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# Oxygen tolerance capacity of upflow anaerobic solid-state (UASS) with anaerobic filter (AF) system

Yao Meng[a, b], Carsten Jost[b], Jan Mumme[c], Kaijun Wang[a,*], Bernd Linke[b]

[a] State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, PR China

[b] Department Bioengineering, Leibniz Institute for Agricultural Engineering (ATB), Max-Eyth-Allee 100, 14469 Potsdam, Germany

[c] UK Biochar Centre, School of GeoSciences, University of Edinburgh, Crew Building, King's Buildings, Edinburgh EH9 3JN, U.K.

*Corresponding author. Tel.: +86-010-62789411; fax: +86-010-62793065. E-mail address: wkj@tsinghua.edu.cn

## Abstract

In order to investigate the oxygen tolerance capacity of upflow anaerobic solid-state (UASS) with anaerobic filter (AF) system, the effect of microaeration on thermophilic anaerobic digestion of maize straw was investigated under batch conditions and in the UASS with AF system. Aeration intensities of 0–431 mL $O_2$ $g_{VS}^{-1}$ were conducted as pretreatment under batch conditions. Aeration pretreatment obviously enhanced anaerobic digestion and an aeration intensity of 431 mL $O_2$ $g_{VS}^{-1}$ increased the methane yield by 82.2%. Aeration intensities of 0–355 mL $O_2$ $g_{VS}^{-1}$ were conducted in the process liquor circulation of the UASS with AF system. Dissolved oxygen (DO) of UASS and AF reactors kept around 1.39±0.27 and 0.99±0.38 mg $L^{-1}$, respectively. pH was relatively stable around 7.11±0.04. Volatile fatty acids (VFAs) and soluble chemical oxygen demand (SCOD) concentration in UASS reactor were higher than those in AF reactor. Methane yield of the whole system was almost stable at 85±7 mL $g_{VS}^{-1}$ as aeration intensity increased step by step. The UASS with AF system showed good oxygen tolerance capacity.

**Keywords:** Anaerobic digestion; Oxygen tolerance capacity; UASS; Maize straw; Microaeration; Solid-state.

## 1 Introduction

Anaerobic digestion (AD) is a promising and competent technology for treating various types of organic wastes and simultaneously producing biogas as a renewable energy carrier (Li et al., 2011). Unavoidable oxygen would be taken into anaerobic digesters unintentionally as the reactors are operated within an aerobic open environment, especially through interactions with the surroundings such as by feeding and mixing

(Kato et al., 1997). Some enzyme synthesizing of strict anaerobes can be inhibited and rapid cell lysis of obligatory anaerobic species can occur in the presence of oxygen so that oxygen is thought to be an inhibitor to anaerobic process (Botheju and Bakke, 2011). Methanogens will be inhibited by oxygen in anaerobic digesters (Ren and Wang, 2004). On the other hand, the rate-limiting step, hydrolysis of particulate matter in AD can be enhanced (Ramos and Fdz-Polanco, 2013) because oxygen can promote facultative microorganisms excrete a higher amount of enzymes hydrolysis (Johansen and Bakke, 2006; Sheets et al., 2015) and limited aeration could increase synthesis and activity of cellular hydrolytic enzymes (Zhu et al., 2009). Lim and Wang (2013) reported that microaerobic treatment could reduce the formation of toxic metabolites (e.g. lactic acid and ethanol) as well as promote the synthesis of certain lipids required for the stability of anaerobes cell membrane. Previous studies about microaeration pretreatment were conducted under batch conditions (Charles et al., 2009; Mshandete et al., 2005). However, the effect of oxygen on anaerobic digestion under semi-continuous conditions was still unclear.

A UASS with AF system was first described by Mumme et al. (2010), and worked well with maize silage (Mumme et al., 2010), wheat straw (Pohl et al., 2012; Pohl et al., 2013), horse manure (Böske et al., 2014; Böske et al., 2015), and maize straw (Meng et al., 2016). Different from other anaerobic reactors, this system included a UASS reactor in which solid feedstock was digested in a plug-flow mode and an AF reactor in which most methanogens existed as biofilm. The effect of oxygen on anaerobic digestion in UASS and AF system can not be predicted from performance data of other reactors.

Therefore, the overall aim of this research is to investigate the oxygen tolerance capacity of the UASS with AF system. Further aims are to investigate the effect of microaeration on maize straw anaerobic digestion under batch conditions; to investigate the effect of oxygen on maize straw anaerobic digestion in two-stage semi-continuous reactors.

## 2 Materials and Methods

### 2.1 Substrates and inoculum properties

Maize straw was collected from a farm in Cadenberge, Germany. After harvest, the straw was chopped to a final average cutting length of 2–5 cm. Afterwards, it was air-dried to achieve a moisture content of less than 10% and stored at room temperature in a woven bag prior to the experiment.

The inoculum was obtained from previous biogas experiments, which were incubated under thermophilic (55℃) conditions at the Leibniz Institute for Agricultural Engineering (ATB). The inoculum were stored at room temperature for several months without feeding, in order to remove biodegradable chemical oxygen demand (COD). It was removed from solids with a sieve of 1 mm before inoculating. Detailed properties of substrates and inoculum are presented in Table 1.

### 2.2 Batch experiments setup

5 experimental treatments were labelled as B1–B5. Glass bottles (capacity 1 L) were filled with 900 g of inoculum. 11.34 g straw was added in each bottle so that the initial VS ratio of substrates to inoculum was kept at 1:2. All the bottles were placed in an incubator (55℃) without stirring. Afterwards, a peristaltic pump (air flow: 7 mL $min^{-1}$) with air stone was used to aerate the treatments B1–B5 and the different aeration intensities of B1-B5 are shown in Table 2. After 0–2 days of aeration, the bottles were

immediately sealed and connected to gas collecting tubes to conduct anaerobic digestion. The controls (without straw) were run in duplicates and the treatments in triplicates. The anaerobic digestion last 59 days until daily biogas yield of each bottle was less than 1% of the total cumulated biogas yield as stated in the VDI guideline 4630 (VDI, 2006). Methane fraction was analyzed from time to time according to produced biogas yield which were enough for biogas analyzer GA 2000 (ansyco GmbH Germany) to measure.

## 2.3 The UASS with AF system setup and operation

A modification of the UASS with AF system described by Mumme et al. (2010) was used in this work. The schematic of the system is shown in Fig. 1. The straw 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 (Pohl et al., 2012) so that the reactor was named upflow anaerobic solid-state reactor. According to previous operation experience (Pohl et al., 2012; Böske et al., 2014), the digestates compact can lead to clogging and can interfere with liquor circulation (Mumme et al., 2010). Therefore, 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 straw, 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 $m^2$ $m^{-3}$. 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 liquor circulation of both system was set to a flow rate of 11.7 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).

Aeration was conducted in the buffer tank for process liquor from the AF reactor using an aeration pump and an air stone. Two drum-type gas meters (TG05/5 Ritter, Germany) were used to measure the biogas production of the UASS and AF reactor. A combined pH-temperature-probe (InPro4260, Mettler-Toledo, USA) was equipped to AF reactor (at effluent outlet) for continuous online measurement. Maize straw was fed daily at an organic loading rate (OLR) of 4.5 $g_{VS}$ $L_{UASS}^{-1}$ $d^{-1}$ from the feeding pipe and the digestates were removed from the top of the UASS reactors every 5 days. After the digestates being removed, a volume of about 9.4 L (height: 20 cm) of solid organic matter remained in the UASS reactor. The solid retention time (SRT) was about 9.7 days. After the biogas production became stable, the system was operated for 50 days meanwhile the UASS reactors were operated at five different aeration intensities as shown in Table 2.

### 2.4 Analytical methods

The biogas composition of each reactor was measured every day using an industrial biogas analyzer (SSM 6000, Pronova, Germany). The DO of effluent of both reactors were measured with a DO meter (Hanna 9147, USA). The determination of total solids (TS) and volatile solids (VS) was conducted according to DIN standard methods (DIN, 2001). 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 (Van Soest et al., 1991). Total ammonium nitrogen (TAN) was analyzed according to the VDLUFA method (VDLUFA, 2007). 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 µm) 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 ($CH_4$ and $CO_2$) was analyzed using the portable gas analyzer GA 2000 (Ansyco GmbH, Germany) equipped with infrared detectors.

## 2.5 Calculations

The calculation of equivalent aerated $O_2$ intensity of the batch experiment is shown as Eq. (1).

$$I_b = Q \cdot \varphi \cdot t \cdot f / (\mathrm{m} \cdot \mathrm{TS} \cdot \mathrm{VS}) \tag{1}$$

In Eq. (1), $I_b$ is the equivalent aerated $O_2$ intensity of the batch experiment. $Q$ is the air flow (7 mL min$^{-1}$). $\varphi$ is the $O_2$ volume content in air (21%). $t$ and $f$ are the corresponding aeration time and aeration pump working frequency of each treatment (Table 2). m is the straw amount added in each treatment (11.34 g). TS and VS is the total solids and volatile solids of straw shown in Table 1.

The calculation of equivalent aerated $O_2$ intensity of the two-stage system is shown as Eq. (2).

$$I_t = Q \cdot \varphi \cdot f / (OLR \cdot V) \tag{2}$$

In Eq. (2), $I_t$ is the equivalent aerated $O_2$ intensity of the two-stage system. $Q$ and $f$ are the corresponding air flow and aeration pump working frequency of each regime (Table 2). $\varphi$ is the $O_2$ volume content in air (21%). OLR is the organic loading rate of the two-stage system (4.5 $g_{vs}$ $L_{UASS}^{-1}$ $d^{-1}$). $V$ is the working volume of the UASS reactor (35L).

The measured biogas volume was converted to its volume at standard temperature, standard pressure, and dry conditions according to VDI guideline 4630 (VDI, 2006). 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 (VDI, 2006). 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. (2014).

# 3 Results and discussion

## 3.1 Impact of microaeration under batch digestions

The inoculum used was found to be in good condition, as the cellulose reference yielded 600±23 mL $g_{vs}^{-1}$ under thermophilic condition was in accordance with the lower limit stated in the VDI guideline 4630 (VDI, 2006). Biogas yields were calculated from the first day of AD until daily biogas yield was less than 1% of the cumulative biogas yield as stated in the VDI guideline 4630 (VDI, 2006). As shown in Fig. 2a, the biogas yields increased sharply in the first ten days. The start of biogas production was more intensive for the aerated treatments (B2–B5) than the unaerated treatment (B1). This is because aeration enhanced the hydrolysis process of maize straw and increased the concentration of readily available metabolites inside the fermentation bottles compared to the control without aeration. Acidogenesis and methanogenesis reaction rate were higher in the first several days because of higher reactant concentration during the anaerobic step. The result was constant with cumulative methane yield of microaeration pretreatment of maize straw reported by Fu et al. (2015). Díaz et al. (2010) also reported that microaeration pretreatment can reduce the lag-phase time of sludge anaerobic digestion.

The methane fraction increased step by step as shown in Fig. 2b. The overall methane fraction of B1-B5 was 70±3%, 70±1%, 73±1%, 72±1%, and 73±1%. Methane is produced only during the methanogenesis process which was the third step after hydrolysis and acidogensis process (Meng et al., 2016). Most reactions just after aeration in this experiment were hydrolysis and acidogensis so that methane fraction increased step by step as the experiment went on. The cumulative methane yield of B1-B5 was 152±35, 193±12, 219±21, 248±20, and 277±11 mL $g_{vs}^{-1}$. The maximum cumulative methane yield was achieved at the equivalent aerated $O_2$ intensity of 431 mL $g_{vs}^{-1}$ (B5), which was 82.2% higher than that of untreated treatment. It was followed by the equivalent aerated $O_2$ intensity of 216 (B4), 108 (B3), and 54 (B2) mL $g_{vs}^{-1}$, which 62.3%, 44.1%, and 27.0% higher than that of untreated treatment, respectively. This is due to improved degradation of maize straw and growth of methanogenic bacteria (Ahn et al., 2014). In the research of Fu et al. (2015), methane yield of maize straw was improved by 16.24%. Lim and Wang (2013) also found that limited air addition did not inhibit the strictly anaerobic methanogens during co-digestion of brown water and food waste and actually enhanced methane yield by 21%.

### 3.2 Impact of microaeration under two-stage conditions

DO and pH in the reactors were shown in Fig. 3. Average DO of both UASS and AF reactors of the 5 regimes nearly did not change although aeration intensity was increased. This indicates that UASS with AF system has strong oxygen tolerance capacity. The process liquor containing dissolved oxygen firstly passed though solid-state bed in the UASS reactor and then the AF reactor. Oxygen was firstly consumed by facultative microorganisms in the UASS reactor and then by the facultative microorganisms in the biofilm in the AF reactor (Song and Logan, 2004). Therefore, the average DO of effluent of AF reactor (0.99±0.38 mg $L^{-1}$) was lower than that of the effluent of the UASS reactor (1.39±0.27 mg $L^{-1}$). The facultative microorganisms in both reactor provided oxygen tolerance capacity potential of anaerobic digesters such as the UASS and AF reactor. pH was relatively stable around 7.11±0.04 during all the experiment periods which was suitable for anaerobic digestion (Liu et al., 2008).

VFAs and SCOD concentration in process liquor were shown in Fig. 4. In regime 3 (equivalent aerated $O_2$ intensity=89 mL $g_{vs}^{-1}$), both VFAs and SCOD concentration were lower than those of the other 4 regimes. VFAs and SCOD concentration of regime 4 and 5 were higher than regime 3 but still lower than regime 1 and 2. VFAs are the process products in anaerobic digestion, which are necessary for the biogas production. VFAs concentration is also an indicative mark of the working condition of anaerobic process (Li et al., 2014). Compared between UASS and AF reactors, VFAs and SCOD had the similar trend because the hydrolytic retention time (HRT) of process liquor in

UASS or AF reactor was only 3 hours which was short enough for adequate mixing in both reactors. Both average VFAs and SCOD concentration in UASS reactor were higher than those of AF reactor. As aeration intensity increased from regime 1 to regime 3, the whole anaerobic process was enhanced so that VFAs and SCOD concentration were the lowest (Fig. 4) and methane yield of AF was the highest (Fig. 6) in the 5 regimes. As aeration intensity increased from regime 3 to regime 5, inhibition of methanogenesis in the UASS reactor by oxygen became more obvious. At the same time, Ahn et al. (2014) pointed that soluble chemical oxygen demand (SCOD) could be increased through aeration in the research of sewage sludge. Therefore, the VFAs and SCOD concentration increased from regime 3 to regime 5. Similarly, because of the conversion of VFAs and SCOD in AF, the VFAs and SCOD concentration gap between UASS and AF reactors got larger from regime 1 to regime 5 as shown in Fig. 4. The average VS, COD, cellulose and hemi-cellulose contents of the digestages were 86.9 ± 6.0%, 1147.0 ± 68.9%, 39.5 ± 4.0%, and 29.0 ± 3.0%, which were almost stable as aeration intensity increased.

### 3.3 Oxygen tolerance capacity of UASS with AF system

Daily methane production rate and methane yield of the five regimes are shown in Fig. 5 and Fig. 6. Daily methane production rate showed a periodic pattern influenced by the removal of solid residue every five days. Average methane production rate and methane yield of the five regimes had the same trend as OLR was kept constantly at 4.5 $g_{vs}$ $L_{UASS}^{-1}$ $d^{-1}$. Methane yield of the UASS reactor decreased by 26.8% from 54±10 to 40±7 mL $g_{vs}^{-1}$ slightly as aeration intensity increased from Regime 1 to Regime 3. Methane yield of the UASS reactor almost kept the same as aeration intensity increased from

Regime 3 to Regime 5. Methane yield in AF kept almost constant between 38±5 and 47±8 mL $g_{vs}^{-1}$. Meanwhile it increased the maximum to 47±8 mL $g_{vs}^{-1}$ in regime 3 at an equivalent aerated $O_2$ intensity of 89 mL $O_2$ $g_{vs}^{-1}$. The total methane yield of the whole system was relatively stable at 85±7 mL $g_{vs}^{-1}$. Methane fraction of UASS reactor was around 63±2% while methane fraction of AF reactor increased from 63±1% to 68±1%. The methane production rate and methane yield of both UASS and AF reactor were not influenced significantly although aeration intensity increased step by step. This is because facultative hydrolysis microorganisms in UASS reactor and biofilm in AF reactor can relieve the inhibition from oxygen (Shen and Guiot, 1996).

## 4 Conclusions

Aeration pretreatment with an equivalent aeration intensity of 431 mL $O_2$ $g_{vs}^{-1}$ improved methane yield by 82.2%. Aeration pretreatment can enhance methane production of maize straw under batch conditions. Two stage-system showed relative stability as aeration intensity increased. Although methane yield of the UASS and AF reactor were affected by aeration, the total methane yield of the whole system was relatively stable. The UASS with AF system showed high oxygen tolerance capacity.

## Acknowledgements

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## Figure Captions

Fig. 1 - Schematic of the system under semi-continuous conditions. (UASS: upflow anaerobic solid-state reactor; AF: anaerobic filter reactor; Pump1: Liquor circulation pump; Pump 2: Aeration pump; working volume of the UASS reactor, AF reactor, and Buffer tank were 35, 35, and 8L, respectively.)

Fig. 2 – Properties of biogas in the batch experiment. (a: cumulative biogas yield; b: methane fraction; equivalent aerated $O_2$ intensity of B1–5 are 0, 54, 108, 216, and 431 mL $g_{vs}^{-1}$, respectively.)

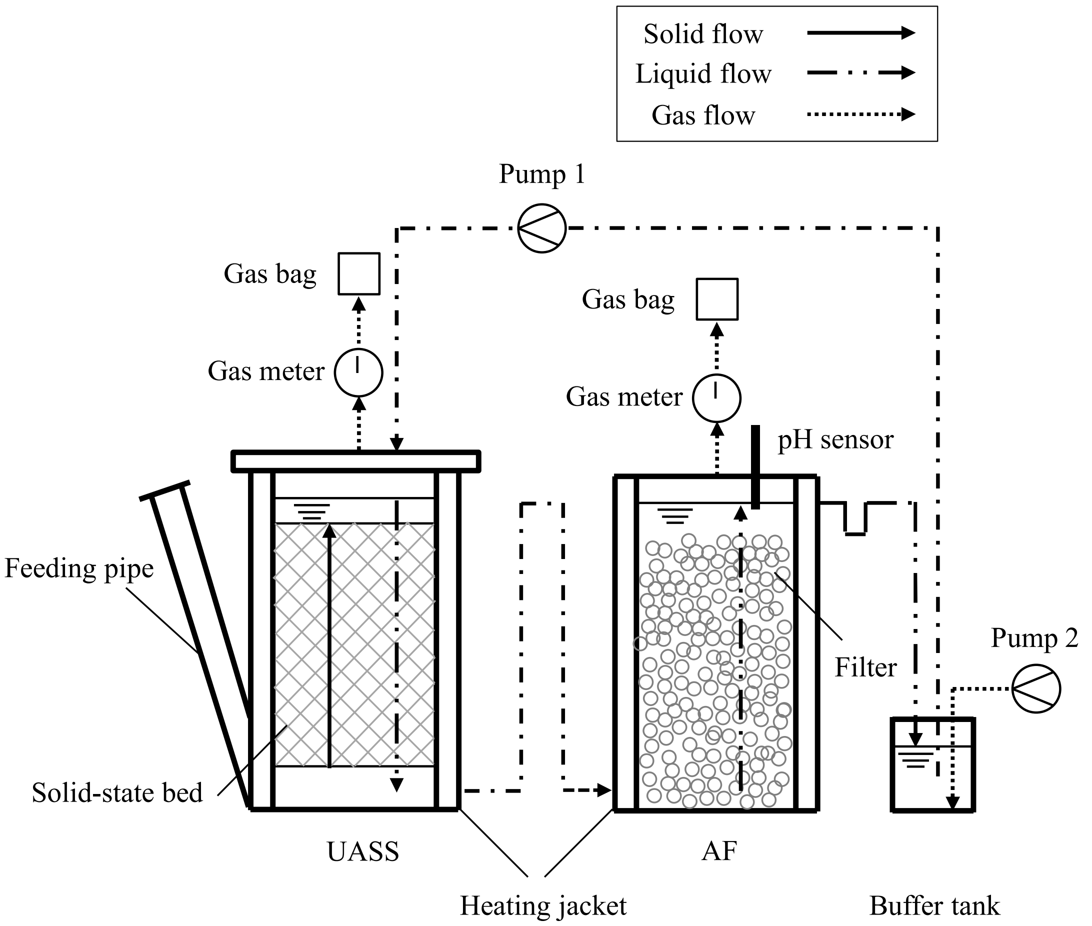
Fig. 3 - pH and dissolved oxygen (DO) of process liquor of upflow anaerobic solid-state (UASS) with anaerobic filter (AF) system.

Fig. 4 - VFAs and SCOD concentration of process liquor of upflow anaerobic solid-state (UASS) with anaerobic filter (AF) system.

Fig. 5 - Methane production rate of the upflow anaerobic solid-state (UASS) and anaerobic filter (AF) reactor.

Fig. 6 - Methane yield of the upflow anaerobic solid-state (UASS) and anaerobic filter (AF) reactor.

## Figures



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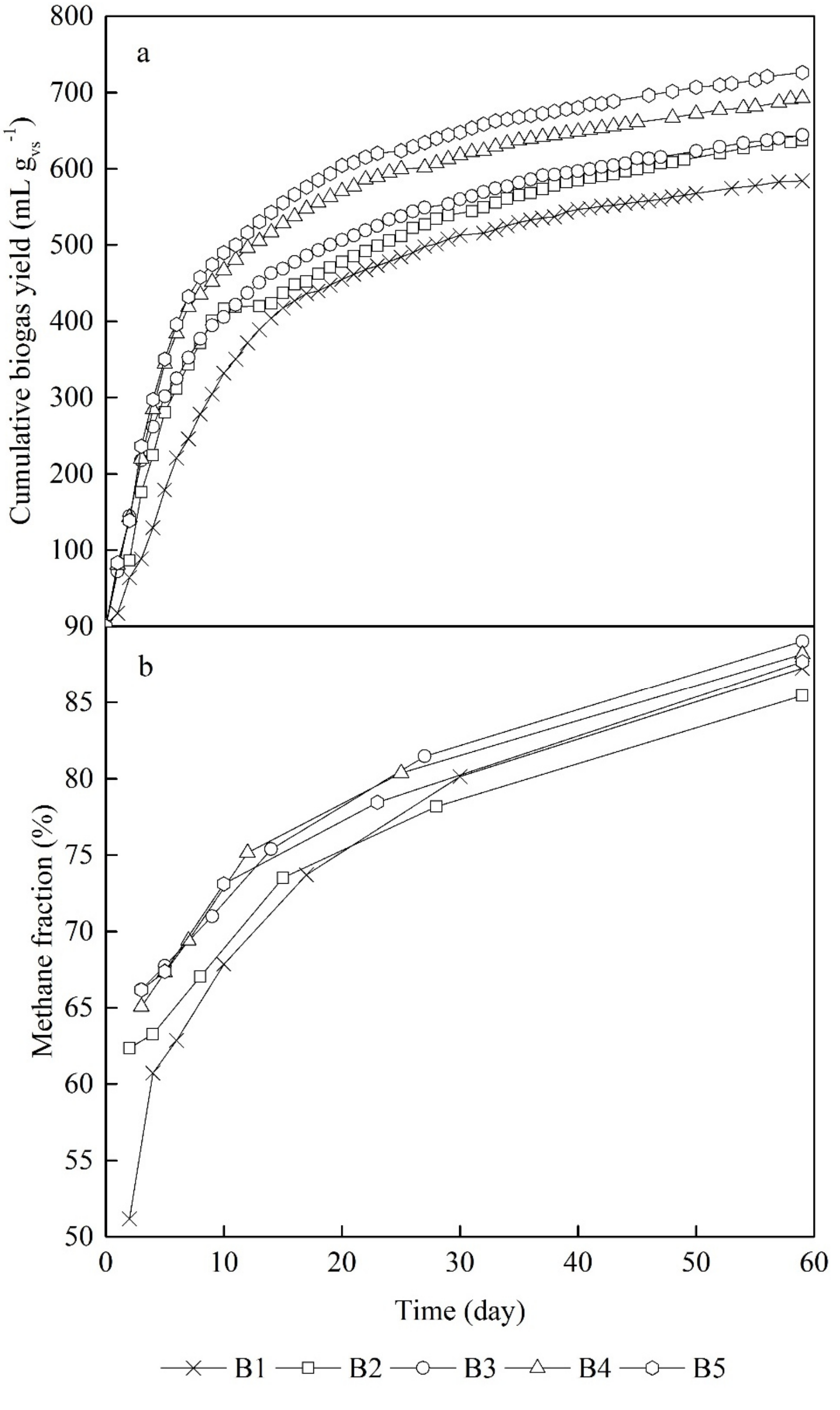

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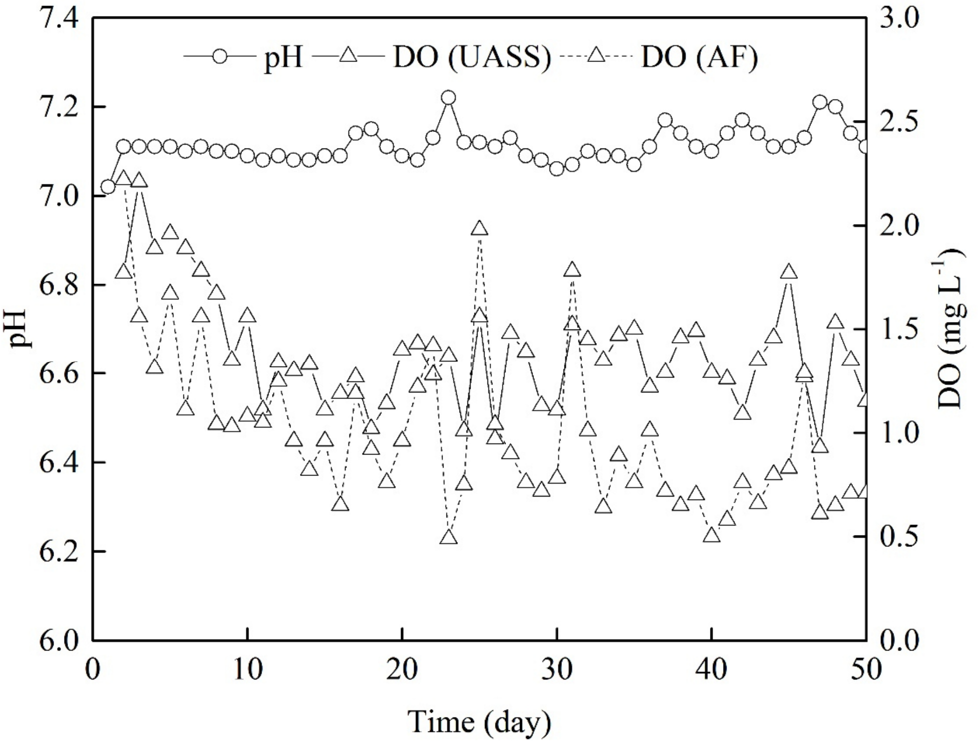

Fig. 3 - pH and dissolved oxygen (DO) of process liquor of upflow anaerobic solid-state (UASS) with anaerobic filter (AF) system.

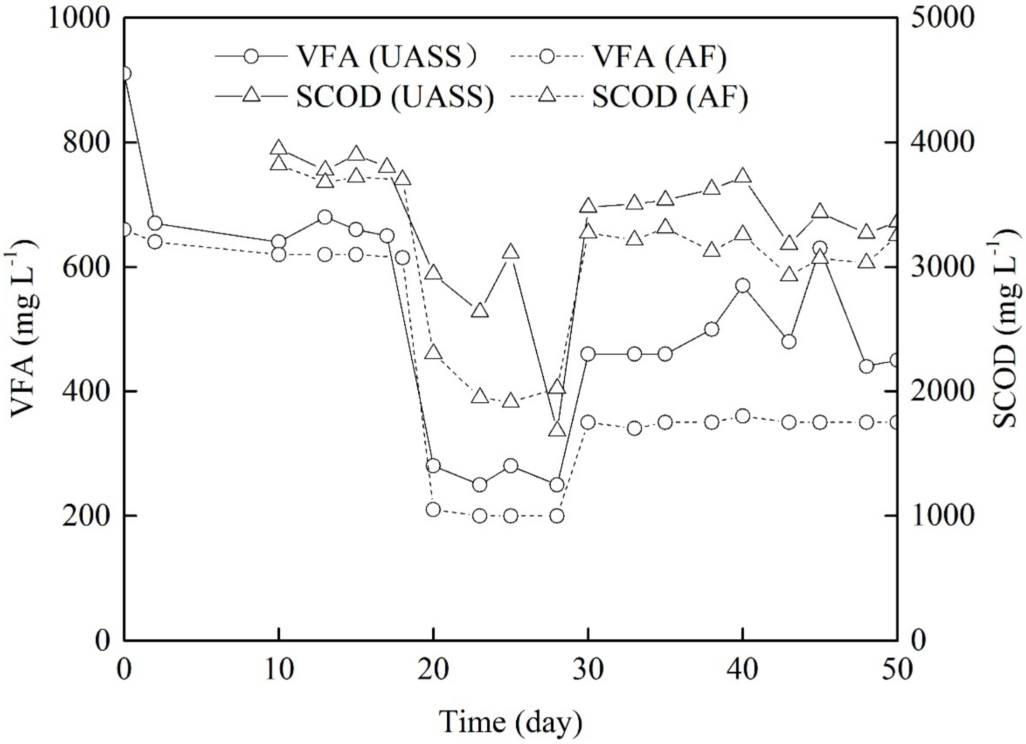

Fig. 4 - VFAs and SCOD concentration of process liquor of upflow anaerobic solid-state (UASS) with anaerobic filter (AF) system.

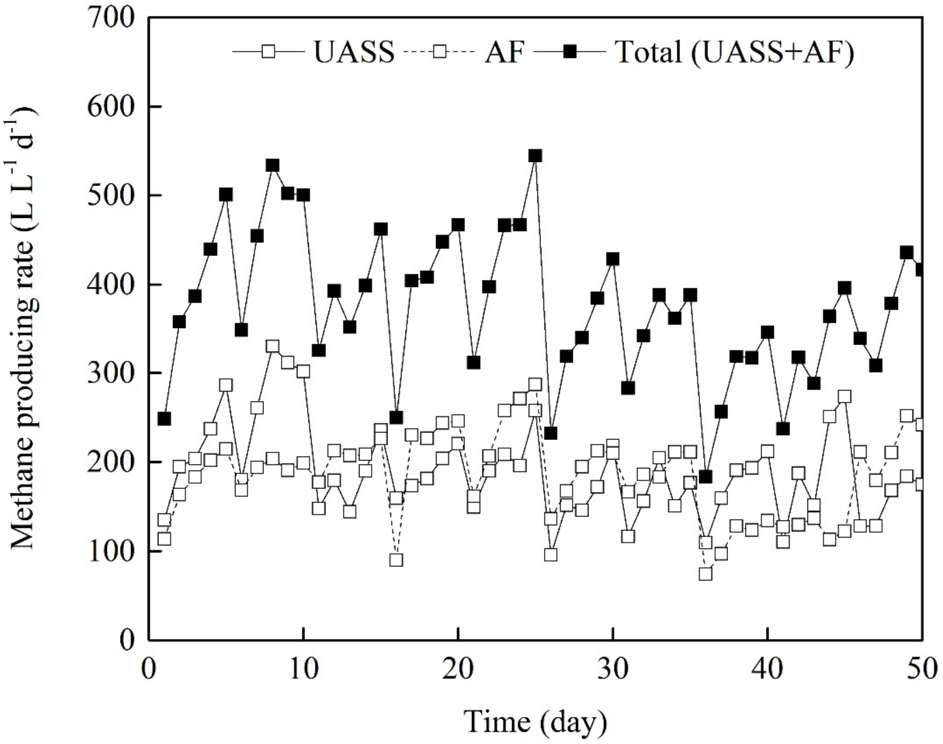

Fig. 5 - Methane production rate of the upflow anaerobic solid-state (UASS) and anaerobic filter (AF) reactor.

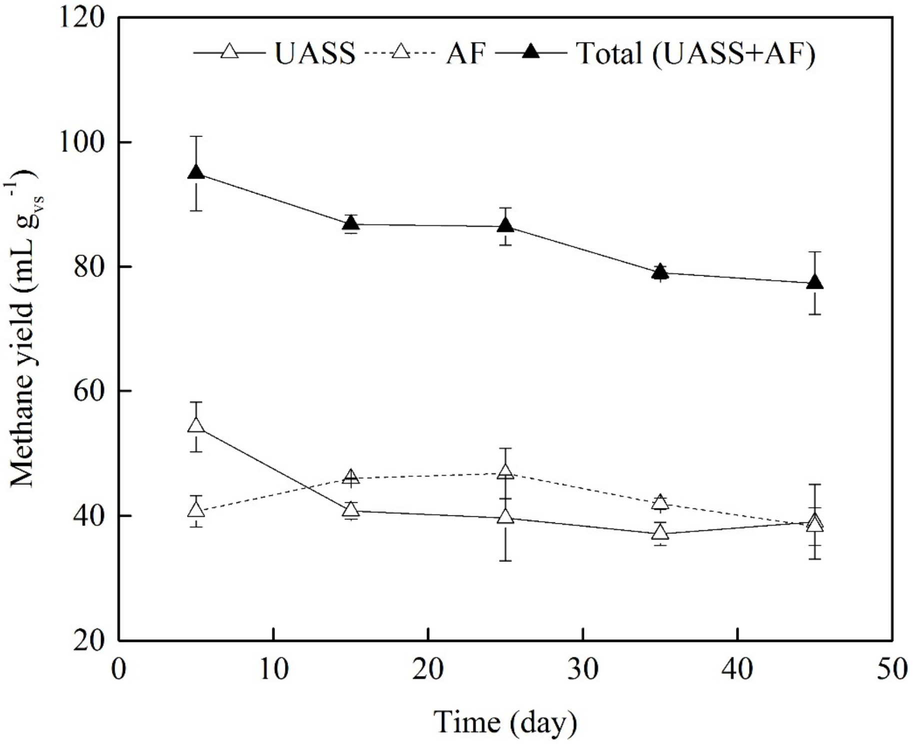

Fig. 6 - Methane yield of the upflow anaerobic solid-state (UASS) and anaerobic filter (AF) reactor.

**Table Captions**

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

Table 2 – Aeration intensity of the batch experiment and the two-stage system

## Tables

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

| Parameter | Unit | Maize straw | Inoculum |
|---|---|---|---|
| TS | %FM | 92.4 | 3.1 |
| VS | %TS | 93.8 | 70.7 |
| COD | g $kg^{-1}$ | 1106 | 38 |
| N | %TS | 0.56 | 4.16 |
| C | %TS | 46.51 | 41.30 |
| S | %TS | 0.08 | 0.50 |
| H | %TS | 6.89 | 6.88 |
| TP | mg $kg^{-1}$ FM | 810.5 | 355.7 |
| TAN | mg $kg^{-1}$ FM | N.D. | 1250 |
| TKN | mg $kg^{-1}$ FM | N.D. | 2493 |
| Crude fat | %TS | 0.8 | N.D. |
| Crude fiber | %TS | 42.9 | N.D. |
| NDF | %TS | 85.3 | N.D. |
| ADF | %TS | 50.3 | N.D. |
| ADL | %TS | 7.3 | 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 – Aeration intensity of the batch experiment and the two-stage system

| Batch experiments | | | | Two-stage system | | | | |
|---|---|---|---|---|---|---|---|---|
| Treatments | Aeration time* | Aeration pump working frequency | Equivalent aerated $O_2$ intensity | Experiment period | Experiment time | Aeration air flow rate | Aeration pump working frequency | Equivalent aerated $O_2$ intensity |
| | hour | | mL $g_{vs}^{-1}$ | | day | mL $min^{-1}$ | | mL $O_2$ $g_{vs}^{-1}$ |
| B1 | 0 | 0 | 0 | Regime 1 | 1-10 | 0 | 0 | 0 |
| B2 | 24 | 15/60min** | 54 | Regime 2 | 11-20 | 47 | 15/60min | 23 |
| B3 | 48 | 15/60min | 108 | Regime 3 | 21-30 | 185 | 15/60min | 89 |
| B4 | 48 | 30/60min | 216 | Regime 4 | 31-40 | 185 | 30/60min | 178 |
| B5 | 48 | 60/60min | 431 | Regime 5 | 41-50 | 185 | 60/60min | 355 |

* Pre-aeration directly before start of the AD experiment

** This means aeration pump worked for 15 min every 60 min (15 min on, 45 min off).