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# **An analysis of single and two stage, mesophilic and thermophilic high rate systems for anaerobic digestion of corn stalk**

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## Abstract

The feasibility of corn stalk anaerobic digestion was investigated in an upflow anaerobic solid-state (UASS) reactor with an additional anaerobic filter (AF) reactor. One single-stage (UASS) and two two-stage systems (UASS + AF) were compared at organic loading rates (OLR) of 2.5, 4.5, and 8.0  $\text{g}_{\text{vs}} \text{L}^{-1} \text{d}^{-1}$ . The single-stage system was operated under mesophilic conditions and the two-stage systems under both mesophilic and thermophilic conditions. Independently of OLR, the mesophilic two-stage system showed higher average methane production rates ( $0.50 \pm 0.01$ ,  $0.63 \pm 0.11$ , and  $0.67 \pm 0.12 \text{ L}_{\text{CH}_4} \text{L}^{-1} \text{d}^{-1}$ ) than the single-stage ( $0.46 \pm 0.02$ ,  $0.46 \pm 0.06$ , and  $0.31 \pm 0.09 \text{ L}_{\text{CH}_4} \text{L}^{-1} \text{d}^{-1}$ ) and thermophilic two-stage ( $0.30 \pm 0.02$ ,  $0.42 \pm 0.05$ , and  $0.66 \pm 0.12 \text{ L}_{\text{CH}_4} \text{L}^{-1} \text{d}^{-1}$ ) systems. Respective methane yields were 84–201, 39–184, and 88–119  $\text{mL g}_{\text{vs}}^{-1}$ . The observed advantage of mesophilic digestion is of particular interest, as degradation of lignocellulosic biomass is usually reported to benefit from higher temperature. The results show the general feasibility of an additional AF reactor after the UASS digestion of corn stalk under mesophilic conditions.

**Keywords:** Corn stalk; Solid-state; Anaerobic digestion; Methane; UASS.

## 1 Introduction

Corn stalk is one of the most abundant agricultural wastes, with an estimated amount of 327.4 million ton produced annually in China [1]. At the same time, because of open burning directly in the field, this has caused heavy environment pollution, including particulate matter (PM), CO, hydrocarbons, etc. which caused serious local

and regional environmental impact [2]. Anaerobic digestion (AD) is a promising and viable technology for treating various types of organic wastes and simultaneously producing biogas as a renewable energy carrier [3]. At the same time, corn stalk is an important lignocellulosic biomass that shows promise as a potential substrate for AD [4].

While the fermentation of liquid materials has reached a high standard with well-proven technologies such as the upflow anaerobic sludge blanket (UASB) reactor or the expanded granular sludge blanket (EGSB) reactor, the fermentation of solids is a relatively young field with several new reactor designs [5]. Although several continuous process reactors including Valorga, Kompogas, and Dranco for solid materials have dominated the market place for solid-state AD systems treating municipal solids waste (MSW), they have not been established for the processing of lignocellulosic biomass or energy crops because of complex process, high maintenance requirements, high parasitic energy loss, etc. [3]. The continuous stirred tank reactor (CSTR) does not function well with lignocellulosic materials such as corn stalk as well. Floating and stratification of solids are common in CSTR, which decreases biogas production and may cause explosion in the reactor or blocking at the connections [6]. Because of the inhibition of accumulated volatile fatty acids (VFAs) and low hydrolysis rate, OLR of solid-state anaerobic reactors still remains unsatisfactory, at  $1-4 \text{ g}_{\text{vs}} \text{ L}^{-1} \text{ d}^{-1}$  [7]. Thus, a new type of anaerobic digestion reactor needs to be developed for lignocellulosic materials like corn stalk.

A UASS reactor was first described by Mumme et al. [8], and worked well with maize silage [8], wheat straw [9,10] and horse manure [11,12], thus providing a promising new approach for corn stalk anaerobic digestion. However, to the authors' knowledge, no previous studies investigated corn stalk in a UASS system or a similar system featuring a solid-state plug flow. Because of its distinct properties, the behavior of corn stalk in the UASS reactor cannot be predicted either from maize silage- or from wheat straw- derived performance data.

Therefore, this work investigates the technical feasibility of corn stalk anaerobic digestion by the UASS process with an additional AF reactor. The objectives were to determine the process performance parameters of solid-state corn stalk digestion with liquor recirculation, to describe the influence of the OLR, and to compare single- and two-stage designs as well as mesophilic and thermophilic conditions.

## **2 Materials and Methods**

### **2.1 Substrates and inoculum properties**

The present study examined corn stalk as a sole substrate. The corn stalk was collected from a farm in Cadenberge, Germany in October 2014. After harvest, it was chopped to a final average cutting length of 2–5 cm as recommended by Ferreira et al. [13] by a mobile chaff cutter (Ralle, Germany). It was then air-dried to achieve a moisture content of less than 10% and stored at room temperature in a woven bag prior to the experiment.

Mesophilic and thermophilic inocula were obtained from previous biogas experiments, which were incubated under mesophilic (37°C) and thermophilic (55°C) conditions at the Leibniz Institute for Agricultural Engineering (ATB). The inocula were stored at room temperature for several months without feeding, in order to remove biodegradable chemical oxygen demand (COD). Before inoculating, they were sieved with a mesh size of 1 mm. Detailed properties of substrates and inocula are presented in Table 1.

## 2.2 Reactor setup

The scheme of reactors is shown in Fig. 1. The main reactor used in this work was a modification of the upflow anaerobic solid-state (UASS) reactor as described by Mumme et al. [8]. The feedstock was fed manually through an inclined feeding pipe to the bottom of the UASS reactor, ascended in the form of a solid-state bed (SSB) in the reactor and was removed manually from the top by removing the reactor's lid as described previously [9]. The feedstock flow in the reactor was upflow so that the reactor was named upflow anaerobic solid-state reactor no matter which direction the flow of the process liquor was. The material density of corn stalk is lower than that of water, thus the feedstock ascends within the UASS reactor. On its way to the top, the solid materials inside the UASS reactor degrade and form digestates. Thereby, the digestates compact, which can lead to clogging and can interfere with liquor circulation [8]. As a deviation from previous UASS experiments [9,11], the liquor flow inside the UASS reactor was changed from upflow to downflow in this work.

The process liquor was applied via the lid of the UASS reactor, passed through the solid-state bed of the digestates, and was removed from the bottom of the reactor.

To relieve the inhibition of accumulated VFAs, an additional AF reactor was added after the UASS reactor to form a two-stage system. The AF reactor was filled with PE biofilm carriers (Bioflow 40, RVT Process Equipment GmbH, Germany) with a surface area of  $305 \text{ m}^2 \text{ m}^{-3}$ . The process liquor in AF was upflow. The working volume of the UASS reactor, the AF reactor and buffer tank was 35 L, 35 L and 8 L each. Process liquid circulation of both system was set to a flow rate of  $11.7 \text{ L h}^{-1}$  using peristaltic pumps (Heidolph, Germany). Both UASS and AF reactors were heated via a thermostatically controlled water jacket (Lauda, Lauda-Königshofen, Germany).

Biogas production was measured using a drum-type gas meter (TG05/5 Ritter, Germany) and was collected in a gas bag. The reactors were equipped with combined pH/temperature probes (InPro4260, Mettler-Toledo, USA) for continuous online measurement.

### 2.3 BMP experiments

The determination of the biochemical methane potential (BMP) of corn stalk and cellulose under mesophilic ( $39^\circ\text{C}$ ) and thermophilic ( $55^\circ\text{C}$ ) conditions was carried out using BMP experiments according to guideline 4630 published by the German Engineering Association [14]. Glass bottles (capacity 1 L) were filled with 900 g inoculum. The initial volatile solid (VS) ratio of substrate to inoculum was kept at 1:2

for all BMP experiments. All the bottles were placed in incubators and connected to gas-collecting tubes outside the incubators. The gas-collecting tubes were filled with a sealing liquid, which was saturated with NaCl salt and acidified with sulfuric acid to a pH of approximately 4. This prevented the dissolving of carbon dioxide during the whole experiment. Two iterations were conducted with blank groups and three iterations were conducted with experimental groups.

#### 2.4 Continuous corn stalk digestion

Three systems were operated in this study, described as: S1 (single-stage system, mesophilic condition), S2 (two-stage system, mesophilic condition) and S3 (two-stage system, thermophilic condition). S1 and S2 were started simultaneously. Temperature was set at 39°C which was proved to be suitable for corn stalk hydrolysis [15] for mesophilic conditions. After inoculation with 3 L mesophilic inoculums, both systems were filled with process liquid from a horse manure-digesting UASS reactor at ATB, Potsdam. Afterwards, dextrose was fed as nutrient with an organic loading rate (OLR) of 0.1 g<sub>vs</sub> LUASS<sup>-1</sup> d<sup>-1</sup> to start the reactor. After a week, corn stalk was fed daily at an OLR of 2.5 g<sub>vs</sub> LUASS<sup>-1</sup> d<sup>-1</sup>. The OLR was calculated according to the volume of the UASS reactor for both single-stage and two-stage systems because the AF reactor was only an additional VFAs digestion device. After another week until gas production became stable, the main experiment was formally initiated. The experiment lasted for 51 days, during which the UASS reactors were operated at three different organic loading rates (OLR): 2.5 g<sub>vs</sub> LUASS<sup>-1</sup> d<sup>-1</sup> (day 1–21), 4.5 g<sub>vs</sub> LUASS<sup>-1</sup> d<sup>-1</sup> (day 22–36), 8.0



$\text{g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$  (day 37–51). After the first experiment, the temperature in the two-stage system was increased to 55°C and inoculated with 3 L of thermophilic inoculum. After methane production became stable, the thermophilic experiments were started. As in the mesophilic trial, the data from the first week were not considered for performance assessment. Operation of S3 was the same as that of S2 except for temperature.

Digestates were removed from the top of the UASS reactors every 7 days ( $\text{OLR}=2.5 \text{ g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$ ), 5 days ( $\text{OLR}=4.5 \text{ g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$ ), and 3 days ( $\text{OLR}=8.0 \text{ g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$ ), respectively. After the digestates were removed, a volume of about 9.4 L (height: 20 cm) of solid digestates remained in each UASS reactor. Distilled water was filled into the UASS reactor each time the digestates were removed, in order to compensate water losses; and to prevent accumulation of high concentrations of organic and inorganic substances, which can inhibit the anaerobic digestion process as described by Nordberg et al. [16].

Due to corn stalk's poor content of trace elements, these were supplemented along with the feeding. Following the recommendation of Abdoun and Weiland [17], 0.01 mL  $\text{g}_{\text{vs-fed}}^{-1}$  of medium No. 144 (containing iron, calcium, copper, zinc, and sodium) was added to the process liquor daily.

## 2.5 Analytical methods

The biogas composition of each reactor was measured daily using an industrial biogas analyzer (SSM 6000, Pronova, Germany). Chemical analyses of the effluents of

UASS and AF reactors were carried out twice in one feeding period. The determination of total solids (TS) and volatile solids (VS) was conducted according to DIN standard methods [18]. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed by a fiber analyzer (ANKOM2000, USA) as described in the literature [19]. Total ammonium nitrogen (TAN) was analyzed according to the VDLUFA method [20]. Total Kjeldahl nitrogen (TKN) and chemical oxygen demand (COD) were measured according to DIN EN 25663: 1993–11 and DIN ISO 15705: 2003–01 respectively. Total carbon (TC) and total nitrogen (TN) were determined by elemental analysis (DIN EN 15104: 2011–04). Volatile fatty acids were measured with a gas-phase chromatograph (Agilent GC 7890A, USA) equipped with a Permabond-FFAP column (length 30 m, diameter 0.32 mm, film thickness 0.5  $\mu\text{m}$ ) and a flame ionization detector. C, N, S, and H fractions were analyzed with a vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Germany), but had only been available for solid samples. For the BMP batch digestion tests, the composition of the produced biogas ( $\text{CH}_4$  and  $\text{CO}_2$ ) was analyzed using the portable gas analyzer GA 2000 (Ansyco GmbH, Germany) equipped with infrared detectors.

## 2.6 Calculations

The measured biogas volume was converted to its volume at standard temperature, standard pressure, and dry conditions according to VDI guideline 4630 [14]. Air was introduced each time when feeding and when removing digestates, which would

potentially disrupt the biogas composition analysis. Therefore, the methane yield was calculated on the assumption that the volumetric fractions of methane and carbon dioxide sum up close to 100% as recommended in VDI guideline 4630 [14]. Each of the two measured values was multiplied by the same factor so that the sum of the two corrected measured values was 100% neglecting trace gases. The detailed calculation was previously described by Böske et al. [11].

### **3 Results and discussion**

#### **3.1 BMP experiments**

The inoculum used was found to be in good condition, as the cellulose reference yielded  $610 \pm 71 \text{ mL g}_{\text{vs}}^{-1}$  of biogas under mesophilic conditions and  $600 \pm 23 \text{ mL g}_{\text{vs}}^{-1}$  under thermophilic conditions (Table 2), which is in accordance with the lower limit of  $600 \text{ mL g}_{\text{vs}}^{-1}$  stated in the VDI guideline 4630 [14]. Methane yield of cellulose in mesophilic and thermophilic conditions were  $278 \pm 34 \text{ mL g}_{\text{vs}}^{-1}$  and  $381 \pm 8 \text{ mL g}_{\text{vs}}^{-1}$  respectively. Cellulose produced more methane under thermophilic conditions. This is consistent with the results of Pohl et al. [9]. However, methane yield from corn stalk under thermophilic conditions ( $152 \pm 35 \text{ mL g}_{\text{vs}}^{-1}$ ) was lower than that under mesophilic conditions ( $256 \pm 12 \text{ mL g}_{\text{vs}}^{-1}$ ). Fu et al. [21] reported methane yield from corn straw of  $296 \text{ mL g}_{\text{vs}}^{-1}$ , which is similar to the present results under mesophilic conditions. In a study using wheat straw [9], methane yield under thermophilic conditions ( $304.29 \text{ mL g}_{\text{vs}}^{-1}$ ) was higher than that under mesophilic conditions

(244.16 mL g<sub>vs</sub><sup>-1</sup>). That result differs from the results of the present work, possible as a result of the special structure of corn stalk and low hydrolysis rate.

Cellulose has higher biomethane potential, while corn stalk has lower biomethane potential under mesophilic conditions. This is due to different reaction speed at different temperatures. Anaerobic digestion can be divided into four steps: hydrolysis, acidification, acetogenesis, and methanogenesis [22]. However, the reaction process from corn stalk to methane is divided into two steps in this article, for the sake of discussion. The first step was from large particles to relatively-easily-hydrolyzed cellulose, and the second step was from the cellulose to methane and CO<sub>2</sub>. The only difference between the production processes for corn stalk and cellulose methane is that corn stalk methane production process requires the first step. Due to the resilient structure and composition of lignocellulosic biomass such as lignin that interlinks cellulose and hemicellulose layers, the conversion efficiency is limited [23,24]. The rate of hydrolysis of particulate organic matter is determined by the adsorption of hydrolytic enzymes onto the biodegradable surface sites [25,26]. Hydrolysis of lignocellulosic biomass is rate-limiting because of the low cellulolytic activity and low specific growth rate of cellulolytic microbes in anaerobic digesters [27,28]. The first step of corn stalk methane production was the rate-limiting step compared to the second step. According to Yao et al. [15], corn stalk showed higher hydrolysis and acidification rate under mesophilic conditions. Usually, the entire reaction is determined by the rate-limiting step. Therefore, although the non-rate-limiting second

step has lower speed, the overall reaction speed of corn stalk anaerobic digestion was still faster under mesophilic conditions.

### 3.2 Properties of process liquor and solid residue

The course of the liquor pH of the three systems is shown in Fig. 2. The pH values in UASS and AF reactors of the two-stage system were similar to each other, as a result of rapid circulation of process liquid. During start-up, the pH in S1 and S2 fluctuated considerably. The average pH values of the three systems during the whole 51-day experimental period were  $6.76 \pm 0.13$  (S1),  $6.98 \pm 0.04$  (S2), and  $7.29 \pm 0.04$  (S3), respectively. Compared with S1, pH levels in S2 and S3 were higher and more stable. When the OLR was increased, the pH in S1 decreased from  $6.83 \pm 0.05$  to  $6.70 \pm 0.19$ , whereas that in S2 was almost stable at around  $6.98 \pm 0.04$ , and that in S3 increased slightly from  $7.25 \pm 0.02$  to  $7.34 \pm 0.03$ . In the last feeding period, pH in S1 decreased very rapidly to less than 6.4. Afterwards, in the phase of decay (no feeding), pH decreased even further to 6.1. In parallel, the VFA concentration in S1 increased from 750 to 2000 mg L<sup>-1</sup> (Fig. 3) after the feeding was stopped.

Nonreversible acid accumulation occurred in S1, whereas no acid accumulation occurred in S2 or S3 during any of the OLR steps.

VFA and soluble chemical oxygen demand (SCOD) in the reactors are shown in Fig. 3 and Fig. 4. VFA concentrations in UASS and AF reactors of S2 and S3 were less than 700 mg L<sup>-1</sup>, which was at the normal level for an anaerobic reactor. VFA concentration in both reactors of S1 was higher than in the corresponding reactors of

S2 and S3. When OLR was increased, VFA and SCOD concentrations in S1 increased. In S1, the average SCOD concentrations during the three OLR steps were 4300, 4900, and 6000 mg L<sup>-1</sup>, respectively. SCOD in the UASS and AF reactors of S2 were around 3500 mg L<sup>-1</sup> and showed little change although OLR was increased. SCOD concentrations in the UASS and AF reactors of S3 were also nearly the same. The VFA and SCOD concentrations were more stable in AF reactors than in UASS reactors.

Ammonia inhibition is uncommon in straw anaerobic digestion because of the high C:N ratio of corn stalk. The C:N ratio of the corn stalk used as feed stock in this study was 83:1. The ammonia concentration of the process liquid was less than 100 mg L<sup>-1</sup> throughout the experiment process, which will not result in ammonia inhibition [29].

Analyses of the chemical properties of corn stalk and digestates throughout the experiment are shown in Table 3. When OLR was increased, the VS, cellulose, and hemicellulose contents increased in S1 and S2 digestates, which indicates relatively low degradation. In contrast, the thermophilic S3 showed almost stable digestate composition, indicating rapid degradation. However, as seen in Table 3, the availability appears lower than that under mesophilic temperatures.

### 3.3 Influence of OLR on reactor performance

When OLR was increased, the digestates had to be removed more frequently from the UASS reactor. The digestate bed heights were 20–30, 20–35 and 20–45 cm at OLR 2.5, 4.5, and 8.0 g<sub>vs</sub> L<sub>UASS</sub><sup>-1</sup> d<sup>-1</sup>. The solid retention times (SRTs) of corn stalk in

UASS reactor at OLR 2.5, 4.5, and 8.0  $\text{g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$  were 18.0, 9.7, and 4.4 days, respectively. The SRT at OLR 4.5 and 8.0  $\text{g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$  were relatively short for methanogenesis, however, they were enough for hydrolysis and acidogenesis [30]. This controlling strategy aimed to produce VFAs mainly in the UASS reactor and produce methane mainly in the AF reactor in two-stage systems, which was proved to achieve more methane and a better process stability by separating the hydrolysis-acetogenesis and methanogenesis phases [31]. Nevertheless, methane production could not be avoided in the UASS reactor.

In S1, methane production rate increased in each feeding period. From the second week onward, methane production rate decreased after digestates were removed.

When OLR was increased from 2.5 to 4.5  $\text{g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$ , the rate of methane production remained unchanged, at around  $0.46 \pm 0.02 \text{ L}_{\text{UASS}}^{-1} \text{d}^{-1}$ . When OLR was increased to 8.0  $\text{g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$ , methane production rate decreased to  $0.31 \pm 0.09 \text{ L}_{\text{UASS}}^{-1} \text{d}^{-1}$ . When OLR was increased to 8.0  $\text{g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$ , the rate of methane production peaked and hydrolysis and acidogenesis rates were higher than methane production rates, such that acid accumulation occurred and the system failed. The OLR of 8.0  $\text{g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$  was too high for the single-stage system, with the result that methane production rate did not increase. As the OLR in S2 increased, methane production rate also increased, from  $0.50 \pm 0.01$  to  $0.63 \pm 0.11$  to  $0.67 \pm 0.12 \text{ L}_{\text{UASS}}^{-1} \text{d}^{-1}$ . More feedstock was fed into the system, so that more methane was produced. The feeding rate of OLR 4.5  $\text{g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$  was 1.8 times that of OLR 2.5  $\text{g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1}$

$\text{d}^{-1}$ , but resulted in only 1.25 times the rate of methane production. The feeding rate of OLR  $8.0 \text{ g}_{\text{vs}} \text{ L}_{\text{UASS}}^{-1} \text{ d}^{-1}$  was 1.78 times that of OLR  $4.5 \text{ g}_{\text{vs}} \text{ L}_{\text{UASS}}^{-1} \text{ d}^{-1}$ , but methane production only increased 1.07 times. In S3 (thermophilic), when OLR was increased, methane production rate showed very rapid increase from  $0.30 \pm 0.02$  to  $0.42 \pm 0.05$  to  $0.66 \pm 0.12 \text{ L}_{\text{UASS}}^{-1} \text{ d}^{-1}$ . The S3 system therefore achieved higher methane production rate relative to rates of OLR increase.

The methane fractions of each system are shown in Table 4. As OLR increased, the methane fraction in the single-stage system (S1) decreased from  $54 \pm 1\%$  to  $50 \pm 1\%$ , whereas the methane fractions in the UASS and AF reactors of S2 and S3 were more and less stable respectively. The methane fraction in the AF reactor was higher than that in the UASS reactor during all of the OLR steps. This is mainly because, after removal of VFAs, more  $\text{CO}_2$  could dissolve in the process liquor. In consequence, the liquor cycle transported  $\text{CO}_2$  from the AF reactor to the UASS reactor, where it was then released due to acidification. This effect was previously described by Böske et al. [11].

#### 3.4 Influence of an additional AF reactor

As shown in Fig. 2, when OLR was increased to  $8.0 \text{ g}_{\text{vs}} \text{ L}_{\text{UASS}}^{-1} \text{ d}^{-1}$ , the pH in S1 decreased to 6.3, acid accumulation occurred, and S1 totally failed. However, throughout the period, pH, VFA and SCOD in S2 were more stable than in S1. S2 (two-stage system) functioned well even when OLR was increased to  $8.0 \text{ g}_{\text{vs}} \text{ L}_{\text{UASS}}^{-1} \text{ d}^{-1}$ . As shown in Fig. 5, when OLR was increased, methane production rate in S1



decreased, whereas that in the two-stage system increased. The two-stage system can thus tolerate higher OLR. Acid accumulation was common to take place in traditional anaerobic digesters. In the research of solid cattle slaughterhouse wastes anaerobic digestion with SCTR reported by Pagés-Díaz et al. [32], VFA concentration reached  $5.9 \text{ g L}^{-1}$  when OLR was increased to  $3 \text{ g}_{\text{vs}} \text{ L}^{-1} \text{ d}^{-1}$ . However, no acid accumulation took place in the system with an additional AF reactor even when OLR was increased to  $8.0 \text{ g}_{\text{vs}} \text{ L}_{\text{UASS}}^{-1} \text{ d}^{-1}$  under both mesophilic and thermophilic conditions in this work.

As shown in Fig. 6, the total methane yield of the two-stage system ( $201 \pm 5$ ,  $140 \pm 24$ , and  $84 \pm 15 \text{ mL g}_{\text{vs}}^{-1}$ ) was higher than that in single-stage system ( $184 \pm 9$ ,  $102 \pm 13$ , and  $39 \pm 11 \text{ mL g}_{\text{vs}}^{-1}$ ) at each OLR step. These results were consistent with the solid conversion rates shown in Table 3. In the two-stage systems, the AF additional reactor shared part of the methanogenesis load with the UASS reactor. In such a system, accumulated acid can be transferred to the AF reactor to be converted to methane, thereby inhibiting acid accumulation in the UASS reactor. The microorganism functions in the two reactors differed due to their different operating conditions. The two-stage system functioned better than being combined into one reactor as in a single-stage system.

As shown in Table 4, the methane fractions of both the UASS reactor and the AF reactor in the two-stage system were higher than that in the single-stage system. This finding is very meaningful for the future biogas industry. Biogas can be collected separately in order to achieve higher quality of biogas. Compared with a single-stage

system, the two-stage system can tolerate higher OLR, is more stable in operation, and produces higher quality and quantity of biogas. Actually, the volume of AF reactor can be decreased in the future research because the OLR (at most  $4.0 \text{ g}_{\text{vs}} \text{ L}^{-1} \text{ d}^{-1}$ ) in this work was really so low for AF reactors [22].

### 3.5 Comparison of mesophilic and thermophilic temperatures

As shown in Fig. 5, the methane production rates of both systems increased gradually as OLR was increased. Under mesophilic conditions, the UASS and AF reactors showed very similar average rates of methane production at the three OLR steps.

However, this result differed from that under thermophilic conditions. In Fig. 5(c), methane production rate in the AF reactors was higher than that in the UASS reactor at lower OLR ( $2.5 \text{ g}_{\text{vs}} \text{ L}_{\text{UASS}}^{-1} \text{ d}^{-1}$ ). The methane production rates of the two reactors were similar at middle OLR ( $4.5 \text{ g}_{\text{vs}} \text{ L}_{\text{UASS}}^{-1} \text{ d}^{-1}$ ), whereas at higher OLR ( $8.0 \text{ g}_{\text{vs}} \text{ L}_{\text{UASS}}^{-1} \text{ d}^{-1}$ ) the UASS reactor showed higher production than the AF reactor.

According to Böske et al. [12], methanogenesis rate was higher under thermophilic conditions, so that VFA was converted to methane immediately after hydrolysis and acidogenesis process in the UASS reactor. Jiménez et al. [33] also reported that specific methane activity (SMA) of thermophilic methanogens was higher than mesophilic methanogens. When OLR was increased, more feedstock was digested and more methane was released directly from the UASS reactor and less methane was released from the AF reactor. Therefore, the UASS reactor showed higher rate of methane production than the AF reactor at higher OLR.

As shown in Fig. 6, methane yield under mesophilic conditions was higher than that under thermophilic conditions at each OLR step. This result differs from UASS digestion of horse manure [11,12] and wheat straw [9,10]. Especially lower OLR was associated with a greater disparity between the methane yields achieved under mesophilic and thermophilic conditions. This is because methanogenesis is the rate-limiting process compared to hydrolysis, and methanogens show greater activity under thermophilic conditions [15]. Usually, the most appropriate temperature for corn stalk hydrolysis and acidogenesis was 39°C [15]. The best temperatures for methanogenesis were 35 and 55°C [22]. When temperature was increased to 55°C, methane production rate and methane yield of easily-hydrolyzed substrate such as cellulose, food waste, and maize silage will increase [34]. As described in Section 3.1, in the present study the process of methane production differed because of the complex structure of corn stalk. The first step of corn stalk anaerobic digestion, which is the rate-limiting process, was inhibited under thermophilic conditions. Therefore, the entire anaerobic digestion process of corn stalk was weaker under thermophilic conditions.

As shown in Fig. 6, the gap in methane yield between the two temperatures was greater at lower OLR. The SRT of the UASS reactor at the lower OLR was longer than that for high OLR such as 4.5 and 8.0  $\text{g}_{\text{VS}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$ . At the same time, methanogenesis was sensitive to SRT. Therefore, at lower OLR, the speed or hydrolysis reaction played a more important role during all of the reactions.

Conversely, methanogenesis played a more important role at higher OLR. Under thermophilic conditions, the advantage of temperature for methanogenesis was not so obvious at higher OLR. Although the temperature was increased to 55°C, methanogenesis was still inhibited by short SRT. Consequently, the methane yield gap between the conditions was smaller at higher OLR.

The methane fractions of both systems are shown in Table 4. Comparing the mesophilic and thermophilic conditions, the methane fractions in the UASS reactors were almost the same. However, the methane fractions in the AF reactors under thermophilic conditions were higher than those under mesophilic conditions.

Hydrolysis, acidification, and methanogenesis occurred in the UASS reactors, such that the advantage of thermophilic condition for high fraction of methane was not obvious. Moreover, less hydrolysis and acidification occurred in the AF reactors than in the UASS reactor. Thus, the advantage of thermophilic conditions for high fraction of methane was more obvious in AF reactors.

#### **4 Conclusions**

The two-stage (UASS + AF) system is promising for anaerobic digestion of corn stalk under mesophilic conditions. Methane yield decreased when OLR was increased. The two-stage system showed more stable performance and produced more methane than the single-stage system. Under thermophilic conditions, the two-stage system produced higher methane fraction but lower yield of biogas than that under mesophilic conditions.

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## Figure Captions

Fig. 1 - Schematic of the single-stage and two-stage systems (UASS: upflow anaerobic solid-state reactor; AF: anaerobic filter).

Fig. 2 - Courses of pH in process liquid of the three systems.

Fig. 3 - Courses of volatile fatty acid (VFA) in process liquid of the three systems.

Fig. 4 - Courses of soluble chemical oxygen demand (SCOD) in process liquid of the three systems.

Fig. 5 - Methane production rate of the three systems analyzed at three organic loading rates (OLR).

OLR 2.5  $\text{g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$  (days 1–21), OLR 4.5  $\text{g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$  (days 22–36), OLR 8.0  $\text{g}_{\text{vs}} \text{L}_{\text{UASS}}^{-1} \text{d}^{-1}$  (days 37–51). (a) S1: single-stage system in mesophilic conditions, (b) S2: two-stage system in mesophilic conditions [separate S2 (UASS) and S2 (AF)], (c) S3: two-stage system in thermophilic conditions [separate S3 (UASS) and S3 (AF)].

Fig. 6 - Methane yield of the three systems at three organic loading rates (OLR).

## Figures

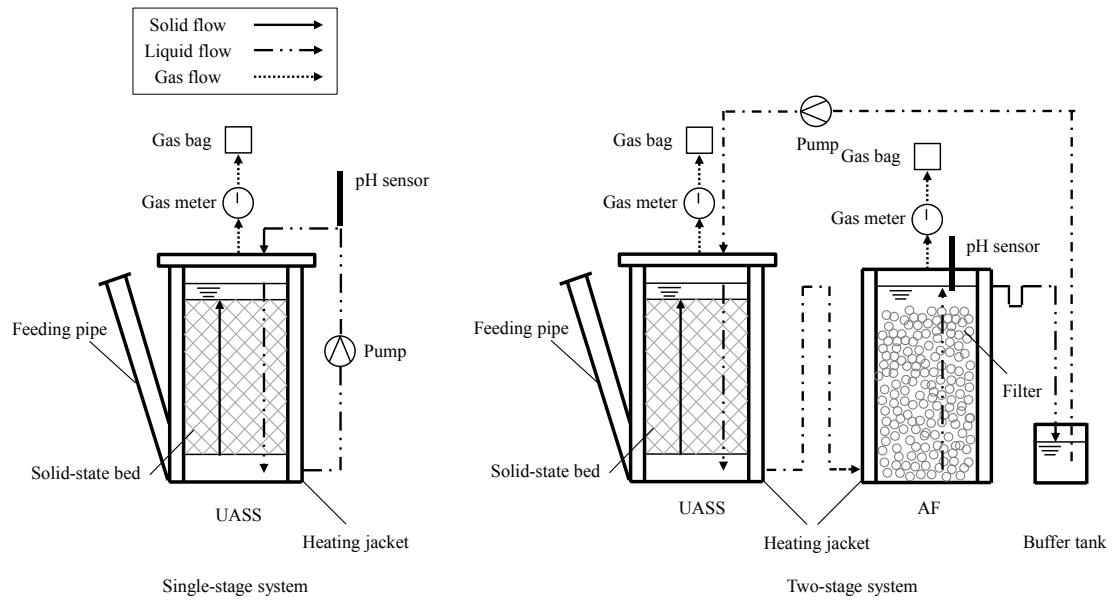


Fig. 1 - Schematic of the single-stage system and two-stage system. (UASS: upflow anaerobic solid-state reactor; AF: anaerobic filter).

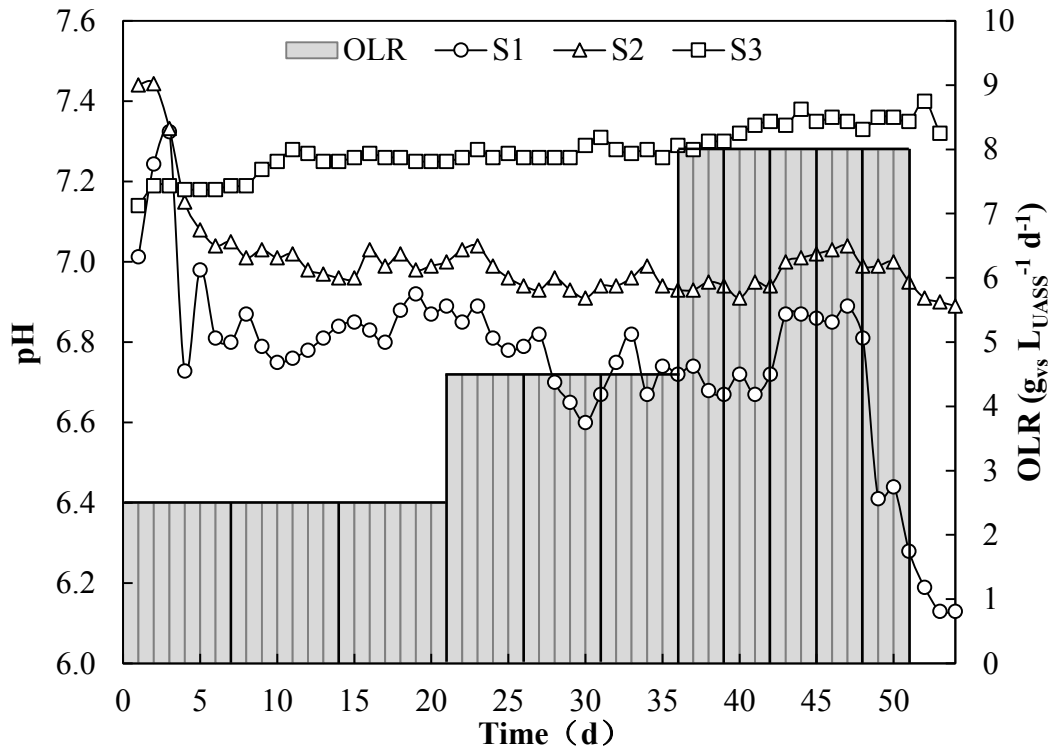


Fig. 2 - Courses of pH in process liquid of the three systems.

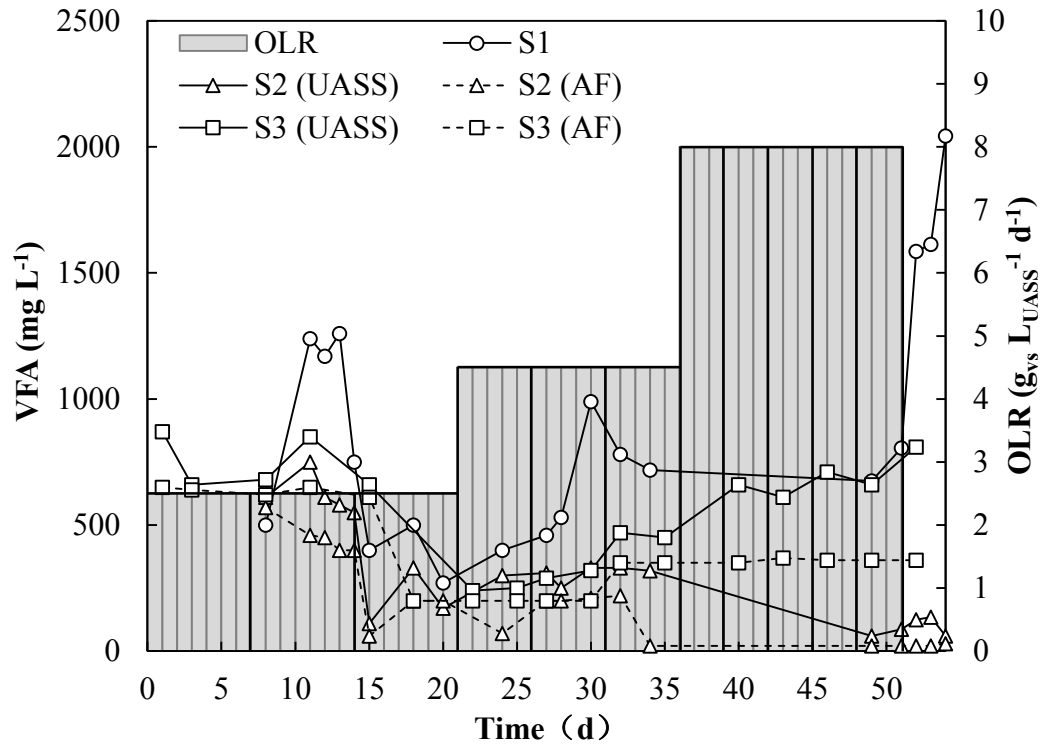


Fig. 3 - Courses of volatile fatty acid (VFA) in process liquid of the three systems.

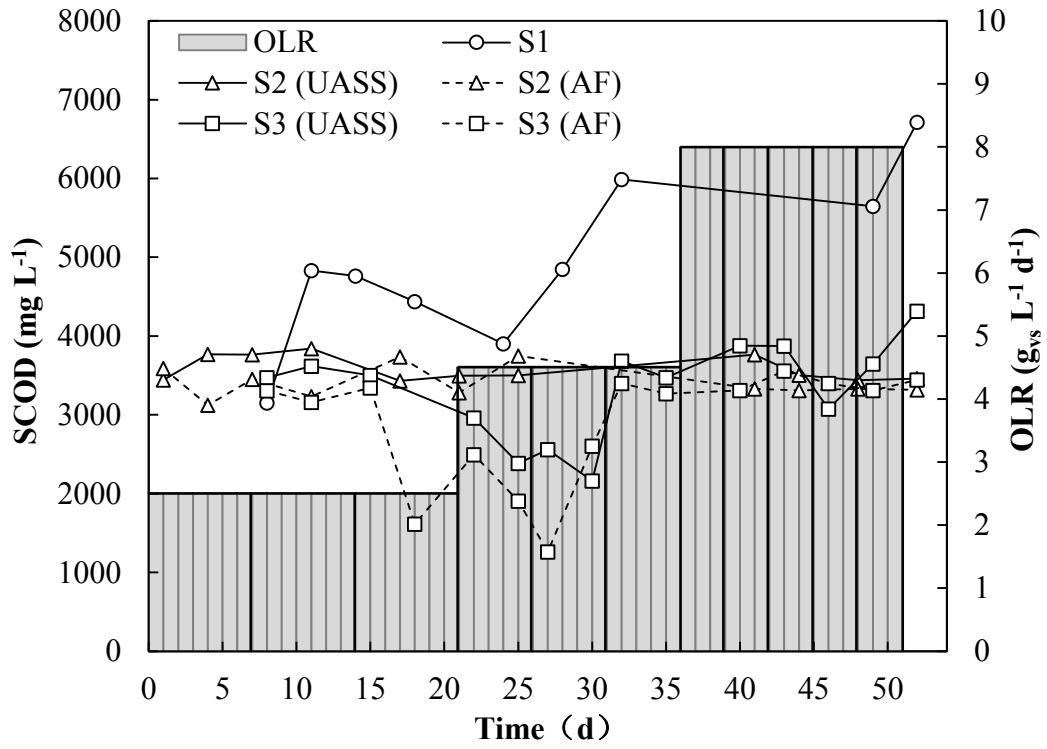


Fig. 4 - Courses of soluble chemical oxygen demand (SCOD) in process liquid of the three systems.

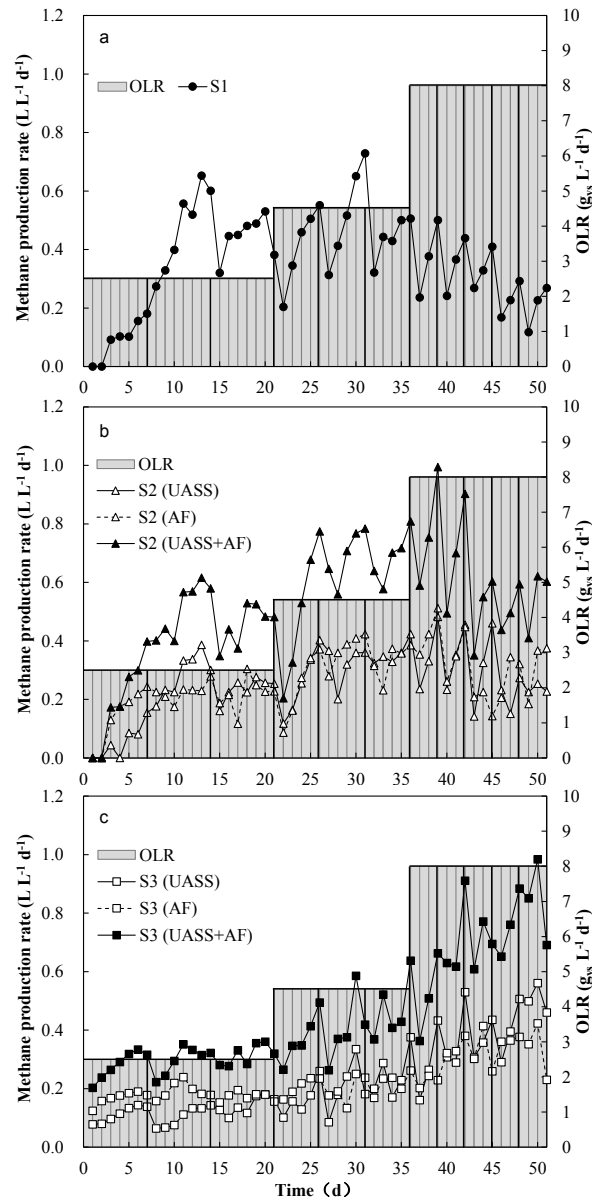


Fig. 5 - Methane production rate of the three systems analyzed at three organic loading rates (OLR).

OLR  $2.5 \text{ g}_{\text{vs}} \text{ L}_{\text{UASS}}^{-1} \text{ d}^{-1}$  (days 1–21), OLR  $4.5 \text{ g}_{\text{vs}} \text{ L}_{\text{UASS}}^{-1} \text{ d}^{-1}$  (days 22–36), OLR  $8.0$

$\text{g}_{\text{vs}} \text{ L}_{\text{UASS}}^{-1} \text{ d}^{-1}$  (days 37–51). (a) S1: single-stage system in mesophilic conditions, (b)

S2: two-stage system in mesophilic conditions [separate S2 (UASS) and S2 (AF)], (c)

S3: two-stage system in thermophilic conditions [separate S3 (UASS) and S3 (AF)].



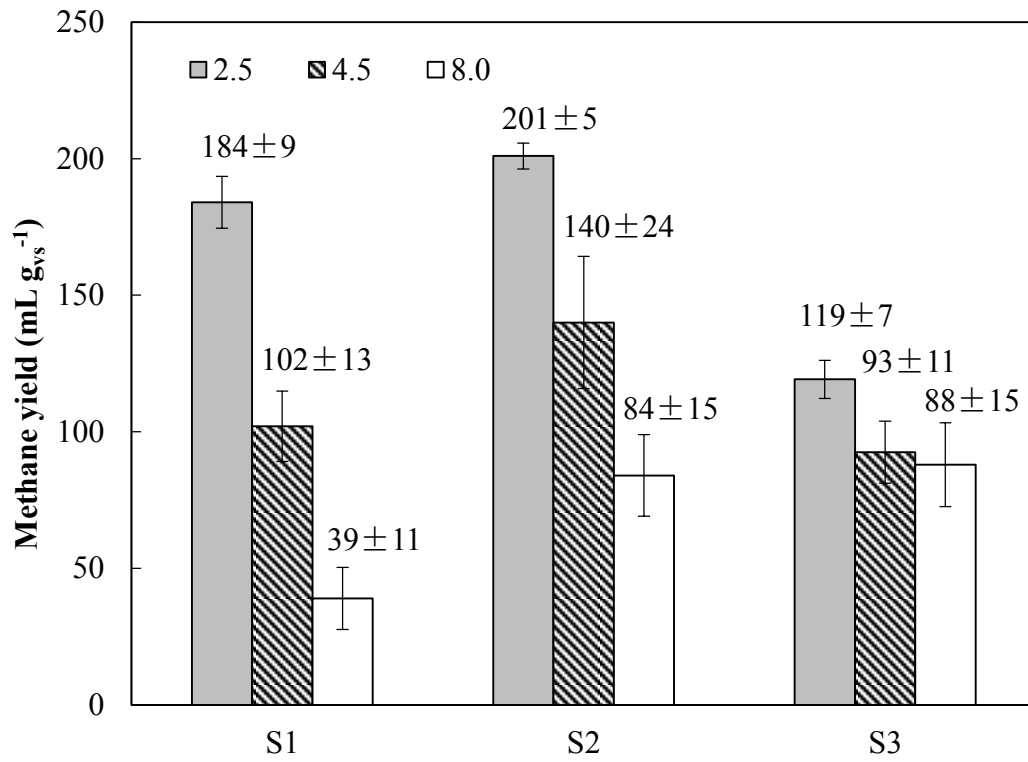


Fig. 6 - Methane yield of the three systems at three organic loading rates (OLR).

## **Table Captions**

Table 1 - Properties of substrates and inoculums used in the experiments.

Table 2 - Methane yield and fraction in BMP experiments.

Table 3 - Properties of digestates from S1, S2, and S3 at different OLRs.

Table 4 - Methane fraction in the three semi-continuous systems.

## Tables

Table 1 - Properties of substrates and inocula used in the experiments.

Parameter	Unit	Substrates	Mesophilic inoculum	Thermophilic inoculum
TS	%FM	92.4	4.9	3.1
VS	%TS	93.8	62.5	70.7
COD	g kg <sup>-1</sup>	1106	48	38
N	%TS	0.56	3.40	4.16
C	%TS	46.51	36.22	41.30
S	%TS	0.08	0.58	0.50
H	%TS	6.89	5.85	6.88
TP	mg kg <sup>-1</sup> FM	810.5	622.7	355.7
TAN	mg kg <sup>-1</sup> FM	N.D.	1188	1250
TKN	mg kg <sup>-1</sup> FM	N.D.	2807	2493
Crude fat	%TS	0.8	N.D.	N.D.
Crude fiber	%TS	42.9	N.D.	N.D.
NDF	%TS	85.3	N.D.	N.D.
ADF	%TS	50.3	N.D.	N.D.
ADL	%TS	7.3	N.D.	N.D.

Note: TS (total solids), VS (volatile solids), COD (chemical oxygen demand), TP (total phosphorus), TAN (total ammonium nitrogen), TKN (total Kjeldahl nitrogen), NDF (neutral detergent fiber), ADF (acid detergent fiber), ADL (acid detergent lignin).

N.D. Not Determined

Table 2 - Methane yield and fraction in BMP experiments.

Samples	CH <sub>4</sub>		Biogas		Methane fraction	
	mL g <sub>vs</sub> <sup>-1</sup>		mL g <sub>vs</sub> <sup>-1</sup>		%	
Units	Mesophilic	Thermophilic	Mesophilic	Thermophilic	Mesophilic	Thermophilic
Cellulose	302 ± 34	381 ± 8	610 ± 71	600 ± 23	56 ± 1	69 ± 1
Corn stalk	256 ± 12	152 ± 35	467 ± 13	267 ± 25	65 ± 1	71 ± 5

Table 3 - Properties of digestates from S1, S2, and S3 at different OLRs.

Units	VS			Cellulose			Hemicellulose			
	%TS			%TS			%TS			
System	S1	S2	S3	S1	S2	S3	S1	S2	S3	
Corn stalk	93.8			43.0			35.0			
OLR	2.5	82.0 ± 2.3	82.7 ± 2.1	90.1 ± 5.1	24.5 ± 1.4	27.0 ± 3.2	36.8 ± 5.3	21.8 ± 1.8	22.5 ± 6.2	28.7 ± 7.9
g <sub>vs</sub>	4.5	86.7 ± 3.7	84.4 ± 1.9	89.2 ± 3.2	34.2 ± 4.5	33.3 ± 0.9	37.5 ± 2.9	27.8 ± 8.1	27.1 ± 0.1	29.7 ± 3.1
L <sub>UASS</sub> <sup>-1</sup> d <sup>-1</sup>	8.0	92.0 ± 1.6	87.1 ± 8.0	88.4 ± 1.6	43.9 ± 1.5	35.7 ± 2.2	38.6 ± 2.8	30.5 ± 2.7	27.5 ± 2.0	30.3 ± 5.6

Table 4 - Methane fraction in the three semi-continuous systems.

Methane fraction %	Systems and conditions	Reactors and substrates	OLR $\text{g}_{\text{vs}} \text{L}^{-1} \text{d}^{-1}$		
			2.5	4.5	8.0
Semi-continuous systems	S1		$53.9 \pm 1.5$	$51.0 \pm 1.2$	$48.5 \pm 4.9$
		UASS+AF	$57.2 \pm 4.3$	$57.1 \pm 1.6$	$56.4 \pm 0.9$
	S2	UASS	$54.4 \pm 6.9$	$55.9 \pm 1.8$	$53.3 \pm 2.1$
		AF	$61.3 \pm 0.2$	$58.3 \pm 1.4$	$59.4 \pm 3.6$
	S3	UASS+AF	$60.8 \pm 1.4$	$58.8 \pm 2.0$	$57.8 \pm 3.3$
		AF	$63.7 \pm 1.4$	$62.9 \pm 0.3$	$63.3 \pm 1.4$