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Enhancement of corn stover hydrolysis with rumen fluid pretreatment at different solid contents: Effect, structural changes and enzymes participation

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ABSTRACT

Different solid contents of corn stover were pretreated with rumen fluid to enhance its hydrolysis and acidification, with whole pretreatment time of 72 h. Results showed that rumen fluid pretreatment effectively enhanced the hydrolysis of corn stover. The concentration of soluble COD for 10% (*w/v*) corn stover was about 2–6.4 times and 1.5–2.5 times of that for 3% and 5% (*w/v*), respectively. The highest reducing sugar concentration for 10% (*w/v*) corn stover was about 20 folds and 5.7 folds of that for 3% and 5% (*w/v*) at 2 h, respectively. Higher solid content of corn stover produced more VFAs, however, VFAs accumulation happened during the 10% (*w/v*) corn stover pretreatment, leading to the higher VFAs concentration for 5% (*w/v*) corn stover than that for 10% (*w/v*) during the 16–36 h. After the pretreatment, the structure of corn stover changed, such as the increase of specific surface area and decrease of crystallinity. Due to the removal of hemicellulose, the relative amount of lignin increased in the treated solids illustrated by the FTIR spectra. The changes of enzymatic activities showed that the enzymes secreted by the rumen microorganisms were quickly adsorbed by the corn stover, participating in the hydrolysis, which resulted in the sharp decrease of enzymatic activities in the fluid at the first 4 h. Though the enzymatic activities increased a little during the pretreatment, they could not restore the original level. This might be caused by the pH decrease and VFAs accumulation. The practical application prospect of this study was also conducted.

1. Introduction

Fossil fuel supply is increasingly tense, with emissions of greenhouse gases and other pollutants during its combustion. Biofuel is green alternative energy sources for fossil fuel, attracting widespread attention. Due to the abundant sources of lignocellulosic biomass, its conversion to renewable energy has come to be promising (Baker and Keisler, 2011). Through anaerobic digestion, the lignocellulosic biomass can be converted to biogas, which is kind of clean energy. As kind of food crop, corn is widely planted around the world. Corn stover is one of the biomass raw materials can be used for the sustainable production of biofuels on a large scale, playing an important role in reducing the dependency on fossil fuels. Anaerobic digestion of corn stover is of great significance in limiting emissions of pollutants, relieving the energy crisis and maintaining the sustainable development.

The corn stover is almost constituted by cellulose, hemicellulose and lignin. The cellulose cell connects with each other through hydrogen bond and Van der Waals forces to form a strong micro fiber. Micro fiber, hemicellulose, polymer and pectin connect with each other, packed by lignin. The cell walls of corn stover fill with matrix formed by cellulose and hemicellulose, and this special, complex structure makes great resistance to fungi, bacteria, enzyme and chemical degradation (Zhu and Pan, 2010). As we all know, anaerobic digestion, including hydrolysis, acidification and

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methane production, can convert complex organic materials first to simple soluble organics, and then to volatile fatty acids (VFAs) and finally to CO_2 and CH_4 (Zhang et al., 2014). In order to effectively use corn stover to produce biogas, the recalcitrant structure of corn stover must be destroyed through hydrolysis, thus the hydrolysis process becomes the limiting step of anaerobic digestion for the production of methane (Jeihanipour et al., 2011).

Different pretreatments have been explored to improve the efficiency of anaerobic digestion, for example, physical, chemical, biological, combined physical and biological pretreatments et al. (Mosier et al., 2005; Zhao et al., 2014; Yuan et al., 2014; Yang et al., 2010; Sun et al., 2011). Wyman et al. (2005) compared the sugar recovery from the pretreatments to corn stover, including ammonia fiber expansion (AFEX), aqueous ammonia recycle, controlled pH, dilute acid, flowthrough and lime. The comparative data showed that pretreatments at lower pH by dilute acid, controlled pH, and flowthrough removed most of the hemicellulose, the flowthrough or partial flow operations removed as much as 75% of the lignin. As higher pH technologies, lime and liquid ammonia recycle removed lignin. However, though AFEX is also a higher pH pretreatment, it did not generate free liquid or separate the lignin or hemicellulose from the cellulose. Jin et al. (2015a) pretreated four different lignocellulosic biomasses by high-pressure homogenization (HPH), which is widely used in food chemical, pharmaceutical and biological processes (Chung et al., 2014; Fang et al., 2015; Xu et al., 2014). The results demonstrated that HPH pretreatment was effective to improve the reducing sugar yield in enzymatic hydrolysis under mild condition without chemical addition or heating. All these mentioned pretreatments can remove hemicellulose or lignin, or increase the specific surface area, and thus improve the contact between the lignocellulosic biomass and anaerobic digestion microorganisms, so as to increase the reaction rate and shorten the reaction period.

Ruminants are animals feed with lignocellulosic biomass, which could return the half-digested food to the mouth after feeding to chew again. They have 4 stomachs: rumen, reticulum, omasum, and abomasum. Rumen is the most important digestive organ. Rumen microorganisms occupy an important position during digestion. In 1 ml rumen fluid, there are about 10^6 bacteria, 10^6 ciliated protozoa and 10^3–10^6 fungus (Sauer et al., 2012). These microorganisms grow rapidly on the lignocellulose entered into the rumen, and secreting digestive enzymes at the same time, such as cellulase, hemicellulase and β-glycosidase et al. Under the help of these enzymes, rumen microorganisms degrade cellulose, hemicellulose and pectin et al. into monosacchride, which could be gradually degraded into VFAs (Fang et al., 2016). The main function of rumen fermentation is to provide VFAs from plant polysaccharide, and methane production is secondary. The anaerobic fermentation of corn stover by rumen microorganisms from a fistulated goat was conducted by Hu and Yu (2005) in batch and semi-continuous cultures. And the results showed that rumen microorganisms were able to rapidly degrade the volatile solids and produce useful VFAs with high yields. Wall et al. (2015) added rumen fluid during the anaerobic digestion of high lignocellulose grass silage, and the results demonstrated that VFAs concentration rose, offering higher 371 ml CH_4 g^{-1} VS and stable operation throughout. Rumen fluid has been sought to potentially enhance the digestion of lignocellulosic biomass by hydrolyzing the linkages between cellulose, hemicellulose and lignin (Yue et al., 2013).

In the past researches, rumen fluid was usually used to enhance the anaerobic digestion of biomass. In our pre-experiment, the anaerobic seed sludge was inoculated immediately after the hydrolysis of corn stover by rumen fluid. The biogas production did not quickly proceed as expected. That might be caused by the lower pH or acid accumulation. Due to its high degradation rate and effective hydrolytic conversion to VFAs we considered the rumen fluid pretreatment as kind of pretreatment focusing on the hydrolysis process instead. Different solid contents need different pretreatment time, and acid accumulation may occur when the solid content is high. In this study, we explored the relationship between pretreatment effect and solid content. Though as reported, rumen microorganisms and the secreted enzymes played an important role during the hydrolysis of lignocellulose (Sauer et al., 2012), few studies were involved in the function of microorganisms and enzymes. The changes of enzymatic activities during rumen fluid pretreatment can tell how the enzymes participate in the hydrolysis of corn stover and the relationship between the enzymes and the hydrolysis products.

In this study, the effectiveness of hydrolysis during rumen fluid pretreatment at different solid contents of corn stover was examined. At the same time, the structural changes of corn stover were also studied. During the rumen fluid pretreatment, the cellulase secreted by rumen microorganisms participated in the hydrolysis process. The changes of enzymatic activities were also evaluated during the pretreatment. Additionally, the application prospect of rumen fluid pretreatment in the reactor was also proposed.

2. Materials and methods

2.1. Corn stover

Corn stover was collected from countryside in Hebei province. After collection, the corn stover was cut short and then triturated by a high-speed universal smashing machine (FW-100, Beijing ever light medical equipment Co., Ltd, China) into particles. Then, the particles were screened, and 20–80 mesh were reserved for the experiments. Before experiment, the corn stover particles were dried at 60 °C for at least 24 h and stored in sealed plastic bag at ambient temperature. The TS and VS of corn stover were 963.4 and 960.4 g kg^{-1}, respectively.

2.2. Rumen fluid

Rumen fluid was collected from Beef Cattle Research Center of China Agricultural University. After collecting from the fistulated beef cattle, rumen fluid was transferred into vacuum cup to keep the activity of the rumen microorganisms. The rumen fluid was filtered through four-layer sterile gauze before determination and experiment. The pH of rumen fluid was 6.2, and TS, VS were 39.6 and 31.3 mg ml^{-1}, respectively.

2.3. Pretreatment of corn stover by rumen fluid

The corn stover was mixed with rumen fluid as 3%, 5% and 10% (*w/v*) in serum bottles, filling with nitrogen to keep the anaerobic environment and sealing with rubber stopper. Each concentration sample was in duplicate, keeping 39 °C in the constant temperature bath. Sampling time was 2, 4, 8, 16, 24, 36, 48 and 72 h.

The samples were centrifuged at 8000 r min^{-1} for 10 min by a refrigerated high-speed centrifuge (3H16RI, Hunan Hersxi instrument & equipment Co., Ltd, China). The solid concentrate produced by centrifugation was washed with water and then dried at 80 °C in a draught drying cabinet for the measurement of FTIR, XRD and specific surface area. The supernatant was filtered through 0.45 μm filter membrane. Filtrate was used for the determination of VFAs, soluble COD (sCOD), reducing sugar concentration, and enzymatic activities.

All the experiments were carried out in duplicate, and the mean and standard deviation values were calculated using the Microsoft Excel program.