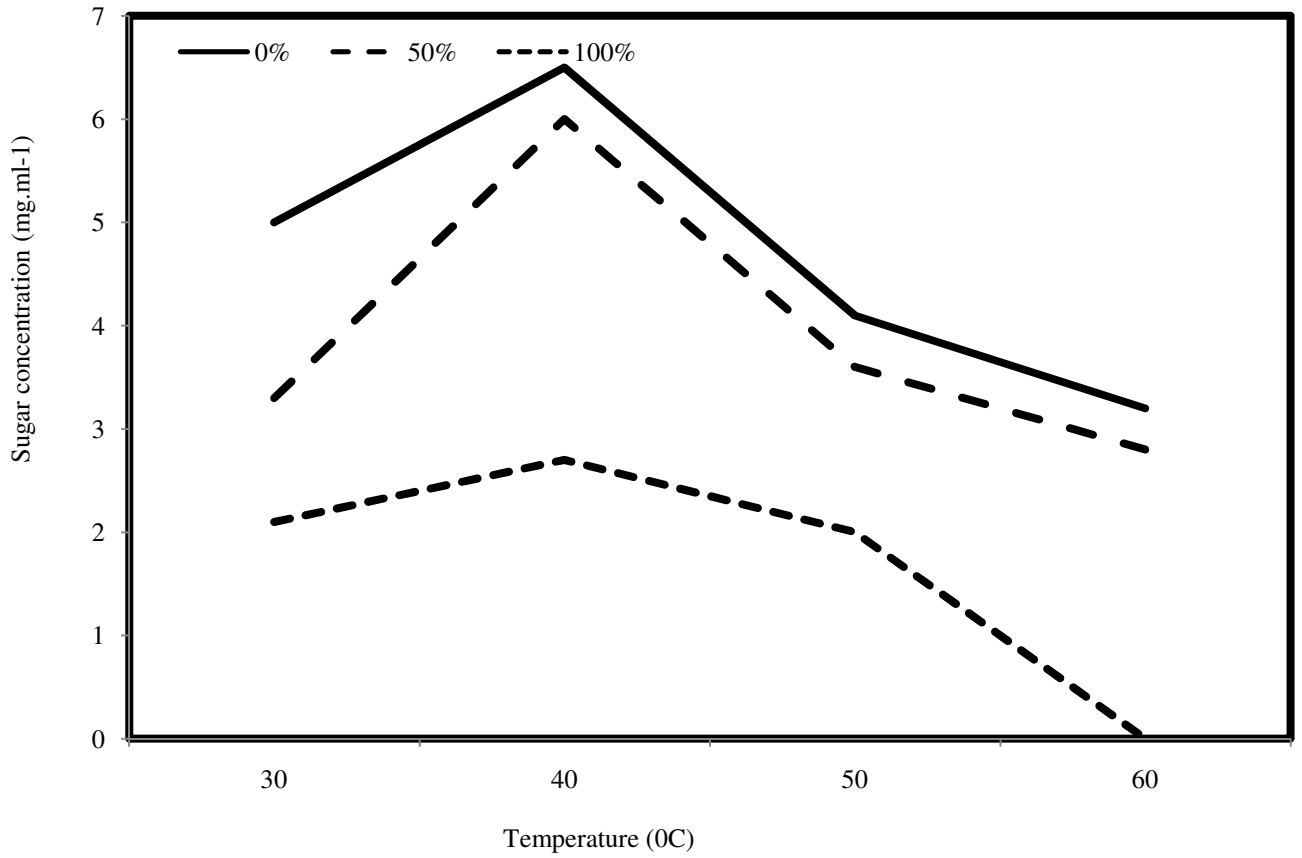

Figure-5
Maximum sugar formation from office paper covered with different amounts of ink while incubated with *T. viride*cellulase at different incubation temperatures

Conclusion

From the investigation it can be concluded that incubation temperature is an important variable to consider during the saccharification of waste office papaer as maximum conversion took place at 40^0C (figure 5) and not at the highest incubation temperature of 60^0C. Like temperature the mass of office paper exposed to biodegradation is also an important variable to optimize for maximum sugar formation during the biodegradation procedure. A mass of 0,2 g per incubation mixture was proved to be the optimum mass during degradation of all types of office paper at the various incubation temperatures. Optimum waste office paper conversion is also dependent on the extent of office paper not covered with ink. Less sugar is released from office paper if the extent of ink coverage is increased. This observation is an important variable to consider when planning a bioconversion process for the saccharification of waste office paper. To increase the susceptibility of waste office paper for cellulase action methods should be applied to remove the ink from waste office paper prior to the cellulase catalyzed degradation of waste office paper into fermentable sugars.

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