Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(10):9-17

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Saccharification of ink covered office paper by different concentrations of cellulase from *Trichoderma viride*

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ABSTRACT

Cellulose, a biopolymer composed of glucose units, is a structural component of waste office paper. Different masses of ink free office paper as well as office paper covered 50 % and 100 % with ink were treated with cellulase from Trichoderma viride releasing fermentable sugars such as glucose thus exploring the renewable energy potential of waste cellulose. Ink covered office paper resulted in less sugar formation compared to ink free office paper. Although an increased amount of sugar was released when increasing masses of waste office paper was hydrolyzed with increasing cellulase concentrations the efficiency of bioconversion was not increasing mass dependent.

Keywords: Waste office paper, Ink, Saccharification, *T.viride*, Efficiency, Sugars.

INTRODUCTION

The search for costly and environmental effective renewable energy resources will intensity as the effect of climate change due to the combustion of fossil fuels becomes evident with increasing droughts and rainfall in different parts of the globe[1]. These changing weather patterns as a result of global warming will not only have an effect on the infrastructure of many countries but will also cause decision makers to re-think issues such as electricity generation, food productionand ways of protection against strong winds and floods.

Biomass has been considered by many as an alternative and renewable energy resource for the production of energy in the form of petroleum, electricity and heat energy[2]. Biofuelssuch as bioethanol obtained from sugarcane is already used by countries such as Brazil as a liquid fuel for motor vehicles [3]. The production of bio-pharmaceuticals from plant materials is also gaining momentum [4]while electricity generation from wood resources is also receiving attention [5].

Another concern besides global warming is the production and accumulation of solid waste of which organic waste is a major component. Millions of tons of organic waste are produced annually occupying valuable land, producing and release dangerous gases during natural fermentation in landfills. Greenhouse gases such as methane and carbon dioxide are produced thus also lower air quality that could stimulate certain bronchial conditions such as allergic respiratory diseasesin peoples living and working in close vicinity of these land fill areas[6]. Solid waste also occupies land that could have been used for agricultural or residential purposes. Organic waste composes of many substances of which paper, food and garden trimmings are major components [7].

An important structural component of organic waste is a natural polymer known as cellulose composed of glucose monomers that are connected by chemical bonds described as β-1,4-glucosidic bonds. Besides acid catalyzed hydrolyses these chemical bonds could also be destroyed by enzymatic action releasing glucose units that could be fermented into bio-products such as bio-ethanol thus render organic waste a potential renewable energy resource. Cellulasea multi-component enzyme system present in bacterial as well as fungal sources plays an important role in releasing glucose from cellulose which then could act as an energy source for these micro-organisms. Cellulose is a structural component of foods, plants and consumer products fromplant originsuch as paper products and when classified and treated as waste all organic materials are discarded with their cellulose sectionsthat is a potential resource of renewable energy[8].

Thousands of tons of paper materials are manufacturedannually from trees and after use treated as part of solid waste. Used paper can only be recycled a number of times before the fibers become too weak to guarantee a paper material of high quality. At this stage recycled paper is landfilled, burnt or incinerated without exploring its renewable energy potential [9,10,11,].

Various types of wastepaper materials are produced with office paper a major component of paper products and as a result a foremost section of organic waste. Waste office paper, representing organic waste,could be developed as a resource of bioenergythrough a cellulase catalyzed process of bio-converting its cellulose component into fermentable sugars such as glucose [12]. Amongst other, most paper products are used in the printing industry causing waste paper to be covered to a certain extent with ink. When exposed to the saccharification action of cellulase the ink covering waste paper could act as a shield preventing a physical interaction betweencellulaseand cellulose fibers that could result in a lower degree of cellulose saccharification. The detailed effect of ink, when covering office paper, on the cellulase catalyzed biodegradation of waste office paper has not received much attention in literature.

This study was aimed at investigating the effect of ink on the relative saccharification of ink free office paper, as well as office paper covered 50 % and 100 % with ink when exposed to the biodegradation action of cellulase from *Trichoderma viride*. The efficiency of the bioconversion process was also concluded.

EXPERIMENTAL SECTION

Enzyme and substrate

Commercial cellulase from *Trichoderma viride* (0,5g) [Merck, EC3.2.1.4., Onozuka R-10]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 atmasses of 0,0038 g, 0,0114 g, 0,019 g, and 0,0266 g were exposed separately to the hydrolytic action of the cellulase enzymes at different concentrations of 2 $mg.ml^{-1}$, 4 $mg.ml^{-1}$, 6 $mg.ml^{-1}$, 8 $mg.ml^{-1}$ and 10 $mg.ml^{-1}$.

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 various enzyme solutions (100 ul). The incubation mixtures were incubated during a period of 2 h at temperatures of 30^0C, 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 usingglucose as a standard [13].

RESULTS AND DISCUSSION

The development of renewable energy resources would become more topical as the effect of climate change as a result of fossil fuelcombustion is experienced, globally. To counteract the effect of global warming traditional fossil fuel sources will have to be replaced with renewable energy resources. Also of importance is a clean environment and air ensuring healthy conditions for humans and animals. Various renewable energies such as solar energy [14], wind energy[15] and bio-energy [16]have been identified as potential substitutes for fossil energy. Waste cellulose has been identified as a major contributor of bio-energy resources as this biopolymer is the major structural component of organic waste such as waste paper, certain waste food substances, garden trimmings and trees [17]. Different types of waste paper like office paper, newsprint, foolscap paper have been implicated in the development

of organic waste as a resource of bioenergy[18]. All these paper materials are used in the printing industry causingwaste cellulose substance to be fully or partially covered with ink with certain sections free of ink.

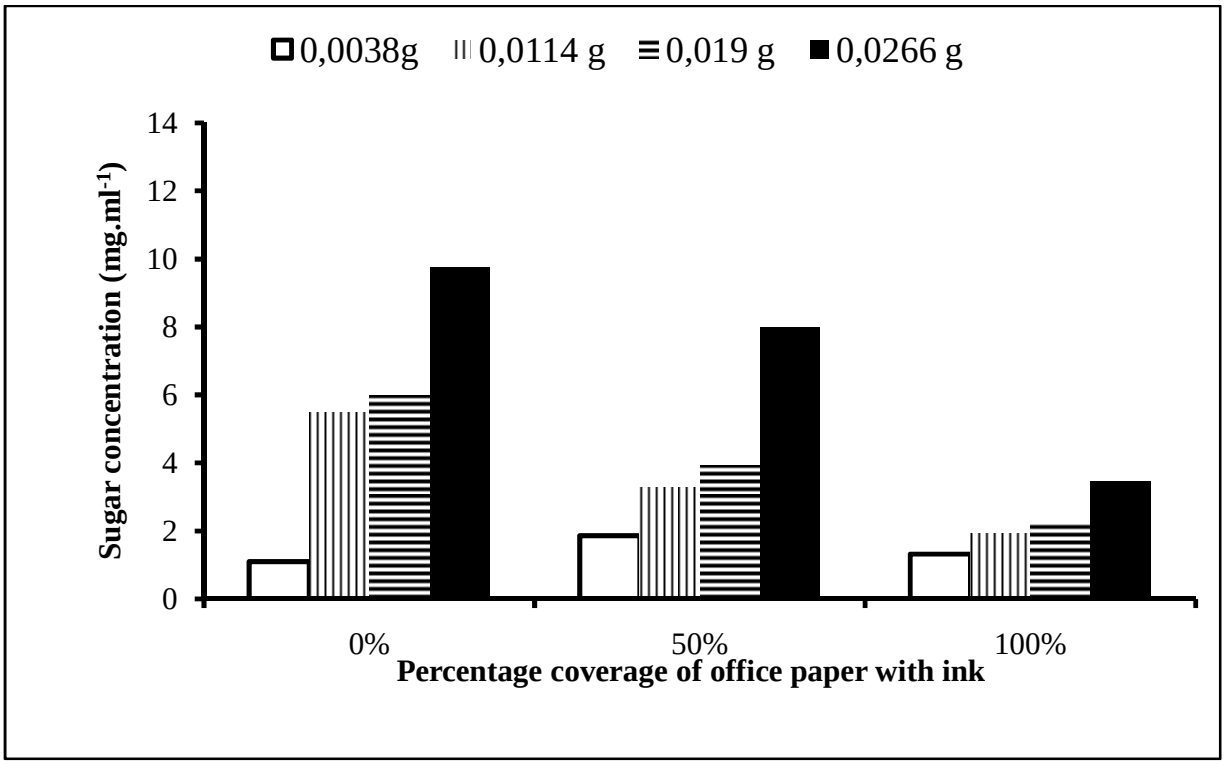

Figure (1):Saccharification of waste office paper with cellulase from *Trichoderma viride*at an enzyme concentration of 2 mg/ml

During the cellulase catalyzed degradation of waste paper it is important that the cellulase enzyme is physically in contact with the surface of the paper materialto perform its hydrolytic acton. The presence of ink on the paper materialcould prevent the enzyme to a certain extent from acting freely on the waste cellulose materials releasing fermentable sugars.

The amount of sugar released from different masses of ink free office paper as well office paper covered 50 % and 100 % with ink when treated with different concentrations of cellulase from *T. viride*were compared in order to determine the effect of ink on the bioconversion of waste office paper.

Saccharification with acellulase concentration of 2 mg.ml^{-1}

When exposed to a cellulase concentration of 2 mg.ml^{-1}all office papersshowed an increase in sugar formation when increasing masses of the respective ink covered paper materials and ink free paper were degraded (Fig. 1). With all masses the ink free paper showed sugar concentrations higher than the amount of sugar produced from the office paper 50 % and 100 % covered with ink except for the sugar released from the lowest mass of 0,0038 g. From this lowest paper mass the highest amount of sugar released was from the paper 50% covered with ink, the paper covered 100 % with ink showed the second highest degree of degradation whilst the ink freeoffice paper was the least saccharified. This observation could be due to the heterogeneous nature of the catalytic reaction and the relative low amount of substrate present in the incubation mixture which limits the possibilities for interaction between cellulase and the substrate. When the masses of paper substrates were increased the degradation exhibits a pattern of increasing sugar production with increasing masses of waste office paper incubated. The amount of sugar released from 0,0114 g ink free paper when degraded was 88 % higher than the amount of sugar produced from the paper 50% covered with ink and 316 % higher than sugar produced from the paper completely covered with ink. When 0,019 g of office paper was treated with the cellulase enzyme the amount of sugar released from the ink free paper was 52 % higher than the amount of sugar released from the paper that was 50% covered with ink and 172 % higher than the amount of sugar released from the paper completely covered with ink. When the highest mass of

0,0266 g office paper was treated with the enzyme concentration of 2 mg.ml^{-1} a sugar concentration of 9,75 mg.ml^{-1} was obtained from the paper not covered with ink and this sugar concentration was 22 % higher than the concentration of sugar formed from the 50 % ink covered paper and 182 % more than the sugar produced from the paper completely covered with ink.

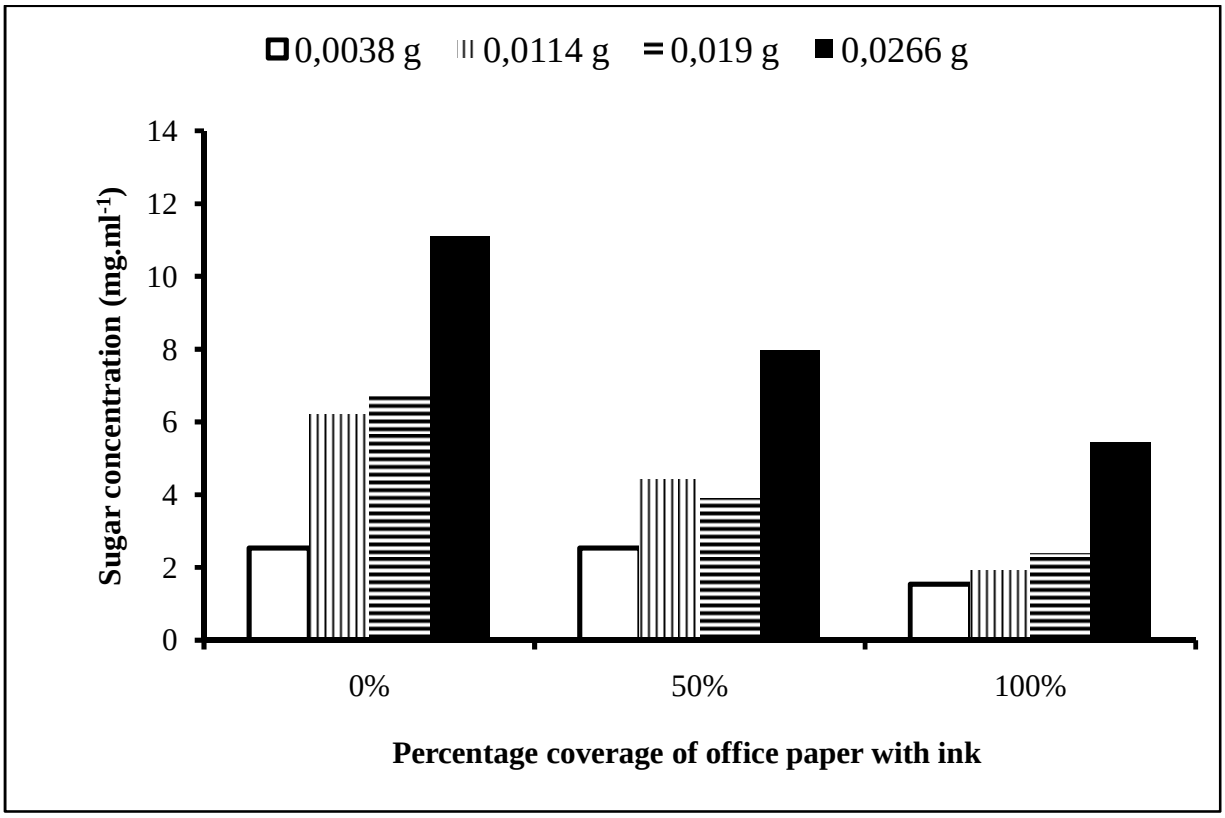

Figure (2):Saccharification of waste office paper with cellulase from *Trichoderma viride*at an enzyme concentration of 4 mg/ml

The amount of sugar produced from 0,0266 g of ink free paper was 786 % higher than the amount of sugar produced at a concentration of 1,10 mg.ml^{-1} during the degradation of the lowest mass of 0,0038 g. In the case of the paper 50 % covered with ink sugars were released from the highest paper mass at a concentration of 7,98 mg.ml^{-1} that was 329 % higher than the concentration of sugars released at concentration of 1,86 mg.ml^{-1} released from 0,0038 g. During the degradation of office paper 100 % covered with ink the amount of sugar released from the highest mass was 161% higher at a concentration of 3,45 mg.ml^{-1} than the amount of sugar released from the lowest mass of the paper material.

Saccharification with a cellulase concentration of 4 mg.ml^{-1}

During biodegradation with an enzyme concentration of 4 mg.ml^{-1}(Fig. 2) similar trends of saccharification was observed as concluded when exposed to an enzyme concentration of 2 mg.ml^{-1}. The highest sugar concentration of 11 mg.ml^{-1}was obtained from ink free office paper when 0,0266 g of the substance was biodegraded and this amount was 337 % higher than the amount of sugar released from the lowest mass of ink free office paper. The maximum sugar value was also 39 % more than the highest amount of sugar released from office paper covered 50% with ink and double the maximum amount of sugar released from office paper fully covered with ink. The saccharification of office paper that was 50% covered with ink showed an increase in sugar formation when an increasing mass of this substrate was exposed to the enzyme solution. The highest conversion was 214 % higher than the lowest concentration of produced sugar.

When all masses fully covered with ink were treated with the enzyme increasing amounts of sugar was released with increasing masses bio-hydrolyzed. The amount of sugar released was also the lowest compared to the corresponding

masses of ink free and half covered with ink office paper when saccharified. Compared to the amount of sugar released when all office paper materials were treated with the lower enzyme concentration of 2 mg.ml^{-1} the enzyme concentration of 4 mg.ml^{-1} resulted in a higher sugar concentration released from all paper materials.

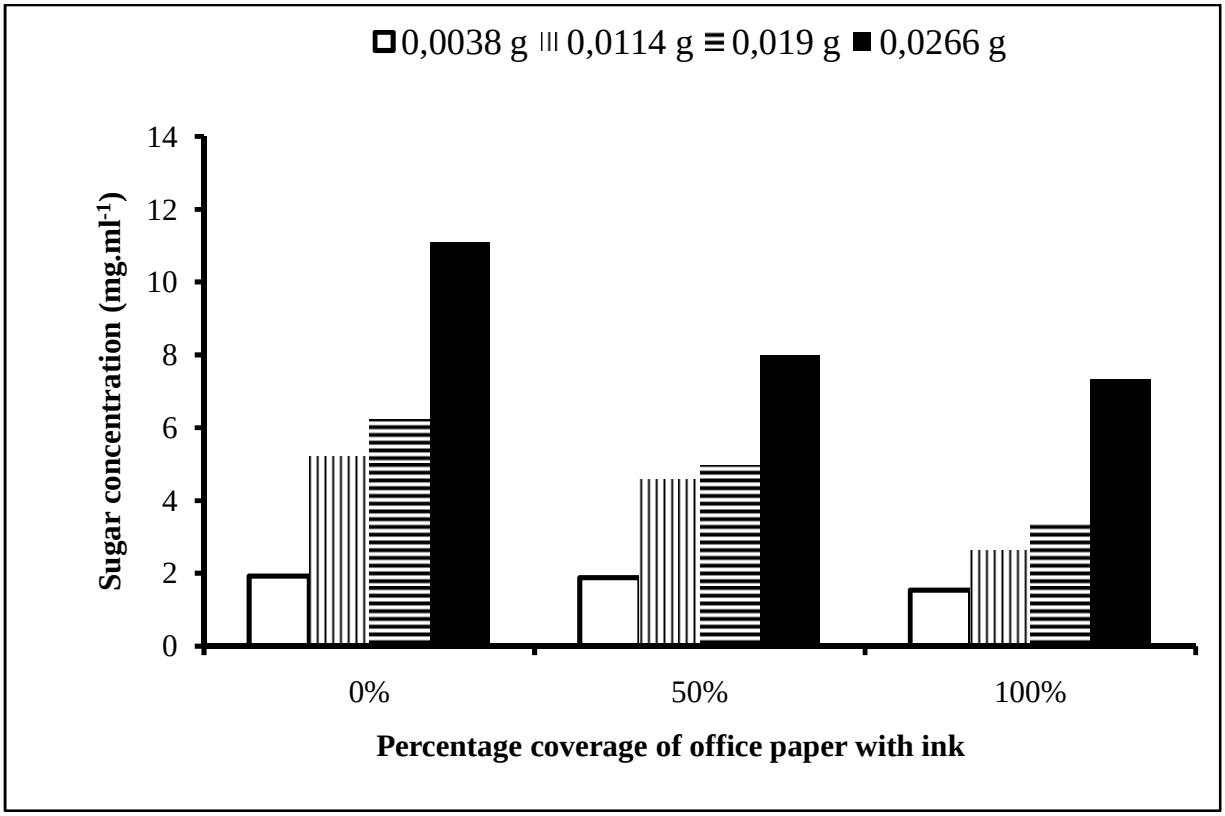

Figure (3): Saccharification of waste office paper with cellulase from *Trichoderma viride*at an enzyme concentration of 6 mg/ml

Saccharification with a cellulase concentration of 6 mg.ml^{-1}

When exposed to a cellulase concentration of 6 mg.ml^{-1}all office paper materials showed the same increasing sugar formation tendency as observed when exposed to the lower enzyme concentrations (Fig. 3). The maximum amount of sugar production was calculated at a sugar concentration of 11,1 mg.ml^{-1} during degradation of 0,0266 g of ink free office paper and this sugar concentration was the same as the amount of sugar released from the corresponding paper mass when exposed to 4 mg.ml^{-1}. The same was observed during the degradation of the 0,0266 g of paper half covered with ink that resulted in a sugar concentration of 7,98 mg.ml^{-1} and this amount of sugar was the same as the sugar concentration released from the corresponding paper when treated with an enzyme concentration of 4 mg.ml^{-1}. The mass of 0,0266 g of fully covered ink released sugar at a concentration of 7,34 mg.ml^{-1} that was 35 % higher than the amount of sugar released during degradation of the corresponding paper when treated with 4 mg.ml^{-1}. Similar to the degradation of these office paper materials with enzyme concentrations of 2 mg.ml^{-1} and 4 mg.ml^{-1} is the observation that the lowest increase in sugar formation was calculated during the degradation of the 0,019 g compared to the degradation of the 0,0114 g of waste paper.

Saccharification with a cellulase concentration of 8 mg.ml^{-1}

As observed during degradation with the lower enzyme concentrationa treatment of increasing masses of office paper resulted in an increasing amount of sugar released when exposed to an enzyme concentration of 8 mg.ml^{-1}(Fig.4). The maximum sugar concentration again was obtained from ink free office paper at a mass of 0,0266 g but this sugar amount was not equal but higher than the sugar released from corresponding masses when treated with lower enzyme concentrations. The maximum sugar concentration of 12,12 mg.ml^{-1} obtained from 0,0266 gink free office paper was 23 % higher than the amount of 9,8 mg.ml^{-1} released from 0,0266 g of office paper half covered with ink and 60 % higher than the sugar released from a similar mass of fully ink covered office paper. The

difference insugar formation between 0,0114 g and 0,019 g is again not to the same extent as the increase in sugar formation between the highest mass and second highest mass when degraded with *T. viride*cellulase.

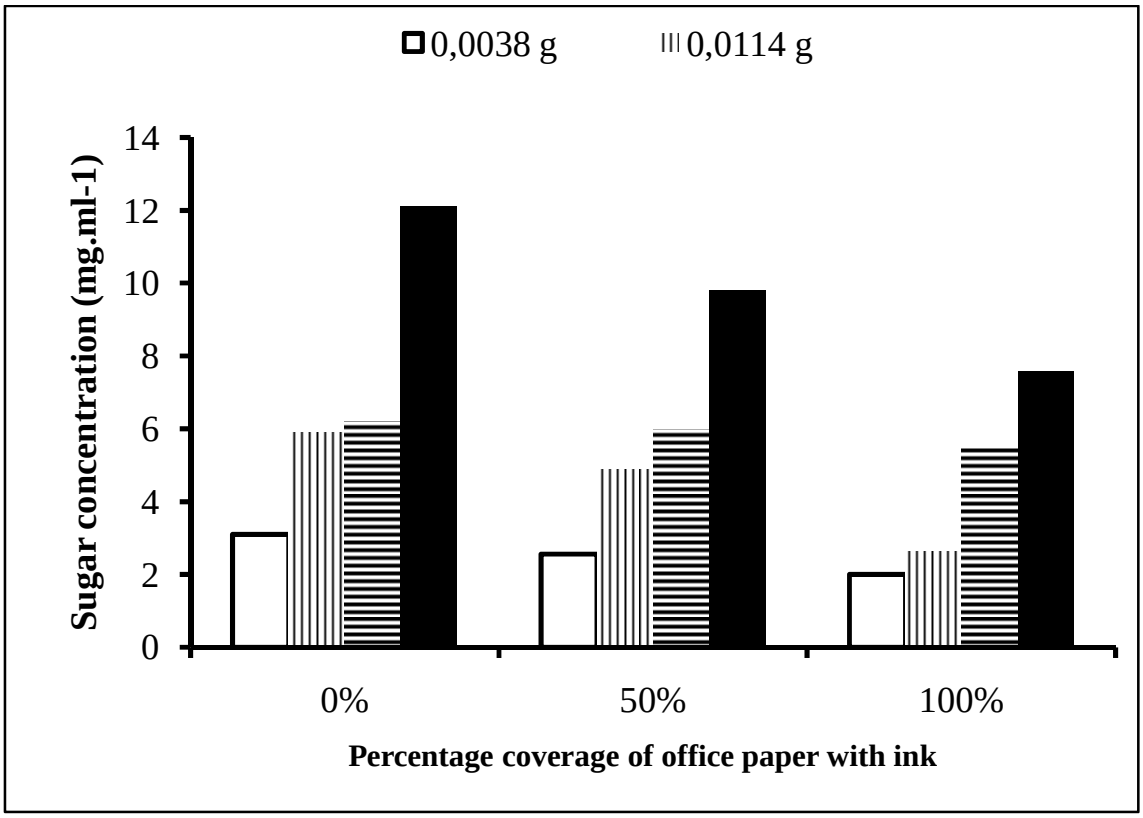

Figure (4): Saccharification of waste office paper with cellulase from *Trichoderma viride*at an enzyme concentration of 8 mg/ml

Saccharification with a cellulase concentration of 10 mg.ml^{-1}

The amount of sugar released from the highest paper masses when exposed to the highest cellulase concentration of 10 mg.ml^{-1}(Fig.5)was more than the amountof sugar released from the corresponding samples and masses when treated with lower enzyme concentrations. The highest sugar concentration of 13,06 mg.ml^{-1} was obtained during saccharification of 0,0266 g ink free office paper at a value 15,5 % more than the maximum amount of sugar released during saccharification of paper 50 % covered with ink and 48 % higher than the maximum sugar concentration released during saccharification of office paper fully covered with ink.

The maximum amount of sugar released during this investigation was obtained from the highest mass of ink free office paper when exposed to the maximum enzyme concentration of 10 mg.ml^{-1}. The sugar concentration of 13,06 mg.ml^{-1} was 7,8 % higher than the second highest sugar concentration of 12,12 mg.ml^{-1} released from 0,0266 g of ink free office paper exposed to an enzyme concentration of 2 mg.ml^{-1}. The highest sugar concentration obtained from ink free office paper was observed when 0,0266g of the material was exposed to an enzyme concentration of 10 mg.ml^{-1}. When exposed to cellulase activity the maximum sugar concentration from office paper 50 % covered with ink was obtained when 0,0266 g of this paper was hydrolyzed with an enzyme concentration of 10 mg.ml^{-1} resulting in a sugar concentration of 11,31 mg.ml^{-1}. Office paper fully covered with ink also resulted in a maximum sugar concentration of 8,8 mg.ml^{-1} when 0,0266 g of this paper was treated with an enzyme concentration of 10 mg.ml^{-1}. From these results it can be concluded that the maximum sugar concentration was obtained when the highest masses of the different office papers were exposed to the highest enzyme concentration but this does not indicate the relative efficiency of the bioconversion process.

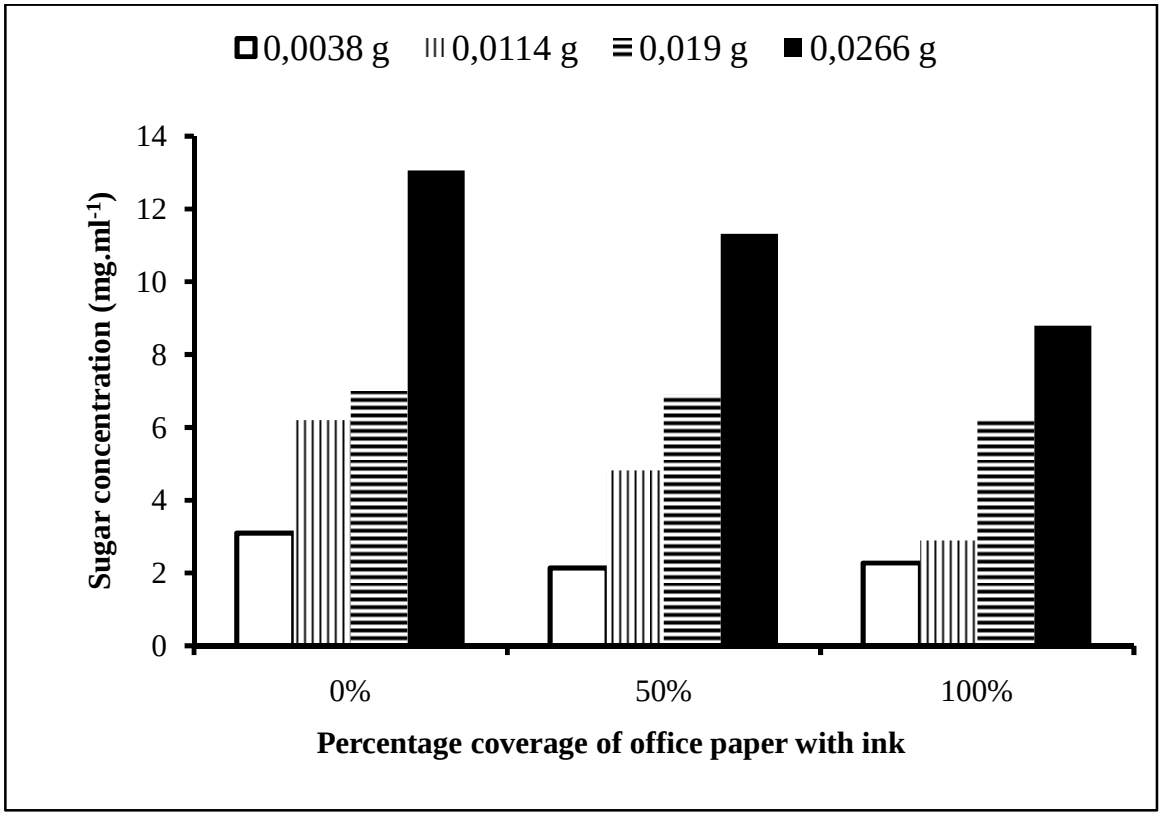

Figure (5): Saccharification of waste office paper with cellulase from *Trichoderma viride*at an enzyme concentration of 10 mg/ml

Relative efficiency of bio-converting waste office paper into fermentable Sugars

It has been shown that when increasing masses of all the office paper materials were exposed to increasing cellulase concentrations the amount of sugar released does also increase. Although this increased sugar formation would eventually result in more fermentation product(s) from glucose it is also important to consider the efficiency of the saccharificationprocess. The efficiency in terms of percentage office paper degraded showed that maximum efficiency was obtained when 0,0038 g of ink free office paper were degraded with a cellulase concentration of 8 $mg.ml^{-1}$ as well as an enzyme concentration of 10 $mg.ml^{-1}$ (Table 1). Both enzyme concentrations resulted in a 41 % saccharification of ink free office paper. The lowest efficiency for ink free paper was14 % saccharificationwhen 0,0038 g of ink free paper was exposed to an enzyme concentration of 2 $mg.ml^{-1}$.

Degradation of the 0,0114 g of ink free office paper showed an efficiency between 23 % and 27 % when exposed to all the enzyme concentrations. When exposed to the various enzyme concentrations a mass of 0,019 g ink free office paper showed an efficiency rate between 16 % and 18 % whilst the highest mass of ink free office paper showed increased percentage of degradation from 18 % to 25 % when increasing enzyme concentrations were used to degrade this amount of office paper.

The biodegradation of office paper 50% covered with ink resulted in the highest efficiency of 37 % saccharification when 0,0038 g of the waste cellulose was exposed to an enzyme concentration of 8 $mg.ml^{-1}$with the lowest degree of saccharification of 10 % calculated when 0,019 g of paper was degraded by enzyme concentrations of 2 $mg.ml^{-1}$ and 4 $mg.ml^{-1}$. A relative high degree of efficiency was observed when the lowest mass (0,0038 g) of this office paper was degraded by all the enzyme concentrations which resulted in an efficiency between 25 % and 37 %. The efficiency of saccharification when 0,0114 g of waste paper was degraded varied between 13 % and 22 % and between 10 % and 17 % when calculated for the degradation of 0,019 g of office paper. Degradation of the 0,0266 g of office paper showed a relative low percentage of degradation between 15 % and 18 %.

When office paper completely covered with ink was exposed to *T.viride*cellulase the maximum efficiency was calculated at a percentageof 30 % when the lowest mass of 0,0038 g was treated with an enzyme concentration of 10 $mg.ml^{-1}$. The lowest efficiency of 5 % was obtained when 0,019 g office paper was treated with 2 $mg.ml^{-1}$cellulase solution. A relative high degree of saccharification was obtained when the lowest mass of this office paper was exposed to all enzyme concentrations which resulted in saccharification rates between 17 % and 30 %. A relative low efficiency was observed when the other three higher masses of this office paper was exposed to the different cellulase concentrations. When 0,0114 g was degraded the efficiency varies between 8 % and 12 % while the mass of 0,019 g resulted in an efficiency rate between 5 % and 16% whilst the highest mass of 0,0266 g resulted in a saccharification rate between 7 % and 16 %.

Opposite to the tendency of sugar formation which revealed that increasing sugar concentration were released when increasing office paper masses were treated with increasing cellulase concentrations is the efficiency of saccharification not related to a positive relationship between increasing mass and increasing enzyme concentration. Higher efficiency of saccharification was observed when lower masses of all types of office paper were incubated with a relative high enzyme concentration.

Cellulase catalyzed bio-conversion of cellulose such as waste office paper into fermentable sugars is a complex process due to the complexity of cellulaseenzymes [19] as well as the heterogeneous nature of the saccharificationprocess[20]. To further complicate the saccharification of waste paper is the partial or completely coverage of paper with ink that could prevent the physical interaction between the cellulase enzyme and the waste cellulose material a phenomenon further hampered by the association of lignin with the cellulose fibers [21].

Table (1): Percentage (%) saccharification of waste office paper with different concentrations of cellulase from *Trichoderma viride*

	Mass of ink free office paper (g)				Mass of office paper covered 50 % with ink (g)				Mass of office paper covered 100 % with ink (g)			
	0,0038	**0,0114**	**0,019**	**0,0266**	**0,0038**	**0,0114**	**0,019**	**0,0266**	**0,0038**	**0,0114**	**0,019**	**0,0266**
2	14	24	16	18	25	15	10	15	17	8	5	7
4	33	27	18	21	33	20	10	15	20	8	6	10
6	25	23	16	21	25	20	13	15	20	11	9	14
8	41	27	16	23	37	22	16	18	26	11	14	14
10	41	27	18	25	30	13	17	17	30	12	16	16

CONCLUSION

The search for alternative energy resources will have to be intensified and the development of waste office paper as a renewable energy resource could assist this process of limiting the negative effect of fossil fuel combustion on the environment. In order to optimize the bioconversion of office paper into fermentable sugars the effect of ink on the saccharification process should be considered when designing a bioconversion process. Also of major importance will be the decrease of solid waste of which office paper is a major component. This step would also result in less land occupied by solid wasteresulting in clean air and more land available for agricultural and residential purposes. The development of waste office paper as a renewable energy resource would also encourage the use of bio-energy thus decreases the dependence on fossil fuels.

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