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Dynamic microwave-assisted alkali pretreatment of cornstalk to enhance hydrogen production via co-culture fermentation of *Clostridium thermocellum* and *Clostridium thermosaccharolyticum*

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ABSTRACT

A novel dynamic microwave-assisted alkali pretreatment (DMAP) of cornstalk (CS) was developed in a microwave pretreatment system, which resulted in the effective removal of lignin and an increased release of soluble compounds more accessible to microorganisms. The key factors affecting the pretreatment process were optimized for enhancing thermophilic hydrogen production by co-culture of *Clostridium thermocellum* and *Clostridium thermosaccharolyticum*. The hydrogen yield reached 105.61 mL g^{-1} of CS when cornstalk was pretreated by DMAP for 45 min with an alkali loading, a liquid/solid ratio and flow rate of 0.12 NaOH g^{-1} of CS, 50:1 (mL:g) and 60 mL s^{-1}, respectively. This was 54.8% higher than that from the untreated cornstalk, with greatly increased hemicellulose and cellulose degradations ranging from approximately 41%–79.55% and 71.28%, respectively. The present work indicates that DMAP has the strong potential to significantly improve the conversion efficiency of lignocellulosic material to renewable biofuel.

1. Introduction

Bio-hydrogen is attracting increasing attention in research fields and industry as a type of renewable energy. Compared with fossil fuels, hydrogen has a high energy yield and generates no pollutants, which makes it an excellent candidate as a clean energy carrier [1–4].

Significant efforts are being made to enhance hydrogen production from lignocellulosic materials. Cornstalk, one of the most abundant low-cost biomass sources, is regarded as a potential long-term alternative feedstock for bio-hydrogen production. However, cellulosic materials are usually not readily fermentable by microorganisms because their complex structure is resistant to enzymatic hydrolysis [5–8]. The co-culture of thermophilic fermentation effectively improved hydrogen yields from cornstalk, with relatively higher degradations of hemicellulose and cellulose than mono-culture [9]. The main factors affecting enzymatic hydrolysis of cornstalk are the crystallinity of the lignocellulosic materials and the lignin content. Therefore, pretreatment is usually required to disrupt the lignocellulosic structure in order to increase the

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accessibility to cellulose and its biodegradability [10,11]. A number of pretreatment methods have been developed for the pretreatment of lignocellulosic biomass over the years including extrusion, steam explosion, dilute acid, lime, wet oxidation, and biological pretreatment. The goal of these methods is to remove or alter the hemicellulose or lignin, decrease the crystallinity of the cellulose, and increase the surface area [7,10,12,13]. In addition, most of these processes require high-temperature or high-pressure; this leads to the formation of degradation products which can act as inhibitors in subsequent stages.

Microwave irradiation has been recently explored as a potential method for lignocellulose pretreatment to enhance bio-fuel production. It is different from conventional heating methods because in microwave processing, energy is supplied directly to the lignocellulosic materials by an electromagnetic field [14–16]. Alkali pretreatment is a typical chemical pretreatment method, and a combination of microwave irradiation with alkali is an alternative pretreatment approach for lignocellulosic materials with higher efficiency [7]. Microwave-assisted alkali pretreatment (MAP) can assist biomass digestion, as has been demonstrated in rice straw, wheat straw, switchgrass, cashew apple bagasse, and herbal-extraction process residues [7,10,17–19]. However, the capacity of these microwave-assisted pretreatment systems limits the practical use of this technology. In addition, the dynamic microwave-assisted alkali pretreatment (DMAP) has clear advantages compared with MAP, such as a large capacity, automated and continuous operation, a short duration and highly efficient lignocellulosic hydrolysis. There continues to be strong interest in the development of novel microwave-assisted pretreatment systems for improving biofuel production from lignocellulosic biomass.

The aim of this study was to investigate the effect of microwave-assisted alkali pretreatment on the digestibility of cornstalk in a scaled-up dynamic microwave pretreatment system, and to evaluate hydrogen production via thermophilic co-culture from the pretreated cornstalk. The alkali loading, microwave irradiation time, solid/liquid ratio and feed flow rate were tested to determine the effectiveness of the pretreatment process.

2. Materials and methods

2.1. Materials and microorganism

Cornstalk, obtained from the Daxing district, in Beijing, China, was dried at 60 °C. The cornstalk material used in all experiments was milled to a 1 mm powder using a plant mill. The main chemical components of the cornstalk were as follows (%, w/w): cellulose 31.5 ± 0.48, hemicellulose 29.8 ± 0.47, lignin 10.8 ± 0.41, and total soluble sugar 12.7 ± 0.36.

Clostridium thermocellum DSM 7072 and *Clostridium thermosaccharolyticum* DSM 869 were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Both stains were cultivated in CM4 medium as follows (g L^{-1}): 1.5 KH_2PO_4, 3.8 $K_2HPO_4 \cdot 3H_2O$, 1.3 $(NH_4)_2SO_4$, 1.6 $MgCl_2 \cdot 6H_2O$, 0.013 $CaCl_2$, 5.0 yeast extract, 1.25 × 10^{-3} $FeSO_4 \cdot 7H_2O$, 1.0 × 10^{-3} resazurin, and 0.5 cysteine-HCl. The *C. thermocellum* culture was subcultured every 3 days into fresh CM4 medium with 10 g L^{-1} microcrystalline cellulose (Sinopharm Chemical Reagent Co., Ltd., China), and the *C. thermosaccharolyticum* culture was subcultured every 24 h into fresh CM4 medium with 10 g L^{-1} xylose. The final pH value of these media was adjusted to 7.2 with sodium bicarbonate [9].

2.2. Apparatus and pretreatment methods

A schematic of the dynamic microwave-assisted pretreatment system is shown in Fig. 1. It was constructed using three stainless steel pretreatment tanks (35 mm i.d. × 350 mm H), and the working volume of each tank was 1 L. A microwave irradiation system with a maximum irradiation power of 2000 W was installed in the pretreatment system. Quartz was installed in the microwave chamber, therefore, the microwaves were able to penetrate the quartz and be subsequently absorbed by the feed liquid. In order to ensure safety, the

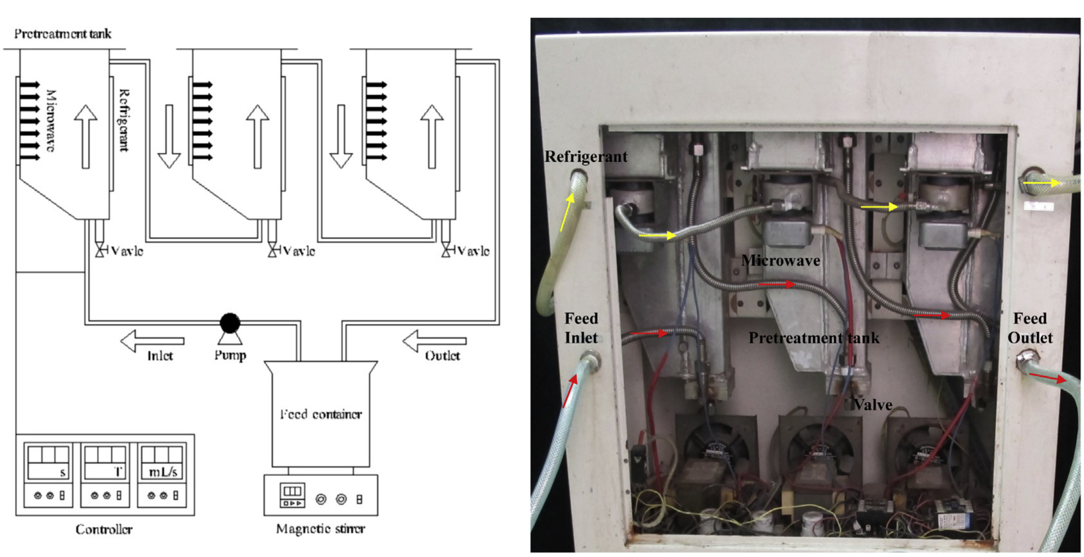

Fig. 1 – Dynamic microwave-assisted pretreatment system. Left: Schematic of reactor system; Right: Photo of reactor layout.

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