Bioresource Technology 147 (2013) 1–6

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Comparative assessment of the methanogenic steps of single and two-stage processes without or with a previous hydrolysis of cassava distillage

Qinghua Zhang *, Xiangdong Zhu *, Lingbao Kong, Ganjun Yuan, Zhijun Zhai, Haoju Liu, Xiaoyan Guo

College of Bioscience and Engineering, Jiangxi Agricultural University, Nanchang 330045, PR China

HIGHLIGHTS

- A two-stage process was employed to degrade cassava distillage.
- A constructed microbial consortium was effectively utilized in two-stage process.
- The methanogensis of two-stage process was very stable with a large range of OLR.
- A higher COD removal ratio can be achieved in the two-stage process.
- A higher specific methane yield can be achieved in the two-stage process.

ARTICLE INFO

Article history:
Received 26 June 2013
Received in revised form 29 July 2013
Accepted 2 August 2013
Available online 9 August 2013

Keywords:
Microbial consortium
Hydrolytic step
Two-stage process
Organic loading rate (OLR)
Cassava distillage

ABSTRACT

In this study, cassava distillage with a high solid content was digested in an anaerobic sequencing batch reactor (ASBR) without or with a previous hydrolytic step by a cellulolytic microbial consortium (i.e., single or two-stage process). The methanogenic steps of these processes were compared and evaluated through observation of the methanogenic stability and methane yield under different organic loading rates (OLRs). It was found the methanogenic reactor can be stably performed with the OLRs lower than 20 g COD L^{-1} d^{-1} in the two-stage process, where a specific methane yield (0.147 L CH_4 g^{-1} $COD_{removed}$) could be achieved, which was 17.6% higher than that of the single-stage process (0.125 L CH_4 g^{-1} $COD_{removed}$). The above results indicated that the degradation of cassava distillage in a two-stage process with a previous hydrolytic step can assure the methanogenic process proceeds with greater stability and generates higher methane yield.

1. Introduction

The increasing amount of organic wastes generated worldwide is a growing concern. With rising energy demands and increasing environmental pollution, interest in renewable methane production from organic wastes has grown (Bruni et al., 2010; Lim and Wang, 2013; Wartell et al., 2012). In general, large amounts of different organic wastes generate from the production of agro-industrial products, creating significant environmental pollution risks. The proper management and disposition of these wastes through anaerobic processes represents a significant opportunity to combine waste treatment and renewable energy production (Esposito et al., 2012). For example, in a typical cassava-based bioethanol production process, up to 15 L of distillage can be generated during fermentation for each liter of bioethanol produced (Zhang et al., 2010), resulting in significant pollution problems. Within these wastes, cassava residues, the main solid portion comprising a large amount of lignocellulosic components are generated. Direct digestion of these wastes will result in low hydrolysis efficiency and consequent reduction of the methane yield due to their recalcitrant structures.

It is well known that the limiting step of anaerobic digestion of solid waste is the first step of hydrolysis or solubilization, where the cell wall is broken down allowing the organic matter inside the cell to be available for biological degradation (Wang et al., 1997). Therefore, many studies have been conducted to enhance the hydrolysis either using two-stage process (Bertin et al., 2013; Chu et al., 2012; Jung et al., 2013; Rincón et al., 2009) or feed pretreatment strategies (Baba et al., 2013; Jackowiak et al., 2011; Strong and Gapes, 2012; Vivekanand et al., 2013; Yao et al., 2013; Zhang et al., 2011a,b). Among these pretreatment strategies, microbial co-cultures have the advantage of avoiding problems with feedback regulation and metabolite repression posed by

---

* Corresponding authors. Tel.: +86 791 83813459.
E-mail addresses: zqh_net@163.com (Q. Zhang), xdzhu815@yahoo.com.cn (X. Zhu).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.08.016

isolated single strains, are attracting the most attention (Wang et al., 2011; Zhang et al., 2011a).

In a previous study, a cellulolytic microbial consortium with high cellulose degradation ability was successfully constructed. This consortium consists of cellulolytic and non-cellulolytic microbes and can completely degrade filter paper within 40 h of incubation, revealing excellent application potential (Zhang et al., 2011a). Therefore, in this study, the anaerobic digestion of cassava distillage with a high solid content is designed to be pre-hydrolyzed by this cellulolytic microbial consortium and then digested in an ASBR. Although the report of anaerobic digestion of cassava distillage using two-stage process was not the first time (Carbone et al., 2002), the majority researches of two-stage process just separated the processes into two-steps, i.e., acidogenesis and methanogensis under anaerobic conditions (Carbone et al., 2002; Nasr et al., 2012), where the microbe resources in these steps only originated from the same inoculums, hence only limited degradation effects could be obtained through such kind of separation, especially degrading the recalcitrant substrates such as lignocellulosic wastes. To enhance the digestion effect of a two-stage process, the application of the hydrolysis of a directional constructed microbial consortium instead of the physical separation of an anaerobic process into two-steps, is scarcely found in recent literatures. Based on an extensive search, there was no single study in the literature that addressed the systemic integration of biological pretreatment of cassava distillage using a constructed microbial consortium and a subsequent anaerobic digestion in an ASBR for methane production in a two-stage process. Such systemic integration may yield extra dividends simultaneously, such as enhanced biodegradation of poorly digestible cassava fibers, decreased VFA concentrations in effluent even at high OLR, increased system stability and methane yields.

In the present study, the anaerobic digestion of cassava distillage containing high solid content in an ASBR combined with a previous hydrolysis step by a constructed cellulolytic microbial consortium was performed with various OLRs to investigate the performance improvements on the CH_4 recovery and stabilization efficiency. As comparison, these cassava distillages without hydrolyzation were directly digested in an ASBR to provide a quantitative comparison based on individual performance parameter. The main goals of this investigation are to verify excellences in unique combination of pre-hydrolysis of cassava distillage by a cellulolytic microbial consortium, sequencing-batch operation and to discuss the applicability of two-stage process for maximum stabilization of cassava distillage with a high-rate production of methane under the wide range of OLR.

2. Methods

2.1. Materials

Cassava residues and distillery wastewater were provided by Taixing fuel ethanol Co. Ltd., Jiangsu Province, China. The major components of cassava residues (obtained as average values of three replicates and expressed as weight percent on a dry basis) were as follows: cellulose, 24.92 ± 0.43; hemicellulose, 17.84 ± 0.28; lignin, 12.28 ± 0.22; total nitrogen (TN), 1.34 ± 0.04; total solids (TS), 95.60 ± 1.41; and volatile solids (VS), 84.32 ± 1.34. The main characteristics of wastewater were (mg/L): sCOD, 24580 ± 264; TN, 288.96 ± 15.34; TS, 19.39 ± 0.06; VS, 14.12 ± 0.13; and pH, 4.25 ± 0.05. The feeds of cassava distillage utilized in this study consisted of cassava residues and distillery wastewater with a ratio of 4% (w/v) on the basis of the optimal result of previous investigation (data not shown). During the previous hydrolytic step of the two-stage process, the pH of these feeds was adjusted to 7.0 by addition of 2 M NaOH.

2.2. The set up and performance of the ASBR

The ASBR, developed by Sung and Dague (1995), is an anaerobic digestion system that works through consecutive cycles of operation, each of which has the following stages: feeding, reaction, settling and discharge. In this study, the methanogenic stage was carried out in an ASBR with an effective working volume of 12 L. One cycle of each batch consisted of four sequences: feeding (0.5 h), reaction (20 h), settling (3 h) and discharge (0.5 h). The 24 h cycle lasted continuously until the end of the experiments. The biogas produced was collected by a water displacement system with 3 M of NaOH solution fitted to the reactor. The volume of methane produced was equivalent to the volume of water collected (Donoso-Bravo et al., 2009). The ASBR was provided with a magnetic stirrer, and the stirring speed was adjusted to 80 rpm in the feeding and reaction steps. The reactor was fed manually on a daily basis by means of an external feeder and the reaction temperature was kept at 55 ± 0.5 °C through a water bath circulator and a built-in water jacket.

The sludge collected from Taixing Jinjiang chemical Co. Ltd., Jiangsu Province of China, was used as the inoculum of the ASBR. The characteristics of the inoculum used was: pH, 7.8; total suspended solids, $32.4 \pm 0.48\ g\ L^{-1}$; volatile suspended solids, $23.6 \pm 0.34\ g\ L^{-1}$; total solids, $35.6 \pm 0.62\ g\ L^{-1}$; and volatile solids, $24.4 \pm 0.25\ g\ L^{-1}$. Before starting the experiments, an adaptation or acclimatization of the inoculum to the substrate studied was carried out. At the beginning of the experiments, 10 L of anaerobic sludge and 2 L of diluted cassava distillage was used for starting up the reactor and performed at 55 °C for two days. Following that, 0.5 L of cassava distillage was fed in and drained out daily respectively until the reactor achieved a steady-state. Once the biomass of the reactor was acclimated, the experiment was started by feeding the hydrolysates pretreated by a cellulolytic microbial consortium or cassava distillage directly.

2.3. The performance of the hydrolytic reactor

The hydrolytic reactor was operated as a continuous stirred tank reactor (CSTR) with an effective working volume of 3 L. During the pretreatment process, 2.85 L of cassava distillage was filled in the reactor and 0.15 L of the cellulolytic microbial consortium (Zhang et al., 2011a) was used as inoculums.

Recently, several research reports indicated that biological hydrolysis of organic wastes conducted under micro-aerobic conditions can enhance the hydrolysis rate and benefit the subsequent anaerobic process (Díaz et al., 2011; Johansen and Bakke, 2006; Lim and Wang, 2013; Mshandete et al., 2005). Similarly, in this study, the hydrolysis of cassava distillage by a cellulolytic microbial consortium was performed with addition of very small amounts of air during the pretreatment process according to the results of previous investigation (data not shown). During the microaerophilic pretreatment process, oxygen concentration in the hydrolytic reactor was maintained between 0.5 and 1 mg/L by bubbling air at 0.01 volume per volume per minute (vvm). The whole hydrolytic process was performed at 55 °C for 24 h with an agitation velocity of 50 rpm. At the end of this process, the resulting hydrolysates were used as feedstocks for next step of anaerobic digestion in an ASBR.

2.4. The single and two-stage process for methane fermentation

In single-stage process, raw cassava distillage was directly digested in an ASBR for methane production. While, a two-stage process consisted of two steps: biological treatment with a previously constructed cellulolytic microbial consortium (Zhang et al., 2011a), and methane fermentation, i.e., cassava distillage