

Effect of Organic Solvents on Enzymatic Hydrolysis of Celluloses

Minoru Kumakura and Isao Kaetsu

Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, Takasaki, Gunma, Japan

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Effect of organic solvents on enzymatic hydrolysis of celluloses was studied. It was found that toluene and xylene can accelerate the rate of the enzymatic hydrolysis reaction of filter paper and cellulose powder. The addition of N,N-dimethylformamide, acetone, and alcohols was negative effect for the enzymatic hydrolysis of solid celluloses. In the addition of alcohols and glycols, the relative glucose yields increased with increasing the carbon number of their solvents. The addition of organic solvents on the enzymatic hydrolysis of celluloses was discussed with swelling effect. It was found that the glucose yield increased as the dipole moment of the solvents decreased.

Introduction

Studies for enzymatic hydrolysis of waste celluloses from point of biomass conversion have been done by many workers [1–3]. Increase of accessibility of cellulose to enzyme has become the important problem because the substrate is solid polymer. It is very difficult to hydrolyze native cellulose effectively with enzyme. To obtain a high yield of glucose at a high reaction rate, various pretreatments of cellulose are required. Alkali swelling, acid pretreatment, removal of lignin, irradiation, and ball milling have been used to increase the availability of cellulose in waste cellulose materials to enzymatic hydrolysis or ruminant digestion [4–11]. Such treatments are done to reduce crystallinity and increase available surface. The authors have been previously studied pretreatment of waste cellulose materials by radiation irradiation [9–11].

In the this work, effect of organic solvents on enzymatic hydrolysis of cellulose materials was studied. Addition effect of organic solvent on enzymatic hydrolysis of cellulose is seemed to be not studied still.

Materials and Methods

Cellulose materials used were cellulose powder (40–100 mesh), filter paper, and carboxymethyl cellulose sodium (CMC). Organic solvents were used without purification. The cellulase used was “ONOZUKA” R-10 Yakult Mfg. Co., Ltd. The

standard enzymatic hydrolysis in the addition of organic solvents were 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 h. The quantity of glucose produced by the hydrolysis was measured with glucose specific reagent (“GOD-PODLK”; Nagase Sangyo co., Ltd.). The addition effect of organic solvents 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 solvents was nomalized for that in non presence of the solvents and shown as the relative glucose yield.

Results and Discussion

Addition effect of various organic solvents

Addition effect of various organic solvents on enzymatic hydrolysis of filter paper was studied. The relation between glucose yield and additive concentration is shown in Fig. 1. The relative glucose yields in the addition of toluence and *m*-xylene increased with increasing additive concentration. On the other hand, the relative glucose yield in the addition of acetone and N,N-dimethylformamide decreased monotonously with increasing additive concentration. In addition of toluene, relative glucose yield curve in Fig. 1 shows a monotonous increase up to ca. 5% additive concentration and after that the curve reached to saturation. Addition of both xylene and toluene gave relative glucose yield of two times. According to this result, it found that the addition of some organic solvents accelerates the rate of the enzymatic hydrolysis reaction of filter paper.

Reprint requests to M. Kumakura.

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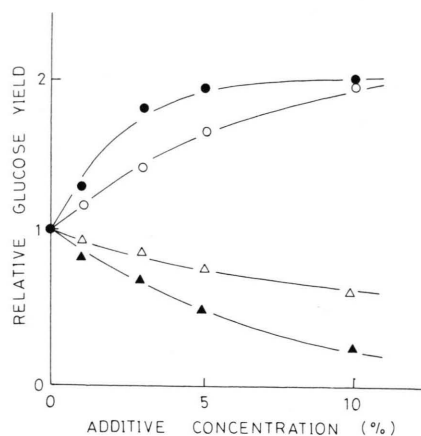


Fig. 1. Effect of various organic solvents on the hydrolysis of filter paper. Organic solvents: ● toluene; ○ *m*-xylene; Δ *N,N*-dimethylformamide; ▲ acetone.

Addition effect of organic solvents on enzymatic hydrolysis of various celluloses

Addition effect of the organic solvents which accelerates the hydrolysis reaction rate in filter paper was studied also on the hydrolysis of other celluloses. Figure 2 shows the result of the addition effect of xylene on the enzymatic hydrolysis of cellulose powder. Addition of xylene in the hydrolysis of cellulose powder increased relative glucose yield as well as that in filter paper. However, the addition effect of xylene in cellulose powder was smaller in comparison with that in filter paper. The relative glucose yield resulting from xylene addition of 10% in cellulose powder was 1.3 times. This difference of

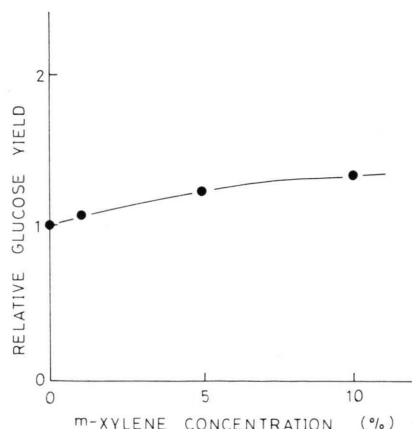


Fig. 2. Effect of *m*-xylene on the hydrolysis of cellulose powder.

addition effect by xylene in both cellulose substances would be due to the physical state of these substances. The crystallinity of cellulose in filter paper consisting of longer cellulose fibrils is higher than that in cellulose fibril decreases by grinding treatment. The polymerization degree of cellulose powder was comparable that of filter paper and these values were ca. 1000 [11]. The enzymatic hydrolysis reaction of solid cellulose should be initiated by immersion of cellulase. It is thought that the solvents such as xylene and toluene act to promote this immersion of cellulase. The immersion of cellulase into cellulose fibril would be increased by swelling action of the solvent for the cellulose. Thus, the acceleration of the hydrolysis reaction rate of the addition of some solvents could be caused to the swelling effect of the solvents. Furthermore, the interaction effect between the hydrophobic group sites of cellulase and hydrophobic solvents might not be neglected, since the hydrophobic group sites of enzyme play a important role on enzyme reaction [12].

Addition effect of organic solvents on the enzymatic hydrolysis of soluble cellulose substance was studied. The result of the addition of toluene, acetone, and ethanol on the hydrolysis of CMC is shown in Fig. 3. In the hydrolysis of CMC, the acceleration of the hydrolysis reaction rate by the solvents was not observed. The addition of the solvents appeared rather to be slightly retardation of the hydrolysis reaction rate. The relative glucose yield in the hydrolysis of filter paper decreased remarkably by the addition of acetone, but that in CMC was slight. The relative glucose yield in the

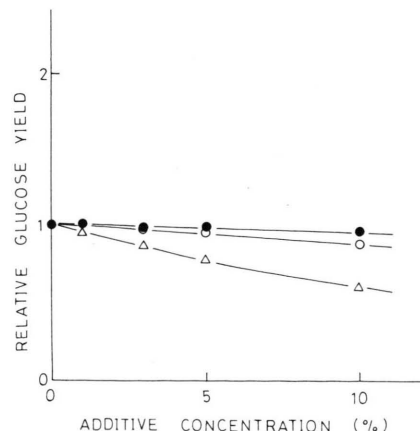


Fig. 3. Effect of various organic solvents of the hydrolysis of CMC. Organic solvents: ● toluene; ○ acetone; Δ ethanol.

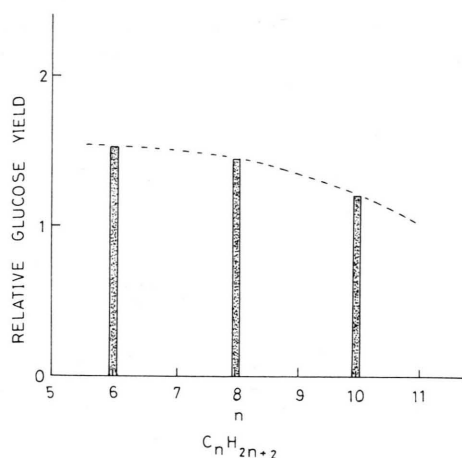


Fig. 4. Effect of hydrocarbons on the hydrolysis of filter paper. Additive concentration; 10%.

addition of toluene was almost constant with increasing additive concentration. These results suggest that the acceleration effect of the solvents such as toluene and xylene on the hydrolysis of solid celluloses is due to a swelling action of these solvents. Toluene and xylene having an accelerating effect on the hydrolysis of solid celluloses are a hydrophobic solvent, and N,N-dimethylformamid and acetone having a retarding effect are a hydrophilic one.

Addition effect of hydrocarbons on the hydrolysis of cellulose

Addition effect of hydrocarbons on the hydrolysis of filter paper was studied. The relation between relative glucose yield and the carbon number of hydrocarbons is shown in Fig. 4. The addition of hydrocarbons of small carbon numbers accelerated the hydrolysis reaction rate, and the hydrolysis reaction rate decreased with increasing the carbon number of the hydrocarbons. Most of hydrocarbons used in this work accelerated the hydrolysis reaction rate as shown in Fig. 4, of which the hydrocarbons are a hydrophobic solvent.

Addition effect of alcohols on the hydrolysis of cellulose

Addition effect of alcohols on the hydrolysis of filter paper was studied. The relation between relative glucose yield and the carbon number of alcohols is shown in Fig. 5. The relative glucose yield increased with increasing the carbon number of

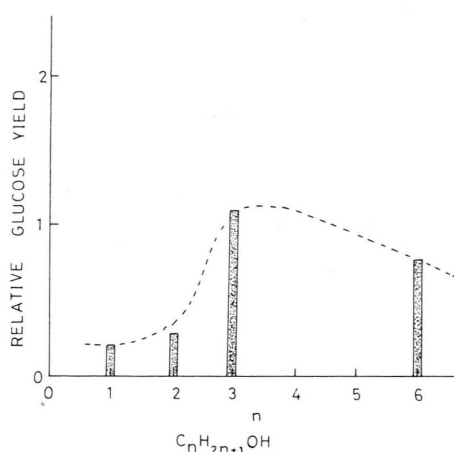


Fig. 5. Effect of alcohols on the hydrolysis of filter paper. Additive concentration; 10%.

alcohols though most of alcohols retarded the hydrolysis reaction rate. Methanol and ethanol of hydrophilic alcohols retarded markedly the hydrolysis reaction rate. Propylalcohol having slightly hydrophobic property did not have the acceleration effect on the hydrolysis of cellulose. In general, the hydrophobic solvents have small dipole moments. Those solvents invade easily into cellulose fibril and would exhibit a swelling action.

Addition effect of glycols on the hydrolysis of cellulose

Addition effect of glycols on the hydrolysis of filter paper was studied. The relation between relative glucose yield and the carbon number of glycols is

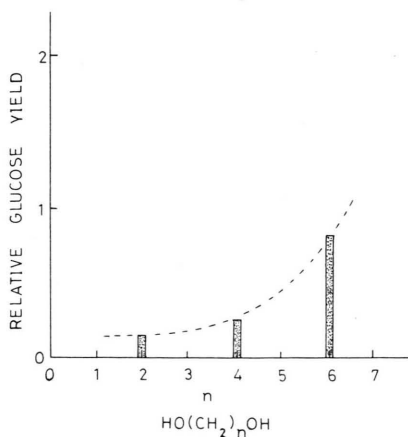


Fig. 6. Effect of glycols on the hydrolysis of filter paper. Additive concentration; 10%.

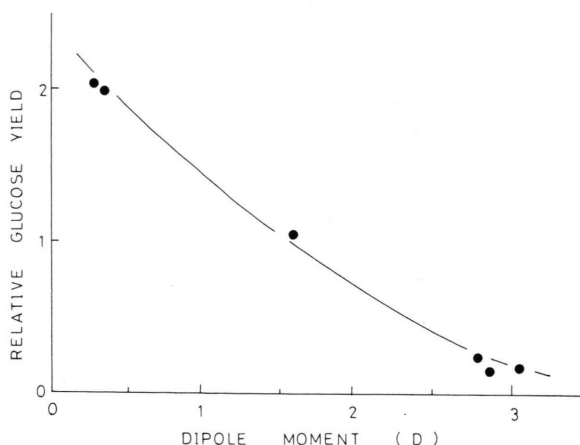


Fig. 7. Relation between the dipole moments of organic solvents and the relative glucose yield on the hydrolysis of filter paper.

shown in Fig. 6. The addition of glycols on the hydrolysis of filter paper gave a retardation effect for the hydrolysis reaction rate though the relative glucose yield increased with increasing the carbon number of glycols. The addition effect of both alcohols and glycols having a hydroxy group on the

hydrolysis gave a similar result. This implies that the molecular structure of organic solvents is concerned to a swelling property leading acceleration effect on the enzymatic hydrolysis reaction of cellulose.

Effect of dipole moment of organic solvents on the hydrolysis of cellulose

In the addition of organic solvents on the hydrolysis of solid cellulose, the hydrophobic solvents having swelling property for cellulose gave an acceleration effect on the hydrolysis reaction rate as mentioned above. The molecular structure of swelling reagents of non polar functional group of which a dipole moment or polarizability is relatively small. Effect of dipole moment of the organic solvents on the hydrolysis of cellulose was studied. The relation between the relative glucose yield and the dipole moment of the solvents is shown in Fig. 7. It was found that the glucose yield increased as the dipole moment of the solvents decreased. The polar solvents having large dipole moments does not give a swelling action, and rather reveals the retardation effect on the hydrolysis reaction. This retardation would result from the degeneration of the enzyme during the hydrolysis reaction.

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