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Evaluation of different types of anaerobic seed sludge for the high rate anaerobic digestion of pig slurry in UASB reactors

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Abstract

Three different types of anaerobic sludge (granular, thickened digestate and anaerobic sewage) were evaluated as seed inoculum sources for the high rate anaerobic digestion of pig slurry in UASB reactors. Granular sludge performance was optimal, allowing a high efficiency process yielding a volumetric methane production rate of $4.1 \text{ L CH}_4 \text{ L}^{-1} \text{ d}^{-1}$ at 1.5 days HRT ($0.248 \text{ L CH}_4 \text{ g}^{-1} \text{ COD}$) at an organic loading rate of $16.4 \text{ g COD L}^{-1} \text{ d}^{-1}$. The thickened digestate sludge experimented flotation problems, thus resulting inappropriate for the UASB process. The anaerobic sewage sludge reactor experimented biomass wash-out, but allowed high process efficiency operation at 3 days HRT, yielding a volumetric methane production rate of $1.7 \text{ L CH}_4 \text{ L}^{-1} \text{ d}^{-1}$ ($0.236 \text{ L CH}_4 \text{ g}^{-1} \text{ COD}$) at an organic loading rate of $7.2 \text{ g COD L}^{-1} \text{ d}^{-1}$. To guarantee the success of the UASB process, the settleable solids of the slurry must be previously removed.

Keywords: Granular sludge, anaerobic sewage sludge, supernatant, volumetric methane production rate, thickened digestate

1. Introduction

The concentration of industrial pig farms involves the increase and concentration of animal waste production. When handled improperly this waste causes, among other environmental problems, greenhouse gas emissions and soil and groundwater pollution by nitrates (Xu et al., 2016).

Intensive pig farms use cleaning systems in which pressurized water is used to carry and handle the waste. This system facilitates the movement of the waste and improves the hygiene and the sanitary conditions of the farms. However, an enormous disadvantage is the complication of the management of increased volumes of slurry, the semi-liquid manure resulting from the mixture of defecations, washing water and residues of food and bedding.

Thermal dehydration in centralized facilities was the most widespread treatment option for pig slurry in Spain in areas with high farming density, due to a strong institutional support. The process used waste heat from combined heat and power plants (CHP), fueled by natural gas. This marginalized other alternatives such as anaerobic digestion. However, the reform of the electricity sector by the Royal Decree 413/2014 (MITT, 2014), which substantially reduced the price of cogenerated kWh, made this technical-financial mechanism economically unsustainable. As a consequence, all of the cogeneration facilities processing pig slurry in Spain were closed in 2014. In this context, future pig slurry treatment systems must be efficient, environmentally friendly and economically sustainable without the help of artificial bonuses that can be withdrawn via a change in legislation.

From an energy-efficient point of view, the anaerobic digestion of pig slurry is much more adequate. When the slurry decomposes in the absence of oxygen it produces methane, a renewable fuel, contributing to environmental protection and energy demand reduction (Zhou et al., 2016). This option, instead of consuming energy, produces it and the resulting energy can be used by the farms themselves, while the digested slurry is usable as fertilizer with improved properties (Antezana et al., 2016; Massé et al., 2011). This practice would imply compliance with one of the basic ecological principles, namely, closing the cycle of raw materials.

Despite the benefits of the anaerobic digestion, the dilution of manure with cleaning water reduces the energy potential in the slurry due to its relative low volatile solids (VS) content. It implies low biogas production rates in continuous stirred tank reactor systems (CSTR) currently used for manure anaerobic digestion (Bergland et al., 2015), making the economic viability of pig slurry biogas plants in conventional wet anaerobic facilities difficult (Vu et al., 2016; Yang et al., 2015).

Under this scenario, if technically viable, the high rate anaerobic digestion technology in upflow anaerobic sludge blanket (UASB) reactor can provide a more efficient solution for the anaerobic treatment of pig slurry. Compared with the CSTR reactor, the main advantages of the UASB reactor are smaller reactor size (lower investment costs) and the fact that mechanical stirrers are not required (lower investment and operational costs). This opens up the possibility of using UASB technology not only in large pig farms, but also in medium and small-sized premises due to small reactor size and the low investment and operating costs.

The UASB reactor concept was created and developed by Gatzke Lettinga and collaborators (Lettinga et al., 1980). The feasibility of the UASB reactor has been sufficiently demonstrated for treating mainly soluble wastewaters. One of the basic conditions for the success of high rate anaerobic digestion process in UASB reactors is the development of granular sludge, a highly settleable sludge with high methanogenic activity (Hulshoff Pol, et. al, 1983). However, there is no consensus about the mechanism and operation conditions triggering granulation (Chong et al., 2012; Hulshoff Pol et. al, 2004). High ammonia nitrogen concentration has been suggested as a factor that prevents granulation in UASB reactors (Hulshoff Pol et. al, 1983). This could be the reason for the lack of granulation in UASB reactors treating animal manure slurry. When granulation does not occur, granules from another UASB reactor can be used to seed the reactor with granular biomass. In this case, availability of granules from another UASB reactor is required. It is also important that granules do not disintegrate due to the change in the feed characteristics and operation conditions (Weiland and Rozzi, 1991).

If granular biomass is unavailable, the UASB reactor can perform efficiently without granules (Chong et al., 2012; Lettinga and Hulshoff Pol, 1991). However, the lower settling capacity of the sludge implies that a lower organic loading rate should be applied, thus resulting in higher hydraulic retention time (HRT) and higher reactor volume. Anaerobic sewage sludge or other types of anaerobic digested sludge can be used to seed the UASB reactor. The main advantage of the anaerobic sewage sludge is the higher availability of this anaerobic biomass. Another type of anaerobic biomass, such as digestate from agro-industrial biogas plants may have the advantage of being adapted to the substrate to be treated in the UASB reactor. Accordingly, digestate from

a conventional biogas plant processing pig manure can provide microorganisms acclimated to the ammonia nitrogen levels of pig slurry.

On the other hand, the presence of suspended solids in the UASB feed can hinder the success of the process by reducing the specific methanogenic activity of the sludge, promoting the formation of scum layers, affecting the stability of granular biomass and leading to its spontaneous and sudden wash-out (Lettinga et al., 1991). In a recent work, Bergland et al. (2015) studied the efficiency, flexibility and stability of pig manure supernatant in UASB reactors seeded with granular biomass, concluding that the treatment of pig manure supernatant in UASB reactors is technically feasible.

The objective of the present work is to evaluate the anaerobic treatment of pig slurry in UASB reactors with three different types of seed sludge with the aim to determine the capacity of the inocula to reach rapid stable operation conditions at high efficiency performance levels. Thickened digestate from an agro-industrial biogas plant, anaerobic sewage sludge and granular biomass were the seed inoculum assayed. The study has been developed with filtered pig slurry and raw pig slurry as UASB feed. The UASB reactor performance was evaluated in terms of methane and biogas production, volatile fatty acids evolution and process stability under increasing organic loading rates. The biochemical methane potential of the filtered slurry and the raw slurry were also determined to evaluate the efficiency in terms of methane yields obtained in the UASB process.

2. Materials and methods

2.1 Pig slurry

The UASB reactors were fed with raw and filtered pig slurry from a closed-cycle pig production farm. The raw pig slurry (RPS) was collected from the storage pit and the filtered slurry (FPS) was the result of filtering the raw slurry through a 100 μm textile filter. Both the filtered and the raw slurry were stored at 4°C until use.

2.2. UASB seed

Three different types of sludge were used to seed the UASB reactors with the aim to compare the performance of the UASB process with different inoculum sources: thickened digestate (TDS) from an agro-industrial biogas plant that processes pig slurry and slaughterhouse wastes (108.7 g TS kg^{-1} sludge and 86.7 g VS kg^{-1} sludge), anaerobic sewage sludge (ASS) from an anaerobic digester of a wastewater treatment plant (33.5 g TS kg^{-1} sludge and 18.4 g VS kg^{-1} sludge) and granular biomass (GR) from an industrial UASB reactor treating wastewaters from a bioethanol production plant (90.2 g TS kg^{-1} sludge and 71.3 g VS kg^{-1} sludge).

2.3. UASB reactor and mode of operation

Lab-scale UASB reactors with an operating volume of 1 L were used (1.3 L total volume). The UASB reactors were cylindrical, made of plexiglass, measuring 65 mm in internal diameter and 450 mm in height. In the upper part, the reactor was provided with a gas-liquid-solid separator device similar to those described in the literature for UASB reactors (Lettinga and Hulshoff Pol, 1991). The feed was prepared daily and entered the reactor by its lower zone and the effluent left the reactor by means of an exit tube on the upper part of the reactor. The reactor was fed in semi-continuous mode in 15-minute cycles by means of a temporized peristaltic pump. A stable reactor temperature was maintained at $36 \pm 1^\circ\text{C}$ in the UASB reactor using a thermostat-controlled electric

heating blanket. Biogas left the reactor through the gas collector by its own pressure. The biogas in the UASB reactor was collected daily in one or two 5-L gas tedlar bags, depending on the amount produced. The volume of biogas was measured by connecting the gas bags to a liquid displacement system device.

The reactors inoculated with granular and thickened digestate sludge were filled with 500 g of biomass (wet weight). On the other hand, due to the lower VS content of the anaerobic sewage sludge, the reactor seeded with this inoculum was filled with 1000 g of sludge (wet weight).

The reactors were first filled with the seed sludge and with the filtered slurry and batch operated for a week to facilitate a progressive acclimatization of the microorganisms and to observe the settling behavior of the sludge. Then, the reactors were started up at 3 days HRT with the filtered slurry as feed. Steady state was assumed when the operation conditions were maintained for three HRTs and the reactor showed stability. At this point, HRT was reduced by increasing the daily feed flow. This operation was repeated until reactor efficiency decreased or operational problems occurred. Operation with raw slurry was only planned in the event of successful operation with the filtered slurry for each of the seed inocula used in the UASB reactors.

2.4. Biochemical Methane Potential tests

Biochemical methane potential (BMP) tests for the raw and the filtered slurry were carried out with the three types of sludge used as UASB seed inoculum. The test was performed for each substrate and inoculum in triplicate in 250-mL serum bottles capped with rubber septum sleeve stoppers, following the methodology described in Valero et al. (2016).

For the BMP determination, each bottle was filled with 150 g (wet weight) mixture of substrate and inoculum with a $VS_{\text{inoculum}}/VS_{\text{substrate}}$ ratio of 2. For each inoculum, the blanks were also assayed in triplicate to measure methane potential of the inocula. Results are expressed as means \pm SD subtracting methane production from the blanks. In order to complete the set-up of the reactors, they were flushed with nitrogen to remove the oxygen in the headspace of the bottles, and then incubated at 37°C for 35 days. Gas production was determined by pressure measurement. The pressure was taken from the headspace of the reactors through the septum with a syringe connected to a digital pressure sensor with a silicon measuring cell (ifm, type PN78, up to 2 bar).

2.5 Analytical techniques

The volatile fatty acids (VFA) were determined using a HP6890 gas chromatograph (GC) fitted with a 2 m 1/8-in glass column, liquid phase 10% AT 1000, packed with the solid-support Chromosorb W-AW 80/100 mesh. Nitrogen was used as the carrier gas at a flow rate of 14 mL/min, and a FID detector was installed. The VFA concentrations are expressed in COD units. The biogas composition was assayed on a 2 m Poropak T column in a HP 6890 GC system with helium as the carrier gas at a flow rate of 15 mL/min with a TCD detector. The biogas and methane production measurements are expressed at 0°C and standard pressure of 760 mm Hg (NCTP) in dry conditions. The influent and effluent pHs were measured from samples with a glass electrode pH meter (WTW, SENTIX 21). Total Solids (TS), Volatile Solids (VS), Chemical Oxygen Demand (COD), Total Ammonia Nitrogen (TAN) and alkalinity were performed according to standard methods (APHA, 1998).

3. Results and discussion

3.1 Characteristics of the filtered and the raw slurry

According to the characteristics of the raw and the filtered slurry (Table 1), it can be said that the characteristics of the latter are quite favorable for the UASB process. Some doubts might arise about the raw slurry due to its particulate matter content. Both substrates present a high ratio $\text{COD}_{\text{VFA}}/\text{COD}$ as well as a high alkalinity content which will result in high COD conversion to methane and protection against acidification. The total ammonia nitrogen (TAN) levels in both substrates are not so suitable for anaerobic digestion, but are below serious inhibition levels reported in the literature. Ammonia inhibition has been reported at TAN concentrations values above 1.7 g L^{-1} for unacclimated biomass (Chen et al. 2008). However, free ammonia (FA) is the toxic agent, and thus pH and temperature play an important role in this kind of inhibition. Thermophilic processes are more sensitive to high TAN levels due to higher FA/TAN ratios. Increased tolerance as a result of biomass acclimatization has allowed anaerobic digestion under TAN concentration as high as 8 g TAN L^{-1} (Parkin et al., 1983). Concerning ammonia inhibition in the anaerobic digestion of pig manure, Hansen et al. (1998) reported a threshold inhibition value of 1.1 g FA L^{-1} , below which the process was uninhibited. Taking into account the TAN content of the pig slurry used in this work (2.9 g TAN L^{-1}), a pH of 8.7 or higher would result in FA values higher than 1.1 g FA L^{-1} at 35°C . Therefore, the TAN content in the pig slurry used in this work should not inhibit the process, but a short period of acclimatization can be expected for the biomass not previously adapted to these TAN levels.

With regards to particulate matter, the filtered slurry should not be problematic for the UASB process. The insoluble COD fraction accounts only for 20% of the total COD and the TSS are below 6 g TSS L^{-1} . With these characteristics the high rate UASB

process should perform successfully at organic loading rates (OLR) between 15 and 24 g COD L⁻¹ d⁻¹ with granular sludge and between 5 and 8 g COD L⁻¹ d⁻¹ with flocculent sludge, according to the criteria reported by Lettinga and Hulshoof Pol (1991). In the case of the raw slurry, the insoluble COD fraction represents 35% of the total COD which calls into question the success of the high rate treatment according to the same criteria.

However, in view of an industrial application at farm scale, raw slurry is a much more profitable substrate. A filtration process implies an additional cost in filtration equipment and operation. In addition, the removal of organic matter through the filtration process would result in a substrate with lower methane potential than that of the raw slurry.

3.2 BMP test of raw and filtered slurry with different inocula

The cumulative methane production results from the BMP tests are depicted in Fig. 1. As expected from the different VS content, the filtered slurry yielded a lower volume of methane per liter of substrate than the raw slurry. The filtered slurry yielded 615 ± 8 mL CH₄ g⁻¹ VS (279 ± 5 mL CH₄ g⁻¹ COD), which results in 6.0 ± 0.1 L CH₄ per liter of filtered slurry. The raw slurry yielded 640 ± 12 mL CH₄ g⁻¹ VS (301 ± 4 mL CH₄ g⁻¹ COD), which results in 8.7 ± 0.1 L CH₄ per liter of raw slurry.

For both substrates, the three inocula attained similar methane potentials after 35 days.

However, the speed at which methane was produced throughout the test was significantly different for the different inoculum sources. Anaerobic sewage sludge was the inoculum that processed both the filtered and the raw slurry the fastest. The granular sludge and the thickened digestate showed a similar methane production rate evolution

throughout the test. The reason for the faster degradation rate observed with the sewage sludge can be attributed to the higher volume of inoculum used (due to its lower VS content), which diluted the ammonia of the pig slurry. Due to the higher volume of sewage sludge inoculum, the ammonia nitrogen levels from pig slurry in the BMP reactors was diminished by dilution with the inoculum (52% ASS inoculum with FPS, 60% ASS inoculum with RPS – wet weight), thus reducing the possibility of methanogenesis inhibition. The higher ammonia nitrogen levels in the BMP reactors with granular sludge can be the reason for the slower degradation rate with this inoculum source (21% GR inoculum with FPS, 28% GR inoculum with RPS – wet weight)). On the other hand, the ammonia nitrogen levels should not be the reason for the slower degradation rates in the bottles containing the thickened digestate inoculum. In this case, the thickened digestate showed an important residual methane yield. It implies that some of the VS that were assumed to be microorganisms, were in reality residual organic matter not processed in the biogas plant, which in turn results in a lower amount of active microorganisms.

Inoculum selection has been pointed out as an important factor for obtaining a reliable estimation of the methane potential of organic substrates (De Vrieze et al., 2015; Holliger et al., 2016). The similar results obtained with the three types of inoculum increase the reliability of the methane potential observed for the raw and the filtered slurry and the capacity of the inocula to process both substrates. These BMP results are particularly useful to evaluate the efficiency of the UASB process in terms of methane yields.

The high values of specific methane yields (SMY) can be explained by the VS determination method, which includes drying at 105°C and incineration (550°C).

During the drying phase, up to 75% of the VFA in the samples can be lost (Derikx et al., 1994). Therefore, the high VFA content in the pig slurry, together with its high contribution to the total organic matter, is the reason why the VS values obtained were lower than the real values, inflating the SMY. In this sense, a wide range of SMY can be found in the literature for pig slurry. Córdoba et al. (2016) observed an SMY of 256 mL CH₄ g⁻¹ VS for pig slurry with 7.21% TS and 6.23% VS content. Kafle and Chen (2016) reported 323 mL CH₄ g⁻¹ VS for pig manure with 31.02% and 26.93% TS and VS content. Møller et al. (2004) reported SMY between 329 and 403 mL CH₄ g⁻¹ VS for different samples of pig manure ranging from 16.7% to 22.3% VS content. With regards to SMY from pig slurry with lower solids content, Cestonaro do Amaral et al. (2016) reported SMY ranging between 170 and 642 mL CH₄ g⁻¹ VS for raw pig slurry samples (VS content between 0.5-2.6%) collected at different departments of two pig farms in Brazil. The same authors also reported a SMY value as high as 737 mL CH₄ g⁻¹ VS for supernatant liquid fraction of pig slurry (0.33% VS) collected from a sow farrowing house. Bergland et al. (2015) reported an SMY of 685 mL CH₄ g⁻¹ VS for pig manure supernatant with 14.5 g TS L⁻¹ and 7.3 g VS L⁻¹ content at continuous operation in UASB reactor at 48 h HRT.

3.3 UASB reactors performance

3.3.1 UASB seeded with granular sludge

The granular sludge occupied approximately 40% of the useful volume reactor and remained settled in the bottom part of the reactor during the batch period. Then, the reactor started to be continuously fed with the filtered slurry at 3 days HRT. The reactor produced biogas from the first operation day and the sludge blanket got expanded.

The performance of the reactor during operation with the filtered slurry in terms of volumetric methane production rate (VMPR) and methane yield is represented in Fig. 2a (days 1-34). During the first operation days, the methane production was lower than expected from the BMP results. Although the process was stable, the methane yield did not exceed $3.5 \text{ L CH}_4 \text{ L}^{-1}$ filtered slurry ($163 \text{ mL CH}_4 \text{ g}^{-1} \text{ COD}$), about 41% lower than the BMP value. From day 5, methane production progressively increased, stabilizing at a value around $5 \text{ L CH}_4 \text{ L}^{-1}$ filtered slurry ($233 \text{ mL CH}_4 \text{ g}^{-1} \text{ COD}$) after 18 operation days at 3 days HRT. When HRT was reduced at 2 days and afterwards at 1.5 days, the reactor rapidly reached steady state conditions increasing not only the VMPR, but also the methane yield, reaching stable values of $3.5 \text{ L CH}_4 \text{ L}^{-1} \text{ d}^{-1}$ and $5.3 \text{ L CH}_4 \text{ L}^{-1}$ filtered slurry ($247 \text{ mL CH}_4 \text{ g}^{-1} \text{ COD}$) at 1.5 days HRT. Taking the BMP value as a reference, the efficiency of the process (CH_4 UASB yield/BMP value) was 88%. At this point, the organic loading rate was $14.3 \text{ g COD L}^{-1} \text{ d}^{-1}$. When the HRT was set at 1.1 days, the reactor suffered biomass wash-out and was stopped after 34 days of operation. As can be deduced from the data in Fig. 2a, the wash-out was not critical because the VMPR was still increasing, reaching $4.0 \text{ L CH}_4 \text{ L}^{-1} \text{ d}^{-1}$. Despite the reactor showing potential to maintain stable operation at 1.1 days HRT, the feed was stopped in order to re-start the process with the raw slurry.

During the operation with the filtered slurry, the effluent pH values, as well as the methane content in biogas, showed symptoms of process robustness. The effluent pH values ranged between 8.6 ± 0.1 and 8.4 ± 0.1 for HRT from 3 to 1.5 days. At 1.1 days HRT, the pH dropped to 8.3 ± 0.1 . The alkalinity of the effluent was also stable with values ranging between 11.0 and $11.7 \text{ g CaCO}_3 \text{ L}^{-1}$. The methane content in the biogas decreased with the increasing organic loading rate, which is a consequence of increased

carbon dioxide production per unit volume of the liquid phase, saturating the liquid phase with CO₂ (Rico et al., 2015). The mean methane content percentage values in the biogas were 86.4 ± 1.3 , 84.4 ± 0.9 , 82.6 ± 0.7 and 81.5 ± 0.5 for 3, 2, 1.5 and 1.1 days HRT.

The VFA concentration in the effluent (Fig. 2b) corroborates the results described above. During the first operation days, the effluent had a concentration value of $5.2 \text{ g COD}_{\text{VFA}} \text{ L}^{-1}$. The increasing methane yield observed indicated a progressive acclimation of the granular biomass to the substrate and operation conditions. When the reactor reached higher methane yields, the VFA concentration in the effluent remained low in value, between 1.3 and $1.8 \text{ g COD}_{\text{VFA}} \text{ L}^{-1}$ at HRT of 3 and 2 days. At higher loading rates, when the HRT was set at 1.5 days, the VFA decreased to concentrations lower than $1 \text{ g COD}_{\text{VFA}} \text{ L}^{-1}$, which coincides with the highest methane yield values and the process efficiency observed. Propionic acid was the predominant VFA, followed by acetic.

After stopping the feed with the filtered slurry at day 34, the UASB reactor was maintained for two days without feeding to allow its degasification. At day 36, the reactor started to be fed with the raw slurry at an HRT of 3 days. The raw slurry formed a layer of sediments in the bottom of the feed vessel. To avoid the entrance of the settled sediments into the reactor, the slurry take-off point was placed above the sediments layer.

The reactor reached rapidly steady state conditions, as can be seen in Fig. 2a (days 36-62). After three days of operation, the reactor yielded a stable value of $6.1 \text{ L CH}_4 \text{ L}^{-1}$ raw slurry ($0.248 \text{ L CH}_4 \text{ g}^{-1} \text{ COD}$). When HRT was reduced to 2 days and afterwards to

1.5 days, the reactor, once again, showed a strong robustness. The methane yield remained at $6.1 \text{ L CH}_4 \text{ L}^{-1}$ raw slurry, whereas the VMPR increased up to $4.1 \text{ L CH}_4 \text{ L}^{-1} \text{ d}^{-1}$ at an HRT of 1.5 days, a value 17% higher than that for the filtered slurry at the same HRT. When the HRT was set at 1.1 days, the VMPR was a constant $4.1 \text{ L CH}_4 \text{ L}^{-1} \text{ d}^{-1}$, but the methane yield decreased to $4.6 \text{ L CH}_4 \text{ L}^{-1}$ pig slurry ($0.187 \text{ L CH}_4 \text{ g}^{-1} \text{ COD}$), indicating a decrease in the efficiency of the process. On the last two days of experimentation the slurry take-off point was placed at the bottom of the feed vessel, causing the entry of the settleable solids into the reactor. This, in turn, provoked a significant sudden wash-out of solids, including the anaerobic biomass. This suggests that the success of the process requires the removal of these sediments.

The effluent pH values were very similar to those observed for the filtered slurry, ranging between 8.6 ± 0.1 and 8.4 ± 0.1 for HRT from 3 to 1.5 days and dropping to 8.36 ± 0.1 at 1.1 days HRT. The alkalinity of the effluent was also stable with values ranging between 12.0 and $12.5 \text{ g CaCO}_3 \text{ L}^{-1}$, which prevents acidification. The methane content in the biogas was a bit lower compared with the filtered slurry, and also decreased with the increasing organic loading rate, the mean percentage values being 83.7 ± 0.8 , 82.7 ± 0.6 , 82.2 ± 0.6 and 80.4 ± 0.7 for 3, 2, 1.5 and 1.1 days HRT.

In this case, there was a bigger difference between the methane yield in the UASB process and the BMP value of the pig slurry: $6.1 \text{ L CH}_4 \text{ L}^{-1}$ raw slurry in the UASB process and $8.7 \text{ L CH}_4 \text{ L}^{-1}$ raw slurry in the BMP test. This can be explained by the fact that the raw slurry was homogenized for the BMP test, so that the settleable solids were introduced in the BMP reactor bottles. On the other hand, settleable solids were not allowed to enter into the UASB reactor, as it was fed just with the supernatant pig slurry. The low VFA content in the effluent corroborates that sediments removal was

the reason for this difference. The minimum presence of VFA in the effluent at HRT of 3 and 2 days shown in Fig. 2b (days 36-62) is indicative of the high process efficiency performance and proves that all the available biodegradable organic matter was being degraded. At 1.5 days HRT, VFA concentration in the effluent reached $1.6 \text{ g COD}_{\text{VFA}} \text{ L}^{-1}$, but at 1.1 days HRT the VFA value increased up to $3.3 \text{ g COD}_{\text{VFA}} \text{ L}^{-1}$, which confirms the decrease in the efficiency of the process. Finally, values of $5.0 \text{ g COD}_{\text{VFA}} \text{ L}^{-1}$ in the effluent were measured when the take-off point was placed at the bottom of the feed vessel.

3.3.2 UASB seeded with thickened digestate

The thickened digestate sludge occupied approximately 40% of the useful volume reactor and experimented sludge flotation from the first day of operation during batch operation. Some of the biogas produced was entrapped under the mass of sludge, causing its flotation. When the reactor started to be continuously fed with the filtered slurry, sludge flotation became an operational problem. The sludge blanket got broken in two parts and the biggest one got trapped under the gas-solid separation device. The first continuous-mode operation days resulted in poor reactor performance, as Fig. 3a shows. Methane yields were quite low compared to those attained with the granular sludge reactor, with values lower than $1.5 \text{ L CH}_4 \text{ L}^{-1} \text{ filtered slurry}$ ($70 \text{ mL CH}_4 \text{ g}^{-1} \text{ COD}$). The reason for the low efficiency of the reactor was probably the channeling caused by the sludge clogging, which hampered the contact between microorganisms and substrate. To cope with this, after six days of operation a propeller-shaped piece of plastic was placed at three-quarters of the reactor height, with the aim to stop the sludge flotation. The piece of plastic worked and stopped the rise of the sludge. Although the big block of sludge remained under the prop and did not settle to the bottom of the

reactor, the efficiency of the reactor improved progressively and after 26 days of operation, the reactor yielded $5.0 \text{ L CH}_4 \text{ L}^{-1}$ filtered slurry ($233 \text{ mL CH}_4 \text{ g}^{-1} \text{ COD}$), a similar value to that attained with the granular biomass. Then, the HRT was reduced to 2 days and the methane yield suffered a small decrease down to $4.3 \text{ L CH}_4 \text{ L}^{-1}$ filtered slurry ($200 \text{ mL CH}_4 \text{ g}^{-1} \text{ COD}$). The HRT was reduced to 1.5 days with the aim to create higher hydraulic turbulence to allow the release of the biogas entrapped in the sludge. However, the shorter HRT increased the operational problems. The methane yield continued to decrease and the reactor was stopped because the sludge rose above the plastic piece and some solids were expelled out with the biogas, ending up in the gas bag.

The effluent pH values ranged between 8.6 ± 0.1 and 8.4 ± 0.1 for HRT from 3 to 2 days. The alkalinity of the effluent ranged between 10.0 and $10.5 \text{ g CaCO}_3 \text{ L}^{-1}$. At 1.5 days HRT, the pH dropped to 8.2 ± 0.1 . The methane content in the biogas was a bit higher compared to the granular sludge reactor, and also decreased with the increasing organic loading rate, the mean percentage values being 87.0 ± 0.5 , 84.2 ± 0.5 and 83.5 ± 0.6 for 3, 2 and 1.5 days HRT.

The VFA levels in the effluent correspond with the methane yields observed during the experimentation. During the first days of operation, before the piece of plastic was installed inside the reactor, the concentration of VFA in the effluent was $8.1 \text{ g COD}_{\text{VFA}} \text{ L}^{-1}$. From then on, as methane yield increased, the VFA concentration in the effluent progressively decreased, dropping down to a value lower than $1.5 \text{ g COD}_{\text{VFA}} \text{ L}^{-1}$ at day 26, on the last day of operation at 3 days HRT. When HRT was set at 2 days, the VFA concentration in the effluent increased to $2.7 \text{ g COD}_{\text{VFA}} \text{ L}^{-1}$. As a significant difference with regard to the granular sludge reactor, the predominant VFA was the acetic acid

with very low levels of propionic acid. It is indicative of the higher capacity of the thickened sludge to process the pig slurry despite the high ammonia nitrogen levels. The higher acetic concentration could possibly be a consequence of a limited contact between microorganisms and substrate caused by sludge flotation and clogging. In fact, the reactor was working more as an anaerobic filter than as a UASB reactor, which, in turn, results in a process with lower efficiency (Lettinga et al., 1983).

Due to the difficulties found in the operation with this type of sludge as inoculum, the reactor was not operated with the raw slurry.

3.3.3 UASB seeded with anaerobic sewage sludge

The anaerobic sewage sludge occupied approximately 75% of the useful volume reactor and remained settled in the bottom part of the reactor during the batch period. When the reactor started to be continuously fed with the filtrated slurry at 3 days HRT, the sludge blanket expanded and some solids were washed-out from the first operation day. The reactor produced biogas from the first operation day and the methane yield progressively increased as Fig 4a shows. After ten operation days, the reactor reached a methane yield of $5.0 \text{ L CH}_4 \text{ L}^{-1}$ filtered slurry ($233 \text{ mL CH}_4 \text{ g}^{-1} \text{ COD}$). Then, the HRT was reduced to 2 days and wash-out increased notably. The VMPR increased, but the methane yield decreased, reaching a stable value of $4.6 \text{ L CH}_4 \text{ L}^{-1}$ filtered slurry ($214 \text{ mL CH}_4 \text{ g}^{-1} \text{ COD}$) after five days of operation at this HRT. When HRT was reduced to 1.5 days, excessive wash-out forced the reactor stop. At this point the methane yield had decreased to $3.8 \text{ L CH}_4 \text{ L}^{-1}$ filtered slurry ($177 \text{ mL CH}_4 \text{ g}^{-1} \text{ COD}$).

Similarly to the previous assays, the effluent pH values ranged between 8.6 ± 0.1 and 8.4 ± 0.1 for HRT from 3 to 2 days. At 1.5 days HRT the pH dropped to 8.3 ± 0.1 . The

alkalinity of the effluent ranged between 9.0 and 9.5 g CaCO₃ L⁻¹. The methane content in the biogas decreased with the increasing organic loading rate, the mean percentage values being 86.0 ± 0.7, 84.2 ± 0.8 for 3 and 2 HRT.

During reactor operation, the system was not able to provide an effluent VFA concentration below 1.5 g COD_{VFA} L⁻¹. As can be observed in Fig. 4b, the lowest VFA concentration reached in the effluent was 1.7 g COD_{VFA} L⁻¹, which was attained at 3 days HRT. At shorter HRTs, the VFA concentration increased, reaching a value of 4.2 g COD_{VFA} L⁻¹ on the last operation day, at an HRT of 1.5 days. As in the case of the granular sludge, the predominant VFA was the propionic acid, followed by acetic.

Due to the lower reactor efficiency performance compared with the granular sludge and the wash-out observed, the reactor was not operated with the raw slurry.

3.4 Comparison of CSTR and UASB systems for the anaerobic treatment of pig slurry.

The reactor performance results of the present work have been compared with previous anaerobic treatment systems reported for pig slurry in CSTR and UASB systems. The UASB process shows improved performance in terms of smaller reactor size (higher OLR), VMPR and methane content in the biogas. As summarized in Table 2, previous studies that processed pig slurry in CSTR systems required HRT equal or longer than 10 days to attain high efficiency performance. Creamer et al. (2008) and Pagilla et al. (2000) processed slurries with higher TS and VS content than those in the present work, thus obtaining higher methane yields (L CH₄ L⁻¹ substrate). It is not possible to know if those slurries would have been suitable for the UASB process due to their higher solids content. However, the similar TS and VS content of the pig slurry processed in

Regueiro et al. (2012), allows its comparison with that in the present work. In this case, the differences are significant. The HRT was 6.7 times higher, the VMPR was 11 times smaller, and the methane content was much lower (55% versus 82%).

In previous UASB studies, Bergland et al. (2015) processed supernatant pig slurry (14.5 g TS L⁻¹ and 7.3 g VS L⁻¹) in UASB reactor (35°C) with granular biomass at OLR between 14 and 400 g COD L⁻¹ d⁻¹, operating at HRT between 48 and 1.7 h. The higher methane yield was obtained at the lowest OLR, reaching an SMY of 685 mL CH₄ g⁻¹ VS (5.0 L CH₄ L⁻¹ slurry) with 81% CH₄ content in the biogas. The highest VMPR (34.0 L CH₄ L⁻¹ d⁻¹) was reached at the highest OLR (400 g COD L⁻¹ d⁻¹). However the efficiency in terms of methane yield at this OLR was 52% lower, (329 mL CH₄ g⁻¹ VS and 2.4 L CH₄ L⁻¹ slurry). Kalyuzhnyi et al. (1999) treated diluted supernatant pig slurry in UASB reactor (35°C) seeded with granular sludge. The slurry processed had a VS content of 6-15 g L⁻¹. The reactor was operated at OLR of 12 g COD L⁻¹ d⁻¹ (1.2 days HRT), reaching a VMPR of 3.2 0 L CH₄ L⁻¹ d⁻¹ (77% CH₄ in biogas) and a methane yield of 3.8 L CH₄ per liter of slurry .

3.5 Process considerations

The results obtained show great potential for pig slurry treatment in UASB reactors.

The process has been successfully performed at high efficiency levels at HRT between 3 and 1.5 days, depending on the type of biomass seed. Another advantage of this process is the high quality of the biogas, with methane contents higher than 80%.

Moreover, the process only requires a relatively small size UASB reactor, a feed pump, a biogas boiler for energy use and a heat exchange system to provide the required process temperature. No mixing devices are required, thus making the process quite

competitive and energetically efficient. This confirms that, when treatment of pig slurry in this type of reactor is possible, the economy and mechanical simplicity of the process can make it economically sound. In this sense, Asam et al. (2011) reported that animal slurries with water content higher than 90% make the economic viability of conventional (CSTR) biogas plants difficult.

It is important to note that the degree of dilution of the pig slurry and the resulting solids content is an important factor for the UASB process. The VS content and the biodegradability of the pig slurry will determine the methane potential of the slurry and, thus, the economic viability of the process. The TSS content will determine the suitability of the UASB process. For instance, the solids content of the pig slurry used in this work were optimal for the UASB process. The organic matter content of the supernatant slurry resulted in a positive net energy production yield, as data in Table 3 shows. It is probable that a slightly higher solids content would result in a higher methane yield without operational problems at a similar HRT, thus improving the energetic balance. But it is also probable that a significantly higher solids content would make the filtration of the pig slurry necessary, thus requiring additional equipment and cost to obtain the filtered slurry suitable for the process. On the contrary, a higher dilution of the pig slurry would result in a lower solids content and lower methane yields that would make the process less attractive from an energetic (and economic) point of view.

With regards to the different types of inocula studied in this work, the best operation conditions for each inoculum are summarized in Table 3. The granular sludge reactor was able to work stably at high efficiency levels at an HRT of 1.5 days and an OLR of $16.4 \text{ g COD L}^{-1} \text{ d}^{-1}$ with the supernatant raw slurry. At higher OLR (shorter HRT),

biomass wash-out started when treating the filtered slurry and also efficiency decreased in the case of the raw slurry. It is probable that a more gradual increase in the OLR might result in stable process performance at higher OLR, which would have allowed successful operation at HRT close to 1 day.

The reactor seeded with the thickened digestate suffered sludge flotation problems that make its use in UASB reactors not recommendable. However, the low propionic acid levels found in the effluent and the high methane content in the biogas suggest a very good adaptation of the microbial reactor community to the pig slurry. Although this kind of sludge is not suitable for the UASB process, it could be useful for other concept of process, such as the bacterial sludge entrapment (anaerobic filter), the bacterial immobilization by attachment to fixed surfaces (anaerobic fixed film reactor) or the mobile particulate surfaces (anaerobic attached film expanded bed).

The anaerobic sewage sludge can be an alternative to inoculate a UASB reactor for the treatment of pig slurry when there is no availability of granular sludge. In such a case, the size of the reactor should be larger in comparison with the granular sludge reactor. The OLR should not exceed the recommended values for flocculent sludge, $8 \text{ g COD L}^{-1} \text{ d}^{-1}$ (Lettinga and Hulshof Pol, 1991). This value is in accordance with the results obtained in the present work, since the best operation conditions were performed at an HRT of 3 days with an OLR of $7.2 \text{ g COD L}^{-1} \text{ d}^{-1}$. Higher OLR applied resulted in excessive biomass wash-out and decreases in reactor efficiency.

4. Conclusions

Pig slurry can be efficiently treated in UASB reactor after removing settleable solids. Granular sludge performance allowed a high efficiency process at 1.5 days HRT,

yielding $6.1 \text{ L CH}_4 \text{ L}^{-1}$ supernatant pig slurry. The thickened digestate sludge was unsuitable for the UASB process due to sludge flotation. The anaerobic sewage sludge can be an alternative for the treatment of pig slurry in UASB reactors when there is no availability of granular biomass. UASB reactor technology is a promising solution for pig slurry due to the small reactor size required, low associated costs and high process efficiency.

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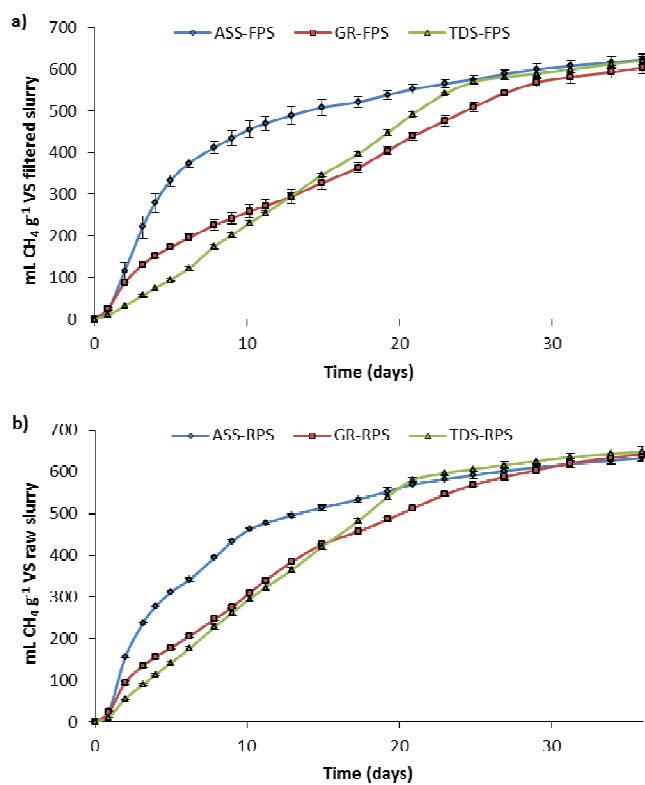
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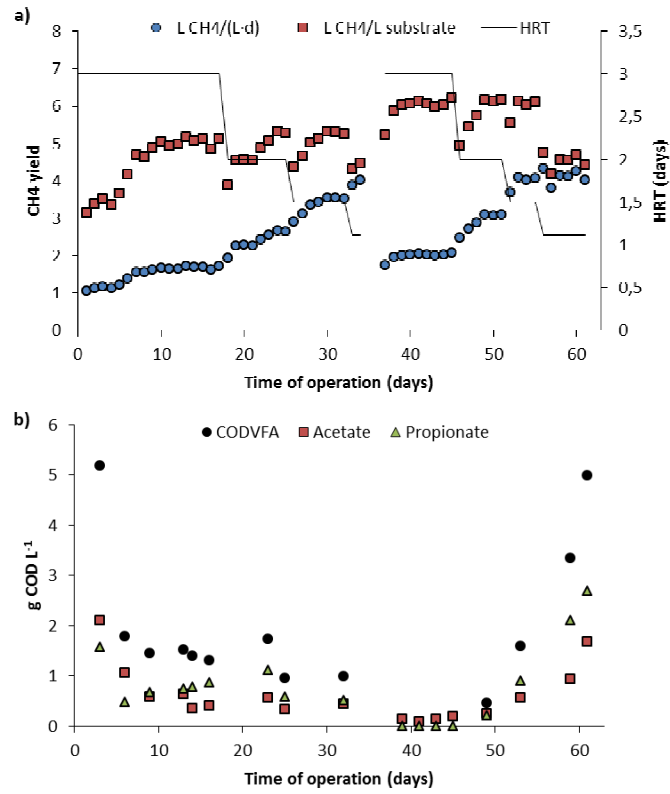
Figure 1. Methane yield curves (BMP test) of the filtered (FPS) a) and the raw (RPS) b) pig slurry substrates for the anaerobic sewage (ASS), granular (GR) and thickened digestate (TDS) sludge inocula. Error bars show standard deviations.

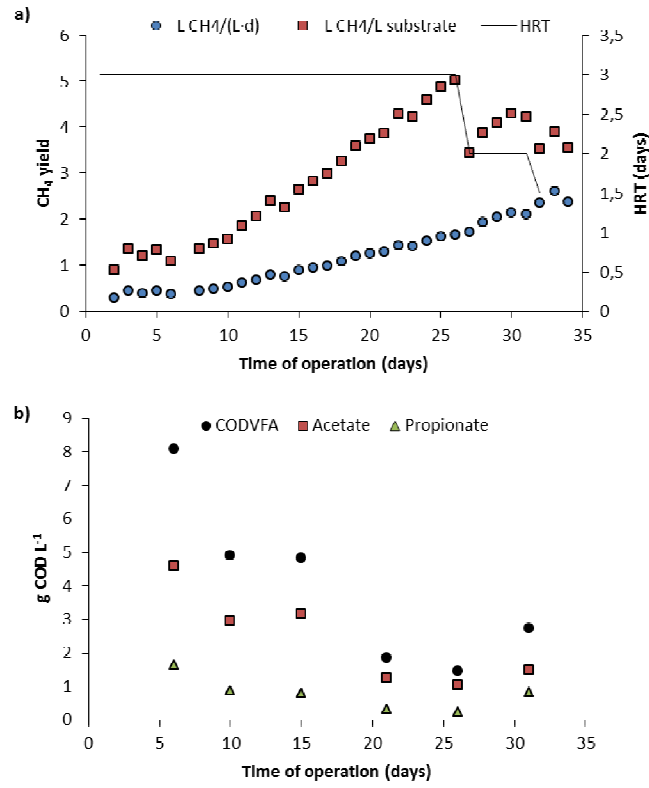
Figure 2. a) Volumetric methane production rate (VMPR) and methane yield at different HRTs and b) VFA in the effluent during UASB operation with granular sludge.

Figure 3. a) Volumetric methane production rate (VMPR) and methane yield at different HRTs and b) VFA in the effluent during UASB operation with thickened digestate sludge.

Figure 4. a) Volumetric methane production rate (VMPR) and methane yield at different HRTs and b) VFA in the effluent during UASB operation with anaerobic sewage sludge.







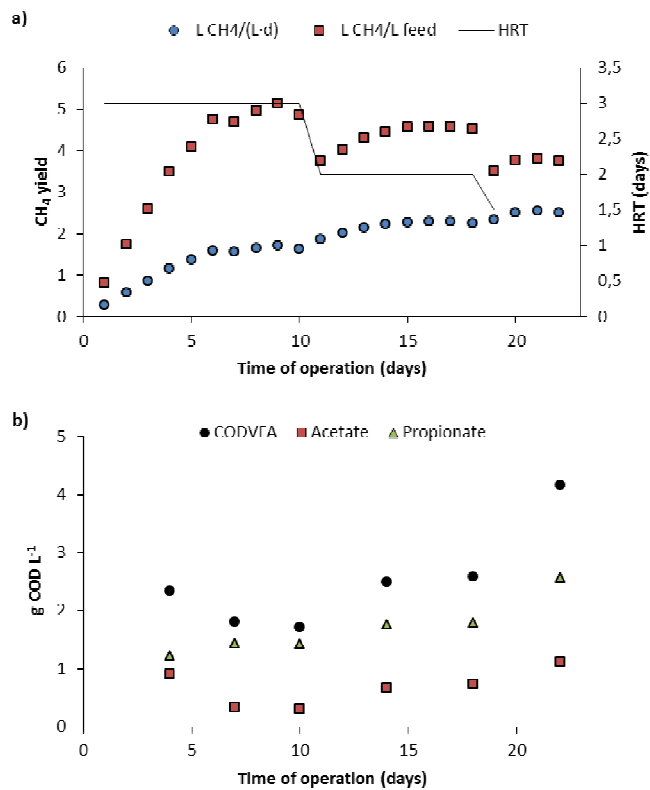


Table 1. Characteristics of the filtered and the raw pig slurry used in this study

Parameter	Filtered Slurry	Raw Slurry
COD (g L ⁻¹)	21.5 ± 1.3	28.9 ± 1.8
COD _{supernatant} (g L ⁻¹)	20.7 ± 0.8	24.6 ± 1.5
COD _{soluble} (g L ⁻¹)	17.2 ± 0.7	18.8 ± 1.1
COD _{VFA} (g L ⁻¹)	13.5 ± 0.6	15.5 ± 0.9
NH ₄ ⁺ -N (g L ⁻¹)	2.8 ± 0.1	2.9 ± 0.2
COD/N ratio	7.7 ± 0.7	8.8 ± 0.8
Alkalinity (g CaCO ₃ L ⁻¹)	8.5 ± 0.1	9.0 ± 0.2
TS (g L ⁻¹)	14.3 ± 0.4	19.2 ± 0.6
VS (g L ⁻¹)	9.7 ± 0.4	13.6 ± 0.6
TSS (g L ⁻¹)	3.6 ± 0.5	8.5 ± 0.4
VSS (g L ⁻¹)	2.9 ± 0.3	7.2 ± 0.5
pH	7.3 ± 0.2	7.3 ± 0.2

Table 2- Operational features of successful anaerobic digestion systems for pig slurry in CSTR and UASB systems

Substrate	TS - VS (g L ⁻¹)	Reactor type Operation Temperature	HRT (days)	OLR (kg COD m ⁻³ ·d ⁻¹)	VMPR (L L ⁻¹ d ⁻¹)	CH ₄ in biogas (%)	CH ₄ yield (L L ⁻¹ substrate)	Reference
Diluted pig slurry	32.0 - 21.5	CSTR 55°C	10	5.5	1.0	74	10.0	Creamer et al. (2008)
Non diluted pig slurry	43 – 28	CSTR 37°C	15	---	0.47	---	7.0	Pagilla et al. (2000)
Filtered pig slurry	17.3 - 11.7	CSTR 35°C	10	2.0	0.37	55	3.7	Regueiro et al. (2012)
Diluted supernatant pig slurry	11.5 - 10.5	UASB- granular 35°C	1.2	12	3.2	77	3.8	Kalyuzhnyi et al. (1999)
Supernatant pig slurry	14.5 – 7.3	UASB- granular 35°C	2	14.2	2.5	81	5.0	Bergland et al. (2015)
Supernatant pig slurry	14.5 – 7.3	UASB- granular 35°C	0.07	400	34.0	76	2.4	Bergland et al. (2015)

Table 3. Summary of the best operation conditions for the UASB process with different types

Type of seed sludge	Granular	Granular	Thickened Digestate	Anaerobic Sewage
Type of pig slurry (substrate)	Filtered slurry	Supernatant Raw slurry	Filtered slurry	Filtered slurry
HRT (days)	1.5	1.5	3	3
OLR (g COD L ⁻¹ d ⁻¹)	14.3	16.4	7.2	7.2
L CH ₄ L ⁻¹ d ⁻¹	3.5	4.1	1.7	1.7
L CH ₄ L ⁻¹ substrate	5.3	6.1	5.0	5.0
%CH ₄ in biogas	82.6	82.2	87.0	86.0
mL CH ₄ g ⁻¹ COD	246	248	236	236
% Efficiency (with respect to BMP value)	88.3	n.d. ^a	83.3	83.3
Biogas gross energy (kWh _{thermal} m ⁻³ substrate)	53	61	50	50
Energy required to heat the substrate ^b (kWh _{thermal} m ⁻³)	17	17	17	17

of seed sludge

Operational problems	No	No	Sludge flotation	Wash-out
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^a BMP for the supernatant raw slurry was not assayed

^b Assuming 1 Kcal/(kg C) as substrate specific heat capacity and a required temperature substrate increase of 15°C

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- Supernatant pig slurry is suitable for UASB at stable high process performance.
- Thickened digestate sludge was inappropriate as seed inoculum source.
- Granular biomass inoculum reactor yielded 248 mL CH₄ g⁻¹ COD at 1.5 days HRT.
- Anaerobic sewage sludge inoculum reactor yielded 236 mL CH₄ g⁻¹ COD at 3 days HRT.

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