Hydrolysis of Napier Grass in Leach Bed Reactor for Methane Production: Effects of Biological Pretreatment and Leachate Temperature

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ABSTRACT

Napier grass is a promising feedstock for methane production. Napier grass biomass contains mostly cellulose, hemicellulose and lignin, but is difficult to degrade without prior treatment in an anaerobic digester. Here we report on the use of a leach bed reactor (LBR), as a first stage reactor of a two-step process for biogas production. The LBR stage is intended for hydrolysis of the biomass and production of volatile fatty acids from the hydrolysate. LBRs are potentially applicate to any solid substrate. Efficiency of the LBRs treating Napier grass under four different operating conditions over a period of 10 days was examined in terms of the hydrolysis achieved and the volatile solids removed. The LBRs were used to examine the effect of temperature of the recirculation leachate and other biological factors to enhance hydrolysis and the removal of volatile solids. Under the best conditions, 23.9% of the feed material was hydrolyzed and the volatile solids removal was 31%.

Key Words: hydrolysis, leach bed reactor, napier grass, biogas

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INTRODUCTION

Anaerobic digestion is a cost-effective method that is used worldwide for commercial production of biogas from organic materials. Biomass of Napier grass (*Pennisetum purpureum*), a highly productive energy crop, is a potential feedstock for producing biogas through anaerobic digestion.

Energy crop biomass and other crop residues typically containing high total solids can be digested alone, or together with other materials, using either wet or dry digestion processes. The digestion process consists of an initial stage in which the carbohydrate and other complex polymers are hydrolyzed and converted to volatile fatty acids (VFAs). Methanogenic bacteria then convert the VFAs to biogas in a second stage, although both stages of the process may occur simultaneously in the same digester. For cellulose-rich substrates such as Napier grass, the initial hydrolysis is a slow rate-limiting step in the digestion process. Leach bed reactors (LBRs), a first stage reactor where hydrolysis and acidogenesis (production of VFAs) occur, have been developed especially for treating solid wastes including the biomass of energy crops (Nizami *et al*., 2010). LBRs are broadly applicable to any structured solid substrate. The solid feedstock is added to the LBR mixed with the inoculum. The solids form a loosely packed bed. The liquid leachate is continuously withdrawn from the bottom of the bioreactor and recirculated to the top (Chanakya *et al.,* 1993; Lissens *et al*., 2001). The organic matter in liquid leachate can be increased *via* several strategies such as biological pretreatment of substrate and high temperature process water (Haruta *et al.,* 2002; Hendriks *et al.,* 2009; Nizami *et al*., 2009). Liquid hot water could increase the hydrolysis rate of lignocellulosic material (Nizami *et al*., 2009) and Hussain *et al*. (2017) found that LBR operating under 50 °C conditions triggered fermentative microbial populations with higher hydrolytic and acidogenic activity in comparison to the operation under 37 °C. The application of LBRs has been studied using different substrates such as Perennial ryegrass (Nizami *et al*., 2010; Xie *et al*., 2012; Wall *et al*., 2016), Timothy grass (Lehtomaki *et al*., 2008), food waste (Browne *et al*., 2013) or cow manure (Shewani *et al*., 2015). However, to the best of our knowledge, the use of LBRs with Napier grass has not been explored. This research focused on the hydrolysis of Napier grass using LBRs with the aim of maximizing the efficiency of hydrolysis in terms of the volatile solids removal from the feed biomass and chemical oxygen demand (COD) production in the leachate.

MATERIALS AND METHODS

1. Materials

1.1 Napier grass

Napier grass (Pak Chong 1) (*Pennisetum purpureum*) from local farm in Nakhon Si Thammarat was harvested after 120 days of previous cutting. Fresh Napier grass was dried until the weight remained constant in a hot air oven at 105 °C to reduce the moisture content. Then, the biomass was ground in a hammer mill and sieved (sieve numbers 8 and 4) to obtain particles in the size range of 2.38–4.76 mm. The ground material was stored at room temperature until used. The measured characteristics of the Napier grass biomass are shown in Table 1.

Table 1 Characteristics of Napier grass, the seed culture and the anaerobic digester effluent

Parameter	Napier grass	Seed culture	Anaerobic digester effluent
Total solid (% w/w)	22.85±0.00	4.34±0.11	1.21±0.01
Total volatile solid (% w/w)	21.46±0.00	3.00±0.06	0.39±0.03
COD (g/g VS)	1.87±0.36	1.58±0.02	0.87±0.02
pH	5.42	6.8	8.2

1.2 Anaerobic digestion effluent and seed culture

Anaerobic digestion effluent was obtained from a mesophilic biogas digester used in treating palm oil mill effluent at the Southern Palm (1978) Co., Ltd., Thailand. The effluent was stored at 4 °C until required.

Seed culture was prepared using sludge from the above mentioned biogas plant. The sludge was mixed with the anaerobic digester effluent and tap water at a ratio of 3:1:4 (sludge: effluent: water by volume). Ground Napier grass (2 g/100 mL) was added and the seed culture was incubated in flasks for 5-7 days at 37 °C. Then, a further portion (2 g/100 mL) of ground grass was added and incubation was continued for a further 3-5 days. The feeding with grass was repeated a total of 4 times to acclimatize the microorganisms to this substrate. The cellulose-degrading bacteria were confirmed by streaking the acclimatized seed culture on the cellulose congo-red agar medium (Gupta *et al.,* 2011). Afterwards, the seed culture was filtered through a 500 micrometer nylon mesh to remove particulate solids and used for inoculation. The measured characteristics of the anaerobic digester effluent and the seed culture are noted in Table 1.

2. Pretreatment of Napier grass

Dry, ground Napier grass with a particle size of 2.38-4.76 mm was pretreated by mixing with the acclimatized seed culture (i.e. the inoculum) with the inoculum/substrate ratio of 0.05 and 0.10 (5 g of volatile solid (gVS) of inoculum/100 gVS of grass, or 10 gVS of inoculum/100 gVS of grass). The inoculum/substrate ratio is expressed as the amount of volatile solid (VS) originating from inoculum per the amount of VS in the substrate. This mixed slurry was incubated for 3 days at 37 °C.

3. Reactor design and operation

Four identical LBRs were used. Each reactor consisted of a cylindrical acrylic column (diameter = 12 cm) and a bottom conical funnel zone made of PVC (Figure 1). The effective volume of the LBR was 2 L. The reactor had a lid at the top. The height of the cylindrical reactor zone was 30 cm and the funnel had a height of 5 cm. A perforated aluminum sheet with 3 mm diameter holes was placed at the bottom of the cylindrical portion of the column to support the bed of the Napier grass substrate.

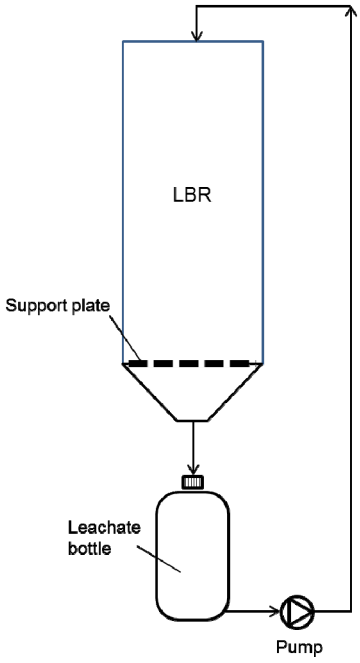

Figure 1 Reactor setup

A mesh with an opening size of 200 micrometers was placed under the perforated aluminum sheet to prevent loss of grass particles and a consequent blockage of the recirculation pipes (Figure 1). A custom made nozzle was placed at the top of the reactor for dispersing the recirculation liquid on top of the bed. The leachate from the bottom of the LBR was collected in a 2-L Duran® laboratory bottle containing a magnetic mixing bar (300 rpm). From this bottle the leached was recirculated to the top of the LBR using a peristaltic pump (model N6-6L Industrial Standard Peristaltic Pump with pump head DZ25-6L) (Figure 1).

4. Leaching experiments

Four separate leach bed reactors (LBR1–LBR4) were operated for 10 days in room temperature (35±1.0 ºC). Anaerobic digester effluent was added to the reactors at an initial liquid/solid ratio of 10 (1000 ml/100 g dry weight) and the recirculation was started. The recirculation pump (Figure 1) operated for 3 h each day with a flow rate of 6 L/h. LBR1 and LBR2 were filled with 100 g dry solids of Napier grass operated at room temperature (LBR1) or at 50 °C (LBR2). Leachate sample was collected each day at termination of recirculation. The volume of the leachate withdrawn in a sample was replaced with an equal volume of distilled water. At the end of each experiment, the dry weight of the grass residue and the total volatile solids were measured.

For LBR3 and LBR4, the reactors were modified to be airtight to assure an absence of aerobic respiration. All connecting points of the reactors were sealed with rubber seals and silicone sealant. The headspace was reduced by filling the reactor with 200 g dry solids of pretreated Napier grass with inoculum-to-substrate ratio (on VS basis) of 0.05 (LBR3), or 0.1 (LBR4). Anaerobic digester effluent at 50 °C was used for recirculation in these two reactors. Details of each leaching experiment are summarized in Table 2.

Table 2 Leaching experiments

Parameter	LBR1	LBR2	LBR3	LBR4
Recirculation rate (L/h)	6	6	6	6
Daily duration of recirculation (h)	3	3	3	3
Leachate temperature (°C)	Room temperature	50	50	50
Inoculum/substrate ratio (g/g)	0.05	0.05	0.05	0.10

5. Analytical methods

Total solids (TS), volatile solids (VS) and the pH were measured using methods 2540B, 2540E, and 4500H (Standard Methods, the American Public Health Association (APHA), 1998), respectively. The COD concentration was determined according to the closed reflux colorimetric method (method 5520D; APHA, 1998). The spectrophotometric absorbance was measured at 600 nm.

The hydrolysis yield is calculated according to Eq. (1) (J.D. Browne et al., 2013):

Hydrolysis yield (%) = Final COD/Initial total COD of grass x 100 (1)

The VS removal is calculated using Eq. (2) (J.D. Browne et al., 2013):

VS removal (%) = [Grass input (gVS) – Grass output (gVS)]/Grass input (gVS) x 100 (2)

RESULTS AND DISCUSSION

The changes in COD and pH over the 10-day duration of operation are shown in Figure 2 for the reactors LBR1–4. In LBR1 and LBR2, COD had declined dramatically at day 3 and continued to decline with time. The hydrolysis yields in LBR1 and LBR2 at the end of the runs were quite low at 3.2% and 4.2% (Table 3), respectively. This indicated a higher rate of COD degradation compared to the rate of hydrolysis. A high rate of COD removal suggested a lack of strictly anaerobic conditions in LBR1 and LBR2 as these reactors were operated under open conditions. Some of the hydrolyzed material was oxidized to carbon dioxide. These results are consistent with Nizami *et al*. (2010) and Browne *et al.* (2013) who observed aerobic respiration in leach bed system. The pH of the leachate in LBR1 and LBR2 continued to drop to below 7.5, but increased near the end of the experiment.

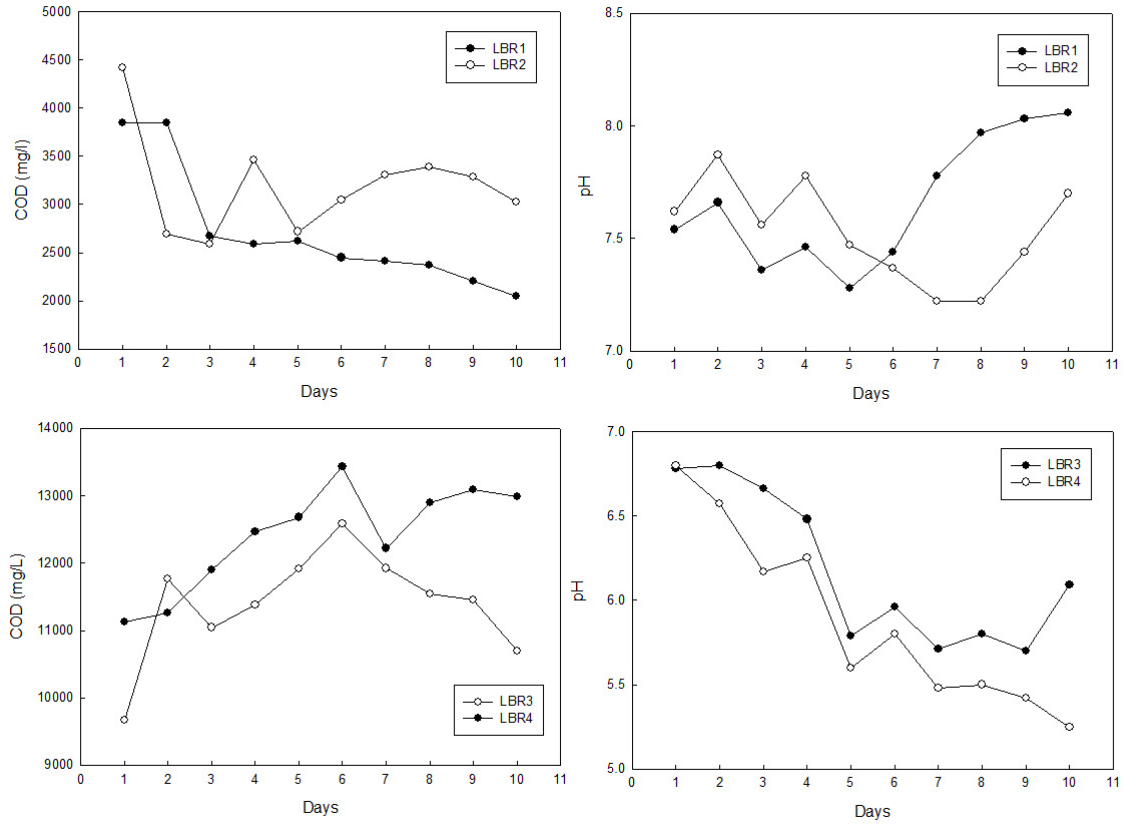

Figure 2 COD and pH variations in LBR1–4

The volatile solids (VS) removal is shown in Table 3. The VS removal in LBR2 was 31.0%, nearly 3% higher than in LBR1, a likely consequence of the higher operating temperature of LBR2. Furthermore, in LBR2, the final COD and the hydrolysis yield were higher compared to LBR1. This indicated that the higher temperature of the recirculating leachate improved the rate of hydrolysis. This is generally consistent with the known temperature effects on enzyme and microbial kinetics of solubilization of cellulose and hemicellulose (Nizami *et al.*, 2010) in substrates such as Napier grass.

To prevent the loss of COD in the reactors LBR1 and LBR2 that is caused by hydrolyzed material oxidizing to carbon dioxide, the reactor design was modified for a rigorous anaerobic operation.The vessels and other connections were sealed to be airtight. In the modified reactors (LBR3, LBR4), COD increased gradually and peaked on day 6 before declining. The hydrolysis yield in LBR3 increased to nearly 18% compared to 4.2% in LBR2. This indicates that when there's no loss of COD, the hydrolyzed material from grass is accumulated in the leachate resulting in higher COD. Therefore, maintaining strictly anaerobic conditions was important for preventing loss of COD and obtaining a high yield of hydrolysis. In both LBR3 and LBR4, the pH gradually declined to around 5.3–6.0 as a consequence of generation of volatile fatty acids (VFAs) (Lehtomaki *et al.*, 2008; Xie *et al.*, 2010; Cadavid-Rodriguez *et al.*, 2014).

Table 3 Summary of the results

Parameter	LBR1	LBR2	LBR3	LBR4
Grass input (g VS)	93.9	93.9	187.8	187.8
Grass output (g VS)	67.5	64.8	130.9	135.9
Final COD in leachate (g)	1.60	2.27	19.24	23.38
VS removal	28.1%[a]	31.0%[a]	30.3%[a]	27.6%[a]
Hydrolysis yield (%)	3.2%[c]	4.2%[c]	18.0%[b]	23.9%[a]

The data were analyzed by Turkey's test. The different superscript letters indicate a statistically significant difference ($p < 0.05$)

The highest hydrolysis yield of 23.9% was obtained in LBR4. A large inoculum (10% by volume) was used in this reactor compared to 5% used in LBR3. The better results in LBR4 are attributed to a larger initial inoculum as microorganisms are the catalysts for the hydrolysis (Yuan *et al.,* 2014).

In this study, the highest hydrolysis yield of 23.9% and the highest VS removal of 31.0% were obtained within 10 days while Xie *et al*. (2012) who evaluated hydrolysis of grass silage in LBR obtained the hydrolysis yield up to 57% and VS removal of 55% within 34 days. Nizami *et al*. (2010) also investigate the efficiency of LBR using grass silage as a substrate and 40 °C recirculation water and reported 67% of the hydrolysis yield and 64% of VS removal in 30 days. Therefore, the longer operation of LBR3 and LBR4 is recommended to help achieve higher hydrolysis yield and VS removal.

CONCLUSION

Leach bed reactors were effective for hydrolysis of Napier grass solids. Maximum hydrolysis yield was obtained under anaerobic conditions. Addition of an acclimatized seed culture and the use of heated leachate (50 °C) in recirculation enhanced the hydrolysis yield and removal of volatile solids. An operating period of longer than 10 days is recommended for improving the extent of hydrolysis.

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