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Relative and efficient saccharification of waste office paper by different concentrations of *Aspergillus niger* cellulase at various temperatures

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ABSTRACT: Waste office paper is a major component of solid waste of which thousands of tons are produced daily. The cellulose section of waste office paper can be hydrolyzed by cellulase enzymes into glucose a fermentable sugar. Relative high and low cellulase concentrations from *Aspergillus niger* have been used to degrade waste office paper that was 100% and 50% covered with ink. Office paper free of ink was also exposed to these enzyme concentrations at incubation temperatures of 30^0C, 40^0C, 50^0C and 60^0C. The ink free office paper showed the highest degree of sugar formation at a concentration of 23 mg.ml^{-1} during an incubation at 50^0C and when treated with the highest enzyme concentration. The highest amount of sugar (18 mg.ml^{-1}) produced from office paper 50 % covered with ink when exposed to the high cellulase concentration was obtained at 40^0C whilst an incubation at the same temperature resulted in the paper 100 % covered with ink to be maximally degraded producing a sugar concentration of 17 mg.ml^{-1}. When exposed to the lower enzyme concentration maximum bioconversion of all office paper materials (100 % as well as 50 % covered with ink and ink free) was obtained at 40^0C with sugar produced at concentrations between 2,8 and 6,6 mg.ml^{-1}.

KEYWORDS: Waste office paper, ink, saccharification, *Aspergillus niger*, efficiency, cellulose.

1 INTRODUCTION

Environmental pollution as a result of increasing volumes of solid waste as well as the effect of global warming are topical issues currently addressed by many scientists and environmental concerning groups [1]. The production of solid waste is mainly the result of household, industrial and agricultural activities [2] while global warming results from fossil fuel combustion [3]. With the effects of climate change becoming more evident the search for alternative and renewable energy resources will intensify [4]. Also of major concern is the land used for landfilling with the consequent release of greenhouse gases from these sites [5].

Bioenergy [6] besides solar [7], wind [8] and geothermal energy [9] has been identified as a resource of renewable energy. All plant materials have a structural component known as cellulose that is composed of glucose units connected by means of β-1,6-glucosidic bonds [10]. Cellulose can be hydrolyzed into glucose a fermentable sugar by an enzyme system known as cellulose [11]. A major component of municipal or agricultural solid waste is of plant origin and examples of these materials are food, paper, garden and plant waste, thus a major amount of cellulose is dumped annually. Most solid waste management procedures include incineration, and landfilling that result in waste cellulose to be degraded with the formation of dangerous substances such as greenhouse gases like carbon dioxide and methane. These chemical compounds have a negative effect on the atmosphere that could be linked to current changes in global climate patterns [12], [13].

Waste cellulose, with glucose as its structural and fermentable building block is considered as a renewable energy resource and when bio-recycled can be converted into biofuels, bio-chemicals or bio-pharmaceuticals [14]. The cellulase catalyzed degradation of cellulose is a complex procedure and the presence of ink on the paper materials could further complicate the degradation procedure as ink acts as a barrier between the paper material and cellulase enzyme. The effective cellulase activity could also be influenced by variables such as enzyme concentration and temperature that could determine the amount of glucose released during hydrolysis of waste cellulose.

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Organic substances are a major component of solid waste with cellulose a structural component thereof. Examples of organic waste include paper and kitchen waste or waste food, garden trimmings as well as many residuals from the agricultural industry. A potential process of bio-recycling waste cellulose is the treatment of these materials with cellulase enzymes converting it into fermentable sugars. Waste paper a major component of organic and solid waste has already been identified as a potential resource of bio-energy [15]. Different types of waste paper such as office paper, newspaper, foolscap paper have been treated with cellulases from different sources in order to saccharified these materials [16].

During this investigation ink free, as well as office paper covered 50 % and 100 % with ink have been exposed to a relative high and low concentration of cellulase from *Aspergillus niger*. These incubations were also performed at different temperatures in order to determine the effect of ink on the bio-degradation of waste office paper. The development of organic waste such as waste office paper as a resource of bioenergy would not only address the issue of an alternative for fossil fuel combustion but will also limit the volumes of solid waste occupying valuable land which then could be used for agricultural purposes or residential development.

2 MATERIALS AND METHODS

2.1 ENZYME AND SUBSTRATE

Commercial cellulase from *Aspergillus niger* (0,5 g) [ICN, Biomedical Inc. 9012-54-8] was prepared in 50 ml, 0,005 M Tris 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 at masses of 0,004 g, 0,012 g, 0,02 g, 0,028g, 0,036 g and 0,044 g were exposed separately to the hydrolytic action of the cellulase enzyme concentrations of 4,7 $mg.ml^{-1}$ and 10,6 $mg.ml^{-1}$.

2.2 CELLULASE INCUBATION, SUGAR AND PROTEIN 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 with the various enzyme solutions (100 ul). The incubation mixtures were incubated at temperatures of 30^0C, 40^0C, 50^0C and 60^0C during a period of 2 h. At the end of the incubation period the sugar concentration of each sample was determined with the DNS-method using glucose as a standard [17]. The concentrations of cellulase solutions were determined by using the biuret test for proteins with BSA as standard [18].

3 RESULTS AND DISCUSSION

The search for alternative and renewable energy resources will have to be intensified in order to limit the growing negative effects which the excess production of greenhouse gases has on the environment. Various waste cellulose products have already been exposed to cellulase catalyzed saccharification [19] and during the current investigation all office paper materials showed an increased amount of sugar production when increasing masses of paper was degraded. The amount of sugar released from the various paper materials increased when increasing masses of paper were hydrolyzed until a unique optimum sugar concentration was obtained that was maintained when further increased paper masses were degraded. During incubations at different temperatures the maximum saccharification was obtained when ink free office paper was degraded with the highest enzyme concentration of 10,6 $mg.ml^{-1}$ at 50^0C (fig. 3) resulting in a sugar concentration of 23 $mg.ml^{-1}$. The second highest sugar concentration (21 $mg.ml^{-1}$) produced from ink free office paper was obtained at 40^0C (fig. 2) while the lowest sugar concentration (3,67 $mg.ml^{-1}$) was formed during an incubation at 30^0C (fig. 1). The second lowest amount of sugar produced (15 $mg.ml^{-1}$) from ink free office paper when treated with the highest enzyme concentration was obtained during incubation at 60^0C (fig. 4). Degradation of office paper 50 % covered with ink showed the highest degree of saccharification (18 mg/ml) when this paper material was incubated at 40^0C with the high cellulase concentration. Office paper completely covered with ink was maximally degraded with the highest cellulase concentration at an incubation temperature of 40^0C that resulted in a sugar concentration of 17 mg/ml (Fig. 2). The maximum amount of sugar released from the ink free paper was 27 % higher than the maximum amount of sugar released during degradation of office paper 50 % covered with ink and 37 % more than the maximum amount of sugar released during degradation of office paper fully covered with ink.

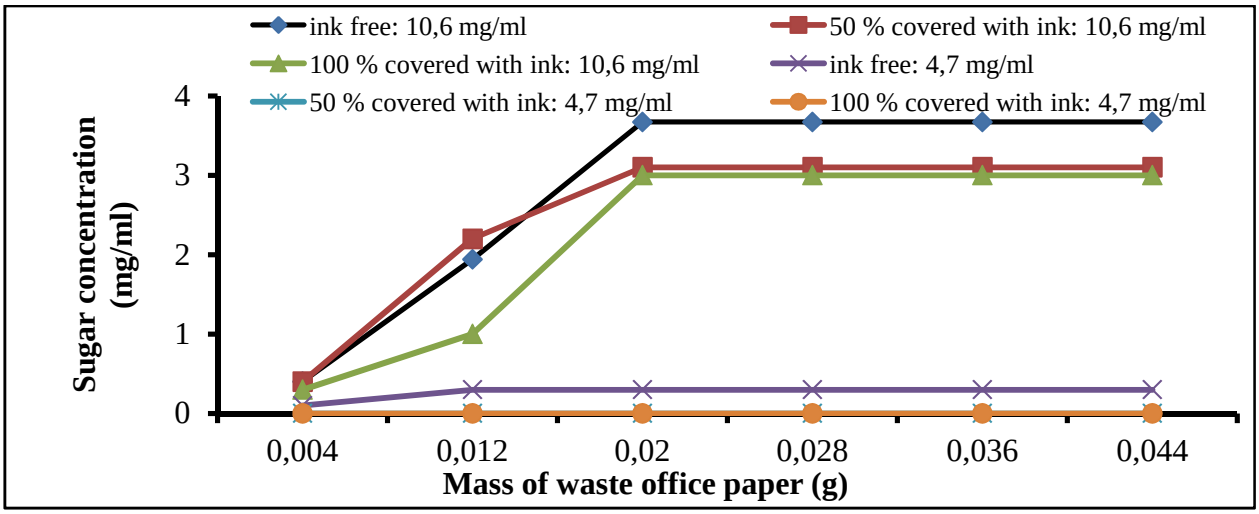

Fig. 1 - Saccharification of waste office paper with cellulase from Aspergillus niger at an enzyme concentration of 10,6 mg.ml^{-1} and 4 mg.ml^{-1} at an incubation temperature of 30^{0}C

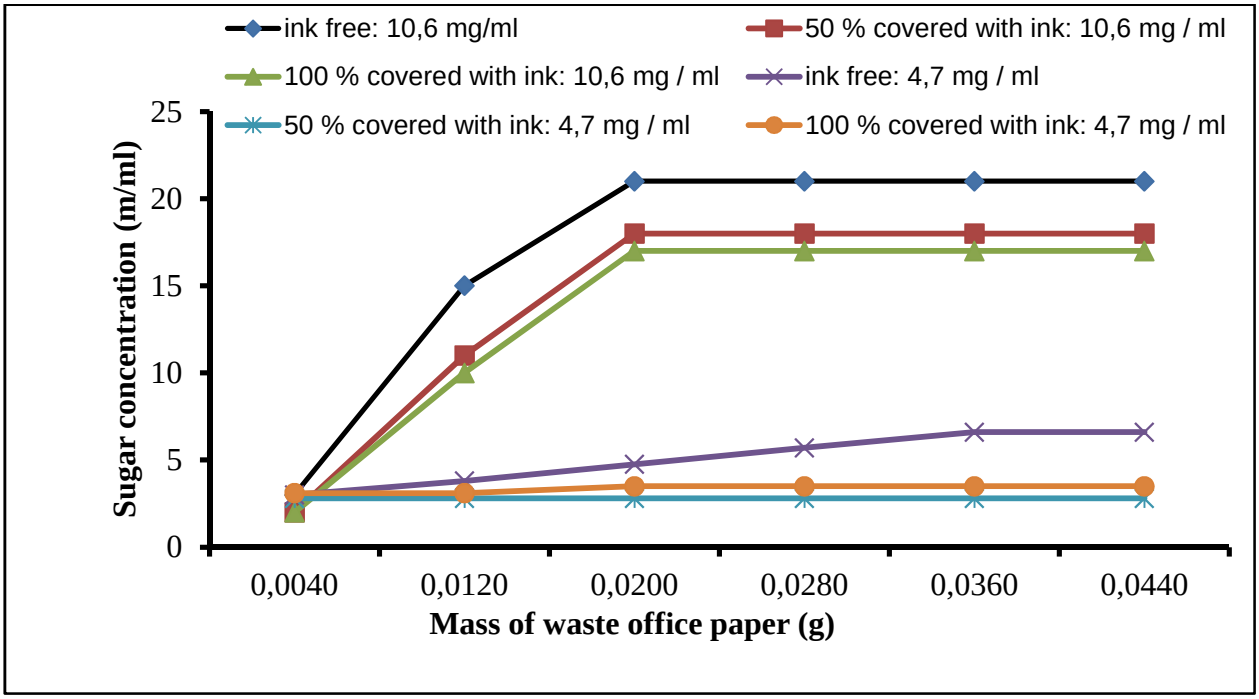

Fig. 2 - Saccharification of waste office paper with cellulase from Aspergillus niger at an enzyme concentration of 10,6 mg.ml^{-1} and 4 mg.ml^{-1} at an incubation temperature of 40^{0}C.

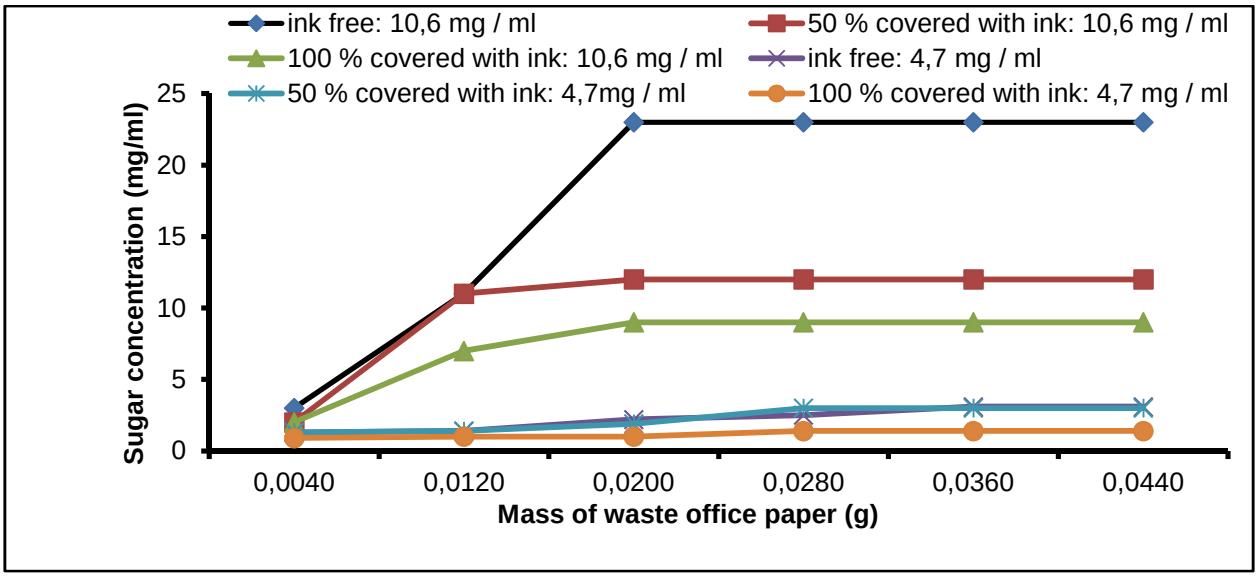

Fig. 3 - Saccharification of waste office paper with cellulase from Aspergillus niger at an enzyme concentration of 10,6 mg.ml^{-1} and 4 mg.ml^{-1} during ml at an incubation temperature of 50^{0}C.

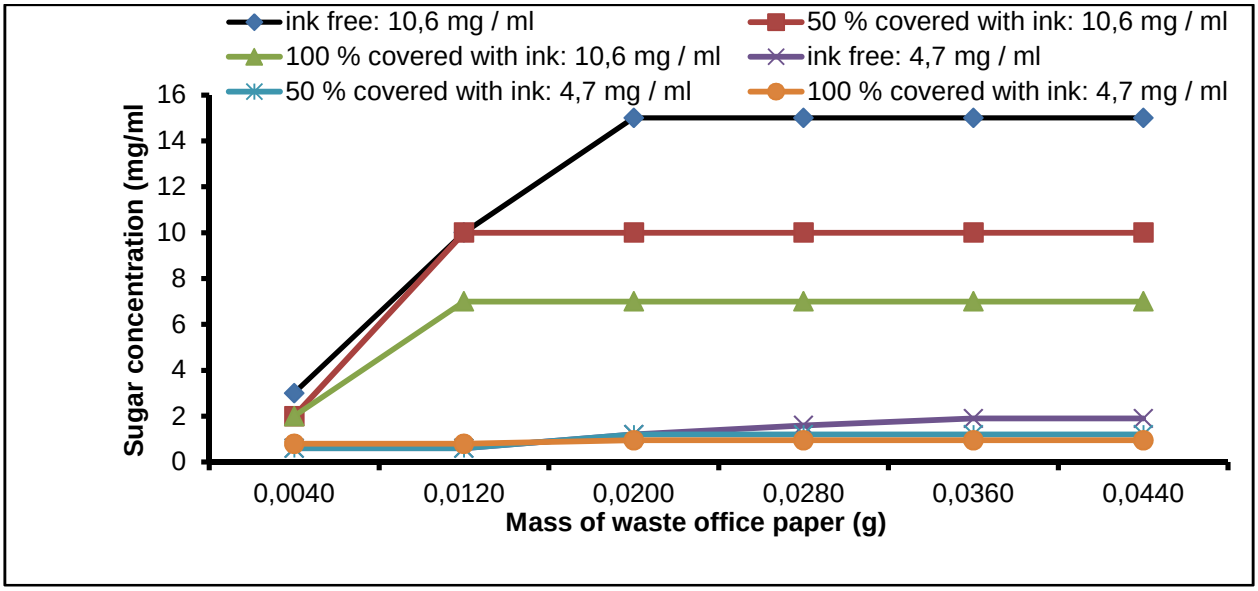

Fig. 4 - Saccharification of waste office paper with cellulase from Aspergillus niger at an enzyme concentration of 10,6 mg.ml^{-1} and 4 mg.ml^{-1} during ml at an incubation temperature of 60^{0}C.

The bio-degradation of the various office papers with the lower enzyme concentration followed the same saccharification tendency as observed when treated with the higher enzyme concentration. Ink free paper proved to be the most susceptible for degradation followed by paper 50% covered with ink while the lowest degree of saccharification was experienced with office paper 100 % covered with ink. When exposed to the lower enzyme concentration a maximum sugar formation was obtained at an incubation temperature of 40^{0}C with the highest sugar concentration obtained from ink free office paper (6,6 mg/ml), followed by paper 50 % covered with ink (5,0 mg/ml) and 3,5 mg/ml sugar obtained from paper fully covered with ink (Fig. 2).

Overall the maximum sugar formation released from the ink free office paper when treated with the higher enzyme concentration was 248 % higher than the maximum amount of sugar released from the same paper when treated with the lower enzyme concentration. The maximum concentration of sugar released from the waste office paper 50 % covered with

ink was 266 % higher than the amount of sugar released from the identical paper when treated with the lower enzyme concentration. An increase of 500 % of sugar formation from the office paper fully covered with ink when treated with the 10,6 mg/ml enzyme solution relative to the sugar formation when this paper was exposed to the lower enzyme concentration.

Although the amount of sugar released from waste office paper is increased when increasing masses of these materials were treated with a fixed enzyme concentration the effectiveness of the saccharification process does not follow the same tendency. The effectiveness of the biodegradation process is expressed in terms of percentage saccharification of waste office papers when treated with the different enzyme concentrations at the different temperatures. The percentage saccharification of waste office paper at an incubation temperature of 30^0C is reflected in table 1 indicating that the highest percentage conversion of ink free office paper with the low enzyme concentration was obtained from a mass of 0.004g and 0,012g whilst the highest cellulase concentration produced maximal percentage saccharification from an office paper mass of 0,02 g producing a 9.1 % saccharification. During the treatment of office paper that was 50 % covered with ink only the high cellulase concentration could affect degradation and at a degree of 9,1 % from 0,012 g paper whilst a mass of 0,02 g of office paper that was fully covered with ink was optimally degraded by the same high enzyme concentration to an extend of 7,5 % degradation.

Table 1 - Percentage (%) saccharification of waste office paper with different concentrations of cellulase from Aspergillus niger during an incubation temperature of 30^0C.

Percentage (%) ink coverage of office paper	Mass of office paper (g)	Percentage (%) saccharification when exposed to the different cellulase concentrations:	
		Low cellulase concentration (4,7 mg.ml^{-1})	High cellulase concentration (10,6 mg.ml^{-1})
0	0.004	1.25	5.0
	0.012	1.25	8.0
	0.02	0.75	9.1
	0.028	0.5	5.0
	0.036	0.4	4.0
	0.044	0.3	2.0
50	0.004	0	5.0
	0.012	0	9.1
	0.02	0	7.7
	0.028	0	5.5
	0.036	0	4.3
	0.044	0	3.5
100	0.004	0	3.7
	0.012	0	4.1
	0.02	0	7.5
	0.028	0	4.1
	0.036	0	3.4
	0.044	0	3.4

During an incubation of ink free office paper at 40^0C (Table 2) the lowest cellulase concentration resulted in the highest percentage saccharification of 37,5 % obtained from a mass of 0,004 g while a 63 % sacchrification was obtained from a higher mass of 0,012 g when exposed to the highest enzyme concentration of 10,6 mg.ml^{-1}. The office paper 50 % covered with ink was 11,6 % saccharified when 0,012 g were treated with the lowest enzyme concentration whilst 0,02 g of this office paper was 47 % degraded when exposed to the highest enzyme concentration. The maximum degradation of paper 100 % covered with ink was 13 % saccharification obtained from 0,012 g of the paper with the low enzyme concentration and 43 % degradation when 0,02 g paper was exposed to the highest cellulase concentration.

Table 2 - Percentage (%) saccharification of waste office paper with different concentrations of cellulase from Aspergillus niger during an incubation temperature of 40^{0}C.

Percentage (%) ink coverage of office paper	Mass of office paper (g)	Percentage (%) saccharification when exposed to the different cellulase concentrations:	
		Low cellulase concentration (4,7 mg.ml^{-1})	High cellulase concentration (10,6 mg.ml^{-1})
0	0.004	37.5	37.5
	0.012	15.8	63.0
	0.02	11.8	52.5
	0.028	10.1	37.5
	0.036	9.1	29.1
	0.044	7.5	23.0
50	0.004	35	25.0
	0.012	11.6	45.0
	0.02	7.0	47.0
	0.028	5.0	33.0
	0.036	3.8	25.0
	0.044	3.5	20.8
100	0.004	12.0	25
	0.012	13.0	41
	0.02	8.7	43
	0.028	6.2	31
	0.036	4.8	25
	0.044	3.9	19

When exposed to 50^{0}C during incubation with cellulase from *A. niger* the maximum degree of saccharification from all three types of office paper when treated with the lower enzyme concentration were obtained during degradation of the lowest amount of paper of 0,004 g (Table 3). During these treatments both ink free office paper and 50 % covered with ink resulted in 16,25 % saccharification, followed by office paper fully covered with ink that were 11,25 % degraded into sugars. When treated with the higher cellulase concentration at 50^{0}C the maximum percentage saccharification of 59 % was obtained from 0,02 g of ink free office paper while 0,012 g of office paper 50 % covered with ink resulted in 45 % saccharification. The paper completely covered with ink was maximally saccharified at 29 % when 0,012 g of this paper was degraded with the high enzyme concentration.

Table 3 - Percentage (%) saccharification of waste office paper with different concentrations of cellulase from Aspergillus niger during an incubation temperature of 50^{0}C.

Percentage (%) ink coverage of office paper	Mass of office paper (g)	Percentage (%) saccharification when exposed to the different cellulase concentrations:	
		Low cellulase concentration (4,7 mg.ml^{-1})	High cellulase concentration (10,6 mg.ml^{-1})
0	0.004	16.25	37.0
	0.012	5.8	45.0
	0.02	5.5	59.0
	0.028	3.4	42.0
	0.036	3.3	32.8
	0.044	3.0	26.8
50	0.004	16.25	25.0
	0.012	5.8	45.0
	0.02	4.75	30.0
	0.028	5.3	21.0
	0.036	4.1	16.0
	0.044	3.4	13.0
100	0.004	11.25	25.0
	0.012	4.1	29.0
	0.02	2.5	22.0
	0.028	2.5	16.0
	0.036	1.9	12.5
	0.044	1.5	10.2

Table 4 reflects the percentage saccharification of the various ink covered office papers when treated with the cellulase enzyme at an incubation temperature of 60^0 C. A mass of 0,12 g of office paper resulted in all three type of papers to be saccharificed to the highest percentage by the higher cellulase concentration with ink free office paper degraded to an extent of 42 % followed by paper covered 50 % with ink bio-converted to an extent of 41 % and finally a 29 % saccharification of office paper fully covered with ink. The lowest mass of 0,004 g of all three types of office paper materials were maximally degraded at this temperature by the lowest cellulase concentration. The percentage saccharification of office paper with both low and high cellulase concentrations at 30^{0}C resulted in the lowest percentage degradation of all incubation temperatures with no degradation observed when office paper 50 % and 100 % covered with ink was exposed to the low cellulase concentration at different temperatures.

Table 4 - Percentage (%) saccharification of waste office paper with different concentrations of cellulase from Aspergillus niger during an incubation temperature of 60^{0}C.

Percentage (%) ink coverage of office paper	Mass of office paper (g)	Percentage (%) saccharification when exposed to the different cellulase concentrations:	
		Low cellulase concentration (4,7 mg.ml^{-1})	High cellulase concentration (10,6 mg.ml^{-1})
0	0.004	7.5	37.5
	0.012	2.5	42
	0.02	3.0	37.5
	0.028	2.8	26.0
	0.036	2.6	21.0
	0.044	2.1	17.0
50	0.004	7.5	25.0
	0.012	2.5	41.0
	0.02	3.0	25.0
	0.028	2.1	17.0
	0.036	1.6	13.0
	0.044	1.3	11.0
100	0.004	10.0	25.0
	0.012	3.3	29.0
	0.02	2.3	17.5
	0.028	1.7	12.5
	0.036	1.3	9.7
	0.044	1.1	7.9

Different organic waste materials such as banana agro-waste [20], waste paper, kitchen waste and animal waste [21] have been identified as possible resources for bio-energy development. These types of bio-developments would become more topical as it is environmental friendly as organic waste is used as a resource for bio-energy production.

4 CONCLUSIONS

The development of renewable energy resources would become more topical as the negative environment effects of fossil fuel combustion are realized. Also of major concern is the accumulation of solid waste of which waste paper is a major component. Waste office paper offers a relative high susceptibly to be developed as a resource of bioenergy due to its cellulose content. In order to optimize the bioconversion of waste office paper as a resource of bioenergy the process needs to be optimized in terms of maximum sugar production that requires exposure of different masses of paper waste to various enzyme concentrations. Bioconversion conditions in terms of pH, temperature and type of cellulase also need to be optimized.

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