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## **Role of the thickness of medium on solid-state anaerobic digestion**

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## Abstract

In solid-state anaerobic digestion, the production of methane is limited compared to liquid-state anaerobic digestion. One of the possible reasons of this limitation could be the reduction of the diffusion of the molecules due to the high total solid content, creating local inhibitory environment. This study investigates the effect of the thickness of the reaction environment and by extension the effect of the diffusion on solid-state anaerobic digestion to evaluate this hypothesis. Two kind of anaerobic reactors with different thicknesses of a wheat-straw based medium (1.0 and 4.2 cm) were investigated for 33 days. The results showed an inhibition of methanogenesis for 4.2 cm height, which generated a gradient of propionate all along the medium thickness. This gradient was likely due to a local accumulation of  $H_2$ , and a high partial pressure of hydrogen can result in an inhibition of the hydrolysis step. This result could explain why a high total solid content in solid-state anaerobic digestion results in an inhibition of the global anaerobic digestion, due to the local accumulation of inhibitory products. Therefore, mixing of solid-state anaerobic digester is mandatory to improve the methane production.

## Novelty statement

Dry or solid-state anaerobic digestion is characterized by a total solid content of at least 15%. This kind of anaerobic digestion processes are highly interesting for waste management-industries. However, the diffusion and gas-liquid transfer of dissolved metabolites are reduced by the high total solid content. In this study, we investigated the effect of the thickness of the reaction environment, and by extension the effect of the gas-liquid transfer kinetics on solid-state anaerobic digestion was investigated to prove that mass transfer limitations could be responsible for a local accumulation of inhibitory intermediates, such as  $H_2$  or VFAs. When the mass transfer was limited, methanogenesis was inhibited locally, which generated a gradient of the fermentative metabolites all along the medium thickness. Therefore, the local accumulation of inhibitory products resulted in a global inhibition of solid-state anaerobic digestion due to the with the decrease of the diffusion and gas-liquid mass transfer. This manuscript presents an original and highly novel research work investigating the effect of a lower mass transfer during solid-state anaerobic digestion processes and the formation of inhibitory gradients.

## Keywords

VFAs; hydrogen; dry anaerobic digestion; diffusion; solid-state anaerobic digestion

## 1 Introduction

Anaerobic digestion corresponds to the degradation of the organic matter into a biogas mainly composed of  $CH_4$  and  $CO_2$  by anaerobic microorganisms. A digested organic matter, so called digestate, is also produced and can be used as fertilizer in agriculture under some sanitary and environmental restrictions. This global biological conversion results from a high number of microbial reactions within a complex microbial ecosystem. Meanwhile, the anaerobic digestion process can be simply described by four main microbial steps: the first step, called hydrolysis, corresponds to the transformation of organic matter into soluble molecules by extracellular enzymes [1]. This step is usually considered as the limiting step for anaerobic digestion using solid matters as a substrate [2]. Then, amino-acids, saccharides and fatty acids are converted into volatile fatty acids (VFAs) or into other organics acids, such as lactate, or in alcohols during acidogenesis step. During acetogenesis, VFAs, alcohols and fatty acids are all converted into  $H_2$ ,  $CO_2$  and acetate by syntrophic acetogens also called Obligate Hydrogen Producing Bacteria (OHPB) [3]. The OHPB are syntrophs of methanogens since the reactions are thermodynamically unfavorable and therefore require the presence of methanogenic archaea consuming the products.  $H_2$  and  $CO_2$  are also converted into acetate by no-syntrophic homoacetogens [3], which is thermodynamically favourable and does not require the presence of methanogenic archaea. During the last step, called methanogenesis, acetate,  $H_2$  and  $CO_2$  are converted into  $CH_4$  by acetotrophic methanogens using acetate as substrate and producing

70% of CH<sub>4</sub> in anaerobic digestion (Pavlostathis and Giraldo-Gomez, 1991) and by hydrogenotrophic methanogens using CO<sub>2</sub> and H<sub>2</sub> as substrates.

Solid-state anaerobic digestion (SS-AD) or dry anaerobic digestion corresponds to anaerobic digestion with total solids (TS) content over 15% [4]. The development of solid-state anaerobic digestion plants in Europe [4, 5] can be related to operational advantages compared to wet anaerobic digestion: e.g., reduction of water, energy demand... However, a high number of hurdles limits the development of solid-state anaerobic digestion such as a decrease of the methane yield, microbial inhibitions or the complexity of the rheological behaviour [5]. Specific researches have been carried out at a macroscopic scale, in batch flask or in continuous laboratory reactors, to evaluate the effect of TS content on solid-state anaerobic digestion. Increasing the TS content always led to a reduction of the hydrogen and methane yields, methane production rates and substrate conversion. High solid content is also associated to a change in metabolic pathways, leading to an increase of the VFAs concentration, a decrease of the pH and an increase of the activity of hydrogenotrophic methanogens [5–10]. Abbassi-Guendouz et al. (2012) and Veluchamy and Kalamdhad (2017) have suggested that the reduction of the performance was due to the decrease of the mass transfer and of the diffusion of soluble molecules within the reacting medium.

Mass transport of soluble compounds (i.e., dissolved gases and soluble metabolites) is driven by two distinct mechanisms: (i) convective transport, related to the digester mixing and (ii) diffusive transport. Depending on the technology, solid-state anaerobic digestion systems are static or difficult to mix. The poor mixing efficiency [12] related to the complex rheological behaviour of the digesting medium [13] can induce low convective transport, and the mass transfer of soluble compounds is thus driven by diffusive transport. The diffusive transport is also strongly affected by the high TS content in solid-state anaerobic digestion systems. Bollon et al. (2013) have determined experimentally the diffusion coefficients in high-solid digested medium and found low diffusion coefficients in the digestate compared to the water. For example, the diffusion coefficients of iodide ion I<sup>-</sup> at 8% TS and 25% TS were 55 to 185 times lower than its diffusion coefficient in water, respectively. Therefore, this low diffusion rate can generate local areas with different local environmental conditions in terms of VFAs concentration (i.e. pH) or dissolved H<sub>2</sub> and CO<sub>2</sub>. For instance, Staley et al. (2011) reported the existence of a correlation between pH, i.e. through VFAs accumulation in the local environment of the microorganisms, and the water content, ranging from 50% to 95%, in landfill. The heterogeneity of the medium may then be responsible for the occurrence of neutral niches which may serve to initiate methanogenesis. Indeed, with a small diffusion rate of soluble compounds, the concentration of inhibitory products can be heterogeneously distributed within the medium, and can conduct to a formation of very distinct zones with concentration gradients of VFAs, pH or even microorganisms for dry solid-state [16–18]. Therefore, the model has 2 different zones: hydrolysis, acidogenesis and acetogenesis would be performed mostly in a first zone with a high concentration of VFAs and an acidic pH, and methanogenesis would be mostly performed in a second zone with a low concentration of VFAs and a basic pH, due to a slow diffusion of soluble VFAs. This model implies a spatialization of the VFAs within the medium, with a gradient concentration of the VFAs from the first zone to the second zone.

The objective of this study was to investigate the effect of the thickness of the medium on the performance of SS-AD. Since the diffusive transport of the soluble compounds is limited under high solid content, a spatialization of local environments is expected from the bottom of the reactor to the interface with the headspace. The effect of two different thicknesses for the medium on the global performance of SS-AD was assessed and the spatialized distribution of metabolites during 33 days of SS-AD was also analysed in the medium.

## 2 Materials and methods

### 2.1 Description of the reactors

Two sets of reactors were operated with the same conditions (inoculation, S/I ratio, TS content, temperature, quantity of the reacting medium). The only difference is the thickness of the medium, and thus the distance for the diffusion of the molecules within the medium.

The first reactors consisted in cylindrical tubes with a height of 8.7 cm and a diameter of 3 cm. 14.5 g of the substrate was mixed with the inoculum to obtain TS of 25%; this mixture was packed to obtain a height of 4.2 cm (Figure 1). The headspace was then flushed with nitrogen. The reactors were incubated in mesophilic conditions (35°C) for 33 days. Twelve reactors were prepared, and four replicates were sacrificed after 8, 20 and 33 days. 7 mm slices of the medium were sampled using the plunger to separate each fraction, and each slice was characterized in composition and microorganisms.

The second set of reactors corresponded to 600 mL flasks filled with 14.5 g of medium, mixed with the inoculum to obtain 25% of TS. This mixture was distributed at the bottom in a thin layer of 1.0 cm (Figure 2). Nine reactors were prepared and incubated under the same conditions than the first reactors (tubes) and three replicates were sacrificed at 8, 20 and 33 days to analyse the composition of the medium in VFAs and microorganisms.

### 2.2 Substrate

Wheat straw (*Triticum aestivum*) was used as a substrate. It was first crushed using a cutting miller through a 1 mm grid, and further sieved between grids of 1 mm and 400  $\mu\text{m}$ . The TS content of crushed wheat straw was 95%. The BMP of this wheat straw was estimated at  $237.3 \pm 1 \text{ mL CH}_4 \cdot \text{gVS}^{-1}$  (measured by BMP flash©; Lesteur et al., 2011).

### 2.3 Operating conditions

Industrial UASB sludge was used to inoculate the batch reactors. The sludge was mixed during 24 h at 35°C and centrifuged (7 841 g, 20 min, and 4°C) to obtain an anaerobic inoculum with TS content of 12%. Both types of reactors were operated with the same conditions. The initial total solid (TS) content was fixed at 25 %, which corresponds to solid-state anaerobic digestion. This TS content should not have any inhibitory effect on AD [6, 20] but corresponds to a limited diffusion due to the high TS content [11]. A substrate/inoculum ratio of 3 (in volatile solids basis) was used, as suggested by Liew et al., (2012). A solution of trace elements (FeCl<sub>2</sub> 2g/L, CoCl<sub>2</sub> 0.5g/L, MnCl<sub>2</sub> 0.1 g/L, NiCl<sub>2</sub> 0.1 g/L, ZnCl<sub>2</sub> 0.05 g/L, H<sub>3</sub>BO<sub>3</sub> 0.05g/L, Na<sub>2</sub>SeO<sub>3</sub> 0.05g/L, CuCl<sub>2</sub> 0.04 g/L, Na<sub>2</sub>MoO<sub>4</sub> 0.01g/L) was added with a volume of 0.2 mL by reactor. A buffer solution of bicarbonate (0.0026 g H<sub>2</sub>CO<sub>3</sub>/g substrate) was used to keep the pH around 8.

### 2.4 Analytical methods

Volumetric biogas production was determined through the variation of the total pressure and the daily biogas composition was measured using a gas chromatograph (Perkin Clarus 580). It was composed of an injector heated at 250°C and two capillary columns heated at 60°C. The first column was an RtUbond for the CO<sub>2</sub>. The second column was an RtMolsieve used for the detection of the O<sub>2</sub>, H<sub>2</sub>, N<sub>2</sub> and CH<sub>4</sub>. The carrier gas was argon at pressure of 350 kPa at 31.8 mL.min<sup>-1</sup>. The detection was ensured by a thermal conductivity detector at 150°C.

Metabolites were quantified by diluting 2 g of medium in 8 g of deionized water for 30 minutes, centrifuged during 20 min at 39 121 g and 4°C, and then filtrated with a 0.2  $\mu\text{m}$  nylon membrane. Then, VFAs were measured with a gas chromatograph Perkin Clarus 580 with an Elite-FFAP crossbond® carbowax® 15 m column connected to a flame ionization detector at 280°C and N<sub>2</sub> at 6 mL.min<sup>-1</sup> as carrier gas [7]. Other metabolites were also quantified using high performance liquid chromatograph. This chromatograph was composed of an automatic sampler (Water 717), a pre-column to filter residues (Micro guard cation H refill

cartridges, Bio-rad) and an Aminex HPX-87H column (300 mm on 7.8 mm, Bio-rad) at 35°C. The eluting solution was sulfuric acid at 0.005 M at 0.4 mL.min<sup>-1</sup>.

## 2.5 Data analysis

R software (version 2.15.2) coupled with the package Rcmdr (version 1.8-4) was used to analyse statistically the data using an ANOVA analysis. Non-significant p-values were fixed at >0.05 and significant p-values were estimated as <0.05; <0.01 and <0.001.

Total substrate degradation (expressed in Chemical Oxygen Demand – COD) was estimated theoretically from a mass balance between the start-up and the sampling date using the protocol of Cazier et al., (2015). A COD mass balance was performed by accounting from the concentration of metabolites (acetate, propionate, butyrate, valerate, caproate, succinate, ethanol, formic acid...) and the cumulated biogas produced. The total substrate degradation was expressed according to initial TS content of wheat straw, as showed in Eq. (1):

$$\text{Total Substrate Degradation} = \frac{A_{H_2,f} + A_{CH_4,f} + A_{met,f} + A_{GC}}{g\ TS} - \frac{A_{met,i}}{g\ TS} \text{ Eq. (1)}$$

with  $A_{H_2,f}$  the final amount of H<sub>2</sub> produced in the headspace,  $A_{CH_4,f}$  the final amount of CH<sub>4</sub> accumulated in headspace,  $A_{met,f}$  the final amount of metabolites accumulated,  $A_{GC}$  the total amount of gas (H<sub>2</sub> and CH<sub>4</sub>) sampled for biogas analyses and  $A_{met,i}$  the initial amount of metabolites in the medium.

## 3 Results

### 3.1 Effect of medium thickness on the performance of solid-state anaerobic digestion

The cumulative production of biogas and methane is shown in Figure 3 for the two different reactor configurations: flasks (medium thickness ~ 1 cm) and tubes (medium thickness = 4.2 cm). After 33 days of reaction, biogas and methane yields were significantly higher in the flasks (117 ± 22 mL.g<sub>TS</sub><sup>-1</sup> and 86 ± 26 mL<sub>CH<sub>4</sub></sub>.g<sub>TS</sub><sup>-1</sup>, respectively) than in the tubes (9.1 ± 2 mL.g<sub>TS</sub><sup>-1</sup> and 9.0 ± 2 mL<sub>CH<sub>4</sub></sub>.g<sub>TS</sub><sup>-1</sup>, respectively). The CH<sub>4</sub> production in the flasks increased from 0 to 26 days (methane yield of 83 ± 18 mL<sub>CH<sub>4</sub></sub>.g<sub>TS</sub><sup>-1</sup> on day 26) and then stabilized from 26 to 33 days (final methane yield of 86 ± 22 mL<sub>CH<sub>4</sub></sub>.g<sub>TS</sub><sup>-1</sup>). The CH<sub>4</sub> yield in the flasks was however lower than the theoretical BMP value for the wheat straw (237 ± 1 mL<sub>CH<sub>4</sub></sub>.g<sub>TS</sub><sup>-1</sup>, measured by BMP flash©, [19]), which might be due to the high TS content (over 20%) which can decrease the overall performances of anaerobic digestion [6, 11]. Carbon dioxide production was higher for the flasks (91 ± 21 mL<sub>CO<sub>2</sub></sub>.g<sub>TS</sub><sup>-1</sup> at 33 days) than for the tubes (0.10 ± 0.01 mL<sub>CO<sub>2</sub></sub>.g<sub>TS</sub><sup>-1</sup> at 33 days).

In the tubes, the CH<sub>4</sub> production was very low from the first days of the experiment (from 3 ± 0.2 mL<sub>CH<sub>4</sub></sub>.g<sub>TS</sub><sup>-1</sup> after one day to 9 ± 2 mL<sub>CH<sub>4</sub></sub>.g<sub>TS</sub><sup>-1</sup> after 33 days). Therefore, CH<sub>4</sub> production was strongly inhibited when the medium was set up in a thick layer compared to a thin layer. The medium thickness thus significantly affected the methane production.

The substrate degradation, estimated from the biogas production and the metabolites production (Eq. (1)), is presented in Figure 4 for the two reactors configurations at three different times (8, 20 and 33 days). The total substrate degradation was higher in the flasks (245 ± 65 g<sub>COD</sub>.kg<sub>TS</sub><sup>-1</sup>) than in the tubes (28 ± 9 g<sub>COD</sub>.kg<sub>TS</sub><sup>-1</sup>) after 33 days of operation. In the flasks, the total substrate degradation increased with time, from 79 ± 18 g<sub>COD</sub>.kg<sub>TS</sub><sup>-1</sup> at 8 days to 245 ± 65 g<sub>COD</sub>.kg<sub>TS</sub><sup>-1</sup> at 33 days. Meanwhile, the substrate degradation was not statistically different in time in the tubes, from 17 ± 7 at 8 days to 28 ± 8 g<sub>COD</sub>.kg<sub>TS</sub><sup>-1</sup> at 33 days, with the similarity of the substrate degradation at 8, 20 and 33 days validated by ANOVA with a p-value > 0.01. Therefore, it can be concluded that the hydrolysis step was inhibited in the tubes.

The composition of the metabolic by-products was different in the flasks and in the tubes. In the flasks, 96% of the substrate was converted into CH<sub>4</sub> and only 4% into VFAs at day 33. In the tubes, the composition evolved during the time of operation. For example, at day 8, 45% of the degraded substrate was converted into CH<sub>4</sub> and 55% into VFAs, which was different from day 33, with 71% converted into CH<sub>4</sub> and 29% into VFAs. Therefore, methanogenesis was the predominant reaction in the flasks but not the predominant reactions in the tubes at 8 days. Moreover, the acetoclastic methanogenesis seems to be the predominant methanogenesis reaction in the tubes since the VFAs concentration decreased between 8 and 20 days to produce methane.

### 3.2 Spatial distribution of VFAs in thick layers (tubes)

The concentrations of metabolites were analysed in the tubes every 7 mm after 8, 20 and 33 days of operation. Profiles of metabolite concentrations are represented in Figure 5. First, no significant gradient of VFAs concentration was detected at 8 days. Indeed, the concentrations of the total metabolites were not statistically different (ANOVA) along the medium depth ( $6 \pm 5 \text{ g}_{\text{COD}}\cdot\text{kg}_{\text{TS}}^{-1}$  for the layer 0-7 mm and  $15 \pm 6 \text{ g}_{\text{COD}}\cdot\text{kg}_{\text{TS}}^{-1}$  for the layer 35-42 mm). However, a strong gradient of VFAs concentration appeared at 20 days, with an increase of metabolites concentration with the depth, from  $4 \pm 1 \text{ g}_{\text{COD}}\cdot\text{kg}_{\text{TS}}^{-1}$  for the layer 0-7 mm to  $23 \pm 8 \text{ g}_{\text{COD}}\cdot\text{kg}_{\text{TS}}^{-1}$  for the layer 35-42 mm. The difference of metabolites concentrations between the top and bottom layers (i.e., 0-7 mm and 35-42 mm) was validated by an ANOVA test, with a p-value of 0.0301. The gradient of concentration was smaller at 33 days than at 20 days, from  $9 \pm 1 \text{ g}_{\text{COD}}\cdot\text{kg}_{\text{TS}}^{-1}$  for the layer 0-7 mm to  $15 \pm 1 \text{ g}_{\text{COD}}\cdot\text{kg}_{\text{TS}}^{-1}$  for the layer 35-42 mm, with also an increase of p-value to 0.0721 (ANOVA). Consequently, a spatialization of the metabolites concentrations was detected in the tubes, with the lowest concentration close to the interface between the gas headspace and the digested medium, especially at 20 days. Gradients of metabolites concentration could be due to the fact that the medium is thicker and diffusion rates of soluble compounds and dissolved gas are lower for high TS content [14], [17, 18].

The total metabolites concentration for the layer 0-7 mm corresponded to the total metabolites concentration in the flasks (thin layer), and they did not change significantly during the experiment, from  $5 \pm 4 \text{ g}_{\text{COD}}\cdot\text{kg}_{\text{TS}}^{-1}$  at 8 days,  $4 \pm 3 \text{ g}_{\text{COD}}\cdot\text{kg}_{\text{TS}}^{-1}$  at 20 days and to  $6 \pm 3 \text{ g}_{\text{COD}}\cdot\text{kg}_{\text{TS}}^{-1}$  at 33 days. This result confirmed that microorganisms behaviour were the same in the thin layer of medium in the flasks and in the 0-7 mm of the tubes, when the diffusion does not have any influence on microorganisms and metabolites.

The composition of metabolites also changed with time in the tubes, especially the percentage of acetate and propionate. At 8 days, the composition of the metabolites was statistically identical (ANOVA, with P-value > 0.05) all along the medium thickness: acetate and propionate corresponded to  $37 \pm 22 \%$  and  $26 \pm 24 \%$  of the total metabolites (in terms of COD), respectively. At 20 days, the composition of metabolites was statistically different (P-value < 0.01) for the layer 0-7 mm ( $34 \pm 9 \%$  acetate and  $19 \pm 10 \%$  propionate, similar to the composition at 8 days), and the layer 35-42 mm ( $21 \pm 16 \%$  acetate and  $47 \pm 16 \%$  propionate). At 33 days, the composition of the metabolites was again statistically identical for all layers (ANOVA, P-value > 0.05) and the percentage of acetate increased compared to 20 days to  $61 \pm 16 \%$  with a decrease of the propionate to  $7 \pm 4 \%$ . Therefore, acetate and propionate were the principal metabolites produced, with graduation concentration visible at 20 days but not at 8 and 33 days. In fact, at 20 days, the propionate concentration varied between 0-7 mm ( $0.8 \pm 0.5 \text{ g}_{\text{COD}}\cdot\text{kg}_{\text{TS}}^{-1}$ ) and 35-42 mm ( $11 \pm 6 \text{ g}_{\text{COD}}\cdot\text{kg}_{\text{TS}}^{-1}$ ) (Figure 5) and was responsible of the increase of the total metabolites concentration with the depth. The increase of the percentage of acetate in the total metabolites between 20 and 33 days was related to the decrease of the propionate concentration during the same period. Indeed, the propionate may be consumed to produce acetate (Eq. (3) in Table 1) [23]. The H<sub>2</sub> and CO<sub>2</sub> produced during this reaction may also be used to produce acetate (Eq. (4) in Table 1). Using Eq. (3) and (4), the propionate consumed during 20 and

33 days (when adding the concentration in each depths in the tubes) may produce at the maximum 7.19 g<sub>COD</sub>.kg<sub>TS</sub><sup>-1</sup> of acetate, which is equal to the acetate measured at 33 days (6.41 ± 3.31 g<sub>COD</sub>.kg<sub>TS</sub><sup>-1</sup>). These results demonstrate a change in main metabolic pathways, from acidogenesis at day 20 to acetogenesis at day 33.

Table 1: Free reaction enthalpies for the production of propionate from H<sub>2</sub> and the consumption of propionate and H<sub>2</sub> to produce acetate [23, 24]

Equations	$\Delta G^0$ (kJ.mol <sup>-1</sup> )	Eq. number
$C_6H_{12}O_6 + 2 H_2 \leftrightarrow 2 C_2H_5COO^- + 2 H^+ + 2 H_2O$	-359	(2)
$C_2H_5COO^- + 3 H_2O \leftrightarrow CH_3COO^- + HCO_3^- + H^+ + 3 H_2$	+76	(3)
$4 H_2 + HCO_3^- + H^+ \leftrightarrow CH_3COO^- + 3 H_2O$	-105	(4)

#### 4 Discussion

Experimental results showed a significant effect of the thickness of the reaction medium on the performance of solid-state anaerobic digestion. In particular, compared to reactors operated with a thin layer, both the methane production and substrate degradation were inhibited when the thickness of the medium was higher, and a VFAs gradient was visible at 20 days in the tubes. The difference of results between the thin and the thick layer could be explained by the limitation of the diffusion in the medium since the total solid content was high (25%) [6, 11] [14], which will also inhibit the methane production and the hydrolysis inhibition [11, 25]. This study showed the effect of the diffusion on the limitation of substrate degradation since it was only inhibited when the thickness of the medium was higher. However, the relationship between the decrease of the diffusion, the inhibition of the hydrolysis and the mechanisms need to be proved.

The decrease of the diffusion may result into a decrease of the pH, an increase of the metabolites concentration or of the local concentration of H<sub>2</sub> which will be directly responsible of the methane production inhibition. However, the pH was the same in the flasks than in the tubes and decreased slightly during the experiment, from 8.73 ± 0.03 at 8 days to 8.13 ± 0.29 at 33 days for the flasks and from 8.74 ± 0.22 at 8 days to 8.52 ± 0.28 at 33 days for the tubes, maybe due to the production of VFAs. The pH had therefore no inhibitory effect on the hydrolysis step since it was always higher than 6 [26]. In addition, the total metabolites concentration (VFAs, ethanol, lactic acid) in the tubes stayed around 13 ± 8 g<sub>COD</sub>.kg<sub>TS</sub><sup>-1</sup>, which corresponded to a maximum of 4.5 g.L<sup>-1</sup> of VFAs. Such VFAs concentration is below the inhibitory level reported in the literature, i.e. 20 g.L<sup>-1</sup>, for CH<sub>4</sub> inhibition at pH 7 [27] and 30 g.L<sup>-1</sup> for hydrolysis inhibition at pH 6 [26]. Therefore, the pH and the metabolites concentration were likely not responsible for the inhibition of the hydrolysis step.

One interesting effect was the apparition of propionate at 20 days in the tubes. Propionate can be produced during acidogenesis by glucose and H<sub>2</sub> (Eq. (2) in Table 1) [24]. Therefore, one hypothesis to explain the gradient of propionate concentration could be the fact that dissolved hydrogen could be produced in the medium without diffusing to the headspace due to the limitation of the diffusion inside the medium [6], i.e. hydrogen was produced and consumed to produce propionate before it could diffuse to the headspace. Moreover, the low concentration of propionate at 0-7 mm could be due to the consumption of H<sub>2</sub> with the CO<sub>2</sub> present in the headspace to produce CH<sub>4</sub>, which would also decrease the H<sub>2</sub> concentration in 0-7 mm compared to 35-42 mm and therefore decrease the propionate produced from dissolved H<sub>2</sub>. Both phenomena could be responsible for the propionate concentration gradient before 20 days, which would then be due to



a concentration gradient of dissolved  $H_2$  due to the limitation of the diffusion of the gas in the medium. Additionally, the hypothesis of the consumption of  $H_2$  with the  $CO_2$  present in the headspace may explain why the  $CO_2$  present in the headspace was very low in the tubes compared to the flasks, since  $CO_2$  is the limiting reactive agent when  $P_{H_2}$  is high [22]. Moreover, the metabolic pathways using propionate to produce methane may be limited for solid-state anaerobic digestion compared to liquid anaerobic digestion; as showed by Zhou et al., (2019) with the decrease of the conversion of propionate to methane by 45% with 28%, which could also explain the accumulation of propionate in the tubes during the experiment.

Moreover, the hypothesis of the presence of a  $H_2$  gradient would also be an explanation for the hydrolysis limitation with medium thickness of 4.2 cm. Indeed,  $H_2$  is known to be an inhibitor of the hydrolysis step in solid-state anaerobic digestion [22]. Previous experiments showed an inhibition of the hydrolysis for an inhibitory atmospheric  $P_{H_2}$  over 800 mbars for wheat straw [22], with a decrease of the methane production for atmospheric  $P_{H_2}$  over 500 mbars. Moreover, when the hydrogen slowly diffuses in the medium, due to the limitation of the diffusion at high TS, its concentration will be lower and therefore may be consumed by the microorganisms faster than the diffusion rate needed to go through 4.2 cm of medium, which would explain why there was no hydrogen in the headspace during 8 to 33 days in the tubes, with the dissolved hydrogen used to produce methane and propionate. It would also explain why the substrate degradation increased between 8 and 20 days, with a limitation of the hydrolysis step but not between 20 and 30 days, due to the time needed to accumulate  $H_2$  in a high concentration which will inhibit the hydrolysis. Therefore, a gradient of dissolved  $H_2$  in the medium for 25% TS may be indirectly proved by a gradient of propionate concentration (as seen as 20 days), but also global metabolites. The accumulation of dissolved  $H_2$  would be higher at 35-42 mm, due to the limitation of the diffusion in the tubes compared to the flasks. A gradient of metabolites concentration was still visible at 33 days, mostly of acetate but the difference of metabolites concentration between 0-7 cm and 35-42 cm was reduced, due to a change of the main reaction in the medium from acidogenesis at 20 days to acetogenesis at 33 days.

## 5 Conclusion

This study showed an inhibition of the substrate degradation in the tubes compared to the flasks, due to the increase of the thickness of the medium. The limitation of the diffusion had also an effect on the VFAs with a gradient of propionate at 20 days and acetate at 33 days in the medium. This gradient could be due to local hydrogen accumulation in the tubes, also forming a concentration gradient in the tubes. Therefore, solid state anaerobic digestion performance seems limited by the thickness and the diffusion, responsible for local accumulation of  $H_2$  and VFAs. Mixing the medium therefore seems to be necessary to improve solid-state anaerobic digester.

### Declarations:

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**Authors's contributions:** E.A. Cazier did the experimentation and the writing of the paper, E. Trably, R. Escudié supervised and reviewed the paper, J.P. Steyer reviewed the paper.

### Data availability statement:

Authors can confirm that all relevant data are included in the article and/or its supplementary information files

### Figures list

Figure 1: Experimental design of the tubes, with a working volume of 0.118 L and a total volume of 0.197L, to analyse the composition of metabolites and microorganisms every 7 mm in the medium during anaerobic digestion at 25% of TS content, 35°C and pH 8.

Figure 2: Experimental design of the flasks, with a working volume of 0.254 L and a total volume of 0.6 L, for the higher specific surface (thin layer of medium) during anaerobic digestion at 25% of TS content, 35°C and pH 8.

Figure 3: Cumulated methane production in  $\text{mL.g}_{\text{TS}}^{-1}$  in tubes (a 4.2cm thick layer of medium) and flasks (a layer of medium < 1cm) during 33 days at 25% TS, 35°C and pH 8.5

Figure 4: Total substrate degradation in  $\text{g}_{\text{COD.kg}_{\text{TS}}^{-1}}$  in tubes ( a 4.2cm thick layer of medium) and flasks (a layer of medium < 1cm) at 8, 20 and 33 days of anaerobic digestion at 25% of TS content, 35°C and pH 8.

Figure 5: Metabolites concentration in  $\text{g}_{\text{COD.kg}_{\text{TS}}^{-1}}$  in 7mm slices according to the depth of the medium at 8, 20 and 33 days. 0 corresponds to the surface near the headspace, and 42 to the bottom pf the reactor.

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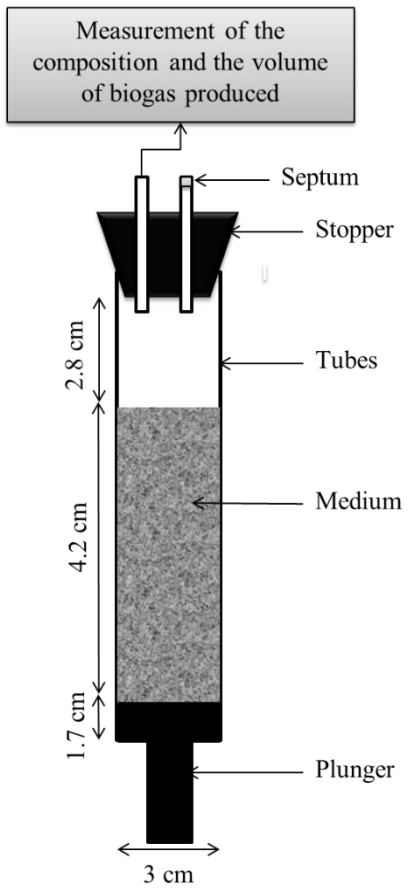


Fig 1.

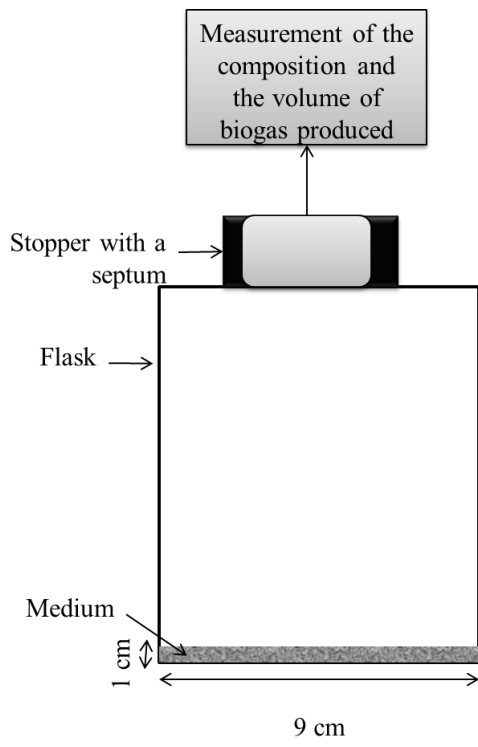


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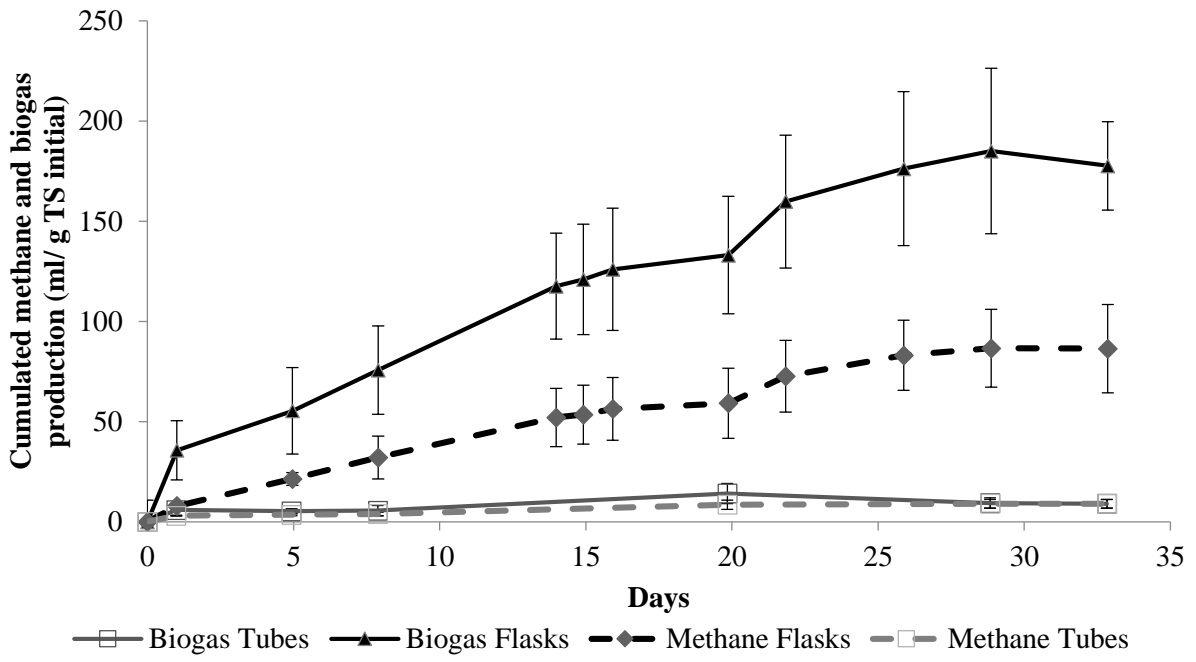


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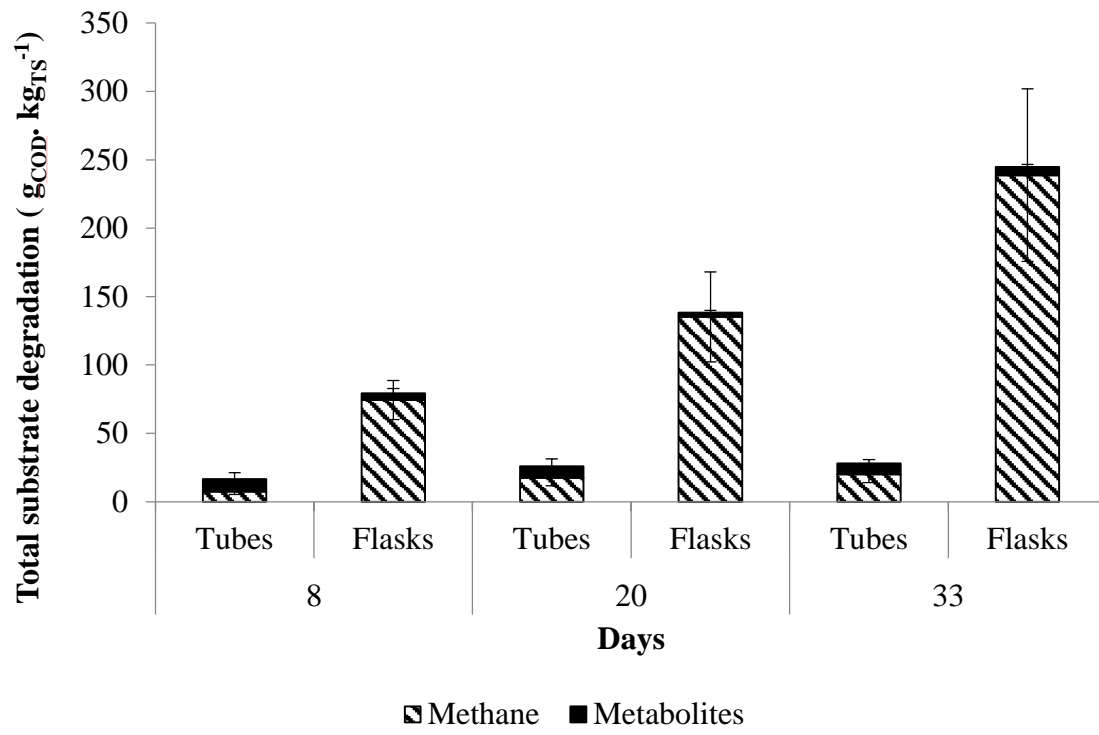


Fig 4.

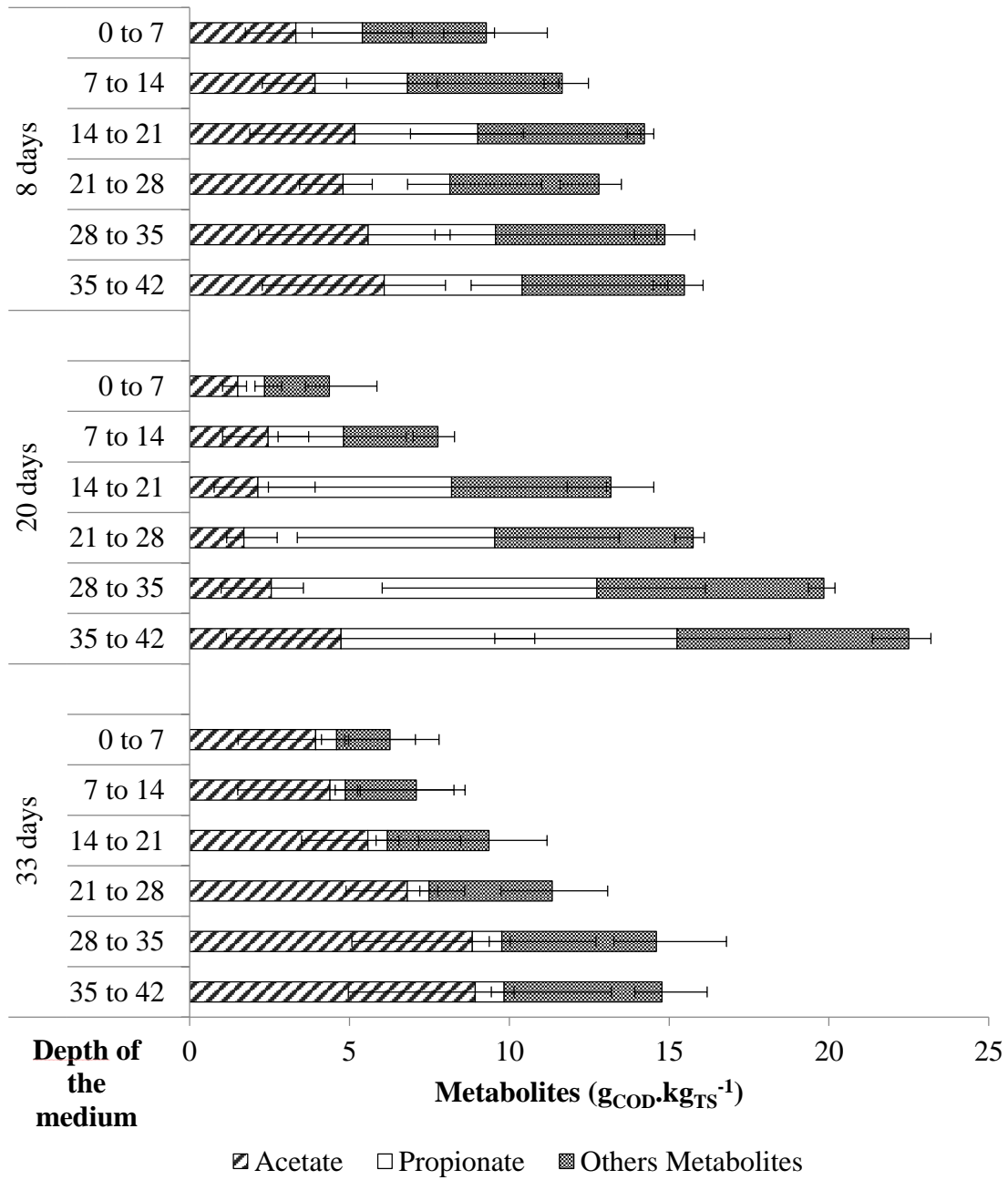


Fig 5.