Bioresource Technology 134 (2013) 391–395

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Short Communication

A simple methodology for rate-limiting step determination for anaerobic digestion of complex substrates and effect of microbial community ratio

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HIGHLIGHTS

- A method was developed to evaluate limiting steps for complex substrate digestion.
- The microbial community ratio concept was proposed and determined by ATP analysis.
- The effect of microbial community ratio on kinetics and limiting step was studied.

ARTICLE INFO

Article history:
Received 30 November 2012
Received in revised form 30 January 2013
Accepted 1 February 2013
Available online 13 February 2013

Keywords:
Rate-limiting step
Kinetics
Microbial community ratio
Anaerobic digestion
Dairy manure

ABSTRACT

Anaerobic digestion (AD) of complex substrates is a multi-step process, which is kinetically controlled by an individual rate-limiting step. A methodology for determining the rate-limiting step during AD of complex substrates was developed by supplementation of metabolic intermediates from each digestion step with dairy manure as an emblematic complex substrate. This method elucidated that hydrolysis of dairy manure was the rate-limiting step when normal anaerobic sludge was used as inoculum. Furthermore, the concept and effect of microbial community ratio was introduced by manipulating two different inocula, i.e. normal anaerobic sludge and heated anaerobic sludge, so that varying ratios (r) of hydrolytic and methanogenic bacteria could be studied. Results revealed that the rate-limiting step changed with the variation of r. For dairy manure, results indicated a critical ratio $r^* = 24$ between hydrolytic bacteria and methanogens, whereby as r decreased or exceeded from this value, hydrolysis or methanogenesis limited the AD process, respectively.

1. Introduction

The anaerobic digestion (AD) of complex organic material is a multi-step process that consists of four stages in series: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Batstone et al., 2002). In such a multi-step, complex process, the kinetics of the slowest step will account for the overall kinetics. AD systems are limited by two major steps depending on the nature of the substrate. Hydrolysis is often limited if the substrate is complex organic solids while the rate-limiting step in the digestion of soluble organic matter is methanogenesis (Tomei et al., 2009). Determination of a rate-limiting step is critical for AD process design treating a specific feedstock, establishment of a stable process performance, and management AD process. Hence, there is a need for an experimental method capable of determining precisely which of the different steps is limiting for a complex substrate.

Anaerobic degradation process is dependent not only on the physic-chemical characteristics of the substrates, but also on the concentration and quality of anaerobic microbial community composed of symbiotic microbes responsible for each disparate metabolic step. The stability of the process is dependent on the inoculum mass and critical balance of different trophic groups (Angelidaki et al., 2009; Ma et al.). The impact of inoculum concentration on biochemical methane potential (BMP) for various organic wastes has been widely studied (Chen and Hashimoto, 1996; Liu et al., 2009; Lopes et al., 2004). Relatively high hydrolysis rates were reached in anaerobic biodegradability tests with a high inoculum to substrate ratio, showing some degree of dependence of hydrolysis to biomass concentration or activity (Fernandez et al., 2001). Similarly, it is believed that inoculum concentration has influence on methane production rate and methane yield (Chen and Hashimoto, 1996). Equally important is the population or composition of the microbial community. For examples, the microbial community ratio, defining the ratio of microbial concentration of each symbiotic growth group, is a key parameter governing the AD process. Balanced microbial population can be translated into

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http://dx.doi.org/10.1016/j.biortech.2013.02.014

proper microbial community ratio. However, research on microbial community ratio and its effect on kinetics of AD is limited and is the focus of this research paper.

The aim of the present work was to develop a simple method for rate-limiting step evaluation through kinetic characteristics from a series of batch tests supplemented with metabolic intermediates generated from the conversion of macro-molecules to methane and carbon dioxide. Moreover, a new concept, microbial community ratio, i.e. hydrolytic bacteria to methanogens ratio, was first proposed in this paper, and its effect on the kinetics of AD process was investigated with two different sources of inocula.

2. Methods

2.1. Substrate and inoculum

Flushed dairy manure, a representative complex substrate with undetermined hydrolysis/methanogenesis rate-limiting step, was collected from the Washington State University Dairy Center in Pullman, WA, USA and stored at 4 °C prior to use. Before adding into reactors, manure was screened through a 2 mm sieve to remove coarse solids, which resulted in mixed liquor containing 9.1 g/L total solids (TS) and 7.6 g/L total volatile solids (VS). Anaerobic sludge was sampled from an anaerobic digester at the Pullman Wastewater Treatment Facility with TS of 17.1 g/L and VS of 11.7 g/L. Two different inocula, namely normal anaerobic sludge (NS) and heated anaerobic sludge (HS), were used in this study. The HS was anaerobic sludge heated in an oven at 105 °C for 2 h in order to kill non-spore-forming methanogens (Logan et al., 2002) then cooled to room temperature in a desiccator. As a result, NS included all three groups of microorganisms, namely hydrolytic bacteria, acidogens, and methanogens while HS consisted of hydrolytic bacteria and acidogens only.

2.2. Rate-limiting step evaluation

The methodology developed for rate-limiting step identification is based on a modified biochemical methane potential (BMP) assay. The aforementioned dairy manure (DM), the basic substrate, was added to serum bottles with working volume of 200 ml. Glucose (GL) was considered as the main intermediate of hydrolysis of carbohydrate complex organics while sodium acetate (SA) was used as model intermediate of acidification (Supplementary Table S1). DM, GL, SA and NS were added into bottles at the concentration of 3 gCOD/L, 3 gCOD/L, and 1 gVS/L, respectively. A bottle filled with dairy manure only served as a control while a bottle with NS only served as a blank. Each bottle was magnetically stirred at a speed of 300 rpm and maintained at 35 °C. Methane production was monitored using a respirometer (Challenge technology AER-200 respirometer, Springdale, AR). Sodium hydroxide was used as scrubbing media to purify methane by adsorbing carbon dioxide and hydrogen sulfide, so that only methane production was recorded. Triplicate analysis was carried out to ensure reproducibility of results. To identify the rate-limiting step during AD process, the following assumptions were made: (1) if glucose increased the methane production rate while acetate did not, then hydrolysis would be the rate-limiting step; (2) if acetate increased the methane production rate while glucose did not, then acidification would be the limiting step; and (3) if both glucose and acetate did not affect methane production rate, then methanogenesis limits the overall process. Usually, the AD process has been considered either hydrolysis or methanogenesis limited, with the acidification step not expected to be a rate-limiting step since acidogenesis is usually the fastest reaction in the anaerobic conversion of complex substrates (Mosey and Fernandes, 1989).

2.3. Effect of microbial community ratio

Experiments for the influence of microbial community ratio on AD process of dairy manure were conducted using a respirometer and maintained at the same operating conditions as the rate-limiting step evaluation experiment. Dairy manure was autoclaved for 30 min in order to sterilize all of the microorganisms. Different volumes of NS and HS were added into bottles giving rise to a wide spectrum of hydrolytic bacteria (H) to methanogens (M) ratios (Table S2). The total sludge concentration in each bottle was 2 gVS/L. Triplicate analysis was also carried out to ensure reproducibility of results.

2.4. Analytical methods

Analyses for TS, VS were done according to Standard Methods (APHA, 1998). Biomass concentration was indirectly determined by measuring adenosine 5′-triphosphate (ATP) concentration using a method detailed in (Wang et al., 2011).

2.5. Kinetics model

2.5.1. Biogas production simulation

The modified Gompertz Eq. (1) was developed to describe the cumulative methane production curve in a batch culture (Zwietering et al., 1990).

$$G = P\ \exp\left\{-\exp\left[\frac{R_{m^e}}{P}(\lambda - t) + 1\right]\right\} \tag{1}$$

where G is cumulative methane production (mL), λ is lag time (d), P is methane production potential (mL), R_m is maximum methane production rate (mL/d), and $e = 2.718281828$.

When glucose was supplemented, a biphasic methane production curve was presented. To characterize each phase, Eq. (1) was used twice to separately best-fit the two methane production phases which resulted in two sets of kinetic parameters. Cumulative methane production exceeding 95% of methane potential (P_1) for the first phase was taken to be the end of the first phase and as well as the beginning of the second phase.

2.5.2. Kinetic model for microbial community ratio (r) affecting rate-limiting step

By definition, r represents the ratio of hydrolytic bacteria to methanogens in the digester and significantly affects the AD process. There should be a critical r^* for the H to M ratio; when $r < r^*$, hydrolysis should be rate-limiting step; while when $r > r^*$, methanogenesis should be rate-limiting step, which can be represented as Eq. (2) (Rittmann and McCarty, 2001).

$$v = \begin{cases} q_H \cdot X_H, & r < r^* \\ q_M \cdot X_M, & r > r^* \end{cases} \tag{2}$$

in which

$$X_M = \frac{1}{r}\alpha_H X \tag{3}$$

$$X_H = \alpha_H X \tag{4}$$

$$q_M = \frac{\mu_M}{Y_M} \tag{5}$$

$$q_H = \frac{\mu_H}{Y_H} \tag{6}$$

where v is methane production rate (mL/L/d), q_H is specific substrate utilization rate when hydrolysis is rate-limiting step (gCOD/gVS), q_M is specific substrate utilization rate when methanogenesis is rate-limiting step (gCOD/gVS), X is total biomass concentration (gVS), X_H is concentration of hydrolytic bacteria (gVS), X_M is concen-