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Intensification of enzymatic hydrolysis of waste newspaper using ultrasound for fermentable sugar production

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ABSTRACT

An effective conversion of lignocellulose into fermentable sugars is a key step in producing bioethanol in an eco-friendly and cost effective manner. In this study, the effect of ultrasound on enzymatic hydrolysis of newspaper, a potential feedstock for bioethanol production due to its high cellulosic content, was investigated. The effect of substrate loading, enzyme loading, temperature, ultrasonic power and duty cycle on the hydrolysis has been studied. Optimum conditions for conventional enzymatic hydrolysis were substrate loading of 5% (w/v), enzyme loading of 0.14% (w/v), temperature of 323 K, and under these conditions and 72 h of hydrolysis, reducing sugar yield of 11.569 g/L was obtained. In case of ultrasound-assisted enzymatic hydrolysis approach, optimum conditions obtained were substrate loading of 3% (w/v), enzyme loading of 0.8% (w/v), sonication power of 60 W, duty cycle of 70%, hydrolysis time of 6.5 h and the reducing sugar yield obtained under these conditions was 27.6 g/L. Approximately 2.4 times increase in the release of reducing sugar concentration was obtained by the ultrasound-assisted enzymatic hydrolysis approach. Results indicate that there is a synergistic effect obtained from the combination of ultrasound and enzymes which lowers the diffusion-limiting barrier to enzyme/substrate binding and results in an increase in reaction rate. The experimental data were also fitted in a simple three parameter kinetic model.

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1. Introduction

The increasing demand for energy and declining fossil fuel reservoirs directed the remarkable attention in finding substitute renewable energy sources. Lignocellulose is the principal renewable source of carbon and is the most abundant polysaccharide on planet [1,2]. Biological conversion of lignocellulosic biomass such as forestry residues, portions of municipal solid waste, and agricultural residues to fermentable sugars reduces the waste disposal expenditure and concurrently meets the increasing demand for energy [3,4]. Even though cellulosic biomass can be hydrolyzed by non enzymatic methods, the utility cost of enzymatic hydrolysis is very less in comparison to the substitute methods such as acid hydrolysis. The important governing reason is that enzymatic hydrolysis is carried out at milder reaction conditions, offers higher yield of products, fewer side reactions and requires less energy [5,6]. Use of ultrasound offers an interesting intensification approach for enzymatic reactions [7,8]. By application of ultrasound, powerful jet streams are created in the liquid which lowers the diffusion-limiting obstruction surrounding the substrate and increases the rate of enzyme-substrate binding. Subsequently after completion of enzymatic hydrolysis, ultrasound also aids in the removal of hydrolysis product from the substrate.

Waste paper is a plentiful and one of the economical substrates for the production of glucose by enzymatic hydrolysis. On an average, waste paper contributes to about 50% municipal solid waste, and in that newspaper alone constitutes 14% [9]. At present, majority of waste paper is land filled or incinerated; but, both of these methods are of increasing environmental concern. The other approach of recycling paper gives lower grade paper products as the fiber length in the paper becomes shorter and this shortening of paper fibers decreases the quality of paper. This means that a large portion of paper would always be sent to disposal, which contains a substantial and underutilized source of sugar [10].

The potential of bioethanol production from waste paper using various process designs has been investigated in the past [11–14]. The studies have been mainly concentrated on understanding the effects of specific operating variables such as surfactants on the enzymatic hydrolysis of newspaper [11–13]. Kuhad et al. [14] studied the fed-batch saccharification of newspaper and reported 38% sugar yield. In all these studies, the yield of reducing sugars from newspapers was very less and hence it is very essential to use a technique which can enhance the yield of reducing sugars

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from newspaper and will make it an attractive feedstock for bioethanol production. Also, enzymatic saccharification of lignocellulosic biomass using cellulase enzyme typically possesses low efficiency and slow reaction rate and thus it is important to investigate the intensification aspects. Hence, approach of newspaper as a substrate with intensification studies using ultrasound has been selected in the present work. The study tries to ascertain the nature of the synergistic effect of ultrasound and enzymes for the specific application. The effect of parameters such as substrate loading, enzyme concentration, power and duty cycle of the ultrasound has been investigated in the present work. The overall purpose was to develop an efficient ultrasound-assisted enzymatic hydrolysis process for converting cellulose into reducing sugar which is one of the important steps in the bioconversion of lignocellulosic material into bioethanol.

2. Materials and methods

2.1. Materials

Newspaper as a substrate was chosen as a lignocellulosic biomass for the enzymatic hydrolysis and was collected from a local supplier. Its composition based on the dry substrate was: 41.02% cellulose, 24.85% hemicellulose, 23.07% lignin, 5.99% ash and 3.89% moisture. The composition was determined by using standard method described by NREL. 3,5-Dinitrosalicylic acid (DNSA), citric acid, sodium citrate, phenol, sodium hydroxide, sodium potassium tartrate and other chemicals were procured from S.D. Fine Chemicals Pvt. Ltd., Mumbai. Cellulase enzyme (EC number 3.2.1.4) was obtained as a gift sample from Advance Biotechnologies, Mumbai, India.

2.2. Experimental methodology

2.2.1. Pretreatment of newspaper

A series of pretreatment processes were undertaken prior to the enzymatic hydrolysis of the newspaper for reducing sugar production. Newspaper was air dried for 2 h at 105 °C and mechanically grinded using electric mixer grinder in distilled water. Pretreatment of newspaper consists of ultrasound assisted alkaline treatment for delignification of the biomass. Newspaper was treated with 1 N NaOH solution at 100 W power for 70 min with 80% duty cycle. After delignification reaction, repeated water washing of the newspaper was carried out to remove the traces of alkali. 80% delignification of the biomass was obtained with this pretreatment as established in our previous work [15]. After water washing, biomass was centrifuged at 10,000*g* for 20 min and dried in oven at 105 °C for 6 h. This dried biomass was used as a raw material for enzymatic hydrolysis for the production of reducing sugars. After pretreatment, the material loss was 37% due to the removal of lignin and dissolution of some part of the hemicellulose and cellulose.

2.2.2. Enzymatic hydrolysis of biomass

Cellulase enzyme with CMCase activity of 205 IU/ml was used for the hydrolysis experiments. A completely baffled, 2.0 L jacketed glass reactor equipped with mechanical stirrer, was used for the enzymatic hydrolysis reaction. A uniform suspension of delignified newspaper (5% w/v) in citrate buffer (pH 4.8) was transferred to the reactor, which was then followed by the addition of cellulase enzyme (0.14% w/v). The total amount of newspaper suspension was 1.4 L. The temperature of the reaction was controlled by circulating water though the outer jacket of the reactor. The hydrolysis reaction was carried out for 72 h. The samples were withdrawn periodically after each hour for analysis. All the samples withdrawn were centrifuged at 10,000 rpm for 10 min. After centrifugation, the supernatant was removed for reducing sugar content analysis. The variables studied during enzymatic hydrolysis were substrate loading, enzyme loading, and temperature of reaction (the specific range of the variables has been mentioned in the discussion later). All the experiments were repeated two times to check the reproducibility and average values have been reported in the figures.

2.2.3. Ultrasound-assisted enzymatic hydrolysis of biomass

A three neck completely baffled, 2.0 L jacketed glass reactor equipped with mechanical stirrer was used for the reaction. Ultrasonic device used for ultrasound-assisted enzymatic hydrolysis was a probe sonicator obtained from Dakshin Ultrasonics, Mumbai. The ultrasonic irradiation at a frequency of 20 kHz was transferred through a titanium cylindrical horn, introduced vertically into the reactor through the side neck and submerged 2.0 cm into the reaction mixture. The ultrasonic horn has a maximum rated power output of 120 W and diameter of 1.1 cm. The schematic representation of experimental set up has been shown in Fig. 1. The

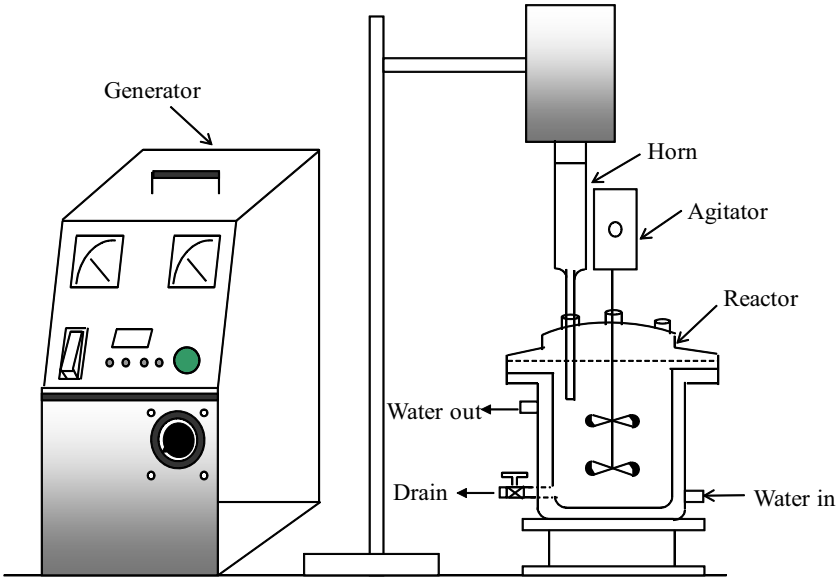

Fig. 1. Experimental setup for ultrasound-assisted enzymatic hydrolysis.

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