Enhancement Effect of Hydrophobic Vinyl Compounds in Enzymatic Hydrolysis of Waste Papers

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Enzymatic hydrolysis of waste papers in the presence of various vinyl compounds was carried out, and the addition of the compounds such as methyl methacrylate, styrene, and vinyl acetate increased glucose yield resulting from the acceleration of the rate of the enzymatic hydrolysis. The optimum conditions existed for the acceleration of the rate of the enzymatic hydrolysis. The magnitude of the acceleration effect appeared to be considerably depended on the nature of the compounds. The acceleration effect of the hydrophobic vinyl compounds was explained according to a dispersion and swelling action.

Introduction

Enzymatic hydrolysis of cellulosic materials is potentially an attractive alternative to processes which utilize nonrenewable resources for the production of fuels and chemicals. Most of cellulosic materials is a crystalline polymer associated in a matrix with cellulose, hemicellulose, and lignin, so that the accessibility of enzymes to cellulose is often prevented and then the rate of enzymatic hydrolysis is lower. To increase enzyme accessibility and hence the rate of the hydrolysis, pretreatment of cellulosic materials is necessary. Several studies have been performed to find methods for pretreating these materials [1–6]. For enzymes to catalyze the hydrolysis of cellulose there must be direct contact between the cellulosic fibrils and the enzyme complex; therefore the rate of the hydrolysis of cellulose is affected by the structure of the cellulosic materials. We have studied radiation and chemical pretreatment of cellulosic wastes to increase enzyme accessibility [7, 8].

In this work, enhancement effect of hydrophobic vinyl compounds in enzymatic hydrolysis of waste papers was studied.

Materials and Methods

Waste papers used were filter paper and newspaper. Methyl methacrylate (MMA), styrene (ST), vinyl acetate (VA), glycidyl methacrylate (GMA), ethyl acrylate (EA), trimethyrolpropane trimethacrylate (TMPT), 2-hydroxyethyl methacrylate (HEMA), hydroxyhexyl methacrylate (HHMA), and methacrylic acid (MA) were used as vinyl compounds. The cellulase used was "ONOZUKA" R-10 obtained from Yakult Mfg. Co., Ltd.

The standard enzymatic hydrolysis in the addition of vinyl compounds was carried out by 0.5% substrate and 0.25% cellulase concentration in 0.1 M acetate buffer solution (pH 4.5) at 40 °C for 1.0 h. The quantity of glucose formed by the hydrolysis was measured with a glucose specific reagent ("GOD-PODLK"; Nagase Sangyo Co., Ltd.). The addition effect of vinyl compounds in the hydrolysis was evaluated by the comparison of the glucose yield on the standard enzymatic hydrolysis, in which the glucose yield in the presence of the compounds was normalised for that in non presence of the compounds and shown as the relative glucose yield.

Results and Discussion

Addition effect of various vinyl compounds on enzymatic hydrolysis of filter paper

The enzymatic hydrolysis of filter paper in the presence of various vinyl compounds was carried out and the relative glucose yield was measured. The relationship between relative glucose yield and additive concentration of the compounds is shown in Fig. 1. As additive concentration increased, the relative glucose yields in the addition of MMA, ST, and VA increased and but that in GMA decreased. From this result, it is found that the addition of

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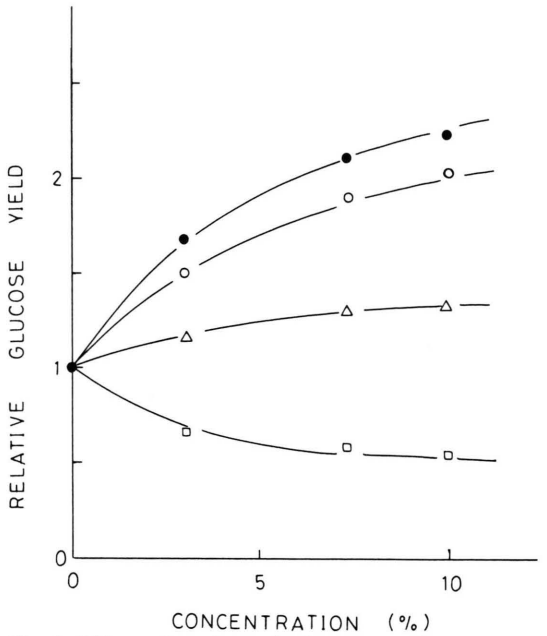

Fig. 1. Effect of various vinyl compounds on enzymatic hydrolysis of filter paper. ● MMA; ○ ST; △ VA; □ GMA.

MMA, ST, and VA accelerates the rate of the enzymatic hydrolysis of filter paper. The order of the acceleration effect in these compounds is as follows; MMA > ST > VA.

In the enzymatic hydrolysis of solid cellulose, the enzyme is immersed into the structure of cellulose fibrils and initiated by its physical contact to cellulose chains. In general, the immersion of the enzyme is rate-determing step on the enzymatic hydrolysis of cellulose. The addition of vinyl compounds played to accelerate the immersion and physical contact of the enzyme. The compounds used in Fig. 1 have a hydrophibic property and hence are not dissolved in water, giving a suspension state, in which the compounds can immerse into the structure of cellulose fibrils by a dispersion (swelling) effect. It is, thus, proposed that an intramiceller swelling in the cellulose fibrils of filter paper takes place by the addition of hydrophobic vinyl compounds such as MMA, ST, and VA. For such a swelling effect, the compounds such as MMA and ST having a small dipole moment (0.10–0.17 D)[9] appeared to be effective. The dipole moment (1.75 D)[10] of VA is larger than those of MMA and ST, indicating that the acceleration effect of VA on the enzymatic hydrolysis is lower than that of MMA and ST.

The acceleration of the enzymatic hydrolysis by the addition of MMA reached to a satulation at a certain concentration (50%) of MMA. The relative glucose yield increased till 20% MMA concentration, and decreased markedly at 70% MMA concentration with increasing MMA concentration. This result shows that optimum addition concentration exists for acceleration of the enzymatic hydrolysis resulting from a swelling effect. The negative effect at high additive concentrations above 70% seems to be a disturbance for enzyme reaction.

Effect of temperature on enzymatic hydrolysis of filter paper in the addition of MMA

The enzymatic hydrolysis of filter paper in the addition of 10% MMA was carried out at various temperatures, and the effect of the temperature was studied. The relationship between relative glucose yield and hydrolysis temperature is shown in Fig. 2. The relative glucose yield increased, reached a maximum, and then decreased with rising hydrolysis temperature. This result shows that optimum temperature exists for the acceleration of the rate of the enzymatic hydrolysis in the presence of MMA. This temperature dependence seems to be related to the action mechanism of MMA for cellulose, which leads to the acceleration of the enzymatic hydrolysis.

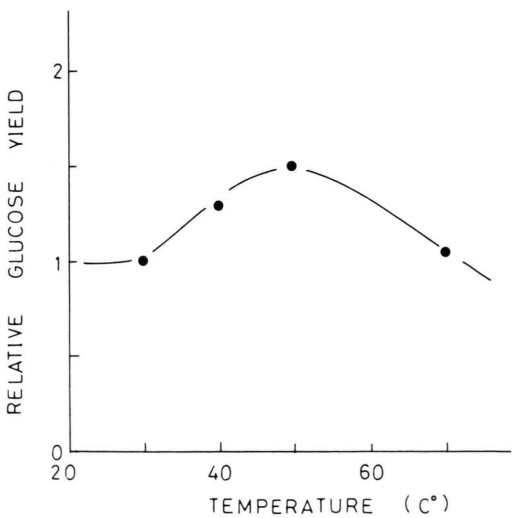

Fig. 2. Effect of hydrolysis temperature on enzymatic hydrolysis of filter paper in the addition of MMA. MMA concentration: 10%.

Since the dielectric constant and dipole moment of organic compounds varies usually with temperature, the interaction of MMA with cellulose leading to the swelling of cellulose fibrils would vary with hydrolysis temperature. A certain treatment temperature (50 °C) is necessary for the swelling of the cellulose fibrils resulting from dispersion. However, the decrease of the relative glucose yield at high temperatures above 60 °C in Fig. 2 would be due to thermal inactivation of the enzyme.

Coexistence effect of MMA and acetone on enzymatic hydrolysis of filter paper

The coexistence effect of hydrophobic vinyl compounds and hydrophilic acetone on the enzymatic hydrolysis of filter paper was studied at 40 °C in the presence of 5% MMA. The relationship between relative glucose yield and addition concentration of acetone is shown in Fig. 3. The relative glucose yield in the coexistence of MMA and acetone decreased with increasing addition concentration, though the magnitude of the decrease in the coexistence is smaller than that in the presence of acetone. This result indicated that the acceleration effect by MMA in the enzymatic hydrolysis was depressed by the addition of acetone. In the addition of acetone only, the relative glucose yield decreased markedly with increasing addition concentration. From this result, it is proposed that acetone is interacted to disturb the enzyme reaction at low concentration even.

Addition effect of various vinyl compounds on enzymatic hydrolysis of newspaper

The addition effect of various vinyl compounds such as MMA, ST, EA, TMPT, HEMA, HHMA, and MA on the enzymatic hydrolysis of newspaper was examined at 40 °C. The relationship between relative glucose yield and addition concentration is shown in Fig. 4. The relative glucose yield in the addition of MMA and ST increased but those in TMPT, HEMA, HHMA, and MA decreased with increasing addition concentration. While, the relative glucose yield in the addition of EA did not vary with increasing addition concentration. From this result, it was found that hydrophobic vinyl compounds such as MMA and ST accelerates the rate of the enzymatic hydrolysis of newspaper containing cellulose, hemicellulose, and lignin as well as filter paper containing of cellulose only. This suggests that MMA and ST can invade into the structure of cellulose fibrils in the

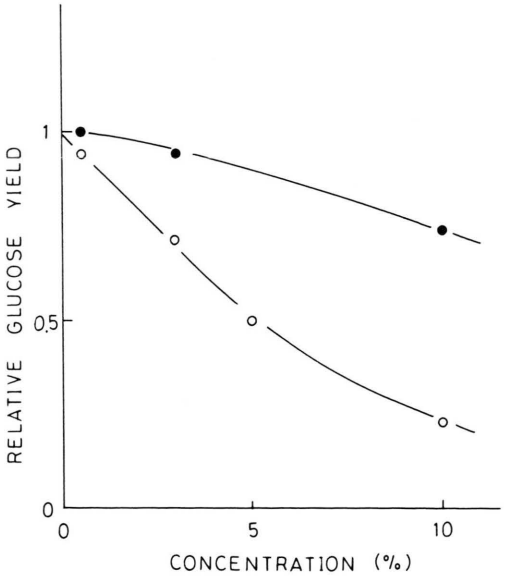

Fig. 3. Relationship between relative glucose yield and acetone concentration in the presence of MMA. MMA concentration: ● 10%, ○ 0%.

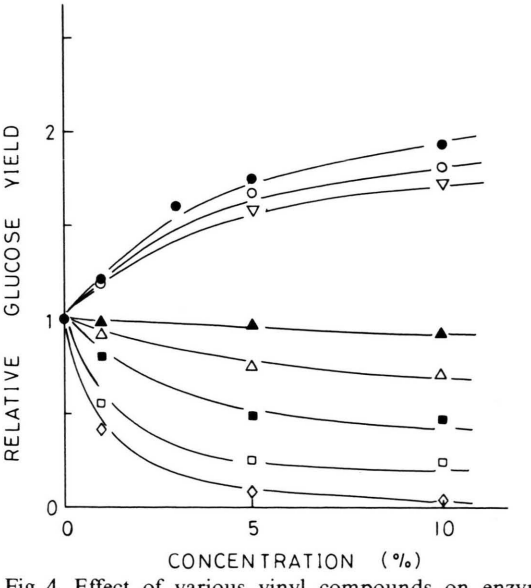

Fig. 4. Effect of various vinyl compounds on enzymatic hydrolysis of newspaper. ● MMA; ○ ST; ▲ EA; △ TMPT; ■ HEMA; □ HHMA; ◇ MA; ▽ MMA (irradiated newspaper, 100 Mrad).

newspaper and then expands the structure by swelling effect. On the other hand, hydrophobic vinyl compounds such as TMPT and HHMA decreased rather the rate of the rate of the enzymatic hydrolysis, though TMPT and HHMA are hydrophobic methacrylate compounds as well as MMA. This difference would be due to the difference of dispersion or swelling ability between MMA and TMPT or HHMA. The depression of the enzymatic hydrolysis in the addition of hydrophilic HEMA and MA is probably due to a disturbance effect for the enzyme reaction, in which MA having carboxyl group might be inactivator for enzyme.

The addition effect of MMA on the enzymatic hydrolysis of irradiated newspaper was examined, in which newspaper was preirradiated with irradiation dose of 100 Mrad by γ-ray from ^{60}Co source prior to enzymatic hydrolysis. The relative glucose yield increased with increasing addition concentration of MMA though its acceleration effect was slightly smaller than that in non-irradiated newspaper as shown in Fig. 4. Irradiated newspaper becomes to a very fragile state by radiation degradation and is converted to smaller molecular products containing oligosaccharides; degree of polymerization is about 10^7. Since such products dissolve in water, greater acceleration effect would not be observed in comparison with non-irradiated newspaper. However, it was found from this result that hydrophobic vinyl compounds such as MMA gave acceleration effect for the enzymatic hydrolysis of waste papers consisting of oligosaccharides.

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