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Biodevelopment of Wastepaper as a Resource of Renewable Energy: Influence of Enzyme Concentration and Paper Amount on the Bioconversion Process

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Renewable energy would become more topical as natural energy resources are depleted. Also a major global issue is the extent of solid waste generation of which biowaste is the main component and development of its bio-energy potential can address issues such as renewable energy and environmental pollution, simultaneously. Used paper is a primary component of biowaste with cellulose its dominant structural biopolymer. Various used paper materials have been treated with a cellulose hydrolyzing enzyme from *Penicillium funiculosum* to bioconvert their cellulose component into sugars such as glucose that could be utilized as feedstock for the synthesis of biodegradable products such as bio-ethanol. These paper materials exhibited nonsimilar susceptibilities toward this cellulase enzyme and consequently different sugar releasing and bioconversion efficiency patterns were obtained when changing quantities of each paper were treated with a fixed amount of enzyme. When a fixed amount of each paper material was treated with increasing enzyme concentrations, different bioconversion and efficiency profiles were also constructed for each paper material biodegraded.

Introduction

Biomass, one of the most underutilized renewable energy resources is biosynthesized during photosynthesis when solar energy is stored as carbohydrates in plants. This resulting green energy utilizes the energy content of items such as agricultural residues (e.g., bagasse from sugarcane, corn fiber, and rice straw), wood waste (e.g., sawdust, timber slash, and mill scrap) as well as paper trash and urban yard clippings in municipal waste.[1,2,3] For millennia, humans have exploited the solar energy stored in chemical bonds by burning biomass as fuel and eating plants for the nutritional energy. More recently, during the last few hundred years, fossilized biomass has been exploited in the form of coal. Fossil fuel formation is the result of very slow chemical transformations that convert the sugar polymer fraction of plants into a chemical composition. These additional chemical bonds in coal represent a more concentrated source of energy and since it takes millions of years to convert biomass into coal, fossil fuels are not renewable in the time frame over which humans use it.

Lately the use of biomass as a renewable energy resource has become topical as the burning of fossil fuels aggravates the greenhouse effect and simultaneously depletes a nonrenewable energy resource, namely coal.[4] The renewability of biomass combustion lies in the fact that the released carbon dioxide can be used as feedstock when biomass is replanted. Biomass combustion is not the only renewable application as it can also be utilized as feedstock for biosynthetic pathways in which case the chemical composition of biomass is important.[5] Biomass varies among species, but consists of about 25% lignin and 75% carbohydrates or sugars with the carbohydrate fraction consisting of two large biopolymers, namely, cellulose composed of glucose units, and hemi-cellulose, a xylose-based polymer.[6,7,8] The lignin fraction acts like a “glue” that holds the cellulose and hemicellulose fibers together.[9]

One of the applications for biomass as a renewable energy resource is the biomass-to-ethanol process whereby the cellulose component of biomass is converted into sugars for successive fermentation into bio-ethanol. The cellulose conversion can be executed by means of acid-catalyzed hydrolysis or an enzyme-catalyzed bioconversion performed by a multicomponent enzyme system called cellulase.[10,11] Three major classes of cellulase enzymes—specifically, endoglucanases, exoglucanases, and β-glucosidases—are present in this enzyme system, making its hydrolytic action quite unique and complicated.[12] This complex enzyme could

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not only be applied to biodegrade pure cellulose but can also degrade cellulose while it is present as a structural component of biowaste. As biowaste is a major part of solid waste its bioconversion into fermentable sugars could be utilized as feedstock for renewable energy development.[5]

Used paper materials have been identified as major components of biowaste and various aspects of office paper, foolscap paper, filterpaper, newspaper, and cardboard bioconversion with *P. funiculosum* cellulase have been described.[13,14] This investigation had as its aim to determine the optimum bioconversion when different paper amounts were bioconverted as well as maximum sugar production when changing enzyme concentrations were applied. Sugar-producing profiles have been constructed reflecting the influence of enzyme concentration and increasing paper amounts on the extent of bioconversion and efficiency of the degradation process.

Experimental Section

Source of Enzyme and Paper Materials. Cellulase (Sigma; E. C. 3.2.1.4.) from *P. funiculosum* was prepared at a concentration of 20.0 mg/mL in 50 mM sodium acetate buffer adjusted with HCl to a value of pH 4.5. Used foolscap paper (unglazed ruled manuscript paper), office paper (used for photocopying and printing), filter paper (Whatman no. 1), cardboard (packaging material), and newspaper were cut into pieces of 0.5 cm × 0.5 cm and treated with the cellulase enzyme.

Cellulase Treatment and Analytical Methods. The used paper materials were mixed and fully covered in separate test tubes with 1.0 mL of the cellulase−buffer solution and incubated at 50 °C for a period of 2 h. Different paper amounts of 2.5 mg, 5.0 mg, 7.5 mg, 10.0 mg, 20.0 mg, 40.0 mg, and 60.0 mg were incubated with the enzyme solution. A fixed amount of each paper material (40 mg) was also treated with increasing enzyme concentrations ranging from 0.2 mg/mL to 0.8 mg/mL. To terminate the cellulase action after the incubation period all test tubes were placed in boiling water for 5 min. The cooled incubation solutions were filtered and the filtrate used to determine the total amount of reducing sugars produced due to the enzyme action. The DNS-method using glucose as standard was used to determine the amount of sugar produced, while the enzyme concentrations were calculated by using the Lowry method with BSA as a standard.[15,16] All incubations were performed in triplicate.

Results and Discussion

Even though biowaste, as a major resource for bioenergy, is not being fully utilized the enzymatic catalyzed bioconversion of waste cellulose into fermentable sugars such as glucose can be illustrated by Figure 1. The bioconversion process can also be influenced by variables such as cellulose pretreatment, cellulase mixtures, and cellulase nature while the composition of biowaste would also determine the amount of sugar produced by a specific cellulase system.[17−19]

When increasing amounts of paper materials were biodegraded with a fixed enzyme concentration, all paper materials (except filterpaper) demonstrated an initial increase in sugar production until a level of maximum bioconversion was reached. Under these reaction conditions maximum bioconversion was obtained with foolscap paper, office paper (Figure 2), and cardboard (Figure 3) at an amount of 10.0 mg per incubation while newspaper (Figure 3) showed optimum saccharification at 7.5 mg per incubation. With filterpaper (Figure 3), however, no maximum level of bioconversion was obtained as an increasing trend of bioconversion was maintained. These constructed sugar-

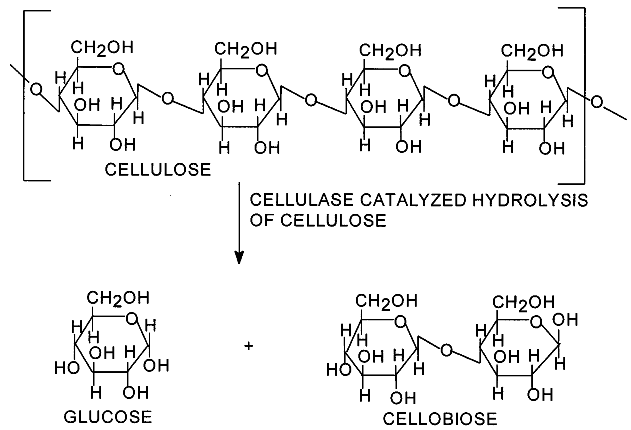

Figure 1. Cellulase-catalyzed biodegradation of cellulose into reducing sugars, glucose, and cellobiose.

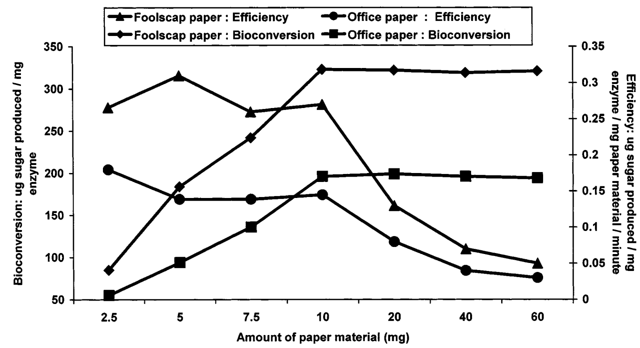

Figure 2. Bioconversion and efficiency tendencies of used foolscap paper and office paper when increasing amounts of these materials were treated with a fixed concentration of *Penicillium funiculosum* cellulase.

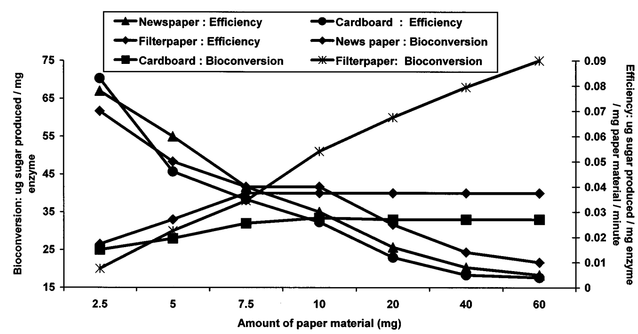

Figure 3. Bioconversion and efficiency tendencies of used newspaper, filterpaper, and cardboard when increasing amounts of these materials were treated with a fixed concentration of *Penicillium funiculosum* cellulase.

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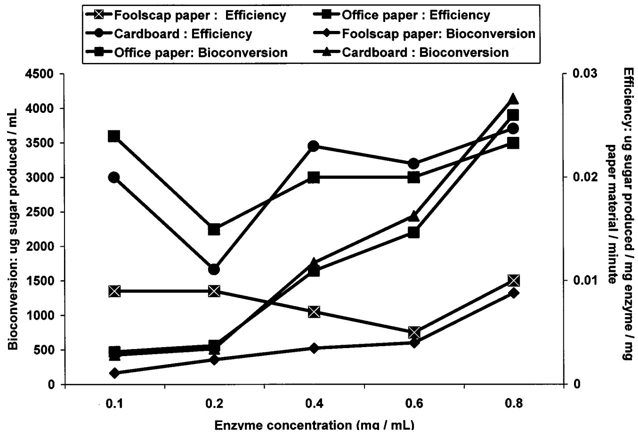

Figure 4. Bioconversion and efficiency tendencies of foolscap paper, cardboard, and office paper when a fixed amount of each paper material was treated with different concentrations of *Penicillium funiculosum* cellulase.

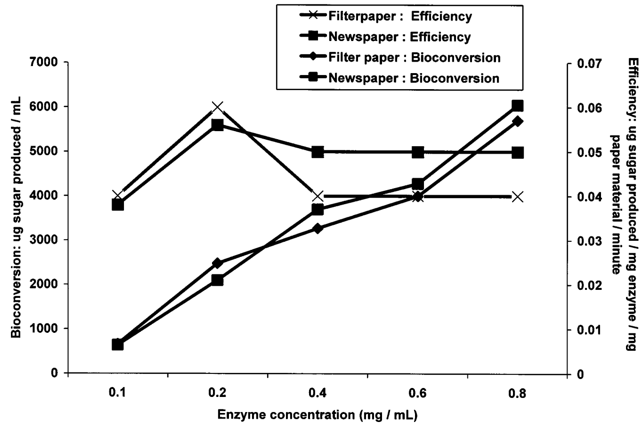

Figure 5. Bioconversion and efficiency tendencies of filterpaper and newspaper when a fixed amount of each paper material was treated with different concentrations of *Penicillium funiculosum* cellulase.

releasing patterns are an important feature reflecting the enzyme to wastepaper combination for optimum bioconversion. Besides the diverse sugar-releasing patterns, the amount of sugar produced from each paper material also differed. At maximum bioconversion and when a fixed enzyme concentration was used, foolscap paper produced the highest rate of bioconversion at a magnitude of 320 μg sugar/mg enzyme, followed by office paper (195 μg sugar/mg enzyme). These bioconversions were followed by filterpaper (75 μg sugar/mg enzyme), newspaper (40 μg sugar/mg enzyme), and cardboard (30 μg sugar/mg enzyme) offering the strongest resistance toward this biological way of degradation.

The efficiency of bioconversion which reflects the relationship between sugar production, enzyme concentration, amount of paper exposed to biodegradation, and period of bioconversion is an important factor that determines the efficiency of renewable development. All the paper materials showed a general trend of decreasing efficiency when increasing amounts of these waste paper materials were biodegraded, indicating the importance of developing procedures to increase the susceptibility of biowaste materials for enzyme-catalyzed bioconversion.

When increasing enzyme concentrations were used to bioconvert a fixed amount of waste paper material, a trend of increasing bioconversion was concluded for all paper materials. At the highest enzyme concentration of 0.8 mg/mL, newspaper showed the highest extent of bioconversion at a concentration of 6050 μg sugar per milliliter incubation volume followed by filterpaper which produced 5700 μg sugar/mL (Figure 5). At the same high enzyme concentration, cardboard and office paper produced 50% less sugar than obtained during the biodegradation of filter paper and newspaper. Foolscap paper exhibited the highest resistance during bioconversion with this relatively high enzyme concentration and the extent of bioconversion was two times less than experienced with newspaper and filterpaper (Figure 4). With regard to bioconversion efficiency, newspaper and filterpaper showed an initial increase in bioconversion efficiency followed by a decline and final constant level of efficiency. With office paper and cardboard the opposite tendency were experienced as an initial decrease in efficiency was followed by a general increase thereof. The bioconversion efficiency of foolscap was very low relative to profiles constructed for the other paper materials as a general increasing trend was observed.

Factors contributing toward the differentiation in bioconversion of different paper materials are the composition of the *P. funiculosum* cellulase system as different waste cellulose materials could demand the action of certain enzyme components absent from a specific enzyme system.[20] The cellulose structure is also an important variable as one part of cellulose is a crystalline section that is difficult to hydrolyze and needs specific components of the cellulase complex while another part of cellulose, called the amorphous section, is more susceptible for cellulase-catalyzed degradation. The relative content of these two components could also vary in the different paper materials with the materials manufactured in unlike ways containing dissimilar additives making its biodegradation more complex.

Conclusions

With the growing global population, a subsequent increased solid waste production and stronger demand for energy are challenges to be addressed by all world communities. Wastepaper, a major component of solid waste materials, offers an opportunity to be developed as a renewable energy resource, limiting the enormous amounts of waste produced daily, decreasing the necessity for fossil fuel products, and lessening the demand for landfilling. The bioconversion process is, however, complicated as each waste material exhibits its own characteristic conversion profile as concluded from this investigation and the conversion of each biowaste material would need to be investigated separately. The use of biomass energy offers unique qualities such as minimizing the production of greenhouse gases that will eventually change the earth's climate, disrupting the entire biosphere that currently supports life. Although the biodevelopment of biowaste is a complex process and different waste materials exhibit nonsimilar biodegradation tendencies, biomass materials such as wastepaper could serve as a renewable energy resource, offering a way of producing clean energy.

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