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

3

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15

1 **Abstract**

2 In order to investigate the oxygen tolerance capacity of upflow anaerobic solid-state
3 (UASS) with anaerobic filter (AF) system, the effect of microaeration on thermophilic
4 anaerobic digestion of maize straw was investigated under batch conditions and in the
5 UASS with AF system. Aeration intensities of 0–431 mL O₂ g_{vs}⁻¹ were conducted as
6 pretreatment under batch conditions. Aeration pretreatment obviously enhanced
7 anaerobic digestion and an aeration intensity of 431 mL O₂ g_{vs}⁻¹ increased the methane
8 yield by 82.2%. Aeration intensities of 0–355 mL O₂ g_{vs}⁻¹ were conducted in the process
9 liquor circulation of the UASS with AF system. Dissolved oxygen (DO) of UASS and
10 AF reactors kept around 1.39±0.27 and 0.99±0.38 mg L⁻¹, respectively. pH was
11 relatively stable around 7.11±0.04. Volatile fatty acids (VFAs) and soluble chemical
12 oxygen demand (SCOD) concentration in UASS reactor were higher than those in AF
13 reactor. Methane yield of the whole system was almost stable at 85±7 mL g_{vs}⁻¹ as
14 aeration intensity increased step by step. The UASS with AF system showed good
15 oxygen tolerance capacity.

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

18 **1 Introduction**

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

1 (Kato et al., 1997). Some enzyme synthesizing of strict anaerobes can be inhibited and
2 rapid cell lysis of obligatory anaerobic species can occur in the presence of oxygen so
3 that oxygen is thought to be an inhibitor to anaerobic process (Botheju and Bakke,
4 2011). Methanogens will be inhibited by oxygen in anaerobic digesters (Ren and Wang,
5 2004). On the other hand, the rate-limiting step, hydrolysis of particulate matter in AD
6 can be enhanced (Ramos and Fdz-Polanco, 2013) because oxygen can promote
7 facultative microorganisms excrete a higher amount of enzymes hydrolysis (Johansen
8 and Bakke, 2006; Sheets et al., 2015) and limited aeration could increase synthesis and
9 activity of cellular hydrolytic enzymes (Zhu et al., 2009). Lim and Wang (2013)
10 reported that microaerobic treatment could reduce the formation of toxic metabolites
11 (e.g. lactic acid and ethanol) as well as promote the synthesis of certain lipids required
12 for the stability of anaerobes cell membrane. Previous studies about microaeration
13 pretreatment were conducted under batch conditions (Charles et al., 2009; Mshandete et
14 al., 2005). However, the effect of oxygen on anaerobic digestion under semi-continuous
15 conditions was still unclear.

16 A UASS with AF system was first described by Mumme et al. (2010), and worked well
17 with maize silage (Mumme et al., 2010), wheat straw (Pohl et al., 2012; Pohl et al.,
18 2013), horse manure (Böske et al., 2014; Böske et al., 2015), and maize straw (Meng et
19 al., 2016). Different from other anaerobic reactors, this system included a UASS reactor
20 in which solid feedstock was digested in a plug-flow mode and an AF reactor in which
21 most methanogens existed as biofilm. The effect of oxygen on anaerobic digestion in
22 UASS and AF system can not be predicted from performance data of other reactors.

23 Therefore, the overall aim of this research is to investigate the oxygen tolerance
24 capacity of the UASS with AF system. Further aims are to investigate the effect of

1 microaeration on maize straw anaerobic digestion under batch conditions; to investigate
2 the effect of oxygen on maize straw anaerobic digestion in two-stage semi-continuous
3 reactors.

4 **2 Materials and Methods**

5 2.1 Substrates and inoculum properties

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

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

16 2.2 Batch experiments setup

17 5 experimental treatments were labelled as B1–B5. Glass bottles (capacity 1 L) were
18 filled with 900 g of inoculum. 11.34 g straw was added in each bottle so that the initial
19 VS ratio of substrates to inoculum was kept at 1:2. All the bottles were placed in an
20 incubator (55°C) without stirring. Afterwards, a peristaltic pump (air flow: 7 mL min⁻¹)
21 with air stone was used to aerate the treatments B1–B5 and the different aeration
22 intensities of B1-B5 are shown in Table 2. After 0–2 days of aeration, the bottles were

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

8 2.3 The UASS with AF system setup and operation

9 A modification of the UASS with AF system described by Mumme et al. (2010) was
10 used in this work. The schematic of the system is shown in Fig. 1. The straw was fed
11 manually through an inclined feeding pipe to the bottom of the UASS reactor, ascended
12 in the form of a solid-state bed (SSB) in the reactor and was removed manually from the
13 top by removing the reactor's lid as described previously (Pohl et al., 2012) so that the
14 reactor was named upflow anaerobic solid-state reactor. According to previous
15 operation experience (Pohl et al., 2012; Böske et al., 2014), the digestates compact can
16 lead to clogging and can interfere with liquor circulation (Mumme et al., 2010).
17 Therefore, the liquor flow inside the UASS reactor was changed from upflow to
18 downflow in this work. The process liquor was applied via the lid of the UASS reactor,
19 passed through the solid-state bed of the straw, and was removed from the bottom of the
20 reactor.

21 To relieve the inhibition of accumulated VFAs, an additional AF reactor was added
22 after the UASS reactor to form a two-stage system. The AF reactor was filled with PE
23 biofilm carriers (Bioflow 40, RVT Process Equipment GmbH, Germany) with a surface

1 area of $305 \text{ m}^2 \text{ m}^{-3}$. The process liquor in AF was upflow. The working volume of the
2 UASS reactor, the AF reactor and buffer tank was 35 L, 35 L and 8 L each. Process
3 liquor circulation of both system was set to a flow rate of 11.7 L h^{-1} using peristaltic
4 pumps (Heidolph, Germany). Both UASS and AF reactors were heated via a
5 thermostatically controlled water jacket (Lauda, Lauda-Königshofen, Germany).

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

18 2.4 Analytical methods

19 The biogas composition of each reactor was measured every day using an industrial
20 biogas analyzer (SSM 6000, Pronova, Germany). The DO of effluent of both reactors
21 were measured with a DO meter (Hanna 9147, USA). The determination of total solids
22 (TS) and volatile solids (VS) was conducted according to DIN standard methods (DIN,
23 2001). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent

1 lignin (ADL) were analyzed by a fiber analyzer (ANKOM2000, USA) as described in
2 the literature (Van Soest et al., 1991). Total ammonium nitrogen (TAN) was analyzed
3 according to the VDLUFA method (VDLUFA, 2007). Total Kjeldahl nitrogen (TKN)
4 and chemical oxygen demand (COD) were measured according to DIN EN 25663:
5 1993–11 and DIN ISO 15705: 2003–01 respectively. Total carbon (TC) and total
6 nitrogen (TN) were determined by elemental analysis (DIN EN 15104: 2011–04).
7 Volatile fatty acids were measured with a gas-phase chromatograph (Agilent GC
8 7890A, USA) equipped with a Permabond-FFAP column (length 30 m, diameter
9 0.32 mm, film thickness 0.5 µm) and a flame ionization detector. C, N, S, and H
10 fractions were analyzed with a vario EL III elemental analyzer (Elementar
11 Analysensysteme GmbH, Germany), but had only been available for solid samples. For
12 the BMP batch digestion tests, the composition of the produced biogas (CH₄ and CO₂)
13 was analyzed using the portable gas analyzer GA 2000 (Ansyco GmbH, Germany)
14 equipped with infrared detectors.

15 2.5 Calculations

16 The calculation of equivalent aerated O₂ intensity of the batch experiment is shown as
17 Eq. (1).

$$18 I_b = Q \cdot \varphi \cdot t \cdot f / (m \cdot TS \cdot VS) \quad (1)$$

19 In Eq. (1), I_b is the equivalent aerated O₂ intensity of the batch experiment. Q is the air
20 flow (7 mL min⁻¹). φ is the O₂ volume content in air (21%). t and f are the
21 corresponding aeration time and aeration pump working frequency of each treatment
22 (Table 2). m is the straw amount added in each treatment (11.34 g). TS and VS is the
23 total solids and volatile solids of straw shown in Table 1.

1 The calculation of equivalent aerated O₂ intensity of the two-stage system is shown as
2 Eq. (2).

$$3 \quad I_t = Q \cdot \varphi \cdot f / (OLR \cdot V) \quad (2)$$

4 In Eq. (2), I_t is the equivalent aerated O₂ intensity of the two-stage system. Q and f are
5 the corresponding air flow and aeration pump working frequency of each regime (Table
6 2). φ is the O₂ volume content in air (21%). OLR is the organic loading rate of the two-
7 stage system (4.5 g_{vs} L_{UASS}⁻¹ d⁻¹). V is the working volume of the UASS reactor (35L).

8 The measured biogas volume was converted to its volume at standard temperature,
9 standard pressure, and dry conditions according to VDI guideline 4630 (VDI, 2006). Air
10 was introduced each time when feeding and when removing digestates, which would
11 potentially disrupt the biogas composition analysis. Therefore, the methane yield was
12 calculated on the assumption that the volumetric fractions of methane and carbon
13 dioxide sum up close to 100% as recommended in VDI guideline 4630 (VDI, 2006).
14 Each of the two measured values was multiplied by the same factor so that the sum of
15 the two corrected measured values was 100% neglecting trace gases. The detailed
16 calculation was previously described by Böske et al. (2014).

17 **3 Results and discussion**

18 3.1 Impact of microaeration under batch digestions

19 The inoculum used was found to be in good condition, as the cellulose reference yielded
20 600 ± 23 mL g_{vs}⁻¹ under thermophilic condition was in accordance with the lower limit
21 stated in the VDI guideline 4630 (VDI, 2006). Biogas yields were calculated from the
22 first day of AD until daily biogas yield was less than 1% of the cumulative biogas yield

1 as stated in the VDI guideline 4630 (VDI, 2006). As shown in Fig. 2a, the biogas yields
2 increased sharply in the first ten days. The start of biogas production was more intensive
3 for the aerated treatments (B2–B5) than the unaerated treatment (B1). This is because
4 aeration enhanced the hydrolysis process of maize straw and increased the concentration
5 of readily available metabolites inside the fermentation bottles compared to the control
6 without aeration. Acidogenesis and methanogenesis reaction rate were higher in the first
7 several days because of higher reactant concentration during the anaerobic step. The
8 result was constant with cumulative methane yield of microaeration pretreatment of
9 maize straw reported by Fu et al. (2015). Díaz et al. (2010) also reported that
10 microaeration pretreatment can reduce the lag-phase time of sludge anaerobic digestion.

11 The methane fraction increased step by step as shown in Fig. 2b. The overall methane
12 fraction of B1-B5 was $70 \pm 3\%$, $70 \pm 1\%$, $73 \pm 1\%$, $72 \pm 1\%$, and $73 \pm 1\%$. Methane is
13 produced only during the methanogenesis process which was the third step after
14 hydrolysis and acidogenesis process (Meng et al., 2016). Most reactions just after
15 aeration in this experiment were hydrolysis and acidogenesis so that methane fraction
16 increased step by step as the experiment went on. The cumulative methane yield of B1-
17 B5 was 152 ± 35 , 193 ± 12 , 219 ± 21 , 248 ± 20 , and 277 ± 11 mL $\text{g}_{\text{vs}}^{-1}$. The maximum
18 cumulative methane yield was achieved at the equivalent aerated O_2 intensity of 431 mL
19 $\text{g}_{\text{vs}}^{-1}$ (B5), which was 82.2% higher than that of untreated treatment. It was followed by
20 the equivalent aerated O_2 intensity of 216 (B4), 108 (B3), and 54 (B2) mL $\text{g}_{\text{vs}}^{-1}$, which
21 62.3%, 44.1%, and 27.0% higher than that of untreated treatment, respectively. This is
22 due to improved degradation of maize straw and growth of methanogenic bacteria (Ahn
23 et al., 2014). In the research of Fu et al. (2015), methane yield of maize straw was
24 improved by 16.24%. Lim and Wang (2013) also found that limited air addition did not

1 inhibit the strictly anaerobic methanogens during co-digestion of brown water and food
2 waste and actually enhanced methane yield by 21%.

3 3.2 Impact of microaeration under two-stage conditions

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

16 VFAs and SCOD concentration in process liquor were shown in Fig. 4. In regime 3
17 (equivalent aerated O₂ intensity=89 mL g_{vs}⁻¹), both VFAs and SCOD concentration
18 were lower than those of the other 4 regimes. VFAs and SCOD concentration of regime
19 4 and 5 were higher than regime 3 but still lower than regime 1 and 2. VFAs are the
20 process products in anaerobic digestion, which are necessary for the biogas production.
21 VFAs concentration is also an indicative mark of the working condition of anaerobic
22 process (Li et al., 2014). Compared between UASS and AF reactors, VFAs and SCOD
23 had the similar trend because the hydrolytic retention time (HRT) of process liquor in

1 UASS or AF reactor was only 3 hours which was short enough for adequate mixing in
2 both reactors. Both average VFAs and SCOD concentration in UASS reactor were
3 higher than those of AF reactor. As aeration intensity increased from regime 1 to regime
4 3, the whole anaerobic process was enhanced so that VFAs and SCOD concentration
5 were the lowest (Fig. 4) and methane yield of AF was the highest (Fig. 6) in the 5
6 regimes. As aeration intensity increased from regime 3 to regime 5, inhibition of
7 methanogenesis in the UASS reactor by oxygen became more obvious. At the same
8 time, Ahn et al. (2014) pointed that soluble chemical oxygen demand (SCOD) could be
9 increased through aeration in the research of sewage sludge. Therefore, the VFAs and
10 SCOD concentration increased from regime 3 to regime 5. Similarly, because of the
11 conversion of VFAs and SCOD in AF, the VFAs and SCOD concentration gap between
12 UASS and AF reactors got larger from regime 1 to regime 5 as shown in Fig. 4. The
13 average VS, COD, cellulose and hemi-cellulose contents of the digestages were $86.9 \pm$
14 6.0% , $1147.0 \pm 68.9\%$, $39.5 \pm 4.0\%$, and $29.0 \pm 3.0\%$, which were almost stable as
15 aeration intensity increased.

16 3.3 Oxygen tolerance capacity of UASS with AF system

17 Daily methane production rate and methane yield of the five regimes are shown in Fig.
18 5 and Fig. 6. Daily methane production rate showed a periodic pattern influenced by the
19 removal of solid residue every five days. Average methane production rate and methane
20 yield of the five regimes had the same trend as OLR was kept constantly at $4.5 \text{ g}_{\text{vs}}$
21 $\text{L}_{\text{UASS}}^{-1} \text{ d}^{-1}$. Methane yield of the UASS reactor decreased by 26.8% from 54 ± 10 to 40 ± 7
22 $\text{mL g}_{\text{vs}}^{-1}$ slightly as aeration intensity increased from Regime 1 to Regime 3. Methane
23 yield of the UASS reactor almost kept the same as aeration intensity increased from

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

10 **4 Conclusions**

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

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- 16

1 **Figure Captions**

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

6 Fig. 2 – Properties of biogas in the batch experiment. (a: cumulative biogas yield; b:
7 methane fraction; equivalent aerated O₂ intensity of B1–5 are 0, 54, 108, 216, and 431
8 mL g_{vs}⁻¹, respectively.)

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

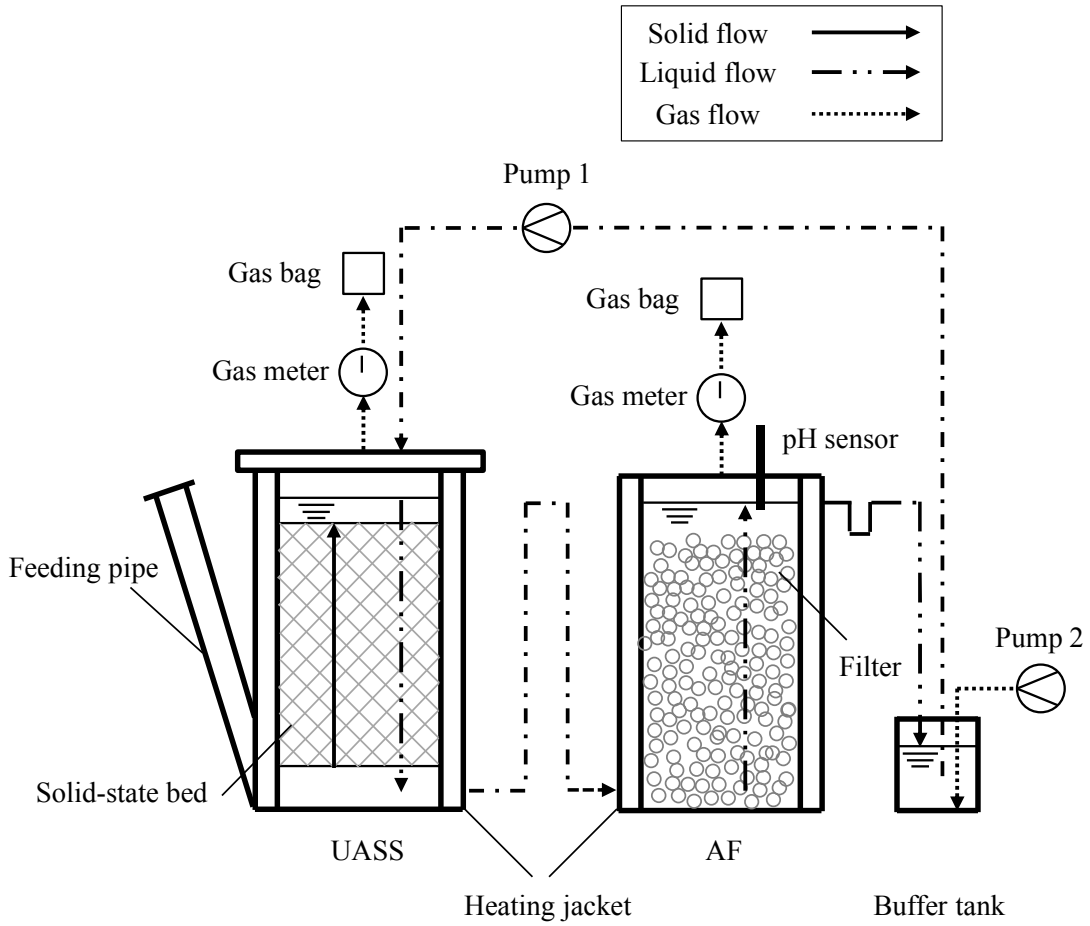
11 Fig. 4 - VFAs and SCOD concentration of process liquor of upflow anaerobic solid-
12 state (UASS) with anaerobic filter (AF) system.

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

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

1 **Figures**

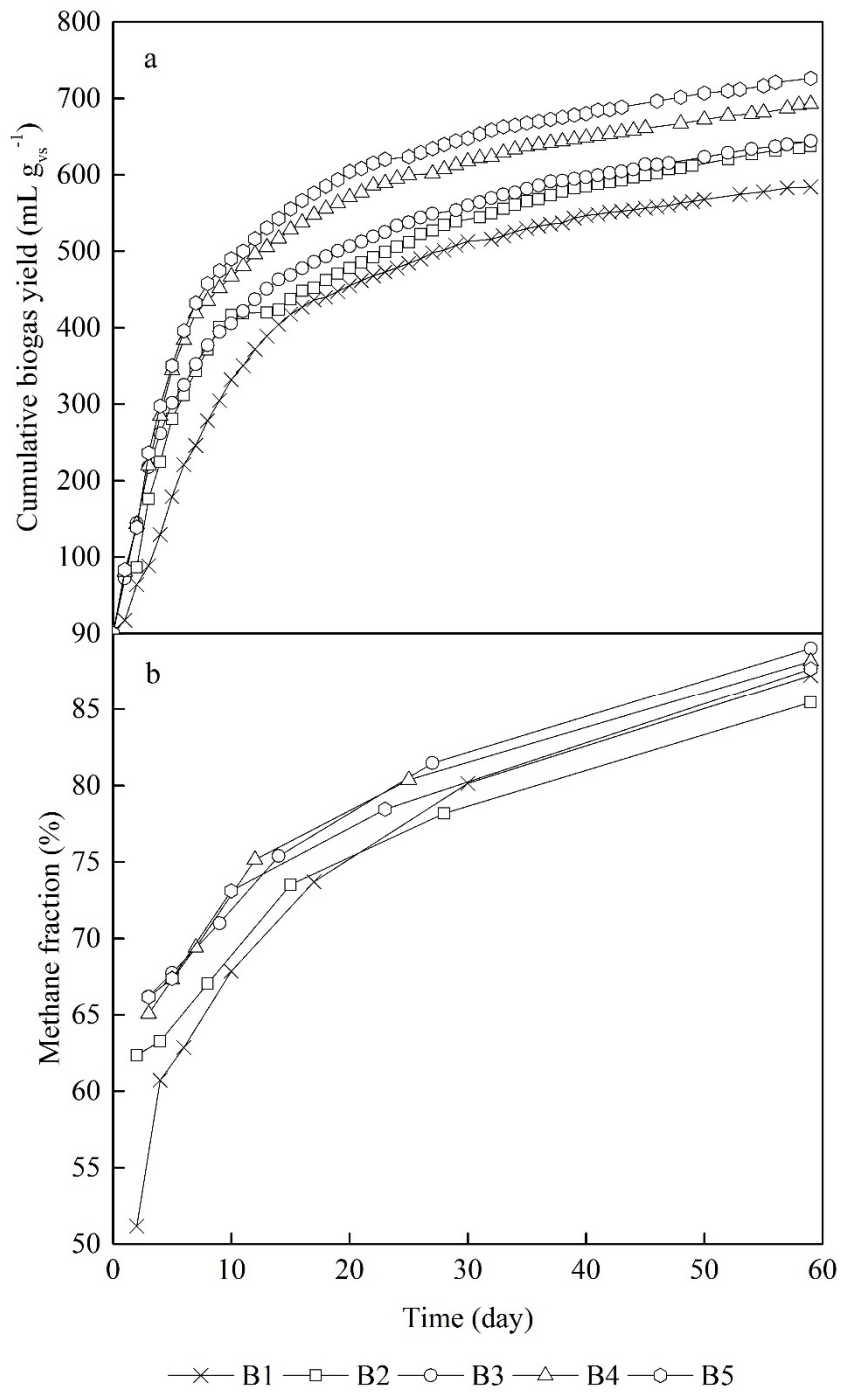
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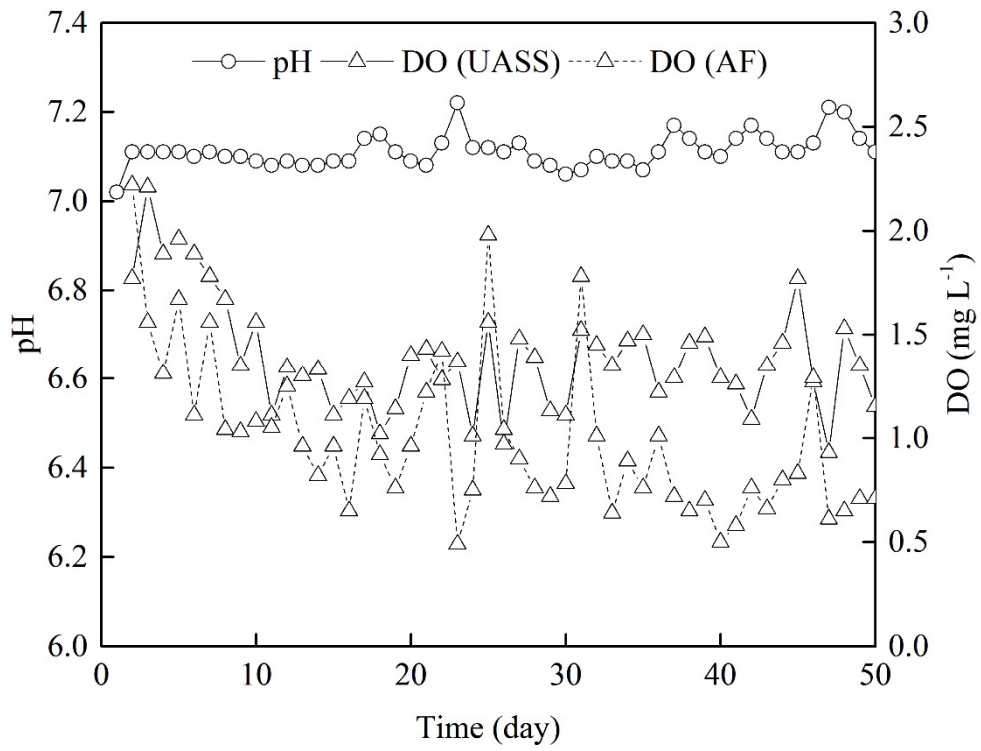
4 Fig. 1 - Schematic of the system under semi-continuous conditions. (UASS: upflow
5 anaerobic solid-state reactor; AF: anaerobic filter reactor; Pump1: Liquor circulation
6 pump; Pump 2: Aeration pump; working volume of the UASS reactor, AF reactor, and
7 Buffer tank were 35, 35, and 8L, respectively.)

8



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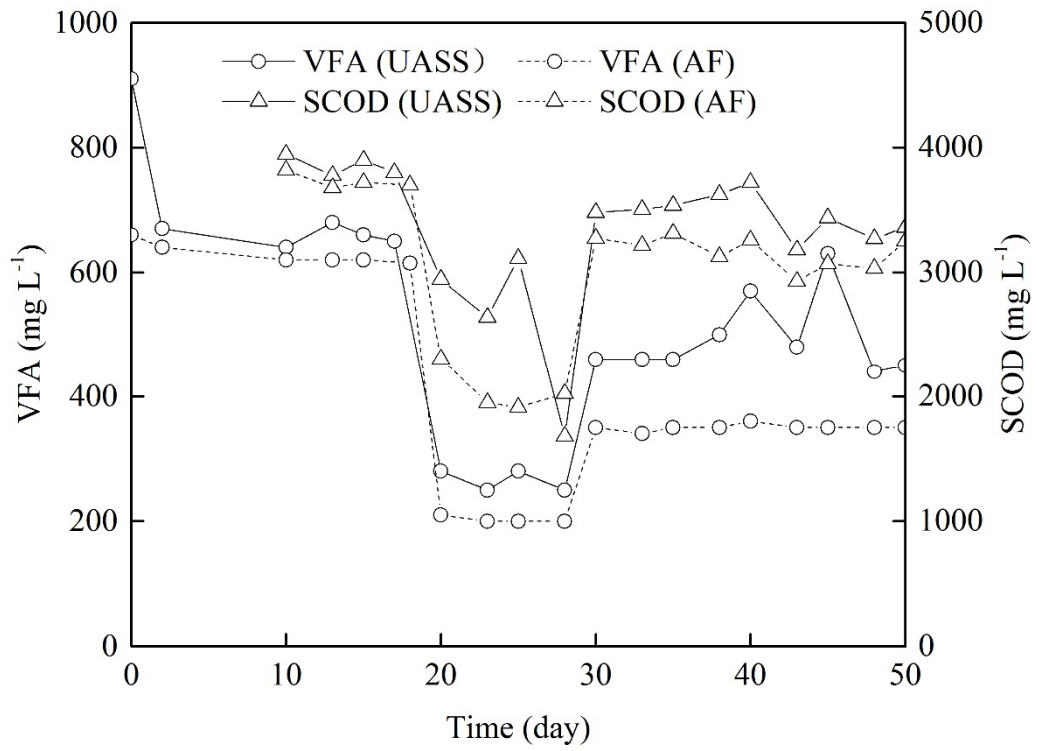
2 Fig. 2 – Properties of biogas in the batch experiment. (a: cumulative biogas yield; b:
 3 methane fraction; equivalent aerated O₂ intensity of B1–5 are 0, 54, 108, 216, and 431
 4 mL g_{vs}⁻¹, respectively.)



1

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

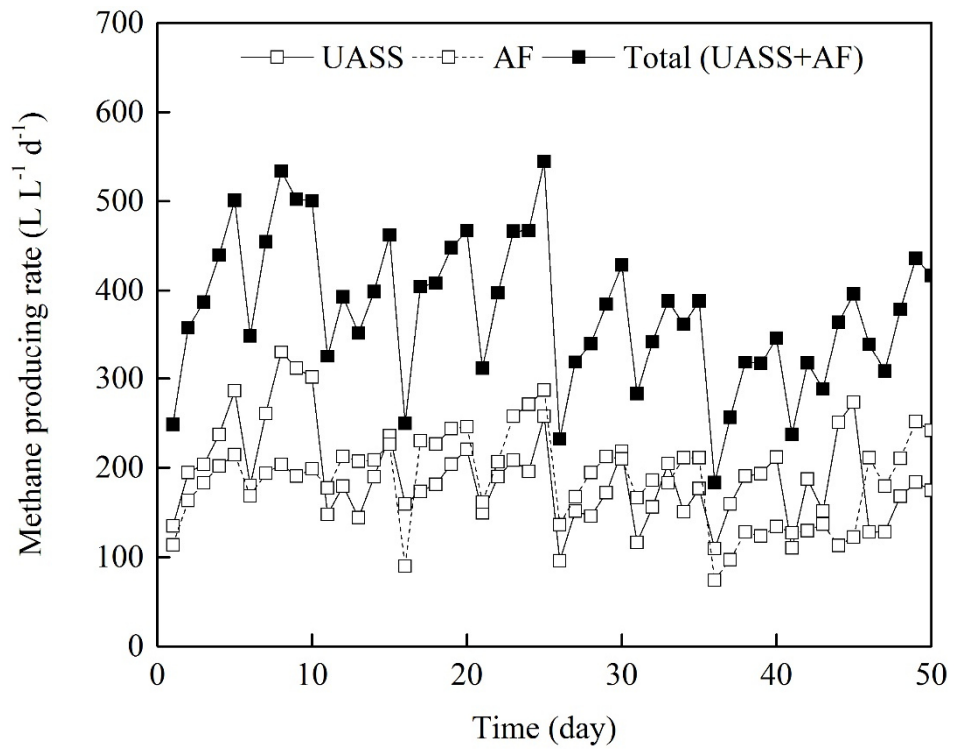
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1

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

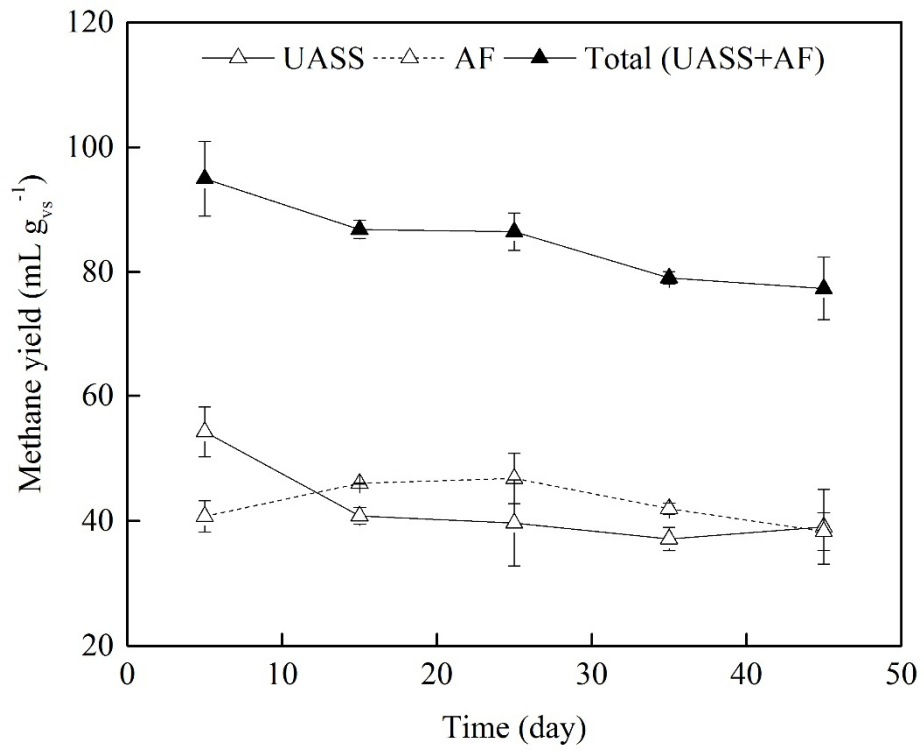
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2 Fig. 5 - Methane production rate of the upflow anaerobic solid-state (UASS) and
 3 anaerobic filter (AF) reactor.

4



1

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

3 (AF) reactor.

1 **Table Captions**

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

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

4

1 **Tables**

2 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. |

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

6 N.D. Not Determined

7

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

| Treatments | Batch experiments | | | Experiment period | Experiment time | Two-stage system | | |
|------------|------------------------|---------------------------------|---|-------------------|-----------------|--|---------------------------------|--|
| | Aeration time* hour | Aeration pump working frequency | Equivalent aerated O ₂ intensity mL g _{vs} ⁻¹ | | | Aeration air flow rate mL min ⁻¹ | Aeration pump working frequency | Equivalent aerated O ₂ intensity 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 |

2

3 * Pre-aeration directly before start of the AD experiment

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