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Effects of ultrasonic intensity and reactor scale on kinetics of enzymatic saccharification of various waste papers in continuously irradiated stirred tanks

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Abstract

Based on the enzymatic saccharification of the various pulps in the previous 0.8 l ultrasonic stirred tank reactor, the ultrasound-enhanced saccharification of waste papers such as newspaper, carton paper, office paper etc. was carried out in the same reactor as well as larger scale stirred tank reactors of size 3.2 and 6.4 l. The saccharification of each waste paper was less enhanced in the larger reactor at a given ultrasonic intensity. This could be attributed to the decrease in the ultrasonic intensity per reaction volume, i.e., the specific ultrasonic intensity. Most waste papers were more efficiently hydrolyzed with increasing specific ultrasonic intensities, although newspaper was less efficiently done for a too high specific intensity. Such an adverse effect might be due to the fact that some impurities in the newspaper such as lignin were activated by an intensive ultrasonic irradiation to form a rigid and closed network, which inhibited the access and adsorption of cellulase on to the substrate surface. The previous kinetic model was found to be applicable to analyze and simulate the saccharification of each waste paper in the different ultrasonic reactors. The ultimate conversion of a substrate based on the total sugar concentration estimated for an infinite reaction time could be correlated as a function of the ratio of initial substrate to enzyme concentrations at a fixed specific ultrasonic intensity. Either the apparent rate constant or the ultimate conversion increased and tended to approach a constant with an increase in the specific ultrasonic intensity except for the case of newspaper, while neither the apparent Michaelis constant, product inhibition constant nor glucose formation equilibrium constant was influenced by the specific ultrasonic intensity.

Keywords: Enzymatic saccharification; Waste paper; Ultrasonic stirred tank; Scale-up; Kinetics

1. Introduction

Waste reutilization has become a matter of great interest since the increased waste emission is threatening the limited resources and living spaces on the Earth. Among the various components of the municipal solid wastes, waste papers rank first with a percentage of 38% [1]. Waste papers can be reutilized several times through manufacturing recycled paper before cellulose fibers become too short and weak to make paper [2]. On the other hand, waste papers may be typical of abundant renewable cellulosic resources for producing useful bioproducts such as soluble sugars [3]. Moreover, bio-ethanol that is ethanol made microbially from biomass, such as cellulose has been approved as a promising alternative energy source for increasing energy security and reducing air pollution from contaminants such as NO_x [4]. To achieve an efficient conversion of waste cellulose to soluble sugars, the enzymatic hydrolysis of cellulose is suggested to be preferred to the various acid-catalyzed processes using inorganic acid, subcritical or supercritical waters, etc. since the former not only offers a bioconversion process under the simpler and milder

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operating conditions but also produces no by-products detrimental to fermentative microorganisms [5].

Despite the numerous researches on the enzymatic hydrolysis of cellulose over the last few decades, the process has remained of low efficiency and not been fully understood because of the heterogeneity of substrate as well as enzyme [6,7]. Many processes have been developed to improve the conversion of cellulose hydrolysis, such as ball mill reactors [8,9], aqueous two-phase systems [10,11], various explosion pretreatment processes [2,12] and so on. The steam explosion pretreatment process is reported to be the most economical process among these processes because it makes the substrate more susceptible to enzymes with a lower energy requirement and environmental protection cost [2,3]. However, the process cannot prevent cellulase from adsorbing irreversibly onto the substrate during the hydrolysis, which has been proposed to be one of the mechanisms responsible for cellulase deactivation [13–15].

In our previous work on the enzymatic hydrolysis of alkali-treated lignocellulosics [16], it was suggested that the continuous ultrasonic irradiation enhanced not only the accessibility of the substrate for enzyme adsorption but also the desorption of the inactively adsorbed enzyme to cause its reactivation resulting in an enhanced hydrolysis. Recently, we have observed a remarkable enhancement for the enzymatic hydrolysis of the paper pulps in a 0.8 l stirred tank reactor with continuous ultrasonic irradiation. The saccharification of the pulp called NUKP under ultrasonic irradiation proceeded as a two-staged reaction, and the enhancement effect of the ultrasonic irradiation on enzymatic hydrolysis of cellulose was evaluated based on the simplified kinetic model proposed. It was also found that the variation in the property of pulp as well as ultrasonic intensity exerted an effect on the apparent rate constant and ultimate conversion of the substrate, but no effect on the apparent Michaelis and competitive product inhibition constants [5]. Although many researches have focused on the ultrasound-enhanced microbial reaction during the last decade [17], there have been few kinetic studies on the effects of ultrasonic intensity and reactor size on the enzymatic reaction enhanced by ultrasonic irradiation for design and scale-up of ultrasonic bioreactors.

The purpose of this work is (a) to extend the enzymatic saccharification of the pulps enhanced by the continuous ultrasonic irradiation to that of the waste papers including newspaper, carton paper and office paper, (b) to scale-up the stirred tank reactor for the saccharification of waste papers with continuous ultrasonic irradiation, (c) to examine the applicability of the previous simplified kinetic model to the present process in the varied scale reactors and (d) to evaluate the effects on the saccharification of ultrasonic irradiation, scale-up and substrate property based on the kinetic model.

2. Materials and methods

2.1. Enzyme and substrates

Cellulase (Meicelase®, 238 FPU/g) from *Trichoderma viride* was provided by Meiji Seika Kaisha Ltd. (Tokyo, Japan) and used without further purification. The cellulase activity was assayed according to the method suggested by Mandels et al. [18]. Newspaper, carton paper and office paper, which had been shredded into pieces (6×12 mm) and then washed with deionized water and dried at 50 °C, were used as model substrates of waste cellulose. NUKP pulp supplied by Daio Paper Corporation (Tokyo, Japan) was used as a reference.

2.2. Determination of cellulose availability of waste papers used

A piece of a waste paper with a size of 20×60 mm was weighed, shaped into a spiral sheet and hydrolyzed in 6 ml of 0.001 M acetate buffer solution of pH 4.8 in a borosilicate test tube at 45 °C for 144 h under an initial cellulase concentration of 4 $g l^{-1}$, which was 10 times higher than that of 0.4 $g l^{-1}$ for the enzymatic saccharification of the waste paper in the ultrasonic stirred tank reactors described below. All the enzymatic hydrolyses of the various waste papers under such a high cellulase concentration were assumed to give the available amount of cellulose present in the respective substrates used. The cellulose availability of a waste paper was therefore calculated as 90 T_f/S_0 %, where T_f ($g l^{-1}$) and S_0 ($g l^{-1}$) represent the observed final total sugar concentration and initial substrate concentration, respectively.

2.3. Ultrasonic stirred tank reactors employed

Fig. 1(a) shows a schematic diagram of the 0.8 l ultrasonic reactor made of semitransparent PVC resin, which was the same as that used previously for the paper pulps [5]. Fig. 1(b) represents larger scale ultrasonic reactors having a reaction volume of 3.2 and 6.4 l. The larger stirred tank reactors made of PVC resin having a round cross-section are equipped with the same design of impeller and ultrasonic horn system as in the case of the 0.8 l ultrasonic reactor, being installed vertically into the tank. The ultrasonic generator used in this work was Sonifier® Model 250D (Branson Ultrasonics Corporation, USA), which is the same as that in the previous work [5] and can produce monofrequency ultrasound of 20 kHz with an adjustable output power up to 250 W to irradiate variable ultrasonic intensities.