Journal of Bioscience and Bioengineering
VOL. 121 No. 5, 557–560, 2016

www.elsevier.com/locate/jbiosc

Impacts of inoculum pre-treatments on enzyme activity and biochemical methane potential

Bing Wang,[1,*] Sten Strömberg,[1] Ivo Achu Nges,[1] Mihaela Nistor,[2] and Jing Liu[1,2]

Department of Biotechnology, Lund University, Getingevägen 60, SE-221 00 Lund, Sweden[1] *and Bioprocess Control Sweden AB, Scheelevägen 22, SE-223 63 Lund, Sweden*[2]

Received 3 July 2015; accepted 5 October 2015
Available online 30 October 2015

Biochemical methane potential (BMP) tests were carried out to investigate the influence of inoculum pre-treatments (filtration and pre-incubation) on methane production from cellulose and wheat straw. First-order model and Monod model were used to evaluate the kinetic constants of the BMP assays. The results demonstrated that fresh inoculum was the best option to perform BMP tests. This was evidenced by highest enzyme activity (0.11 U/mL) and highest methane yields for cellulose (356 NmL CH_4/gVS) as well as wheat straw (261 NmL CH_4/gVS). Besides, high biodegradability (85.8% for cellulose and 61.3% for wheat straw) was also obtained when the fresh inoculum was used. Moreover, a kinetic evaluation showed that inoculum pre-incubation at 37°C or storage at 4°C introduced a lag-time whereas the effects on hydrolysis rate were less consequent. In summary, pre-treatments affected the enzyme activity of the inoculum, and further on, significantly influenced the methane production and the degradation kinetics of the investigated substrates. It is recommended that filtration of inoculum should be avoided unless in case too large particles therein.

© 2015, The Society for Biotechnology, Japan. All rights reserved.

[**Key words:** Anaerobic digestion; Biochemical methane potential; Biodegradation; Enzyme activity; Kinetic parameters; Pre-treatment]

Anaerobic digestion (AD) has gained increasing attention in recent years, and is considered an efficient and low cost approach for renewable energy production. AD process contains four main steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis. During the hydrolysis step, the large organic compounds are broken down into small ones, e.g., amino acids, long chain fatty acids, sugars, by hydrolytic bacteria, which hydrolyse the substrate by secreting extracellular enzymes. For example, cellulase decompose cellulose into glucose, while starch is broken down into glucose by amylase enzymes (1,2). When microorganisms are able to produce suitable enzymes, the hydrolysis step is going to speed up, however, in some other conditions, hydrolysis may become a rate-limiting step if the substrate is hardly accessible by the enzymes because of some rigid structural composition (3).

Biodegradability (BD) of a substrate and its potential to produce methane via AD can be studied preliminarily by biochemical methane potential (BMP) test. Standard protocols for anaerobic biodegradability tests are extensive, such as ISO standards (4), ASTM standards (5,6) and VDI standard (7). However, these guidelines cover only general experimental protocol without providing strict requirement on how data should be monitored, calculated and presented, which makes the results obtained from different labs difficult to compare because of many factors. One of the most important and complex factor is the inoculum, since there are big variations in origins, metabolic activities and residual biodegradable substrates and so on (8). Furthermore, the variations of bacteria population and their metabolic activities also influence the level of extracellular enzyme and their activities, which subsequently affects anaerobic digestion of substrate. The characteristics of the inoculum, i.e., the kind of microorganisms and their enzymatic activity depend mostly on its origin, pre-treatment procedure (mechanical, thermal and chemical) (9,10) and storage conditions. However, up to date, several studies on enzyme activity of the inoculum in relation to hydrolysis and methanogenesis have been found in literature (11,12).

Therefore, the purpose of this study was twofold: (i) to evaluate the influences of inoculum pre-treatments on a BMP test, for this purpose, inoculum filtration and pre-incubation were applied prior to the BMP test; (ii) to find out the effect of enzyme activity of inoculum on methane production and kinetic degradation of the substrate. In this light, the carboxymethyl cellulase activity (CMCaseA) of inoculum was analysed before and after pre-treatment. The results of this study may provide useful information for the optimization of BMP assays.

MATERIALS AND METHODS

Inoculum and substrate The inoculum used to carry out the BMP test was collected from a mesophilic biogas digester (Ellinge, Eslöv, Sweden) which treats sewage sludge from the wastewater treatment line for municipal wastewater and industrial wastewater from a local potato-processing factory.

Microcrystalline cellulose (Alfa Aesar, Germany) and pre-dried wheat straw (5-mm particle size) were used separately as substrates for the BMP test.

Inoculum pre-treatments Inoculum pre-treatment was performed in terms of either filtration or pre-incubation or combination of both. The filtration was carried out with the aid of a sieve (2-mm mesh) to remove particles and homogenise the inoculum. Thereafter, both fresh inoculum (F-I, used for BMP test within 24 h after collecting from plant) and sieved fresh inoculum (F-SI) were stored at 4°C (4°C-I and 4°C-SI) in cold room or pre-incubated at 37°C (37°C-I and 37°C-SI) in water bath under anaerobic conditions for 5 days, respectively, prior the BMP tests.

* Corresponding author. Tel.: +46462223672; fax: +46462224713.
E-mail address: bing.wang@biotek.lu.se (B. Wang).

1389-1723/$ – see front matter © 2015, The Society for Biotechnology, Japan. All rights reserved.
http://dx.doi.org/10.1016/j.jbiosc.2015.10.004

TABLE 1. The amount of substrate and total amount of mixture added in each bottle.

Inoculum	Cellulose (g)	Wheat straw (g)	Total amount (g)
F-I	2.59	2.86	400
F-SI	2.52	2.78	400
4°C-I	2.45	2.70	400
4°C-SI	2.54	2.80	400
37°C-I	2.48	2.74	400
37°C-SI	2.44	2.69	400

Total amount of mixture = amount of inoculum + substrate.

BMP tests The BMP tests of cellulose and wheat straw by using fresh or pre-treated inocula were performed at 37°C in triplicates in automatic methane potential test system II (AMPTS II, Bioprocess Control AB, Sweden) until daily gas production was less than 1% of the accumulated volume of gas (approximate 30 days) according to VDI 4630 (7). A more detailed description of the system can be found in Stömberg et al. (13).

Inoculum and substrates were added in 500 mL standard bottles at a ratio of 2:1 based on volatile solids (VS). The amount of inoculum and total amount of mixture (i.e., inoculum and substrate) added in each BMP test is presented in Table 1. Blank bottles were set by adding inoculum only. The experiment was performed according to the protocol was previously reported by Koch et al. (14). For determination of methane yield, the methane production from the inoculum was subtracted from the total methane production of the test bottle.

Analytical methods Wheat straw was pre-dried before milled by Grindomix (GM 200, Retsch, Germany) at 6000 rpm for 3 min. The total solids (TS) and VS of inocula and substrates were determined according to standard protocols (15). The pH of inoculum was measured using the electrode from TitraLab 80 titrator (Radiometer, Copenhagen, Denmark).

Carboxymethyl cellulase activities (CMCaseA) of the inocula were evaluated prior the BMP tests by spectrophotometry (Ultrospec 1000, Biochrom, UK) as reported by Zhou et al. (16).

The accumulated gas volumes of inocula during the pre-incubation step were collected in gas bags which were made by a gas-tight and impermeable material (FlextrusTransofoil EL-OPET/PE). The gas volume was measured by a graduated 100 mL gas-tight glass syringe (Fortuna, Germany). The methane content of biogas produced was determined by gas chromatography (GC, Agilent 6890N, TCD) (17).

During the BMP test, methane production was recorded automatically by AMPTS II. At the end of the process, a report containing normalized (standard temperature and pressure, STP: 273.15 K, 101.32 kPa; compensation of the water vapour content) accumulative methane production and flow rate was generated for further data analysis.

A traditional first-order model (Eq. 1) and the Monod model alternative (Eq. 2) (18) were used to evaluate the kinetic parameters of substrates. The first-order model was used to describe the fast and abruptly stopping degradation of cellulose whereas the Monod model, which describes better the slowly declining gas production at the end of the process, was applied for the degradation of wheat straw. As both cellulose and wheat straw showed signs of a lag-phase, with lower productions at the start, a delay time (θ) was introduced for both models (19).

$$\mathrm{BMP}\,(t) = \mathrm{BMP}_{\max}(1 - exp(-k(t-\theta))) \quad (1)$$

$$\mathrm{BMP}\,(t) = \mathrm{BMP}_{\max} k(t-\theta)/(k(t-\theta)+1) \quad (2)$$

In Eqs. 1 and 2 BMP (t) is the cumulative methane yield (NmL CH_4/gVS) at given time t (d), BMP_{max} is the maximum or ultimate methane yield (NmL CH_4/gVS) of the substrate, k is the rate constant or hydrolysis rate constant (d^{-1}), θ is the delay time or lag phase time (d).

Theoretical methane potential (BMP_0) is generally used to predict the methane yield of a specific substrate. It is frequently expressed as NmL CH_4 at STP conditions per amount of organic material added (NmL CH_4/gVS_{added}). The BMP_0 of compounds can be calculated according to Buswell's equation (20). The extent of anaerobic biodegradability of substrates was calculated by experimental methane yield (BMP_{exp}, i.e., BMP_{max}) in comparison with the BMP_0 according to the Eq. 3:

$$\mathrm{BD}(\%) = (\mathrm{BMP}_{exp}/\mathrm{BMP}_0)\cdot 100 \quad (3)$$

All the BMP tests were performed in triplicates, and the results were expressed as mean ± standard deviation (SD). The statistical difference in methane yields and enzyme activities was evaluated by analysis of variance (Single-factor ANOVA, $p \leq 0.05$) in Excel (Microsoft Excel, 2010).

RESULTS AND DISCUSSIONS

Characterization of inocula and substrates Table 2 presents the characteristics of inocula and substrates. It can be clearly seen that the TS, VS and pH of inocula were rarely affected by filtration or pre-incubation at 4°C or 37°C.

TABLE 2. Characteristics of inocula and substrates.

Inoculum/ substrate	TS% (w/w)	VS% (w/w)	VS/TS (%)	pH	Methane yield (NmL CH_4/gVS)
F-I	2.08 ± 0.03	1.26 ± 0.02	60.58	7.49	ND
F-SI	2.03 ± 0.00	1.23 ± 0.00	60.59	7.58	ND
4°C-I	1.94 ± 0.03	1.19 ± 0.02	61.34	7.53	1.68
4°C-SI	2.02 ± 0.00	1.23 ± 0.00	60.89	7.57	0.52
37°C-I	2.00 ± 0.01	1.21 ± 0.01	60.50	7.50	22.08
37°C-SI	1.99 ± 0.00	1.18 ± 0.00	59.30	7.52	22.60
Cellulose	96.45 ± 0.12	96.42 ± 0.14	99.96	ND	ND
Wheat straw	91.12 ± 0.19	87.38 ± 0.16	95.90	ND	ND

ND means not determined.

The VS/TS ratio of inoculum fluctuated between 59.3% and 61.34%. The 37°C-SI showed lower VS/TS ratio among all the inocula. This was probably as a result of higher degradation of residual organic matter. This was also evidenced by high methane production during the pre-incubation.

CMCaseA of inocula Enzyme activity was used to assess the hydrolytic potential of the inocula. One unit (U) of enzyme was defined as the amount of the enzyme which liberated one micro mole of reducing sugar per minute (21).

Fig. 1 shows that CMCaseA of inocula decreased slightly after filtration, because some of microorganisms might be bound to particles and removed by filtration (22). The 37°C-SI has a significant lower ($p < 0.05$) CMCaseA, probably because the microorganisms are active at mesophilic condition and some of bacteria start lysis due to starvation (23). This result was also in agreement with the lowest VS/TS ratio and highest methane production during the pre-incubation (Table 2).

Methane yields of substrates Fig. 2 shows the methane yields of cellulose and wheat straw. The data obtained from this study is similar to those found in works by Kreuger et al. (24), Jackowiak et al. (25) and Kaparaju et al. (26).

The 37°C-SI showed significantly lower ($p < 0.05$) methane yields for both substrates. This observation correlated positively to the low CMCaseA obtained for the corresponding pre-treated inoculum. The highest residual methane production was obtained from 37°C-SI during the pre-incubation; this was also in agreement with the lowest methane yield achieved when this inoculum was used.

Correlation between CMCaseA of inocula and methane yields of substrates As discussed previously the obtained methane yields of cellulose and wheat straw correlated positively with the CMCaseA of the inocula (Fig. 3).

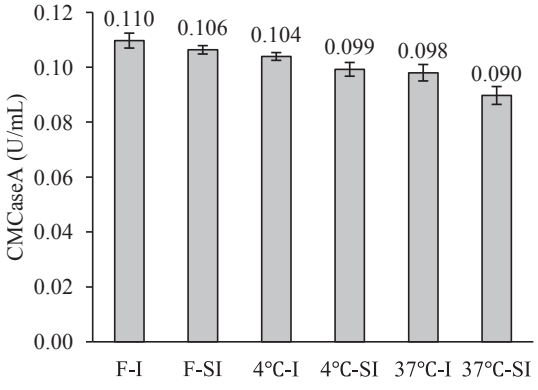

FIG. 1. Enzyme activity (CMCaseA) of inocula.

دانلود مقاله

http://daneshyari.com/article/20117

- ✓ امکان دانلود نسخه تمام متن مقالات انگلیسی
- ✓ امکان دانلود نسخه ترجمه شده مقالات
- ✓ پذیرش سفارش ترجمه تخصصی
- ✓ امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
- ✓ امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
- ✓ دانلود فوری مقاله پس از پرداخت آنلاین
- ✓ پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات