# Comparison of Reactor Configurations for Biogas Production from Rapeseed Straw

Xinmei Fu * and Yuan Hu

To investigate the effects of reactor configurations on the anaerobic digestion performance of agricultural residue rapeseed straw, semi-continuous, one-stage, continuously stirred tank reactors (CSTR) and batch, two-stage, leach bed-upflow anaerobic sludge blanket (UASB) reactors were operated at the same hydraulic retention time (HRT) (30 days) and target organic loading rate (OLR) (3.0 gVS/L.d). In the continuously loaded CSTR, the specific methane yields did not substantially change as the OLR increased from 1.5 gVS/L.d to 3.0 gVS/L.d during the four periods. Conversely, the specific methane yields in the batch-fed leach bed-UASB increased considerably with the increase in OLR. The leach bed reactor contributed 75% of the total yield, while the UASB reactor contributed only 25% of the total yield. The total specific methane yields were 108 mL/gVS and 160 mL/gVS for the leach bed-UASB and CSTR, respectively, while the volatile solid (VS) reductions of the rapeseed straw were 27.1% and 36.6%, respectively. The results indicated that the process performance was more efficient in the CSTR than in the leach bed-UASB for the digestion of rapeseed straw. Biogas production was clearly affected by the reactor configurations.

*Keywords: Anaerobic digestion; Biogas; Continuously stirred tank reactor (CSTR); Leach bed-upflow anaerobic sludge blanket (UASB); Rapeseed straw*

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## INTRODUCTION

Lignocellulosic biomasses, particularly energy crops, agricultural residues, and forest residues, have gained increasing attention as feedstocks for producing bioenergy, especially biogas produced through anaerobic digestion (Cavinato *et al*. 2010; Sawatdeenarunata *et al*. 2015; Niu *et al*. 2015). However, lignocellulosic biomass consists primarily of cellulose, hemicellulose, and lignin, which interact to form a highly resistant and recalcitrant biomass structure, precluding microbial attack and resulting in low biodegradation. Consequently, the hydrolysis of lignocellulosic biomass is a major limitation for its anaerobic digestion because of its complex structure. The lack of appropriate digesters to handle the high solids of lignocellulosic biomasses as feedstocks is another limitation in digestion. The currently available anaerobic digesters were mainly developed for handling low-solid feedstocks, such as agricultural and industrial wastewater, livestock manure, waste activated sludge, food waste, *etc.* (Mao *et al*. 2015). Continuously stirred tank reactors (CSTR) have been one of the most common types of digesters applied in industrial-scale biogas plants for treating high-moisture organic waste in recent decades (Mao *et al*. 2015). Continuously stirred tank reactors for digesting lignocellulosic biomass have been developed in both academic research and industrial-scale practice (Demirel and Scherer 2008; Cavinato *et al*. 2010). Although a CSTR is simple to operate, applying such a digester to digesting the high solids of lignocellulosic biomass is energy and water intensive, as a result of mixing the biomass, expensive grinding of the biomass prior to digestion, and diluting the feedstocks (Nges and Björnsson 2012). In recent research, the leach-bed-based single-stage and two-stage reactor systems were tested in the digestion of lignocellulosic feedstocks (Lehtomäki and Björnsson 2006; Lehtomäki *et al*. 2008). In the two-stage system, one or several leach beds, functioning as the first stage, were connected with high-rate digesters, such as the upflow anaerobic sludge blanket (UASB) (Lehtomäki *et al*. 2008) or anaerobic filter (Lehtomäki and Björnsson 2006), for the second stage. Several disadvantages of the CSTR mentioned above do not exist in the leach bed-based two-stage system. However, complicated operation and control, as well as underdeveloped technology, hinder the two-stage system application in industrial practice. Comparable data is lacking in the literature in regards to which reactor configuration is better for both high methane yield and low energy input when using lignocellulosic biomass as the substrate.

Unlike energy crops, agricultural residues do not directly compete with food or feed production. China is a country with a large population and limited farmland to produce food. Therefore, an effective solution for this problem is to use the agricultural residues as feedstocks for biogas production instead of the energy crops. When used for biogas production, agricultural residues have some advantages, such as the prevention of environmental pollution, a relatively low feedstock cost, *etc.* It was reported that 0.75 billion tons of agricultural waste straw were produced in 2012 in China (Shiwei *et al*. 2015).

It has been reported that anaerobic digestion of lignocellulosic biomasses alone for biogas production is prone to instability and process failure (Scherer *et al.* 2009; Weiland, 2010). In some literature (Cuetos *et al.* 2011; Li *et al.* 2014), co-digestion of agricultural residues with manure has been reported to greatly improve the anaerobic digestion of agricultural residues due to the various nutrients in manure. However in China, developing large-scale and intensive livestock farms is the main trend at present, resulting in a reduced total number of farms, although household-scale farms or small-scale farms still exist in some places. Sometimes there are long distances for the manure to be transported from the intensive livestock farms to some biogas plants for the digestion of agricultural residues. Expensive transportation would result in increased cost. So the scarcity of manure has led to some biogas plants being operated without, or with little, manure. Nges and Björnsson (2012) reported that digesting energy crops supplemented with macro- and micronutrients instead of manure showed the same stimulatory and stabilising effects as manure with high methane yields achieved. Rincón *et al.* (2012) also reported a study using winter wheat silage as mono-substrate with added micronutrients in long-term anaerobic digestion trials and obtaining the high VS destruction and methane yields. Therefore, it is feasible to digest the lignocellulosic biomasses alone without adding manure.

Rapeseed straw is a potential candidate for biogas production because it exists in vast quantities in China (Song *et al*. 2009). The aim of this study was to examine the effects of reactor configurations (*i.e.*, semi-continuous one-stage CSTR and batch, two-stage leach bed-UASB) on the performance of rapeseed straw anaerobic digestion in mesophilic conditions. In this study, the rapeseed straw would be digested with the addition of supplementary nutrients instead of manure.

## EXPERIMENTAL

### Materials and Inocula

The substrate, rapeseed straw, was collected from the farmland around the Southwest University of Science and Technology (SWUST). The straw was cut to a length of 200 mm to 500 mm, stored at -18 °C, and thawed before the experiments.

Two different inocula were used. For the CSTR and methane potential experiments, mesophilic anaerobic sludge from a full-scale biogas plant treating pig manure was used (Jiangyou, Sichuan, China). For the UASB reactor experiments, mesophilic granules from a mesophilic UASB reactor treating wastewaters from a distillery (Mianyang, Sichuan, China) were used as inoculum.

**Table 1.** Characteristics of Rapeseed Straw and Inocula in the Experiments

| Parameter | TS (% ww) | VS (% ww) | Cellulose (% TS) | Hemicellulose (% TS) | Lignin (% TS) |
|---|---|---|---|---|---|
| Rapeseed Straw | 81.0 ± 0.08 | 75.1 ± 0.07 | 35.1 ± 0.2 | 22.5 ± 0.3 | 11.7 ± 0.2 |
| Granules | 9.3 ± 0.4 | 7.1 ± 0.3 | - | - | - |
| Anaerobic Sludge | 3.6 ± 0.2 | 2.1 ± 0.2 | - | - | - |
| * Standard deviation on triplicate samples; ww: wet weight; - : No analysis | | | | | |

The characteristics of the rapeseed straw and the inocula are given in Table 1. The fiber composition was calculated on a total solids (TS) basis and expressed as % TS.

### Nutrients

For feedstock preparation, the substrate was diluted with a mineral medium to ensure that the nutrient conditions were not limiting. For the mineral medium, the macronutrients and micronutrients were prepared in stock solutions as described by Yanling (1998) and diluted with tap water. Briefly, nutrient concentrations in the stock solutions were: 170 g/L $NH_4Cl$, 37 g/L $KH_2PO_4$, 100 g/L $Na_2S\cdot9H_2O$, 2 g/L $FeCl_3\cdot4H_2O$, 2 g/L $CoCl_2\cdot6H_2O$, 0.5 g/L $MnCl_2\cdot4H_2O$, 30 mg/L $CuCl_2\cdot2H_2O$, 50 mg/L $ZnCl_2$, 50 mg/L $H_3BO_3$, 90 mg/L $(NH_4)_6Mo_7O_{24}\cdot4H_2O$, 100 mg/L $Na_2SeO_3\cdot5H_2O$, 90 mg/L $AlCl_3\cdot6H_2O$, 50 mg/L $NiCl_2\cdot6H_2O$, 1 g/L ethylenediaminetetraacetic acid (EDTA), and 500 mg/L resazurin. For the mineral medium preparation, 1 mL of the stock solution with the above composition was diluted with 1 L of tap water.

### Biochemical Methane Potential (BMP) Experimental Set-up

Biochemical methane potential (BMP) assays were carried out in 500-mL glass bottles with a working volume of 350 mL. The rapeseed straw was ground in a homogenizer (JJ-2, Jintan Xinhang Instrument Factory, Jintan, China) until it passed through a 4-mm mesh. An inoculum (anaerobic sludge) to substrate ratio of 2:1 on a volatile solid (VS) basis was used in the BMP experiment, which was performed in triplicate. Nutrient media was added to a final volume of 350 mL. The assays were flushed with pure $N_2$ (99%) for 2 min and incubated in a water bath at 37 °C ± 1 °C. The bottles were manually shaken three times every day. The results of the control assays with inoculum alone were subtracted from the results of the sample assays. The gas composition and total gas volume were monitored every other day during the assays.

## Reactor Experiments Set-up and Operation

The effects of the reactor configurations on biogas production from the rapeseed straw were evaluated in one-stage CSTR and two-stage, leach-bed USAB reactors.

*One-stage CSTR experimental set-up and operation*

Two 4-L digesters, each with a working volume of 3 L, were used for the one-stage CSTR experiment. These reactors were constructed of a Plexiglas tube with gastight top and bottom plates and a heating jacket through which water was circulated. Impellers (JJ-1A, Changzhou Guoyu Instrument Manufacturing Co., Ltd., Jintan, China) inserted from the top plate were used to mix the reactor content. The reaction temperature was controlled at 37 °C by circulating hot water inside the reactor water jacket from water baths (HH-501, Changzhou Guohua Electric Appliance Co., Ltd., Jintan, China). Digestate (*i.e.*, digested residue) was removed through an outlet port in the lower half of the reactors, and feed was added *via* the top inlet port. Feeding and removing was carried out manually in duplicate reactors with the aid of a 100-mL plastic syringe. The biogas produced was collected from the top of the reactors in gastight aluminum bags.

For feedstock preparation, the substrate was ground to a particle size of less than 4 mm in the same manner as mentioned above and then diluted with mineral medium. A semi-continuous feeding type was adopted, and digesters were fed once per day following the removal of the same volume of effluent. The reactors were initially inoculated with 3 L of the mesophilic inoculum (anaerobic sludge).

The CSTR experiment was divided into four experimental periods, which are presented in Table 2, along with the different operational parameters. The hydraulic retention time (HRT) was 30 days throughout the experimental period. The target organic loading rate (OLR) of 3.0 gVS/L.d for the CSTR was based on previous reports by Nges and Björnsson (2012) and Rincón *et al.* (2012). During each period, a minimum of 1 HRT was maintained, and the OLR was changed only when a steady-state condition was achieved. In the preparation of feedstocks, the substrates were diluted to give VS concentrations of 45 gVS/L.d, 60 gVS/L.d, 75 gVS/L.d, and 90 gVS/L.d in the feedstock. Gas and liquid samples were taken daily to monitor biogas volume, methane content, and pH. The volatile fatty acid (VFA) values in the liquid samples were determined twice every week. Liquid samples for the determination of TS, VS, and composition of the digestate were withdrawn at the end of the experiment. The values for the last 10 days of each period were analyzed.

**Table 2.** Operational Parameters in the Experimental Periods of the CSTR Reactors

| Experimental Period (days) | I (1−41) | II (42−75) | III (76−108) | IV (109−148) |
|---|---|---|---|---|
| OLR (gVS/L.d) | 1.5 | 2.0 | 2.5 | 3.0 |
| HRT (days) | 30 | 30 | 30 | 30 |
| Feedstock (gVS/L) | 45 | 60 | 75 | 90 |

*Two-stage leach bed-UASB experimental setup and operation*

The two-stage reactor setup consisted of a leach bed reactor and a UASB reactor. The leach bed reactor, as the hydrolysis stage, had a total volume of 1 L, and it was made of thermostated glass vessels with an inner diameter of 60 mm with a gastight removable rubber plug and a fixed bottom with mesh, which supported solids and avoided blockage of the drainage tubes.

The UASB reactor, as the methanogenic stage, was a 1.3-L total volume thermostated glass vessel with a working volume of 1 L. The produced biogas was collected from the top of both reactors into gas-tight aluminum bags (Fig. 1). Before starting the experiment, the UASB reactors were inoculated with 200 mL of granular sludge and 800 mL of nutrient media, and they were operated for one month with glucose solution (glucose diluted with nutrient media) at an OLR of 1.5 g chemical oxygen demand (COD)/L.d. In this experiment, two parallel setups were used and operated at 37 °C in a batch manner. The rapeseed straw was cut to a length of 10 mm to 20 mm before use. The leach bed reactor was filled with 100 g of substrate and 500 mL of nutrient media. Background methane production from the granules was reduced by incubation at 37 °C for 8 days prior to the start of the experiment. All of the reactors were flushed with nitrogen before closing. All of the leach bed reactors were operated initially with internal recirculation, and then the transfer of the leachate to the UASB reactor was started. The leachate from the bottom of the leach bed reactor was either circulated to the UASB reactor or recycled back to the top of the leach bed. The effluent from the UASB reactor was also either recirculated back to the leach bed or to the bottom of UASB reactor. The exchange of liquid between the leach bed and the UASB reactor was done manually once or twice per day, and the volume exchanged was determined by the soluble chemical oxygen demand (SCOD) of the leachate from the leach bed and the OLR from the leach bed reactors to the UASB reactors. Gas and liquid samples from both the leach bed and UASB reactors were taken daily for the values of biogas volume, methane content, and pH. The SCOD values in the leachate of the leach bed reactors were determined every other day. The VFA values in the UASB reactors were determined twice every week. In order to characterize the changes in the leach bed material, the TS and VS of the digestate in the leach bed reactors were determined after they were terminated, and then the extent of the VS reduction was calculated.

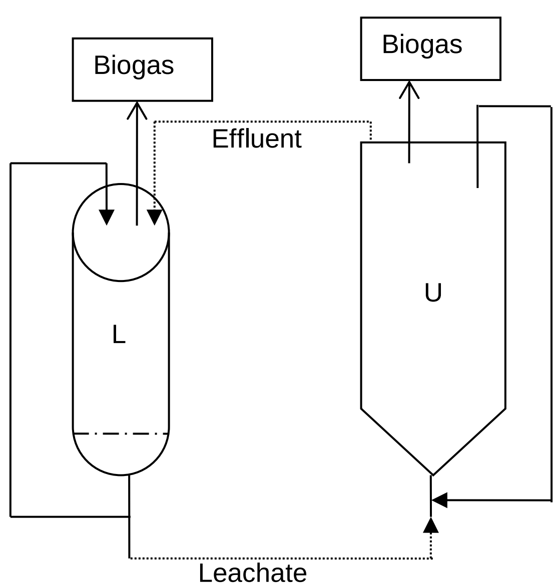


**Fig. 1.** Two-stage anaerobic digestion reactor (L: leach bed; U: UASB)

## Analytical Methods

Biogas volume was measured with a 100-mL gastight syringe, and its composition was analyzed with a gas chromatograph (GC-CP3800 with thermal conductivity detector, Varian, Palo Alto, CA , USA) equipped with a TDX-01 column (2 m, Shimadzu, Kyoto, Japan). Argon was the carrier gas. Volatile fatty acids were analyzed using a gas chromatograph (GC-CP3800 with flame ionization detector, Varian, Palo Alto, CA, USA) equipped with a DB-FFAP capillary column (30 m × 0.323 mm × 1.00 µm, Agilent, Santa Clara, CA, USA). Nitrogen was the carrier gas. The pH was measured with a pHS-2F pH meter (INESA Scientific Instrument Co. Ltd, Shanghai, China). The TS and VS were determined according to standard methods detailed by the APHA (1998). The SCOD was measured in accordance with the standard method of water quality HJ/T 399 (2007).

For fiber compositional analysis, both the milled initial substrate and the digestates after anaerobic digestion were dried at 40 °C for two days, finely ground (< 1 mm particle size) in a mill (FS500Y-3, Guangzhou Laymax Machinery Equipment Co. LTD, Guangzhou, China), and then analyzed for the contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) according to Van Soest's method (Liying 2007) with a cellulose analyzer (Fibertec 2010, Foss Analytical, Hillerød, Denmark). The hemicellulose (% TS) and cellulose (% TS) contents were calculated as described by Eqs. 1 and 2,

$$Hemicellulose = NDF - ADF \quad (1)$$

where *NDF* is the composition of neutral detergent fiber (% TS), and *ADF* is the composition of acid detergent fiber (% TS),

$$Cellulose = ADF - ADL \quad (2)$$

where *ADF* is the composition of acid detergent fiber (% TS), and *ADL* is the composition of acid detergent lignin (% TS).

# RESULTS AND DISCUSSION

## Biochemical Methane Potential (BMP) Experiment

To establish the maximum expected methane yield of rapeseed straw, a BMP experiment was carried out. A VS-based BMP yield of 193 mL/gVS was obtained for rapeseed straw after 30 days of incubation. This BMP value was comparatively less than that reported for rapeseed straw by Søndergaard *et al.* (2015) (258 mL/gVS). This could be due to the use of a higher temperature (53 °C) and a much smaller particle size for the substrate (0.1 mm to 0.4 mm) in the BMP assays by Søndergaard *et al*. (2015). Cuetos *et al.* (2011) reported a BMP yield of about 120 mL/gVS for rapeseed straw. The lower BMP yield could be because of a higher lignin content (16.6% TS) in the substrate, compared to the lignin content of 11.7% TS in this study. Among all of the substrate characteristics, the lignin content was reported to be the most important factor affecting methane production (Sawatdeenarunata *et al*. 2015). As reported previously (Amon *et al.* 2007; Kruse *et al.* 2008; Kreuger *et al.* 2011), the lignin content not only varied between species, but could also have varied in the same species due to different growth conditions and maturation, which affected the methane yields of biomass.

## One-stage CSTR Process

The process performance of the CSTR reactors throughout the experiment, evaluated in terms of specific methane yield at an HRT of 30 days, is presented in Fig. 2. Each OLR was operated until a steady-state condition, characterized by less than 10% variation in the methane yield, gas production rate, and pH within 10 successive days, was reached (Søndergaard *et al*. 2015).

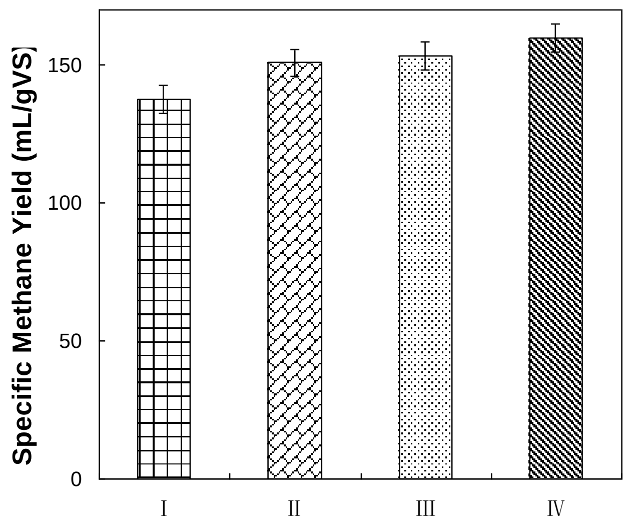


**Fig. 2.** Specific methane yields during the four periods of digestion in CSTR reactors at 37 °C. The error bars are standard deviations. (I: 1.5 gVS/L. d; II: 2.0 gVS/L. d; III: 2.5 gVS/L. d; IV: 3.0 gVS/L. d).

The CSTR experiment was initiated with an OLR of 1.5 gVS/ L.d in period I. The average specific methane yield (based on added VS of the substrate) during the last 10 days of this period was 138 mL/gVS. A slightly gradual increase in methane yields ranging from 138 mL/gVS to 160 mL/gVS was observed from period I to IV when OLRs were also increased from 1.5 gVS/L.d to 3 gVS/L.d. The reactors were initially started up with an adaptation period of acclimatizing the anaerobic microorganisms to the lignocellulose-rich feedstocks. Then during the process from period I to IV, the adaptability could continuously be improved, resulting in increased methane yields.

The average methane content of the biogas produced during stable operation of the four periods for the CSTR process ranged between 51% and 58% (data not shown). This range of methane content was in agreement with the values reported by Nges and Björnsson (2012) (52% to 58%), Rincón *et al.* (2012) (53%), and Kacprzak *et al.* (2010) (53%), in which the carbohydrate-rich substrates were also used alone, without adding any manure as the co-digestion substrates in the CSTR systems. Some studies (Kaparaju *et al*. 2009; Cuetos *et al*. 2011; Søndergaard *et al*. 2015) conducted in CSTR have shown that co-digestion of the carbohydrate-rich biomass with materials such as agricultural residues and manures not only can improve the quantity, but also the quality of the biogas. Kaparaju *et al.* (2009) noted that the methane percentage was 59% when the reactor was fed with only wheat straw hydrolysate, but it increased to 62.3% to 66.6% during co-digestion with cow manure. Cuetos *et al.* (2011) and Søndergaard *et al.* (2015) also reported methane contents of 63% to 66% and 64% to 65%, respectively, in biogas produced by the co-digestion of crop residues and manure. The reason for this may be attributed to the composition of the substrates. As reported by Weiland (2010), the theoretical methane contents of carbohydrates and raw protein are 50% and 70% to 71%, respectively. Manure usually contains a higher protein content than carbohydrate-rich biomass, resulting in a higher methane content in biogas when manure was added as a co-substrate, compared to the carbohydrate-rich biomass digested alone.

The VFAs and pH in the reactors during stable operation of the four periods are shown in Fig. 3.

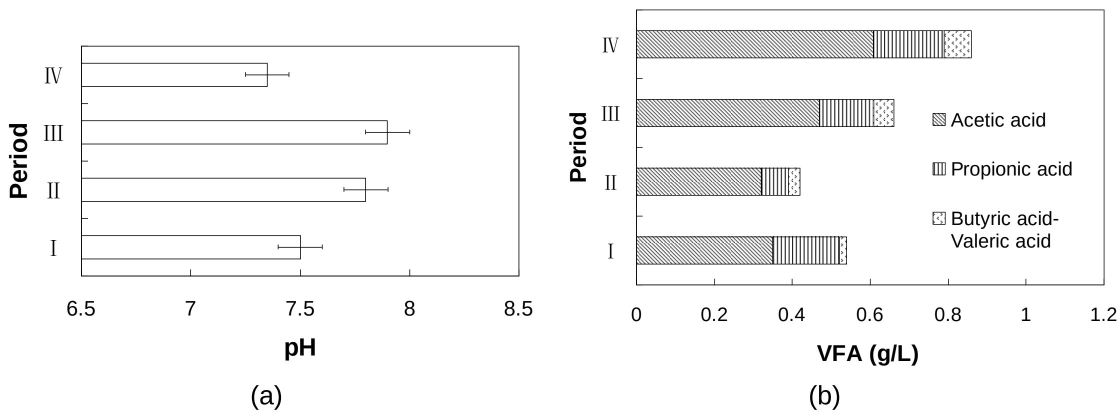


**Fig. 3.** Concentration of total pH (a) and VFAs (b) during the four periods of operation of the CSTR reactor experiment. The error bars are standard deviations. (I: 1.5 gVS/L. d; II: 2.0 gVS/L. d; III: 2.5 gVS/L. d; IV: 3.0 gVS/L.d).

The total VFAs increased from 0.42 g/L to 0.86 g/L with the increasing OLR from period II to IV. At a lower OLR of 1.5 gVS/L.d in period I, however, the value of total VFAs was higher than that at a higher OLR of 2.0 gVS/L.d in period II. This result may have occurred because period I was a period of adaptation of the inoculum to the carbohydrate-rich substrates and inhibitor compounds, resulting in some accumulation of VFAs. This result was in agreement with the results reported by Nges and Björnsson (2012). The total VFA levels were below 1000 mg/L, indicating high stability of the operation conditions (Kacprzak *et al.* 2010; Nges and Björnsson 2012). Acetic acid was the main VFA, followed by propionic acid and butyric acid or valeric acid, successively.

The pH in the reactors ranged from 7.4 to 7.9 during stable operation throughout all four periods, demonstrating a healthy anaerobic digestion. The pH values (between 7.0 and 8.0) and VFA concentrations (below 1000 mg/L) were within the ranges favorable for methanogenesis and considered indicative of stable processes. This result could be attributed to the addition of macro- and micronutrients. It was found in a few studies (Hinken *et al.* 2008; Scherer *et al.* 2009; Pobeheim *et al.* 2010; Weiland 2010) that macronutrients such as, nitrogen (N), phosphorus (P), sulphur (S) and micronutrients such as, iron (Fe), nickel (Ni), molybdenum (Mo), cobalt (Co), tungsten (W), selenium (Se) played a crucial role in the growth and metabolism of anaerobic microorganisms. Macronutrients can act as buffering agents, balancing carbon (C):N ratio, or as integral part of enzymes involved in methane production (Scherer *et al.* 2009; Weiland 2010) while micronutrients have been reported to be cofactors of enzymes involved in the biochemistry of methane formation (Hinken *et al.* 2008; Pobeheim *et al.* 2010). Therefore, it was reported that the supplementation of macro- and micronutrients instead of manure could improve methane production and process stability in the mono-digestion of energy crops. (Nges and Björnsson 2012; Nges *et al.* 2012).

## Two-Stage Leach Bed–UASB Process

The leach bed reactors were operated with internal recirculation for the first day, and then liquid transfer to their respective UASBs was started, and an equal volume of liquid was recycled back to their respective leach bed reactors. In this way, no extra water was introduced into the system.

In two of the leach beds, hydrolysis started immediately, as indicated by the pH and SCOD values after 24 h of internal circulation, as shown in Fig. 4.

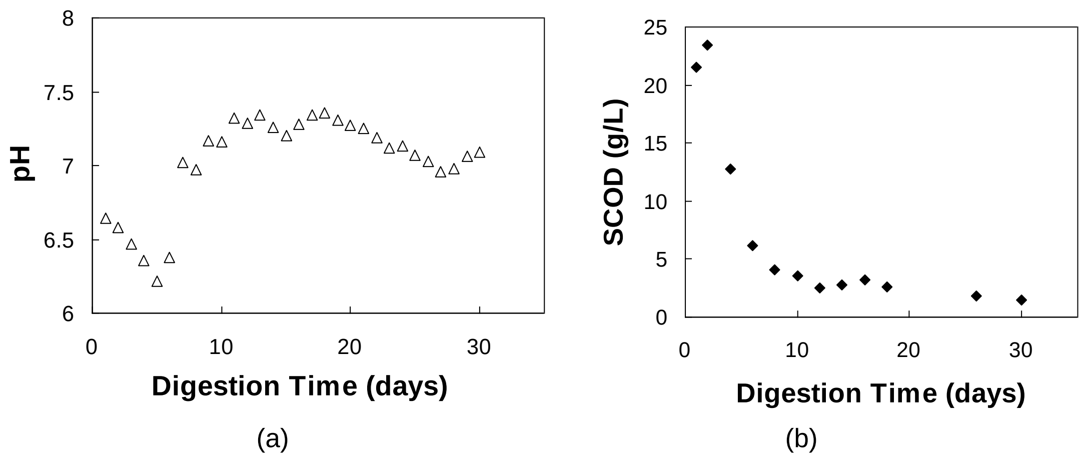


**Fig. 4.** pH (a) and SCOD (b) in the leachate of leach bed reactors in the leach bed-UASB process

The OLR (loaded from the leach bed reactors to the UASB reactors) was initiated at 1 gCOD/L.d on day 2 for all two-stage systems, increased gradually to the target OLR of 3 gCOD/L.d, and then decreased to 1 gCOD/L.d when the SCOD of the leachate in the leach bed was not adequate to maintain the target OLR.

The pH values in the leach bed reactors initially dropped, and then they increased when SCOD levels decreased rapidly with the leachates replaced by the effluent of the UASB reactors (Fig. 4). On days 7 to 8, when the pH and SCOD values of the leachate in the leach bed reactors were above 7.0 and below 6 gCOD/L, respectively, circulation between the leach beds and their corresponding UASBs was terminated. From then on, the leach bed reactors were operated as one-stage reactors until day 30, and the pH values in the leach bed reactors varied between 6.9 and 7.4. During the 7 to 8 days that the UASBs were in operation, the pH in the UASBs ranged from 7.0 to 7.6, and the total VFA values increased from 0.4 g/L to 0.6 g/L (data not shown), indicating that the UASB reactors were operated under stable conditions.

In two of the leach bed reactors, methane production and concentration remained low (less than 10 mL.d and 10%, respectively) until day 4, when methanogenic activity began. Thereafter, these values started to increase slowly. Methane concentration in the gas from the leach bed reactors increased from 26% to 70% from day 4 onwards, while in the UASB reactors, methane content remained between 50% and 75% during the 7 days of operation.

The total accumulated specific methane yield in the leach bed-UASB process is shown in Fig. 5. The specific methane yield achieved for the leach bed-UASB process was 108 mL/gVS. Of these methane yields, 25% originated from the UASB, and 75% originated from the leach bed reactors. This result was similar to those from previous reports by Lehtomäki and Björnsson (2006) and Nkemka and Murto (2013).

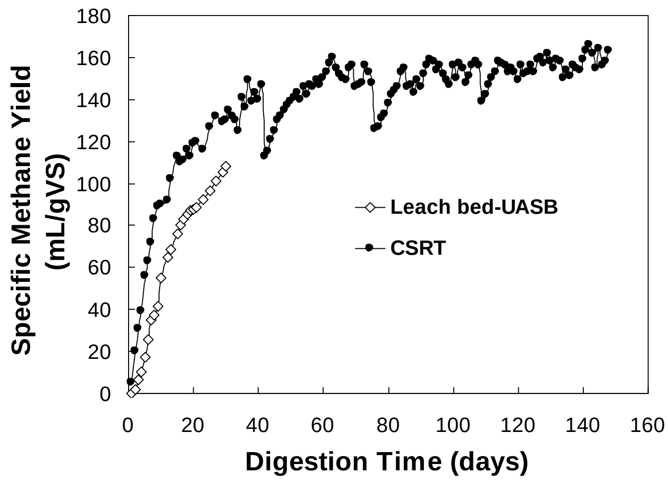


**Fig. 5.** Cumulative specific methane yield in the CSTR and Leach bed-UASB processes

Lehtomäki and Björnsson (2006) found that when willow with a high content of lignocellulose (53.1% TS) was used as the substrate for two-stage anaerobic digestion, 84% of the total methane yield was from the hydrolysis reactor, while for sugar beet with a low content of lignocellulose (8.7% TS), this ratio value was only 17%. As shown in Table 1, the lignocellulose content in the rapeseed straw used in this study was 69.3%. Correspondingly, a similar result to willow was found in the present study.

The composition of the rapeseed straw used in this study was comparable to that of reed investigated by Nkemka and Murto (2013) (28% TS to 34% TS cellulose, 22% TS hemicellulose, and 14% TS lignin). A methane yield of about 140 mL/gVS was obtained (estimated) when the reed was digested in a leach bed-UASB system after 30 days of operation, a comparable methane yield to that of rapeseed straw in the present study at the same HRT. As shown in the report of Nkemka and Murto (2013), the methane yield of the reed increased by 57% with an increase of HRT from 30 days to 107 days, indicating that a long digestion time was needed in a two-stage system for digestion of a lignocellulose-rich material, such as 70 days to 90 days, in order to obtain the maximum biogas potential of the substrate. Similarly, the methane yield would likely have been increased substantially if the digestion time in the present study had been extended to the appropriate time.

## Comparison of Reactor Configurations

In the CSTR, the specific methane yields did not substantially change as the OLR increased from 1.5 gVS/L.d to 3.0 gVS/L.d during the four periods (Fig. 5). In the leach bed-UASB reactors, the specific methane yields increased considerably as the OLR increased from 1.0 gCOD/L.d to 3.0 gCOD/L.d at the same HRT (Fig. 5).

In the four experimental periods of the CSTR, the methane yields all remained above 70% of the BMP yield, which indicated that the CSTR experiment was a well-performing anaerobic digestion process. The total specific methane yield for the leach bed-UASB system was only 56% of the BMP yield, suggesting that incomplete digestion of the rapeseed straw occurred in the two-stage system. However, the values of pH and total VFAs in the UASB reactors were all within a range feasible for methanogenesis, indicating a stable operation in the leach bed-UASB system.

The specific methane yield and VS reduction of the substrates in the CSTR process in the steady state of the fourth period and in the leach bed-UASB process in the end, operated at the same HRT (30 day) and similar OLR (3 gVS/L.d or 3 gCOD/L.d), are shown in Table 3.

**Table 3.** Results of Anaerobic Digestion in Leach Bed-UASB and CSTR Reactors at an HRT of 30 Days and an OLR of 3 gVS/L d or 3 gCOD/L d

| Configuration | Leach Bed-UASB | CSTR |
|---|---|---|
| VS Reduction (%) | 27.1 ± 0.9 | 36.6 ± 1.3 |
| Specific Methane Yield (mL/gVS) | 108 ± 7 | 160 ± 10 |
| * Standard deviation on duplicate reactor samples | | |

The VS reduction and specific methane yield were 35% and 48% higher for the CSTR reactor than the leach bed-UASB reactor, respectively. This result indicates that the reactor configuration had a remarkable influence on the anaerobic digestion of rapeseed straw. The CSTR reactor showed greater efficiency than the leach bed-UASB reactor, at a similar OLR and HRT.

It is generally believed that two-stage anaerobic digestion process is separated into the acidogenic and methanogenic steps in the two reactors, which provides an optimal environment for each of the distinct microbial populations performing these biochemical transformations, thus providing enhanced stability, high organic load and an overall faster reaction. On the other hand, in a one-stage process both the acidogenic step and the methanogenic step are mixed in one reactor, which results low process stability due to susceptible to pH and temperature fluctuations, overloading, acid accumulation, *etc*. Some studies (Vinas *et al.* 1993; Lehtomäki *et al.* 2008; Nasr *et al.* 2012) claimed that a two-stage digestion system would result in a greater yield of methane over the one-stage system due to a larger fraction of the substrates being converted to biogas by the more vigorous activity of the acidogenic microbes in the first stage of the two-stage system. Nasr *et al.* (2012) reported a comparative analysis on the efficiency of single-stage and two-stage digestion systems using thin stillage as the substrate and revealed that an improved energy yield of 18.5% was achieved through two-stage systems. Lehtomäki *et al.* (2008) investigated anaerobic digestion of grass silage in leach bed reactors with and without a second stage UASB reactor and found that 66% of the total methane potential in grass silage was obtained in the two-stage process whereas in the one-stage process only 20% of the methane potential was extracted.

In the studies metioned above (Vinas *et al.* 1993; Lehtomäki *et al.* 2008; Nasr *et al.* 2012), the feedstocks used in the one-stage anaerobic digestion processes were processed in the entirely same way as the ones in the two-stage processes in terms of composition, particle size, and mixing conditions of the working liquid. However, in the present study, the feedstock applied in the one-stage CSTR process was significantly different from that in the two-stage leach bed-UASB process in terms of such variables as particle size, mixing status, *etc*. The much smaller size of feedstock and continuously stirring in the one-stage

CSTR process all favored the anaerobic digestion process, resulting in an efficient performance. Therefore, a contrast result to the ones mentioned by other studies (Vinas *et al.* 1993; Lehtomäki *et al.* 2008; Nasr *et al.* 2012) was achieved in this study.

Some previous reports (Rincón *et al*. 2012; Nges and Björnsson 2012; Nges *et al.* 2012) have shown that more than 3 gVS/L.d to 4 gVS/L.d of OLR could result in failure of the digestion of energy crops and crop residues in one-stage CSTR, while considerably more than 3 gCOD/L.d of OLR (5 gCOD/L.d to 25 gCOD/L.d) could be used successfully for two-stage systems when high-rate anaerobic digestion reactors were applied as the methanogenic stage, such as UASB or anaerobic filter reactors (Andersson and Björnsson 2002; Lehtomäki and Björnsson 2006; Lehtomäki *et al*. 2008). However, if lignocellulose-rich biomass was used as the substrate in two-stage systems, the limited release of organic material from the biomass to the liquid could not support an efficient operation of high-rate methanogenic reactors (Nkemka and Murto 2013). Therefore, easily degradable biomass is more suitable to be digested in two-stage systems than slowly degraded lignocellulose-rich biomass. The former could be expected to get a faster VS removal rate in the two-stage system than in the one-stage CSTR system if high OLRs were applied.

As suggested by Nkemka and Murto (2013), a long digestion time was needed for lignocellulose-rich substrates digested in the two-stage system to obtain the maximum biogas potential. However, a long digestion time in the design of a full-scale plant is not commercially feasible. Therefore, for slowly degraded lignocellulose-rich biomass, the one-stage CSTR system is preferred for the anaerobic digestion process.

After the CSTR and leach bed-UASB processes were terminated, the compositions of the digestates from both processes were analyzed based on % TS, as compared with that of the initial rapeseed straw (Table 4).

**Table 4.** Composition of the Initial Rapeseed Straw and the Digestates from CSTR and Leach Bed-UASB Reactors Based on TS

| Samples | Rapeseed Straw | Digestate of Leach Bed-UASB | Digestate of CSTR |
|---|---|---|---|
| Hemicellulose (% TS) | 22.5 ± 0.3 | 23.8 ± 0.9 | 18.1 ± 0.8 |
| Cellulose (% TS) | 35.1 ± 0.2 | 36.9 ± 1.3 | 27.2 ± 1.1 |
| Lignin (% TS) | 11.7 ± 0.2 | 15.5 ± 0.7 | 27.1 ± 0.7 |
| * Standard deviation on duplicate reactors' samples | | | |

Compared to the rapeseed straw before digestion, the digestates of the leach bed-UASB and CSTR processes showed a 10.0% and 4.5% increase, respectively, in the lignocellulose contents (namely, hemicellulose, cellulose, and lignin). The changes in the composition of the digested fiber material in this study were in agreement with those reported in the study by Surendra and Khanal (2015). The observation of increased lignocellulose contents in the digestates was actually impossible. The composition was calculated on a % TS basis. In fact, TS includes not only the recalcitrant lignocellulose, but also the easily degradable, non-fiber components, such as non-structural sugars, crude proteins, *etc.* (Sluiter *et al*. 2008). Therefore, after the easily degradable, non-fiber contents of the initial substrate were digested and removed, the lignocellulose contents appeared to be increased accordingly.

Even so, the structural carbohydrate content (hemicellulose and cellulose) of the digestate from the CSTR was 21% lower than that of the initial substrate, while the structural carbohydrate content of the leach bed-UASB was 5% higher than that of the initial substrate. This result suggested that the digestibility of hemicellulose and cellulose during anaerobic digestion varied considerably between the two reactor systems. The noticeable difference could mainly be caused by the finely ground substrate (less than 4 mm) in the CSTR, compared to a larger particle size (10 mm to 20 mm) in the leach bed-UASB. Reducing the particle size could disrupt the rigid structure of the lignin around the hemicellulose and cellulose, thereby making hemicellulose and cellulose more accessible for microbial attack, and resulting in the higher digestibility of the structural carbohydrates. The effect of particle size reduction of the substrate on the degradation of hemicellulose and cellulose was also observed in some other fiber materials digestion studies (Xiao *et al*. 2013; Shen *et al*. 2014; Surendra and Khanal 2015). However, the effect of particle size reduction on lignin degradation was not as evident as the effect on structural carbohydrates. Conversely, the lignin content in the digestate from the CSTR was much higher than that of the leach bed-UASB, with both digestates having higher lignin content than that of the initial substrate. It has been generally accepted that lignin is much more recalcitrant to biological degradation than hemicellulose and cellulose, with little value for bioenergy production (Chayanon *et al*. 2015; Surendra and Khanal 2015).

Additionally, the long-term operation of the semi-continuous CSTR reactors (109 days, before period IV) allowed for microbial adaptation to the substrate in the CSTR and thus, for inhibition to be avoided, which also favored the anaerobic digestion of hemicellulose and cellulose, whereas the batch-fed substrate in the leach bed-UASB reactors was inoculated with methanogenic granules for less than 30 days.

The composition of the digestates from the leach bed-UASB reactors and CSTR were highly consistent with their corresponding methane yields.

The results from the present study suggest that reactor configuration has a profound influence on the anaerobic digestion. Although the one-stage CSTR reactor was simpler to operate and more efficient in this study, compared to the two-stage leach bed-UASB reactor, the CSTR had some unfavorable factors, such as considerable energy consumption from grinding the substrates and continuously mixing the digestion liquid, and foaming from time to time on the surface of the reaction liquid. The separation of digested solids and liquid in the effluent after anaerobic digestion from the CSTR also had a high energy demand, while the energy needed for the same separation was almost negligible for the leach bed-UASB system. However, the disadvantages of the difficult control of operation and process parameters for the two-stage leach bed-UASB reactor make it less practical for application in industry.

It is worth considering whether it is possible to combine the strengths of these two different rector systems. When the lignocellulosic biomasses is used as the substrate, the hydrolysis of substrate is the limiting step in the anaerobic digestion of two-stage system. Therefore if the CSTR reactor was used as the first stage instead of the leach bed reactor in the two-stage system, combined with UASB as the second stage, it would improve a larger fraction of the substrates being hydrolyzed in the first stage due to smaller particle size used and continuous mixing and an improved efficient performance would be achieved. As for the more considerable energy consumption in CSTR reactor over the leach bed reactor, it could be compensated by optimizing the operating conditions, such as, stirring the digestion liquid from time to time instead of continuous stirring, applying the high solid contents of feedstocks in a decreased volume of reactor with a declined heating energy demand, *etc.*

## CONCLUSIONS

1. A specific methane yield of 108 mL/gVS for the leach bed-UASB, compared to a yield of 160 mL/gVS for the CSTR at the same HRT (30 day) and a similar OLR (3 gVS/L.d or 3 gCOD/L.d), was achieved, showing the higher efficiency of the CSTR.
2. The anaerobic digestion of rapeseed straw as a sole substrate was stable and feasible in both the CSTR and leach bed-UASB reactors.
3. In addition to the methane yields, some influencing factors relevant to the digestion of lignocellulosic biomass should also be carefully considered when choosing a suitable reactor configuration, such as the energy balance, simplicity of operation and control, pretreatment of the substrate, disposal of the effluent from the digesters after digestion, *etc.*

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