Conversion of Used Paper Materials into Sugars: A Biochemical Process to Limit Environmental Pollution

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Abstract: Many global communities have labeled chemistry as a major culprit responsible for environmental pollution. Looking at the rate at which gases are released into the air, effluents pumped into rivers, and the volumes of solid waste dumped, then this perception seems to be valid. This undergraduate laboratory exercise illustrates that biochemistry can be applied to conserving the environment by limiting pollution through the bioconversion of organic waste into sugars, and thus it assists with the development of alternative and renewable energy resources.

Introduction

Organic waste is a major component of the solid waste produced daily by households, offices, industry, and the agricultural sector. Typical examples of organic waste are grass and tree cuttings, vegetable peels, used paper materials, straw, and residues produced during the harvesting of agricultural products. In most cases, these waste materials are burnt or dumped, thus aggravating environmental pollution. It would, therefore, be of great environmental benefit if these waste materials could be recycled into other useful products relieving the strain on natural resources.

In order to utilize organic waste materials it is necessary to establish their molecular composition before exposing them to a bio-recycling process. The major structural component of organic waste such as used paper materials is cellulose, a biopolymer consisting of thousands of glucose units. These units are linked with *b*-1,4-glucoside bonds, and the resulting biopolymers are associated by means of hydrogen bonding. For this reason, cellulose exhibits structural features such as crystalline sections and amorphous parts [1]. In addition, hemicellulose, a xylose based biopolymer, offers structural support to these organic materials. Thus, organic waste is a source of untapped carbohydrates that are normally lost due to burning or because they become part of the landfill.

Cellulose has already been described as the most abundant source of food, fuel, and chemicals, its usefulness due to its ability to be hydrolyzed to glucose [2]. The degradation of cellulose to glucose, also referred to as saccharification, can be accomplished by a chemical reaction (hydrolysis) involving the addition of a water molecule. Two main methods, enzyme hydrolysis and acid hydrolysis, can affect degradation of cellulose. Many fungi [3] and bacteria [4] secrete a multicomponent enzyme system called cellulase that exhibits the ability to saccharify cellulose. Increasing knowledge of the mode of action of cellulases and their recent applications [5] has greatly increased the prospects of enzymatic hydrolysis (Figure 1) over chemical processes because of its potentially high saccharification efficiency [6] and avoidance of pollution [5].

Cellulase is commercially available and this experiment describes the cellulase-catalyzed bioconversion of various used paper materials to sugars by this enzyme from Penicillium funiculosum and Trichoderma viride. Bioconverting the cellulose component of organic solid waste to glucose, which can subsequently be fermented into ethanol, provides a valuable fuel source. In addition, the disposal of these organic solids is one of the most significant challenges facing environmental scientists, and if these materials can be used as a substrate for the production of ethanol or other bioproducts, then two environmental concerns are addressed at one time.

Experimental

Used Paper Materials and Pretreatment. Used paper materials, such as office paper (photocopying and printing), foolscap paper (unglazed, ruled manuscript), newspaper, and filter paper (Whatman No. 1), were prepared as pieces of 0.5 by 0.5 cm (non-pretreated) prior to enzymatic treatment. They were also milled into a fine powder (pretreated), which increased the total surface area of the cellulose materials, resulting in an increased degree of saccharification.

Enzymes and Incubation. Cellulase from microorganisms such as *P. funiculosum* and *T. viride* was obtained commercially (Sigma, E.C. 3.2.1.4.) and prepared separately at a concentration of 10.0 mg mL–1 in 0.5 M sodium citrate buffer, pH 4.5. A triplicate amount (20.0 mg) of each paper material, non-pretreated and pretreated, was transferred to different labeled test tubes and mixed with 1.0 mL of each enzyme solution. The enzyme-catalyzed bioconversion was performed by incubating the reaction mixture for a period for 1 h at 50 ºC and terminated by leaving the test tubes in boiling water for 10 min. To separate the resulting reducing sugars from nondegraded paper materials, the incubation mixtures were centrifuged at 10,000 rpm for 10 min and the supernatant filtered through a 0.45 um filter. The filtrate was used to determine the nature and concentration of the resulting sugars.

Analytical Methods. The individual sugars were determined by HPLC using a Supelcosil LC-NH2 column linked to a refractive-index detector using methanol/water (30:70) as mobile phase. At a flow rate of 2.0 mL min–1, both glucose and cellobiose were eluted within 5 min and these sugars were quantified by comparing with glucose and cellobiose standards at concentrations between 10 and 50 mg m–1. All analyses were performed in triplicate.

Results and Discussion

Cellulose is major structural component of all wood-related products and an abundance of this biopolymer is contained in

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Table 1. Increased Saccharification (%) of the Different Used Paper Materials by Cellulase from *P. Funiculosum* and *T. Viride* Due To the Pretreatment

Nature of Paper	Increased saccharification (%)			
	P. funiculosum	*SD*	*T. viride*	*SD*
Filter paper	100	7.8	31	10.0
Foolscap paper	130	10.3	175	9.8
Newspaper	34	7.9	20	9.4
Office paper	32	7.8	20	8.9

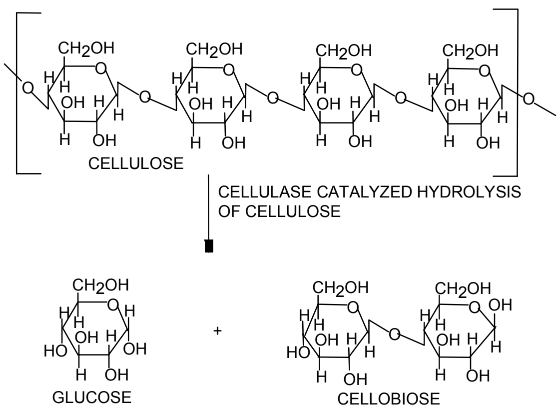

Figure 1. Cellulase-catalyzed hydrolysis of cellulose to sugars such as glucose and cellobiose.

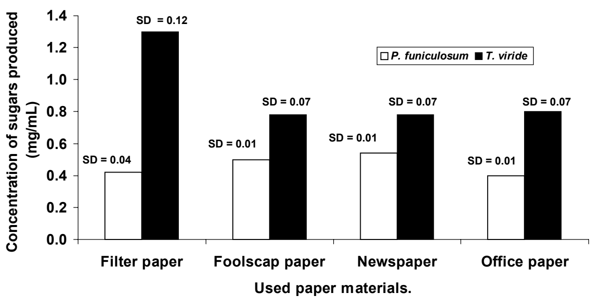

Figure 2. Bioconversion of non-pretreated used paper materials to sugars by cellulase from different microorganisms.

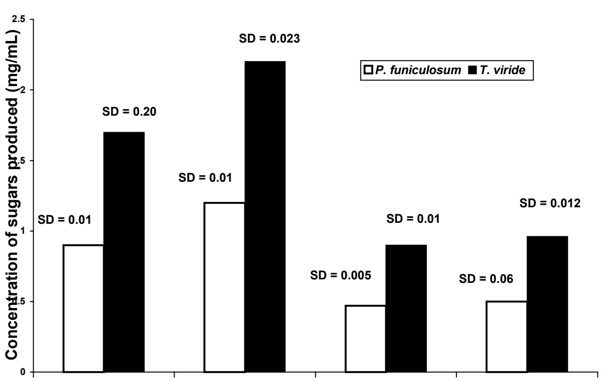

Figure 3. Bioconversion of pretreated used paper materials to sugars by cellulase from different microorganisms.

municipal waste [8] to which used paper materials contribute substantially. The conversion of cellulose to glucose follows the following reaction: $(C_6H_{10}O_5)n$ (cellulose, 162 g mol–1) + n $H_2O \rightarrow n$ $C_6H_{10}O_6$ (glucose, 180 g mol–1), and Figure 2 reflects the total concentration of sugars produced as well as standard deviations for bioconversion of the nonpretreated used paper materials. *T. viride* cellulase was more active on all these materials than *P. funiculosum* cellulase with the highest rate of bioconversion experienced with filter paper, followed by foolscap paper, office paper, and newspaper, while non-pretreated foolscap paper was the best degraded by *P. funiculosum* cellulase. With the pretreated paper substrates, *T. viride* cellulase also showed the highest activity on all materials although the relative extent of bioconversion by both enzymes was higher than observed with the corresponding non-pretreated materials (Figure 3).

The highest increase in saccharification due to pretreatment was calculated during the bioconversion of foolscap paper with both cellulases (Table 1) and the differentiation in extent of bioconversion could be the result of two factors, namely;

1) Cellulase is a multicomponent enzyme system whose composition varies for different microorganisms. These components act synergistically as well as in a concerted way during saccharification of cellulose and their presence or absence would determine the efficiency of the bioconversion process.
2) Cellulose exhibits a complex structure as it has a crystalline section that is difficult to hydrolyze as well as an amorphous part that is more susceptible for cellulase action. The relative content of these sections varies for different cellulose materials and thus would also influence the bioconversion process.

Conclusions

This experiment, which can be performed within a 3-h practical session, proves that the cellulose content of organic waste such as used paper materials can be bioconverted into sugars by the hydrolytic action of an enzyme known as cellulase. Different cellulases show different susceptibilities for organic waste materials [9], and to increase the extent of saccharification, these waste materials can be exposed to pretreatment, such as milling prior to bioconversion. From these observations it can be concluded that biochemistry, or more specifically enzymes, can be applied in the development of uses of organic waste as alternative and renewable resources for energy. This helps to emphasize the positive role that science plays in conserving the environment.

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