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Anaerobic degradation of microcrystalline cellulose: Kinetics and micro-scale structure evolution

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ABSTRACT

The degradation kinetics and micro-scale structure change of microcrystalline cellulose during anaerobic biodegradation were investigated. A modified Logistic model was established to properly describe the kinetics, which showed good fitness and wide applicability for cellulose degradation. A maximum degradation rate of 0.14 g L^{-1} h^{-1} was achieved after cultivating for 51.5 h. This result was in good agreement with the scanning electron microscope and X-ray diffraction analysis. Channels of 400–500 nm size started to occur on the crystalline surface of cellulose at around the inflexion time. Accordingly, the crystallinity significantly decreased at this point, indicating a degradation of the crystalline structure zones by anaerobic bacteria. This study offers direct morphological evidence and quantitative analysis of the biodegradation process of cellulose, and is beneficial to a better understanding of the cellulose degradation mechanism.

1. Introduction

Cellulose is the most abundant renewable natural organic material on earth (van der Zee et al., 1998; Thanakoses et al., 2003). Biodegradation is widely believed as the most attractive and promising avenue for cost-efficient treatment of cellulosic wastes and sustainable resource recovery at the same time (Steven et al., 2001; Ueno et al., 2007; Sun et al., 2009). However, there are severe barriers to such processes at the present stage. Attributed to the unique stable structure, cellulosic materials are inherently recalcitrant to microbe attack (Hu et al., 2004; Huang et al., 2010). Thus, low degradation efficiency (DE) has been a major challenge for cellulosic waste biodegradation. In addition, our understanding about the biodegradation process of cellulose is still rather limited, which further constrains our ability for process optimization.

Kinetic modeling offers a powerful tool to explore into the reaction processes in complex systems. Since the early 1990s, the Logistic equation has been used for describing the growth of pure stains *Streptomyces viridosporus* (Korus et al., 1991), *Coniothyrium minitans* (Ooijkaas et al., 2000) and *Escherichia coli* (Fujikawa et al., 2004). Moreover, this equation has been modified to estimate the degradation of 2,4-dichlorophenoxyacetic acid (Langner et al., 1998). However, no efforts have been made to model substrate utilization in the anaerobic degradation process by using the Logistic equation.

The anaerobic degradation of cellulosic wastes has been intensively studied, and a variety of kinetic models have been developed for the process simulation (Myint et al., 2007; Zhao et al., 2009). However, these kinetic studies are mostly based on macro-scale analysis of reaction rate and cannot reveal the micro-scale changes of cellulose structure. On the other hand, a variety of microscopic inspection technologies such as scanning electron microscopy (SEM) and atomic force microscopy (AFM) have been successfully used for visualized characterization of the micro-scale structures of cellulosic materials (Zhao et al., 2007; Hu et al., 2008), but these studies offer little information on the dynamic morphological/structural changes of cellulosic materials during anaerobic biodegradation. Moreover, the relationships between cellulose morphology/structure and DE are still unclear so far.

Therefore, this study aims to get an insight into the dynamic process of microcrystalline cellulose (MCC) biodegradation and try to find out the links between cellulose structure and degradation performance by taking into account both the reaction kinetics and the morphological/structural changes. A modified Logistic model, which has been widely used for biodegradation process simulation, was adopted for the kinetic study. SEM detection was performed to visualize the morphological evolution. Considering that SEM cannot distinguish amorphous from crystalline regions

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in the same fiber of cellulose, X-ray diffraction (XRD) analysis, which is known to quantitatively characterize the crystallinity (CrI) from two-phase structure theory and amorphous halo correction (Krässig, 1993), was also introduced to offer more structural information. To the best of our knowledge, this is the first attempt to comprehensively investigate the anaerobic biodegradation process of MCC by the combined use of kinetic analysis and SEM, XRD characterization.

2. Materials and methods

2.1. Culture and experimental operations

Fresh cattle manure obtained from a cattle farm in Suzhou, China was used as the inoculum. MCC (analytical grade, from Sinoharm Chemical Reagent) without further purification was used as the sole carbon source. The MCC was obtained from wood and/or cotton pulp after treatment with acid. The MCC powder is insoluble in water and dilute acid, but dispersible in water and partial swelling in dilute acid. Granularity range is 20–100 μm. Moisture is 6%. The other components of the basal medium include (mg L^{-1}): $NaHCO_3$, 5000; NH_4Cl, 280; $CaCl{\cdot}2H_2O$, 10; K_2HPO_4, 250; $MgSO_4$, 100; yeast extract, 100; H_3BO_3, 0.05; $FeCl_2{\cdot}4H_2O$, 2; $ZnCl_2$, 0.05; $MnCl_2{\cdot}4H_2O$, 0.05; $CuCl_2{\cdot}2H_2O$, 0.03; $(NH_4)SeO_3{\cdot}5H_2O$, 0.05; $AlCl_3{\cdot}6H_2O$, 2; $NiCl_2{\cdot}6H_2O$, 0.05; EDTA, 1; resazurin, 0.2; and 36% HCl, 0.001 mL L^{-1}.

The degradation tests were carried out in a 5-L fermentor (Baoxin Biotech., China) at the stirring rate of up to 120 rpm. The working volume of the reactor was 2 L. The temperature and pH were controlled at 39 ± 1 °C and 6.75, respectively.

2.2. Sampling and analysis

At regular intervals, 10 mL of sample mixture was taken from the fermentor for analysis. The supernatants after centrifugation (8000*g*, 15 min) were passed through a 0.45-μm polycarbonate membrane and stored at −20 °C before analysis. Volatile fatty acids (VFAs) were determined by GC (6890N, Agilent., USA). The biogas volume was measured by the water replacement method, and the biogas composition was analyzed by another GC (9790, FuLi, China). Total dissolve organic carbon (DOC) was analyzed by Liqui TOCII (Elementar., Germany) and reduced sugars were measured by the anthrone method (Koeher, 1952). Cell dry weight (CDW) was estimated by measuring microbial protein by the method of Pavlostathis et al. (1988). Protein was determined using the Lowry method with bovine serum albumin as the standard (Raunkjær et al., 1994). Total solids (TS), volatile solids (VS) were analyzed according to standard methods (APHA, 1999).

2.3. SEM analysis

A Quanta 400 FEG SEM (FEI, USA) operated at 5 keV was used to image cellulose samples at different time intervals. Samples were coated with Au by a vacuum sputter-coater to improve conductivity.

2.4. XRD analysis

Crystalline structures of the cellulose samples were analyzed by wide-angle XRD on a X'pert PRO MPD system (Panalytical, Netherland). Ni-filtered Cu Kα radiation (λ_1 = 1.54 Å) generated at a voltage of 40 keV and a current of 40 mA was utilized. The scanning was performed from 5 to 50° at a speed of 2° min^{-1}. Crystalline allomorphs of cellulose were determined by the resolution of the wide-angle XRD curves. CrI values were calculated from the diffraction intensity data using the empirical method proposed by Segal et al. (1959):

$$\mathrm{CrI} = 100\frac{I_{002} - I_{\mathrm{Amorph}}}{I_{002}} \quad (1)$$

where I_{002} is the peak intensity corresponding to the (002) lattice plane, I_{Amorph} peak intensity corresponding to amorphous cellulose at 2θ degrees of 18°, 2θ is Bragg angle.

The average size of crystallite was calculated from the Scherrer equation based on the width of the diffraction patterns. In this work, crystalline size was calculated from the 101, 10−1, 002 and 040 lattice planes of MCC samples (Chen and Yokochi, 2000).

$$D_{(hkl)} = \frac{k\lambda_1}{B_{(hkl)}\cos\theta} \quad (2)$$

where $D_{(hkl)}$ is crystalline size (nm), k is Scherrer constant (0.89), λ_1 is X-ray wavelength (1.5406 Å), $B_{(hkl)}$ is full-width at half-maximum of the reflection *hkl* measured.

2.5. Kinetic model development

A modified logistic equation (Zwietering et al., 1990) was adopted to describe the degradation kinetics:

$$S = S_0\left\{1 - \frac{1}{\left\{1 + \exp\left[\frac{4R_m}{S_0}(\lambda - t) + 2\right]\right\}}\right\} \quad (3)$$

where S is substrate concentration at time t (g L^{-1}), t is incubation time (h), S_0 is initial substrate concentration (g L^{-1}), λ is lag time (d), R_m is maximum substrate degradation rate (g L^{-1} h^{-1}).

By differentiation Eq. (2), the rate of substrate degradation r_s (g L^{-1} h^{-1}) can be described as:

$$r_s = \frac{-4R_m \exp\left[\frac{4R_m}{S_0}(\lambda - t) + 2\right]}{\left\{1 + \exp\left[\frac{4R_m}{S_0}(\lambda - t) + 2\right]\right\}^2} \quad (4)$$

Regression fitting was performed using OriginPro8.0™.

3. Results

3.1. Degradation kinetics

Concentrations of cellulose and its degradation products in all batches were monitored at specific time intervals. Fig. 1 shows the changing profiles of cellulose and produced VFAs, reduced sugar, DOC and microorganisms in a typical run. The initial concentration of cellulose and bacteria were 11.5 g L^{-1} and 1.15 g CDW L^{-1}, respectively.

As shown in Fig. 1a, the cellulose concentration remained almost unchanged during the initial incubation period of 24 h. Then, it declined significantly in 24–84 h. After that, the degradation rate slowed down again and the DE of 65% was obtained at the end of incubation. The DOC and VFA concentrations in the supernatant increased gradually during the degradation. Acetate and propionate were the main degradation products, which account for near 90% of the total VFA concentration. Butyrate and iso-valerate were also detected in the supernatant but at low levels (Fig. 1b). The reduced sugar remained at a relative low level (<110 mg L^{-1}) during the degradation process. Total of 480 mL biogas was obtained at the end of a typical batch. Carbon dioxide, hydrogen and methane were determined as the main components, which accounted for 83%, 2% and 15% of the total biogas volume, respectively. The carbon recovery was determined as 60%.

Eq. (3) was fitted to the experimental data of cellulose concentration vs. time. As shown in Fig. 2a, a high correlation coefficient (R^2) of