

## Effect of inoculum VS, organic loads and I/S on the biochemical methane potential of sludge, buckwheat husk and straw

Mahmoud Elsayed<sup>a,\*</sup>, Yves Andres<sup>b</sup>, Walid Blel<sup>c</sup>, Raouf Hassan<sup>a</sup>, Abdelkader Ahmed<sup>a</sup>

<sup>a</sup>Civil Engineering Department, Faculty of Engineering, Aswan University, 81542 Aswan, Egypt, emails: m.elsayed@aswu.edu.eg, engzezo1111@yahoo.com (M. Elsayed), eng\_r\_m@yahoo.com (R. Hassan), dratahmed@yahoo.com (A. Ahmed)

<sup>b</sup>IMT Atlantique, GEPEA UMR CNRS 6144, 4 rue Alfred Kastler, 44307 Nantes cedex 3, NANTES, France, email: yves.andres@imt-atlantique.fr

<sup>c</sup>CNRS, GEPEA, UMR 6144, CRTT, 37 Bd de l'Université, BP 406, 44602 Saint-Nazaire Cedex, France, email: walid.blel@univ-nantes.fr

Received 17 May 2018; Accepted 15 March 2019

### ABSTRACT

This study aims to evaluate the influence of inoculum volatile solids (VS), organic loads and I/S ratios on methane production from anaerobic co-digestion of sludge (PS), straw (WS) and buckwheat husk (BH). Biochemical methane potential (BMP) tests were conducted in 500 mL digesters under mesophilic conditions. For this paper, two experiments were carried out: in the first experiment, five mixtures of PS, WS and BH were tested depending on their I/S ratios, to investigate the optimum mixture for effective methane production. The highest CH<sub>4</sub> yield was recorded at an I/S ratio of 3, which showed an increase of 1.06–1.74 times more than the other I/S ratios. In the second experiment, six amounts of PS, WS and BH at different inoculum VS organic loads were used to investigate the best VS organic loads for optimum gas generation. The highest methane (CH<sub>4</sub>) yield was recorded at an organic load of 15 g VS/L, which showed higher CH<sub>4</sub> yields of 1.20–1.58 times more than other organic loads, while the lowest CH<sub>4</sub> yield was obtained at an organic load of 10 g VS/L. The results were obtained using a statistical analysis test. The VS removal rate and pH were evaluated. The results indicated the positive effect that inoculum VS organic loads and I/S ratios have on improving CH<sub>4</sub> production.

**Keywords:** Sewage sludge; Agro-wastes; Anaerobic co-digestion; Methane production; Inoculum VS organic loads; Inoculum to substrate ratio

### 1. Introduction

Sewage sludge (SS) is a normal end product of wastewater treatment stages [1]. A huge quantity of SS is produced all over the world, which makes elimination or recycling rather problematic. Every year, huge quantities of SS are composted or disposed of in landfill or on agrarian land. Wei et al. [2] report that the treatment and disposal of SS accounts for more than half of the aggregate cost of sewage treatment. Anaerobic digestion of SS for energetic valorization has been the subject of several studies, and has been implemented in several industrial processes. The amount of sludge produced

in France by the 19,750 wastewater treatment plants (WWTP) gradually increased to 1 million tons of dry matter in 2007 [3]. The sludge produced by WWTPs could be converted to biogas using anaerobic digestion. Biogas production showed a low yield when it was digested alone, due to its low carbon content [4]. Anaerobic co-digestion is useful for generating renewable energy and stabilizing SS and other abundant organic wastes [5].

On the other hand, large amounts of waste are produced from agricultural residues all over the world. Crops wastes are mainly disposed of by incineration or in landfill, which can have harmful consequences for the environment. Wheat

\* Corresponding author.

is considered one of the most important agricultural crops all over the world. The total world production of wheat is 715.91 million tons. Buckwheat is also considered a very important crop, especially in Europe and Asia. The total world production of buckwheat is 2.348 million tons [6]. Lignocellulosic materials such as wheat straw (WS) and buckwheat husk (BH) are composed of carbohydrates, fats and proteins. These components are directly recoverable in biomethane as a result of microbiological activity in an anaerobic environment, while the carbohydrates part of the biomass can be used for ethanol recovery, rather than fats and proteins [7]. Anaerobic co-digestion of SS and agro-wastes helps to improve biodegradability of feedstock and increase methane yields, more than individual digestion of each substrate. Biogas production from waste can be estimated using the biochemical methane potential (BMP) test [8]. The BMP test reveals that the inoculum parameter has a very important influence on methane production, encouraging researchers to devote more attention to the study of it.

Research on the effect of inoculum on methane ( $\text{CH}_4$ ) yields has been conducted by several researchers, but most of these studies have focused mainly on the effect of inoculum to substrate (I/S) ratio on  $\text{CH}_4$  yields using one or two substrates [9]. However, a few studies have been carried out on the effect of I/S ratios using multi-substrate digestion. Akyol et al. [10] studied the effect of I/S ratio and solids content from anaerobic digestion of tannery sludge, and showed the highest recorded methane production at an I/S ratio of 0.5. Raposo et al. [11] reported that the optimal  $\text{CH}_4$  yield was recorded at an I/S ratio of more than 2. Chynoweth et al. [12] found that  $\text{CH}_4$  production was increased at I/S ratios of 0.5–1.0, using the BMP test for co-digestion of biomass with different wastes. Gonzalez-Fernandez and Garcia-Encina [13] studied the effect of I/S ratio on swine slurry, and the results showed that the  $\text{CH}_4$  yield was improved by increasing I/S ratios. In addition, the Hashimoto [14] study on anaerobic digestion of wheat straw at different substrate-to-inoculum ratios showed that the methane yield decreased when the substrate-to-inoculum ratio was higher than 4.0. Forster-Carneiro et al. [15] demonstrated that the highest  $\text{CH}_4$  yield was recorded at the highest percentage of inoculum. Dechrugsa et al. [16] examined the effects of I/S ratio and inoculum source for anaerobic digestion of manure and grass, and the results reported that a high I/S ratio enhanced methane yields.

On the other hand, the optimal values of inoculum volatile solids (VS) organic loads for BMP testing are poorly documented in previous studies. Elsayed et al. [17] studied the effect of VS organic loads on feedstock with the BMP test by anaerobic co-digestion of primary sludge and wheat straw, and the results showed that the optimal methane production was with a VS load of 7.50 g VS/L.

Most previous studies have focused mainly on the influence of inoculum percentage in relation to feedstock percentage on methane yields, using one or two substrates. However, the optimal VS organic loads of inoculum used in the BMP test have not yet been widely estimated. This work has several objectives; the first part is to show the effect of I/S ratio on  $\text{CH}_4$  yields using multi-substrate mixtures. The second part is to determine the optimal VS organic loads of inoculum in the BMP test to increase methane production.

## 2. Methodology

### 2.1. Feedstock

In order to study the effect of inoculum VS and I/S ratios on methane production, anaerobic co-digestion of sludge (PS), straw (WS) and buckwheat husk (BH) have been chosen, first due to the abundance of waste generated either at the end of waste treatment processes or during the valorization of agricultural crops, and second because they allow wide variation in the proportions of VS and I/S ratios studied for this work, which will identify the effect that each parameter has on the production of biogas. In this study, primary sludge (PS) was acquired from the municipal WWTP in Nantes and dried in order to stabilize it. Wheat straw (WS) was collected from a farm and ground using a hammer mill and coffee grinder to obtain a minimal size of under 1.0 mm. According to Yong et al. [18], the best size for the straw is in the range 0.3–1.0 mm for an optimal anaerobic digestion process. A huge quantity of buckwheat is produced in France, which is mainly used for flour. Buckwheat husk (BH) was obtained from mills in the Voltière area of Garnache.

### 2.2. Inoculum

In this study, mushy cow manure (CM) was used as inoculum. The inoculum was stocked in anaerobic conditions to stimulate bacteria and remove dissolved methane. Mesophilic conditions (37°C) were used in this study and an adaptation stage of the microorganisms was carried out in these conditions for more than 30 d before starting the experiments [19].

### 2.3. Analytical measurement techniques

Using Standard Methods [20], the VS, TS and pH were estimated. The determination of the elemental sample composition was done using a Flash EA 1112 (THERMO FINNIGAN, IMT Atlantique) device. CHNS and O were measured separately using different conditions. The total methane production at ambient temperature was recorded using the water displacement method. The composition of the biogas produced was obtained using a Clarus 500 Gas Chromatograph (PerkinElmer, IMT Atlantique) equipped with a thermal conductivity detector and two columns. The cumulative methane production was calculated to STP values (10<sup>5</sup> Pa and 273.15 K). The substrate and inoculum characterization are shown in Table 1.

### 2.4. Purification of methane

The composition of biogas from an anaerobic digestion process contains methane ( $\text{CH}_4$ ), carbon dioxide ( $\text{CO}_2$ ) and small amounts of other gases. The high percentage of carbon dioxide ( $\text{CO}_2$ ) in the produced biogas decreases its heating value and increases its transport costs [21]. Purification of the biogas can be carried out by absorbing the  $\text{CO}_2$ , which increases the  $\text{CH}_4$  content. In this work, the  $\text{CO}_2$  was absorbed from the produced biogas by passing it through 3 M sodium hydroxide (NaOH) solution. The 250 mL glass bottles were filled with NaOH solution and sealed with plastic caps. Each cap contained two plastic tubes, one of which was connected

Table 1  
Substrate and inoculum characterization

Characteristics	PS	WS	BH	Inoculum
VS (TS %)	82.50 ± 0.10	95.64 ± 0.05	97.60 ± 0.08	73.28 ± 0.65
TS (dry wt.%)	81.70 ± 0.15	90.82 ± 0.20	85.00 ± 0.36	5.59 ± 0.84
TC (dry wt.%)	39.90 ± 0.44	47.62 ± 0.58	47.50 ± 0.51	ND
TN (dry wt.%)	6.70 ± 0.25	0.30 ± 0.04	2.30 ± 0.12	ND
TO (dry wt.%)	28.30 ± 0.19	44.10 ± 0.42	43.37 ± 0.30	ND
TH (dry wt.%)	5.40 ± 0.09	5.54 ± 0.17	6.10 ± 0.12	ND
C/N ratio	5.96	158.73	20.65	ND
pH	ND	ND	ND	6.80 ± 0.05

Notes: PS = primary sludge, WS = wheat straw, BH = buckwheat husk, VS = volatile solids, TS = total solids, TC = total carbon, TN = total nitrogen, TO = total oxygen, TH = total hydrogen and C/N = nitrogen to carbon. The data represent the means ± SD,  $n = 4$ .

to a batch reactor bottle and the other to the gas measuring device using the water displacement method.

### 2.5. Batch test

Batch tests were conducted at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$  using the same method as Elsayed et al. [17]. Each configuration was duplicated in order to carry out a statistical analysis. 500 mL batch reactors with a working volume of 400 mL were used. The BMP test set-up is shown in Fig. 1. The conditions selected for the experiment were based on Elsayed et al. [17]. In the present study, two groups of BMP tests were performed. In the first group, five different amounts of PS, BH and WS based on their I/S ratios of 0.5 (B1), 1.0 (B2), 2.0 (B3), 2.50 (B4) and 3.0 (B5) were tested to obtain the optimal I/S ratio for effective methane production (Table 2).

In the second BMP test, different amounts of PS, WS and BH were anaerobically digested in batch reactors labeled A1 to A6, using different VS organic loads of inoculum in the range 3.0 to 18.0 g VS/L, as shown in Table 3. An appropriate amount of feedstock and inoculum was introduced in each reactor, maintaining a VS inoculum/VS substrate ratio equal to 2, in order to decrease prevalence limitation and avert toxic conditions [22]. In both experiments, the C/N ratio in all the reactors was kept at around 10 [17]. Batch tests of PS, WS and BH as single substrates were performed for use as a control; these are recorded as E1, E2 and E3 respectively

(Table 2), where every reactor of the single digestion of the feedstock was filled with the only substrate and inoculum. At the beginning of the batch test, the pH value for all the reactors in the two groups of experiments was adjusted to the value of  $7 \pm 0.1$ . At the end of the BMP test, samples from each bottle were analyzed to measure the TS, VS and pH. The VS removal and biodegradability of the substrates were



Fig. 1. Batch test set-up.

Table 2  
Different mixtures of feedstock in co-digestion of sludge, straw and buckwheat husk based on their I/S ratios

Reactor number	PS (g VS/400 mL)	WS (g VS/400 mL)	BH (g VS/400 mL)	I/S ratio
B1	5.95	2.76	2.75	0.50
B2	2.99	1.39	1.38	1.00
B3	1.48	0.69	0.69	2.00
B4	1.19	0.56	0.55	2.50
B5	1.00	0.46	0.46	3.00
E1	2.90	0.00	0.00	2.00
E2	0.00	2.90	0.00	2.00
E3	0.00	0.00	2.90	2.00

Notes: PS = primary sludge, WS = wheat straw, BH = buckwheat husk, and I/S ratio = inoculum to substrate ratio. The reactors B1 to B5 were fed by mixtures of PS, WS and BH whereas E1, E2 and E3 were fed by a single substrate of PS, WS and BH, respectively.

Table 3  
Different amounts of feedstock in co-digestion of sludge, straw and buckwheat husk at different inoculum VS organic loads

Reactor number	A1	A2	A3	A4	A5	A6
PS <sub>added</sub> (g VS/400 mL)	1.87	1.55	1.25	1.00	0.73	0.31
WS <sub>added</sub> (g VS/400 mL)	0.87	0.73	0.57	0.50	0.34	0.15
BH <sub>added</sub> (g VS/400 mL)	0.86	0.72	0.58	0.50	0.33	0.14
Mixture load (g VS/400 mL)	3.60	3.00	2.40	2.00	1.40	0.60
Inoculum <sub>added</sub> (g VS/400 mL)	7.20	6.00	4.80	4.00	2.80	1.20
Inoculum <sub>added</sub> (g VS/L)	18.00	15.00	12.00	10.00	7.00	3.00
C/N ratio	10.62	10.67	10.58	10.93	10.60	10.70

Notes: PS = primary sludge, WS = wheat straw, BH = buckwheat husk, and C/N = carbon to nitrogen.

then determined. The VS removal was calculated using the following equation:

$$VS_{\text{removal}} = \frac{VS_{\text{in}} - VS_{\text{exit}}}{VS_{\text{in}}} \times 100 \quad (1)$$

where  $VS_{\text{removal}}$  is the volatile solids removal for the feedstock used,  $VS_{\text{in}}$  is the input volatile solids for the feedstock used in g/L,  $VS_{\text{exit}}$  is the exit volatile solids for the feedstock used in g/L.

### 2.6. Statistical analysis

For this research, cumulative methane yields (CMYs) were carried out as the means of two duplicates for the BMP test. Comparisons between the tested conditions were carried out using STATGRAPHICS Centurion XV software and ANOVA analysis. The conditions of methane production are considered significantly different for a  $p$ -value lower than 5%. This procedure carried out a one-way analysis of variance for CMYs at the various levels of inoculum VS

organic loads and I/S ratios were performed at a confidence interval of 95%.

## 3. Results and discussion

### 3.1. Effect of I/S ratio on the anaerobic co-digestion of multi-substrates

#### 3.1.1. Methane yields of a mixture of PS, WS and BH

To enhance methane ( $\text{CH}_4$ ) production from co-digestion of multi-substrates at different I/S ratios, five anaerobic co-digestion BMP tests were carried out. At the same time, three individual digestion control tests of PS, WS, BH and inoculum were performed.

Daily  $\text{CH}_4$  yields from co-digestion of PS, WS and BH at different I/S ratios are shown in Fig. 2. The figure shows that the maximum  $\text{CH}_4$  production from single digestion of PS, WS and BH was 18, 27.2 and 17.20 mL/g VS<sub>add</sub>, respectively, and was recorded after 16, 6 and 14 d from the start of the batch tests. At the 30th day of digestion, the methane production varied between 1 and 5 mL/g VS<sub>add</sub> for the

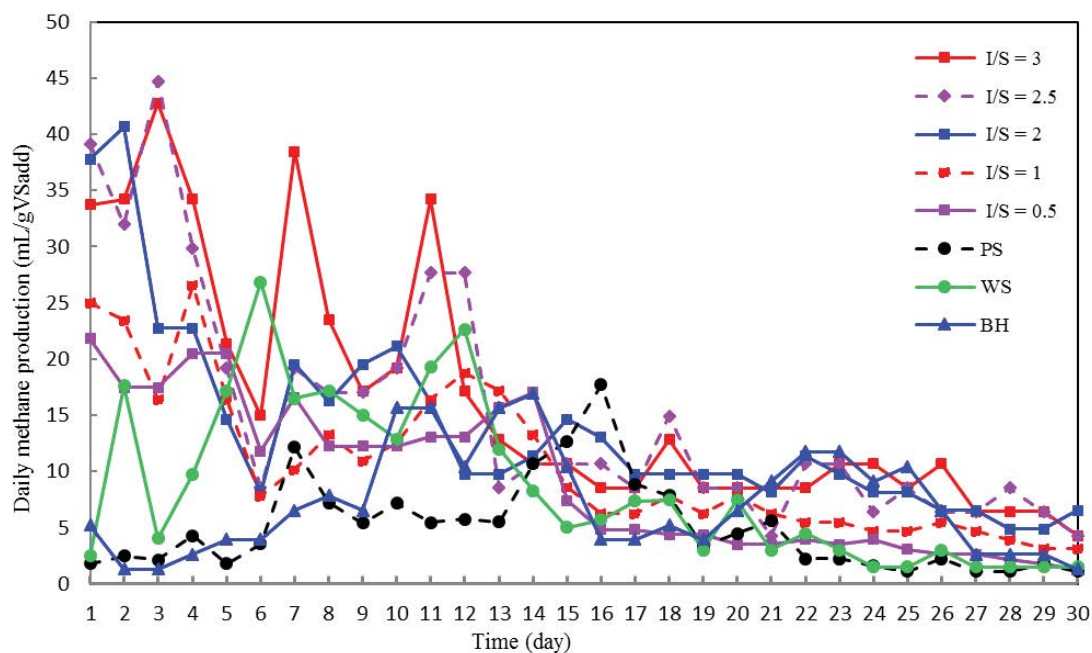


Fig. 2. Daily  $\text{CH}_4$  yields from co-digestions of PS, WS and BH at different I/S ratios.

different samples, with the lowest values for single samples. The sample corresponding to I/S ratio = 3 showed strong production during the first 15 d but a low methane concentration at the 30th day of digestion compared with other mixing, which could be explained by the near-exhaustion of its biodegradable compounds [23].

The cumulative  $\text{CH}_4$  yields from co-digestion of PS, WS and BH at different I/S ratios are shown in Fig. 3. The CMYs at I/S ratios of 0.5, 1.0, 2.0, 2.50 and 3.0 were found to be 282.90, 322.20, 418.40, 464.10 and 493.70 mL/g VS<sub>add</sub>, respectively, which were higher than those obtained from individual substrates of PS, WS and BH. This may be as a result of anaerobic co-digestion of PS, WS and BH that improves biogas production and biodegradability of the feedstock than the single digestion of the feedstock [17].

In addition, the increase in I/S ratio induces the methane yields to increase to the highest rate of I/S ratio = 3 (493.70 mL/g VS<sub>add</sub>), which showed higher CMYs of 1.06, 1.18, 1.53 and 1.74 times the values recorded from the other I/S ratios, at 2.5, 2, 1 and 0.5, respectively. The minimum CMYs were observed at I/S ratios of 0.5 (282.90 mL/g VS<sub>add</sub>) and 1 (322.20 mL/g VS<sub>add</sub>).

The optimal methane production was recorded at I/S ratios of 3 and 2.5, possibly due to the provision of good conditions for bacteria to grow, which achieved the highest VS removal rate [17]. In this study, the pH value ranged between 7.30 and 7.54 during the BMP test for all the reactors, which corresponds to the optimal range given by Sibiya and Muzenda [24] (between 6.5 and 8.0).

The maximum CMY observed from co-digestion of PS, WS and BH using different I/S ratios was 493.70 mL/g VS added at an I/S ratio of 3, which showed a higher value of methane

production than what was observed by Wang et al. [25] and Abdul Razaque et al. [26], at 234.7 and 322 mL/g VS added, respectively. This result is in agreement with those of Raposo et al. [11] and Gonzalez-Fernandez and Garcia-Encina [13] which have shown that the increase of the I/S ratio allows an improvement in the production yield of biogas. Furthermore, Raposo et al. [11] reported that the optimal  $\text{CH}_4$  yield was recorded at I/S ratio of more than 2 which is consistent with the results obtained in this work. However, there is a maximum threshold of I/S ratio from which methane productivity will fall and which is not identified under conditions tested in this work.

The statistical analysis for the observed results was performed using ANOVA analysis software. The effects of five different levels of I/S ratio on methane production were compared. An *F*-test was performed and its value was <0.05, with a confidence level of 95.0% (Fig. 4). An I/S ratio of 3 is considered to be the best optimized ratio statistically, producing high  $\text{CH}_4$  yields.

The volatile solids removal rate from co-digestion of PS, WS and BH at different I/S ratios is shown in Fig. 5. The figure shows that the best percentage of biodegradability is obtained at I/S ratios of 2.5 (76.33%) and 3 (74.22%), which confirms the biogas productivity results. This result confirms that a part of the organic matter is intended for the growth of bacterial flora and not necessarily for the fermentation process [11].

### 3.1.2. Methane percentage in produced biogas

The biogas produced from co-digestion of different wastes consists of 50% to 80% methane ( $\text{CH}_4$ ) and 20% to 40% carbon dioxide ( $\text{CO}_2$ ) [27]. From an economic perspective,

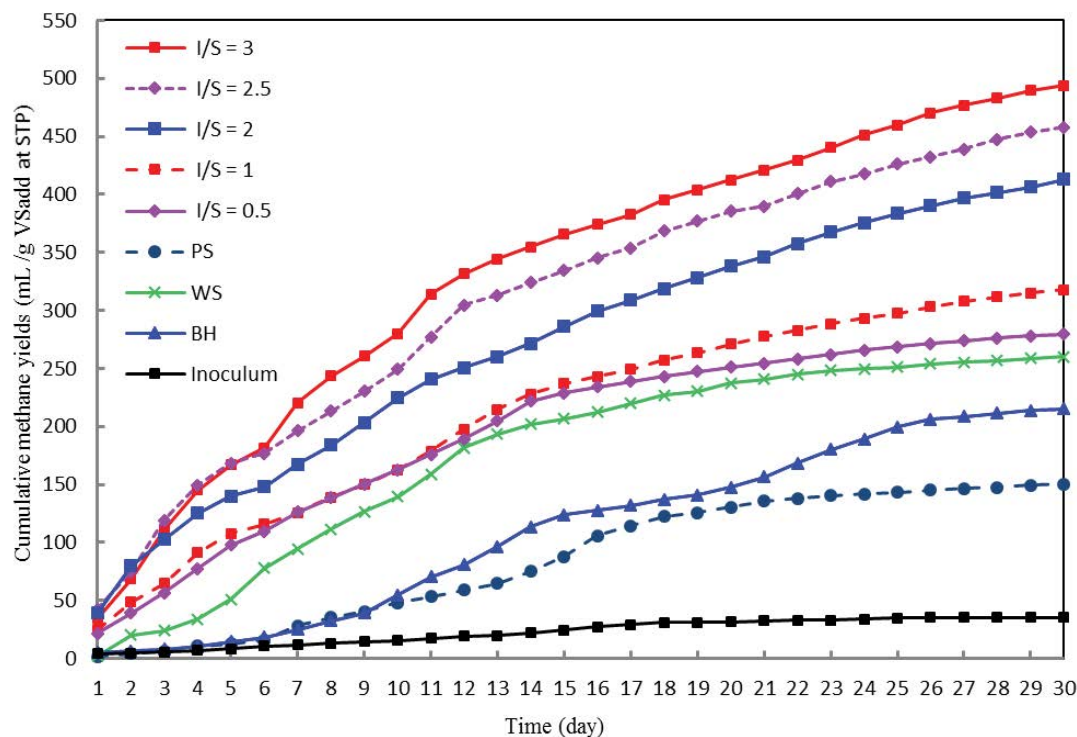


Fig. 3. Cumulative  $\text{CH}_4$  yields from co-digestion of PS, WS and BH at different I/S ratios.



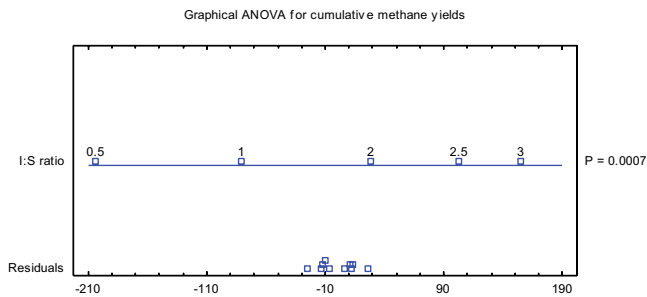


Fig. 4. Graphical ANOVA analysis of I/S ratio factor for cumulative methane yields.

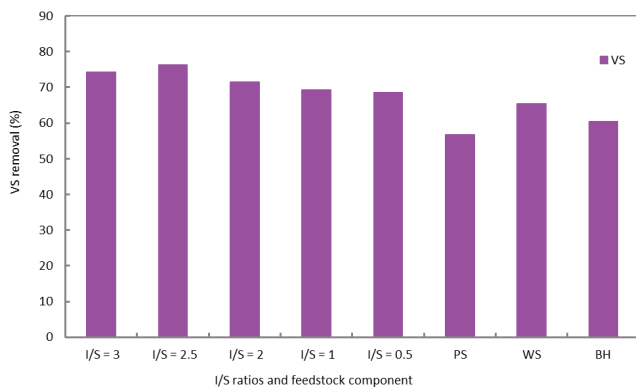


Fig. 5. Average VS removal from co-digestion of PS, WS and BH at different I/S ratios.

increasing the methane percentage in the produced biogas is preferred. The purification process succeeded in absorbing the  $\text{CO}_2$  from the produced biogas using sodium hydroxide. The methane content in the biogas produced from co-digestion of PS, WS and BH under different I/S ratios is plotted in Fig. 6. The figure shows that the highest average methane content of the purified biogas (95.53% and 95.14%) is at I/S ratios of 2.0 and 3.0, respectively, while the lowest value of 92.53% was recorded at an I/S ratio of 1.0. In addition, the average methane content from the co-digestion of PS, WS and BH in the BMP test was higher than the individual digestion of PS,

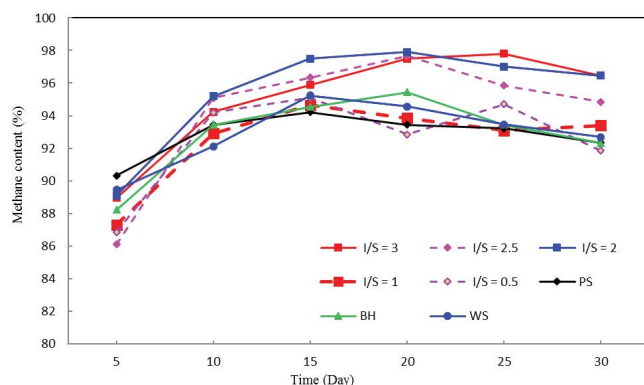


Fig. 6. Average methane content in purified biogas from the co-digestion of PS, WS and BH at different I/S ratios.

WS and BH. It is also observed that for the different conditions tested, there is a peak of methane production. This peak is observed at 25 d for I/S ratio of 3 whereas for both cases of 2 and 2.5, the peak is observed at 20 d. The fall following these peaks corresponds to an increase in  $\text{CO}_2$  production. This fall is greater with the case of I/S ratio of 2.5 than one of 3. The high biodegradability at I/S of 2.5 does not necessarily corresponds to the high methane production. Thus, the increase of the  $\text{CO}_2$  level in this case confirms the high bacterial activity concluded above [28].

As shown in Fig. 7, the purification process for the produced biogas succeeded in increasing the average  $\text{CH}_4$  content from within the range 55.35%–63.45% before the purification process, to within the range 92.53%–95.40% after the purification process.

### 3.2. Effect of inoculum VS organic loads on co-digestion of PS, WS and BH

#### 3.2.1. Daily and cumulative methane production

The daily flow rate of methane yields from co-digestion of WS, PS and BH at different inoculum VS organic loads is shown in Fig. 8. The figure shows that the peak values from individual digestion of PS, WS and BH were 18, 27.2 and 17.20 mL/g VSadd on the 16th, 6th and 14th day, respectively.

The peaks of methane production from co-digestion of PS, WS and BH at different inoculum VS organic loads of 18.0, 15.0, 12.0, 10.0, 7.0 and 3 g VS/L were found to be 32.50, 34.90, 30.50, 35.00, 27.10 and 25.30 mL/g VSadd, respectively. These values are higher than the peaks observed from single digestion of PS, WS and BH.

CMYs from co-digestion of PS, WS and BH at different inoculum VS organic loads are shown in Fig. 9. The figure shows that the CMYs from co-digestion of PS, WS and BH at inoculum VS organic loads of 18.0, 15.0, 12.0, 10.0, 7.0 and 3 g VS/L were 315.80, 487.52, 405.51, 307.62, 350.11 and 339.44 mL/g VSadd, respectively; this represents an increase of 2.07, 3.20, 2.66, 2.02, 2.30 and 2.23 times more than the individual digestion of PS, an increase of 1.20, 1.85, 1.54, 1.17, 1.33 and 1.19 times more than the digestion of WS separately, and higher CMYs of 1.45, 2.24, 1.86, 1.41, 1.61 and 1.56 times more than the individual digestion of BH.

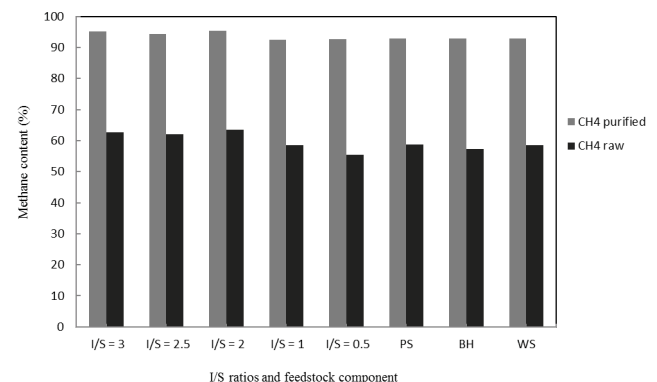


Fig. 7. Average  $\text{CH}_4$  content in biogas produced from co-digestion of PS, WS and BH at different I/S ratios before and after the purification process.

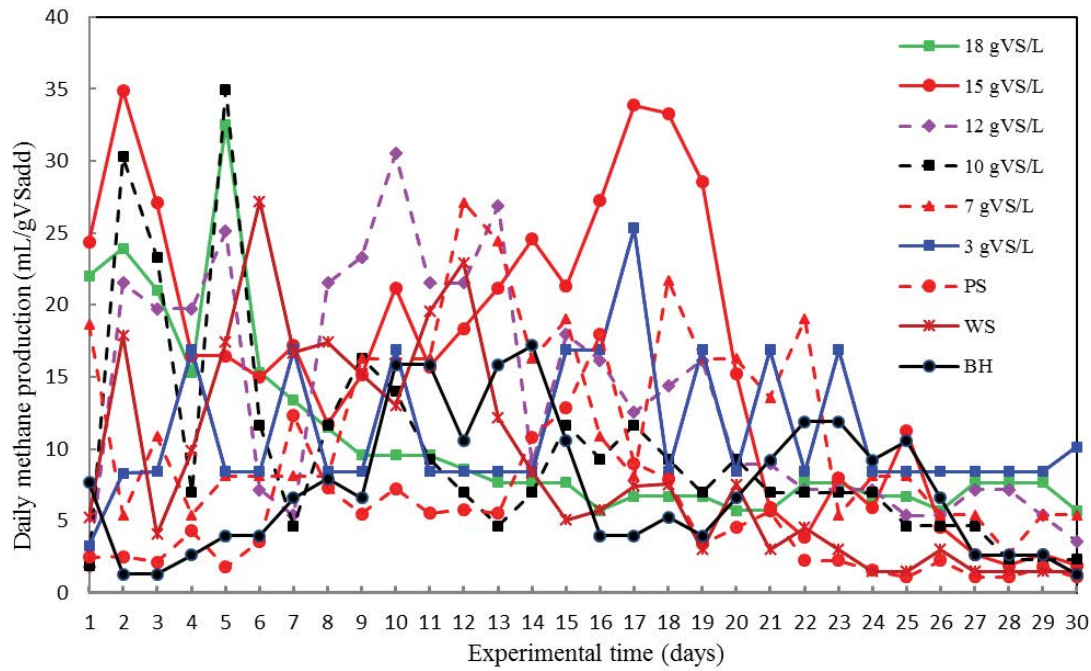


Fig. 8. Variations in daily methane production from the co-digestion of PS, WS and BH at different inoculum VS organic loads.

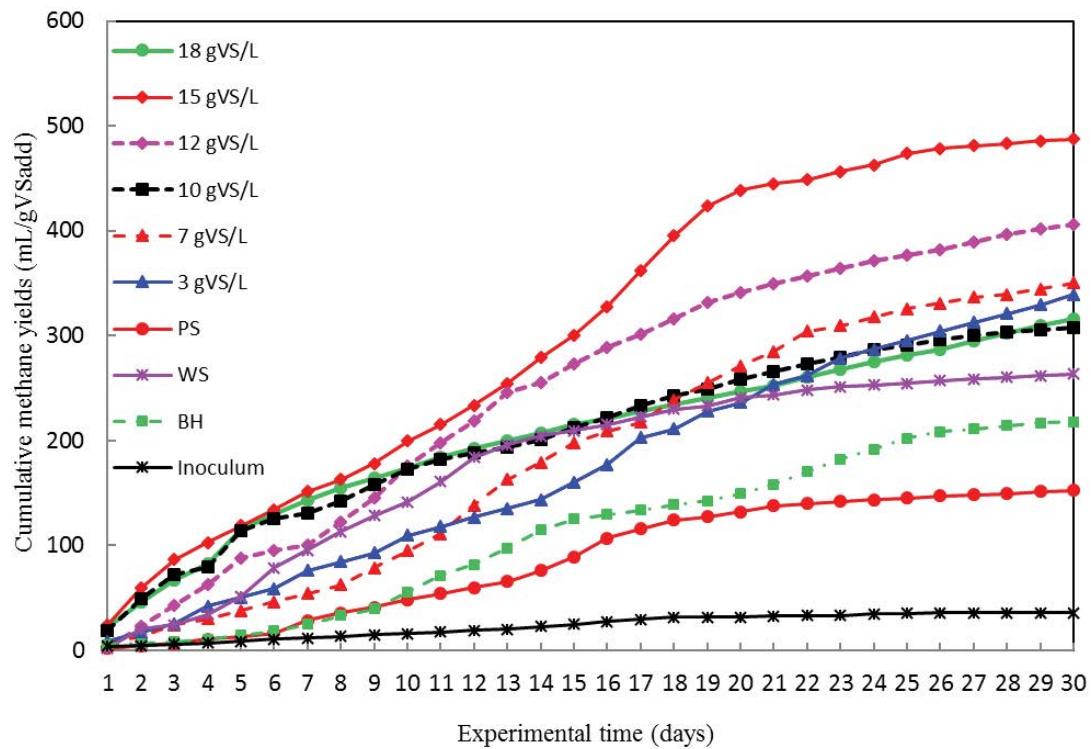


Fig. 9. Cumulative methane yields from the anaerobic co-digestion of PS, WS and BH at different inoculum VS organic loads.

As shown in Fig. 9, when the inoculum VS organic loads increased, the CMYs increased gradually to peak at 15 g VS/L of organic load, and then decreased. The highest CMY was obtained at an organic load of 15 g VS/L (487.52 mL/g VSadd), which showed an increase of 1.54, 1.20,

1.58, 1.39 and 1.44 times more than values recorded with the other organic loads of 18, 12, 10, 7 and 3 g VS/L, respectively; the lowest CMYs were recorded at an organic load of 10 g VS/L (307.62 mL/g VSadd). The results obtained report that anaerobic co-digestion of sludge, straw and buckwheat

husk using optimized inoculum VS organic loads can greatly increase CH<sub>4</sub> yields and the stability of feed materials [25]. In anaerobic digestion, it is known that an increase in inoculum concentration is responsible for the accentuation of substance conversion into methane. The inoculum concentration also affects the adaptive period of methanogenic bacteria and their sensitivity to inhibitory effects [29].

The statistical analysis carried out in this part was to compare the mean values of cumulative methane yields for the six different levels of inoculum VS organic loads (ANOVA test). The *P*-estimation of the *F*-test was under 0.05. The inoculum VS organic load of 15 g VS/L was considered the optimal VS organic load, producing the highest CH<sub>4</sub> yield (Fig. 10).

In this study, the maximum CMY recorded using different inoculum VS organic loads was 487.52 mL/g VSadd (at 15 g VS/L), which was higher than the CMY values reported elsewhere, such as Wei et al. [30] (233.40 mL/g VSadd), Abdul Razaque [26] (322 CH<sub>4</sub>/g VSadded), and Gunaseelan [31] (300.93 mL/g VSadd). The increase in biogas production does not follow a linear evolution vs. the VS organic loads, which confirms the results found in the literature. Indeed, at high concentration of VS organic loads anaerobic digestion inhibition can take place for various reasons. Montes et al. [32] have studied the effect of organic load on biogas production in a UASB reactor and showed that the methane potential increases with the increase in the VS organic loads until values where the opposite phenomenon has occurred. Indeed, an inhibitory effect of the anaerobic digestion is observed by the medium acidification. For Browne and Murphy [33], the methane production reduction can be induced by the increase of the total ammonia nitrogen rate in the digester at high VS organic loads. This phenomenon is accompanied by the pH increase. The same conclusion was reported by Zahan et al. [34] during their co-digestion experiments of chicken litter, food waste, wheat straw and hay grass.

### 3.2.2. Methane percentage in produced biogas

Zhao et al. [21] showed that produced biogas can be used immediately to generate energy. However, the heating value of biogas decreases if the percentage of CO<sub>2</sub> is increased. The average methane content from co-digestion of PS, WS and BH at different inoculum VS organic loads is shown in Fig. 11. The figure shows that the maximum methane content

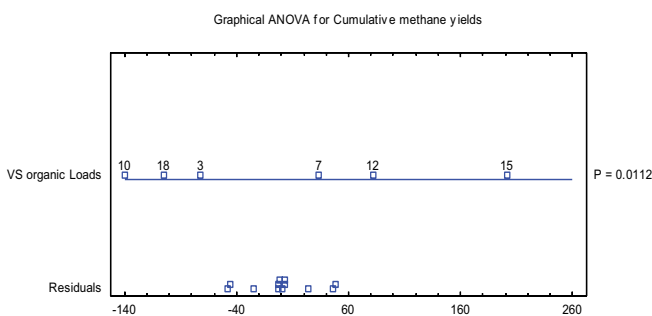


Fig. 10. Graphical ANOVA analysis of VS organic loads factor for cumulative methane yields (mL/g VSadd).

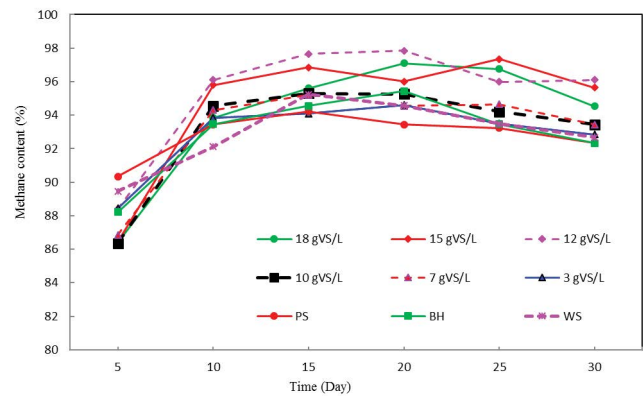


Fig. 11. Average methane percentage from co-digestion of PS, WS and BH based on their different inoculum VS organic loads.

was recorded at inoculum VS organic loads of 12 and 15, while the minimum was reported at inoculum VS organic loads of 3. As shown in Fig. 12, the purification process for the produced biogas succeeded in increasing the average CH<sub>4</sub> content from within the range 53.30%–63.11% before the purification process, to within the range 92.89%–95.35% after the purification process.

### 3.2.3. Characterization of digested feedstocks

pH stands out as one of the most significant parameters affecting the growth of bacteria. It is also an effective way of following the progress of anaerobic digestion and providing a process regulation, and should be kept within the acceptable range of 6.5 to 8 to ensure a successful anaerobic digestion process occurs in the reactor [24]. In this study, the measured pH value for all reactors during the biochemical methane potential test ranged between 7.25 and 7.64, which was considered the acceptable range for a good methanation process.

The volatile solids removal rate from co-digestion of PS, WS and BH at different inoculum VS organic loads is shown in Fig. 13. The maximum VS removal was observed at inoculum VS organic loads of 15 gVS/L (15.33%), while the

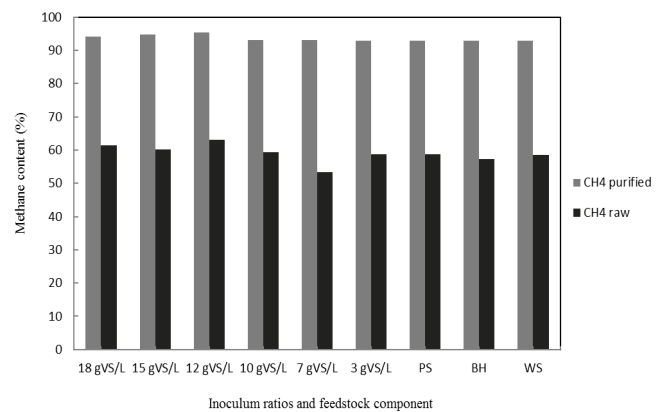


Fig. 12. Average CH<sub>4</sub> content in biogas produced from co-digestion of PS, WS and BH based on their different inoculum VS organic loads before and after the purification process.



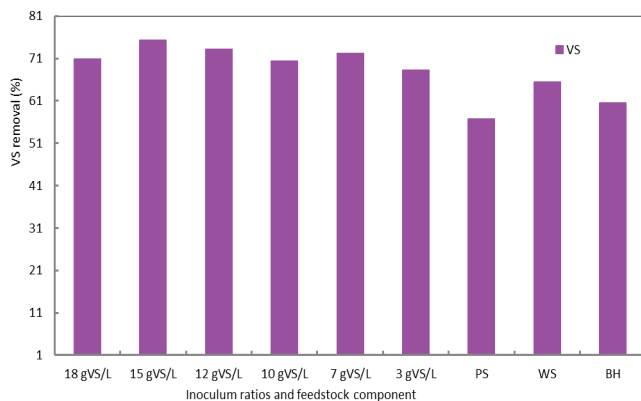


Fig. 13. Average volatile solids (VS) removal rate from co-digestions of PS, WS and BH based on their different inoculum VS organic loads.

lowest value was recorded at inoculum VS organic loads of 3 gVS/L. Which comply with the methane productivity.

#### 4. Conclusions

In this research, two batch tests were carried out. In the first experiment the optimal I/S ratio for multi-substrates was tested using different blends of sludge, straw and buckwheat husk depending on their I/S ratios. The highest CMY was recorded at an I/S ratio of 3 - higher than the single digestion of PS, WS and BH, while the minimum value was observed at I/S ratio of 0.5. The optimal  $\text{CH}_4$  yields, recorded at an I/S ratio of 3, may be due to the percentage of inoculum affecting the stability of the reactor and the feed materials. In the second experiment, the optimal inoculum VS organic loads, which enhance  $\text{CH}_4$  yields, was obtained. The cumulative methane curves of the co-digestion of these substrates show a low production kinetics at the first days (between 5 and 15 d depending on the media composition). This lag-phase time is generally observed with lignocellulosic substrates, which are not easily biodegradable. The maximum CMY and VS removal were observed at an inoculum VS organic load of 15 gVS/L, higher than the individual digestion of sludge, straw and buckwheat husk. However, the minimum value was recorded at an inoculum VS organic load of 10 gVS/L. This may be as a result of providing balance nutrients and a good condition for micro-organisms to grow. It has also been shown that at high VS organic loads and I/S ratios the lag-phase time is more important. Thereafter, the anaerobic digestion is controlled by the internal parameters of the digester such as the pH effect. After this phase, an increase in the methane production is observed. It has also been shown that at high VS organic loads and I/S ratios the lag-phase time is more important. Thereafter, the anaerobic digestion is controlled by the internal parameters of the digester such as the pH effect which can in some cases increased or decreased depending on whether the process evolves towards the production of the volatile fatty acids or the total ammoniac nitrogen. Finally, no pH variations were observed during BMP tests for the different combinations of concentration. The results reported that the anaerobic co-digestion of sludge, straw and buckwheat husk at the best inoculum VS

organic loads with the addition of optimized I/S ratios can improve  $\text{CH}_4$  yields.

#### Acknowledgements

This work was supported by GEPEA UMR CNRS 6144 (IMT Atlantique, France) and Aswan University (Egypt).

#### References

- [1] D. Bolzonella, P. Pavan, P. Battistoni, F. Cecchi, Mesophilic anaerobic digestion of waste activated sludge: influence of the solid retention time in the wastewater treatment process, *Process Biochem.*, 40 (2005) 1453–1460.
- [2] Y. Wei, R.T. Van Houten, A.R. Borger, D.H. Eikelboom, Y. Fan, Minimization of excess sludge production for biological wastewater treatment, *Water Res.*, 37 (2003) 4453–4467.
- [3] M. Pradel, A.L. Reverdy, M. Richard, L. Chabat, Environmental Impacts of Sewage Sludge Treatment and Disposal Routes: A Life Cycle Assessment Perspective, 4th European Conference on Sludge Management, May 26 2014, p. 8.
- [4] M. Elsayed, Y. Andres, W. Blel, A. Gad, Methane production by anaerobic co-digestion of sewage sludge and wheat straw under mesophilic conditions, *Int. J. Sci. Technol. Res.*, 4 (2015) 1–6.
- [5] M. Pohl, J. Mumme, K. Heeg, E. Nettmann, Thermo- and mesophilic anaerobic digestion of wheat straw by the upflow anaerobic solid-state (UASS) process, *Bioresour. Technol.*, 124 (2012) 321–327.
- [6] FAOSTAT, Food and Agricultural Commodities Production/ Commodities by Regions, Food and Agriculture Organization of the United Nations, <http://faostat3.fao.org/compare/E>, Accessed 21 April 2015.
- [7] Y. Yao, A.D. Bergeron, M. Davaritoucheae, Methane recovery from anaerobic digestion of urea-pretreated wheat straw, *Renewable Energy*, 115 (2018) 139–148.
- [8] F. Raposo, V. Fernández-Cegri, M.A. De la Rubia, R. Borja, F. Beline, C. Cavinato, G. Demirel, B. Fernández, M. Fernández-Polanco, R. Frigonv Ganesh, P. Kaparaju, J. Koubova, R. Mendez, G. Menin, A. Peene, P. Scherer, M. Torrijos, H. Uellendahl, I. Wierinck, V. Wilde, Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study, *J. Chem. Technol. Biotechnol.*, 86 (2011) 1088–1098.
- [9] G. Liu, R. Zhang, H.M. El-Mashad, R. Dong, Effect of feed to inoculum ratios on biogas yields of food and green wastes, *Bioresour. Technol.*, 100 (2009) 5103–5108.
- [10] Ç. Akyol, B. Demirel, T.T. Onay, Recovery of methane from tannery sludge: the effect of inoculum to substrate ratio and solids content, *J. Mater. Cycles Waste Manage.*, 17 (2015) 808–815.
- [11] F. Raposo, C.J. Banks, I. Siebert, S. Heaven, R. Borja, Influence of inoculum to substrate ratio on the biochemical methane potential of maize in batch tests, *Process Biochem.*, 41 (2006) 1444–1450.
- [12] D.P. Chynoweth, C.E. Turick, J.M. Owens, D.E. Jerger, M.W. Peck, Biochemical methane potential of biomass and waste feedstocks, *Biomass Bioenergy*, 5 (1993) 95–111.
- [13] C. Gonzalez-Fernandez, P.A. Garcia-Encina, Impact of substrate to inoculum ratio in anaerobic digestion of swine slurry, *Biomass Bioenergy*, 33 (2009) 1065–1069.
- [14] A.G. Hashimoto, Effect of inoculum/substrate ratio on methane yield and production rate from straw, *Biol. Wastes*, 28 (1989) 247–255.
- [15] T. Forster-Carneiro, M. Pérez, L.I. Romero, Influence of total solid and inoculum contents on performance of anaerobic reactors treating food waste, *Bioresour. Technol.*, 99 (2008) 6994–7002.
- [16] S. Dechruga, D. Kantachote, S. Chairapat, Effects of inoculum to substrate ratio, substrate mix ratio and inoculum source on batch co-digestion of grass and pig manure, *Bioresour. Technol.*, 146 (2013) 101–108.

- [17] M. Elsayed, Y. Andres, W. Blel, A. Gad, A. Ahmed, Effect of VS organic loads and buckwheat husk on methane production by anaerobic co-digestion of primary sludge and wheat straw, *Energy Convers. Manage.*, 117 (2016) 538–547.
- [18] Z. Yong, Y. Dong, X. Zhang, T. Tan, Anaerobic co-digestion of food waste and straw for biogas production, *Renewable Energy*, 78 (2015) 527–530.
- [19] V. Hansen, V. Schmidt, V. Angelidaki, V. Marca, V. Mosbæk, V. Christensen, Measurement of methane potentials of solid organic waste, *Waste Manage.*, 24 (2004) 393–400.
- [20] APHA, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington DC, 1998.
- [21] Q. Zhao, E. Leonhardt, C. MacConnell, C. Frear, S. Chen, Purification Technologies for Biogas Generated by Anaerobic Digestion, Compressed Biomethane, CSANR Research Report, 2010.
- [22] C. Rico, D. Diego, A. Valcarce, J. Rico, Biogas Production from various typical organic wastes generated in the region of Cantabria (Spain): methane yields and co-digestion tests, *Smart Grid Renewable Energy*, 5 (2014) 128–136.
- [23] J. Mata-Alvarez, J. Dosta, M.S. Romero-Güiza, X. Fonoll, M. Peces, S. Astals, A critical review on anaerobic co-digestion achievements between 2010 and 2013, *Renewable Sustainable Energy Rev.*, 36 (2014) 412–427.
- [24] N. Sibiya, E. Muzenda, A Review of Biogas Production Optimization from Grass Silage, International Conference on Chemical Engineering and Advanced Computational Technologies, Nov. 24–25, Pretoria, South Africa, 2014.
- [25] X. Wang, G. Yang, F. Yongzhong, R. Guangxin, H. Xinhui, Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw, *Bioresour. Technol.*, 120 (2012) 78–83.
- [26] S. Abdul Razaque, R. Mahar, K. BROHI, Anaerobic biodegradability and methane potential of crop residue co-digested with buffalo dung, *Mehran Univ. Res. J. Eng. Technol.*, 32 (2013) 509–518.
- [27] M. Herout, J. Malafák, L. Kučera, T. Dlabaja, Biogas composition depending on the type of plant biomass used, *Res. Agric. Eng.*, 57 (2011) 137–143.
- [28] F. Müller, G.C. Maack, W. Buescher, Effects of biogas substrate recirculation on methane yield and efficiency of a liquid-manure-based biogas plant, *Energies*, 10 (2017) 325.
- [29] R.R. Birch, C. Biver, R. Campagna, W.E. Gledhill, U. Pagga, J. Steber, H. Reust, W.J. Bontinck, Screening of chemicals for anaerobic biodegradability, *Chemosphere*, 19 (1989) 1527–1550.
- [30] S. Wei, H. Zhang, X. Cai, J. Xu, J. Fang, H. Liu, Psychrophilic anaerobic co-digestion of highland barley straw with two animal manures at high altitude for enhancing biogas production, *Energy Convers. Manage.*, 88 (2014) 40–48.
- [31] V.N. Gunaseelan, Anaerobic digestion of biomass for methane production: a review, *Biomass Bioenergy*, 13 (1997) 83–114.
- [32] J.A. Montes, R. Leivas, D. Martínez-Prieto, C. Rico, Biogas production from the liquid waste of distilled gin production: optimization of UASB reactor performance with increasing organic loading rate for co-digestion with swine wastewater, *Bioresour. Technol.*, 274 (2019) 43–47.
- [33] J.D. Browne, J.D. Murphy, The impact of increasing organic loading in two phase digestion of food waste, *Renewable Energy*, 71 (2014) 69–76.
- [34] Z. Zahan, S. Georgiou, T.H. Muster, M.Z. Othman, Semi-continuous anaerobic co-digestion of chicken litter with agricultural and food wastes: a case study on the effect of carbon/nitrogen ratio, substrates mixing ratio and organic loading, *Bioresour. Technol.*, 270 (2018) 245–254.