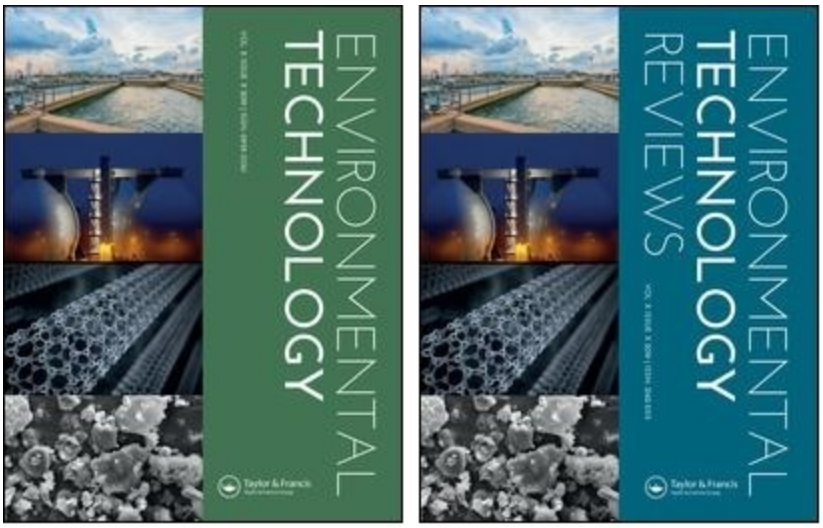
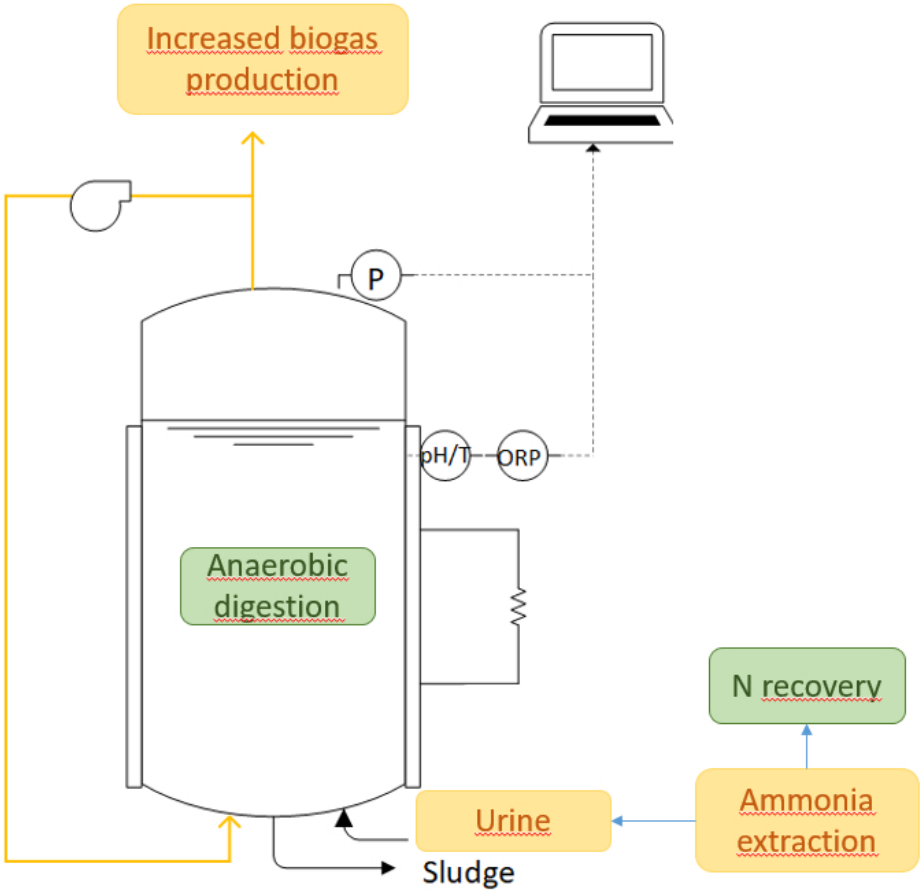


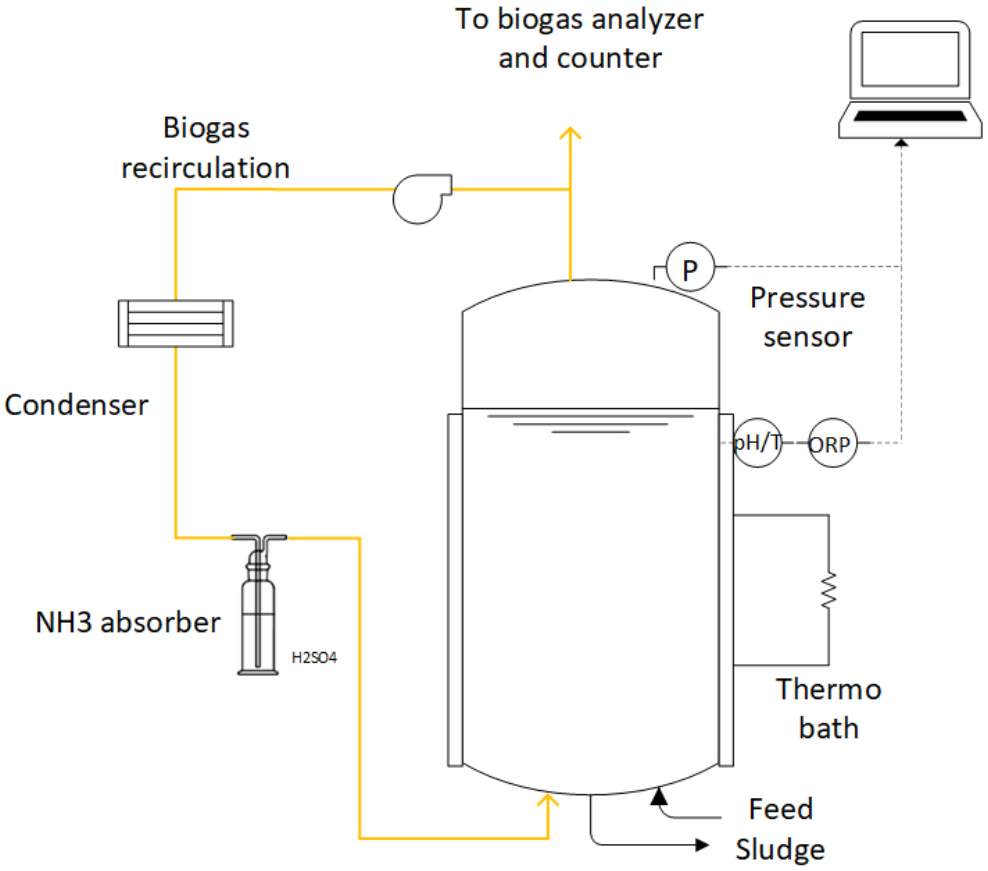
Influence of free ammonia extraction in methane production from human urine

Journal:	*Environmental Technology*
Manuscript ID	TENT-TENT-2023-0118.R2
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Ruiz-Martinez, Ana; Universitat de València, Chemical Engineering Greses, Silvia; Fundación Imdea energía Jimenez, Emerita; FCC Aqualia SA Serralta, J.; Universitat Politecnica de Valencia, Instituto de Ingeniería del Agua y Medio Ambient Claros, Javier; Ciclagua SA Ferrer, J.; Universitat Politecnica de Valencia, Instituto de Ingeniería del Agua y Medio Ambient Seco, A.; Universitat de Valencia
Keywords:	anaerobic digestion, free ammonia inhibition, nitrogen recovery, urine, yellow waters

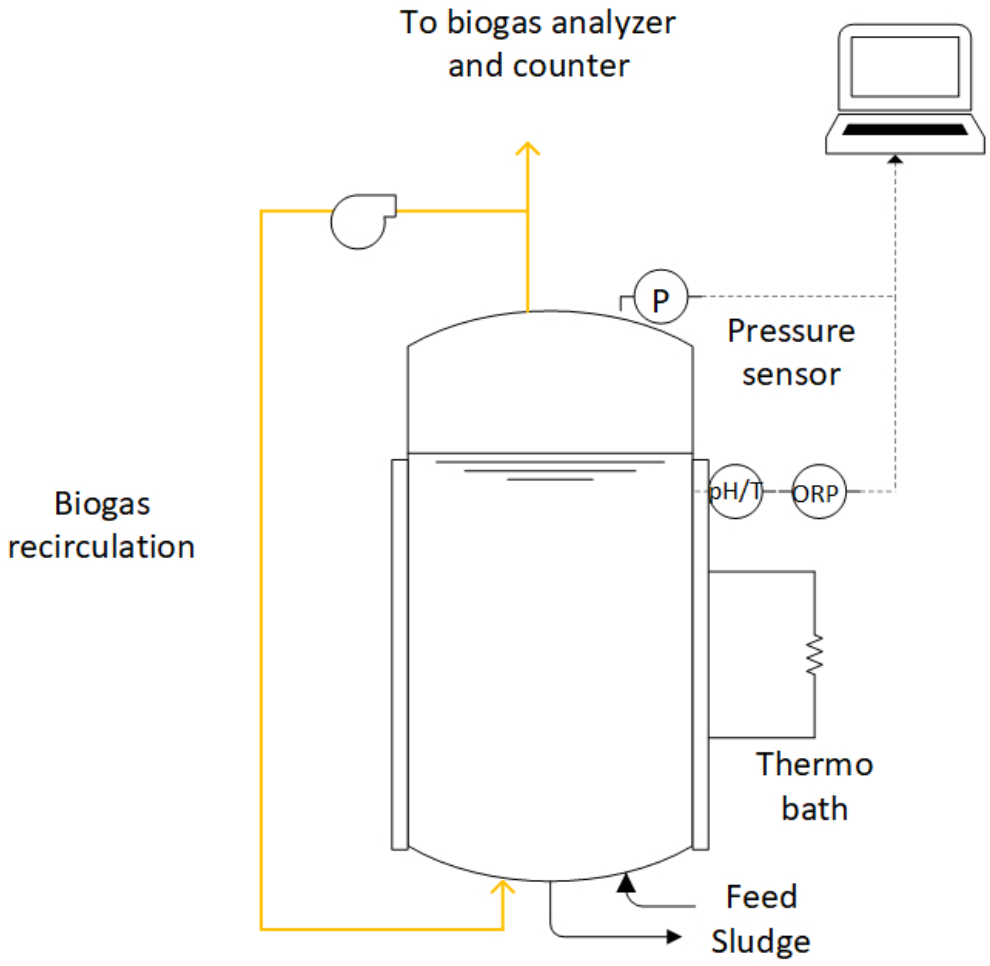
SCHOLARONE™
Manuscripts



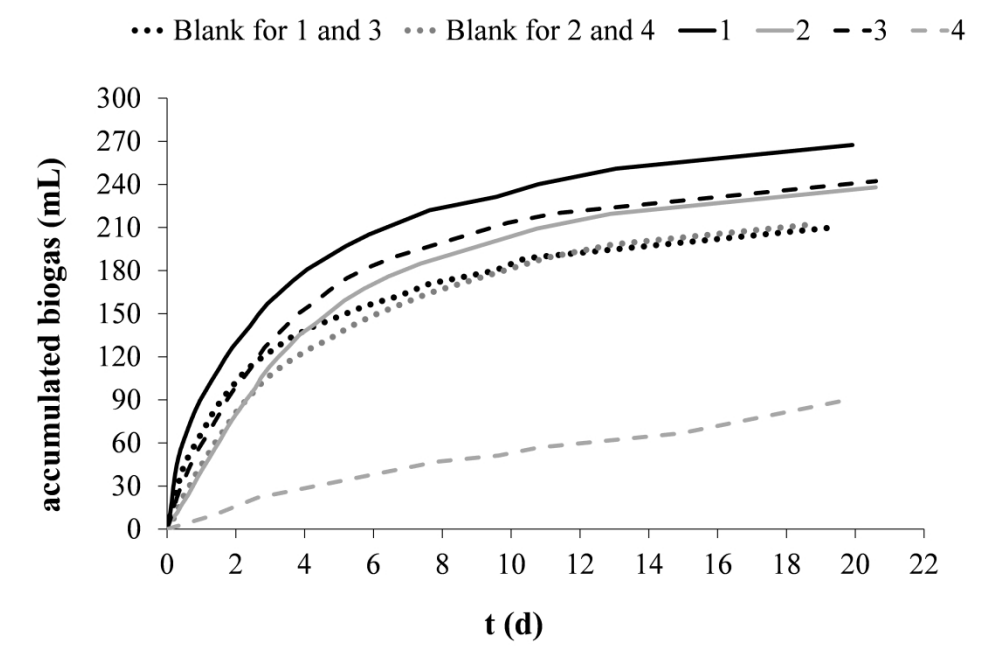
394x372mm (47 x 47 DPI)



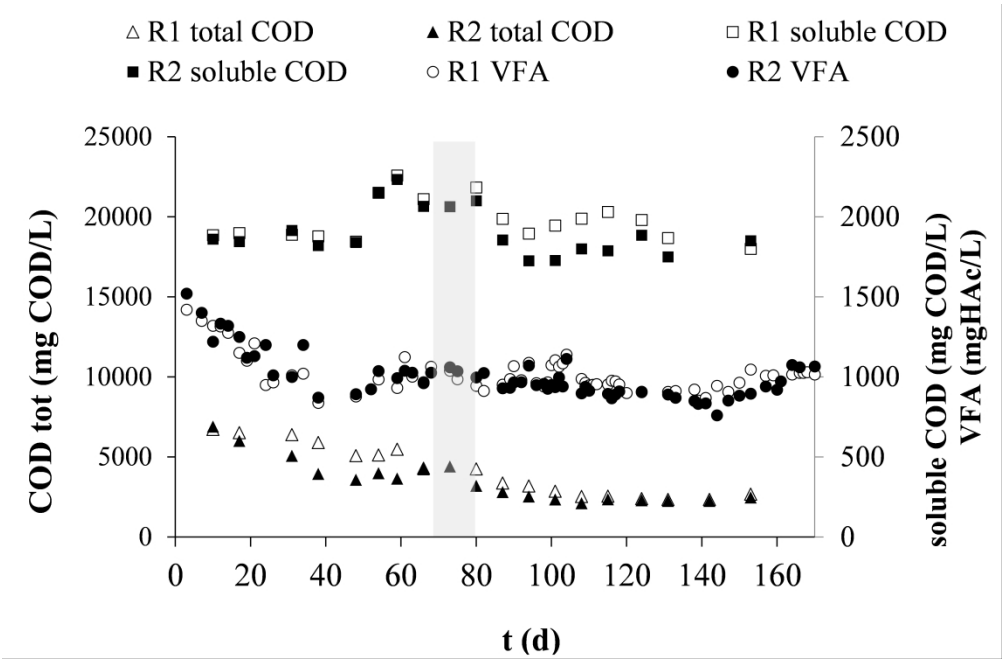
30x27mm (600 x 600 DPI)



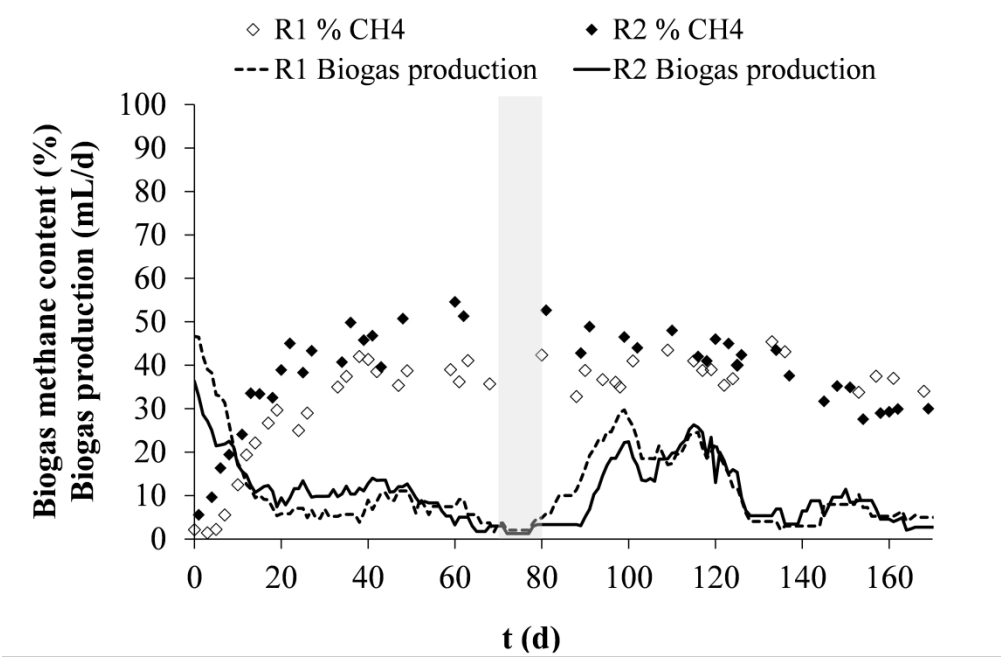
27x27mm (600 x 600 DPI)



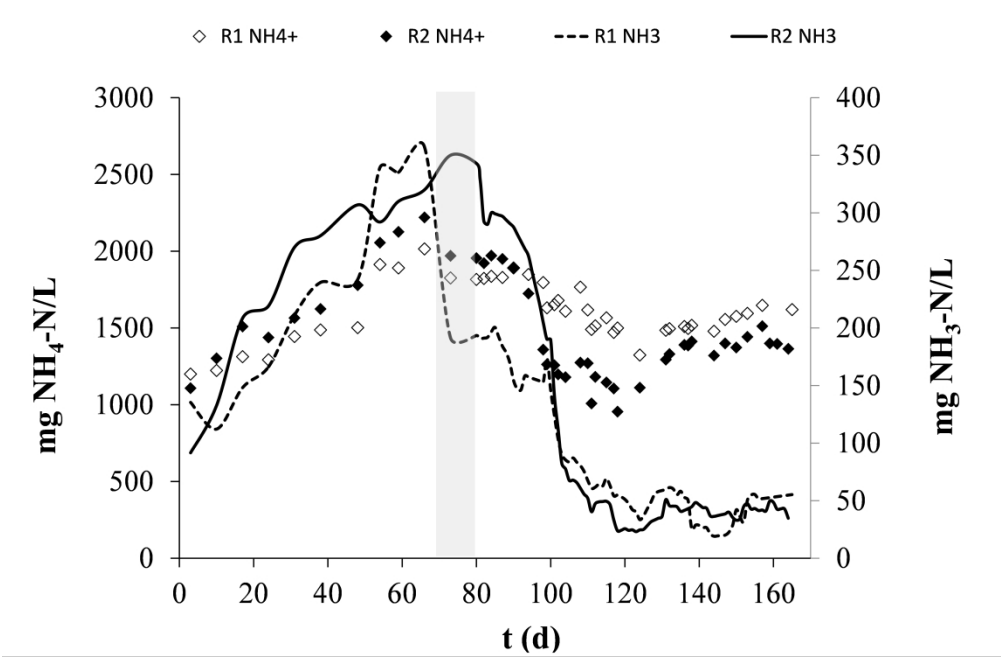
258x169mm (500 x 500 DPI)



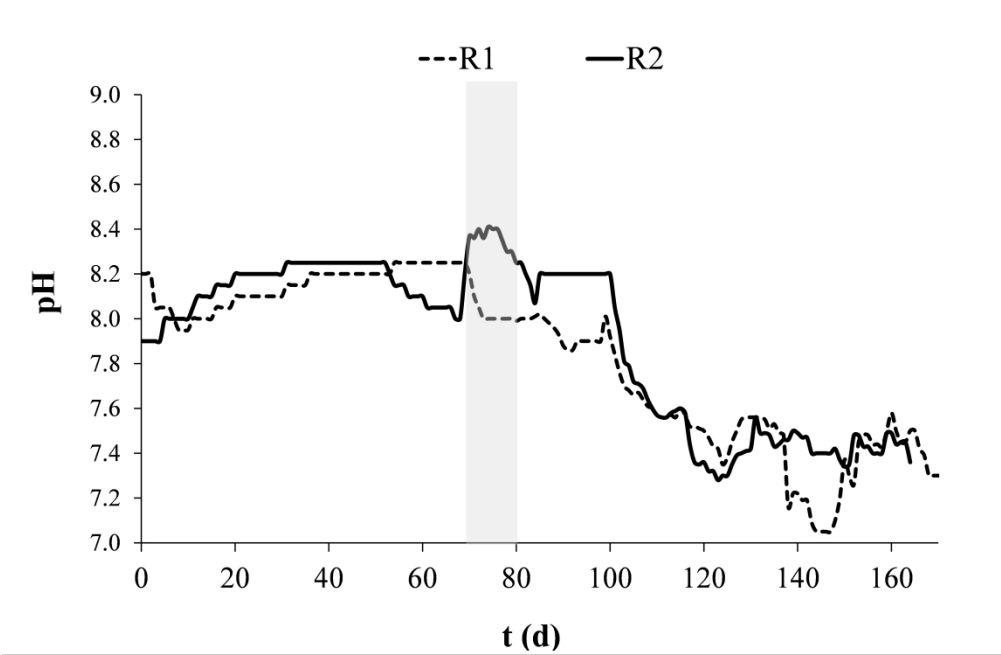
258x169mm (500 x 500 DPI)



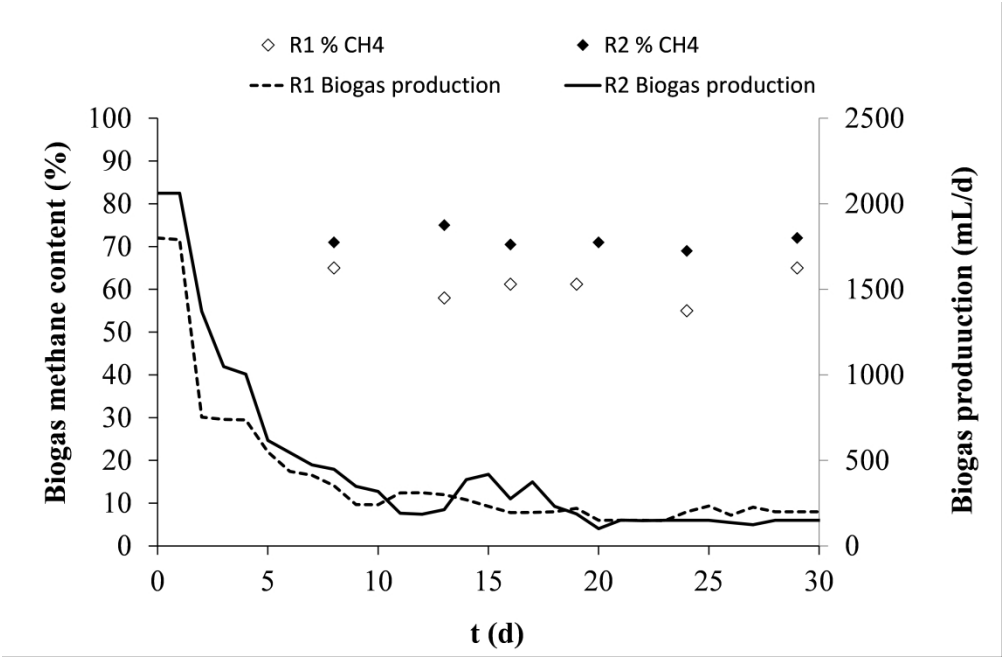
258x169mm (500 x 500 DPI)



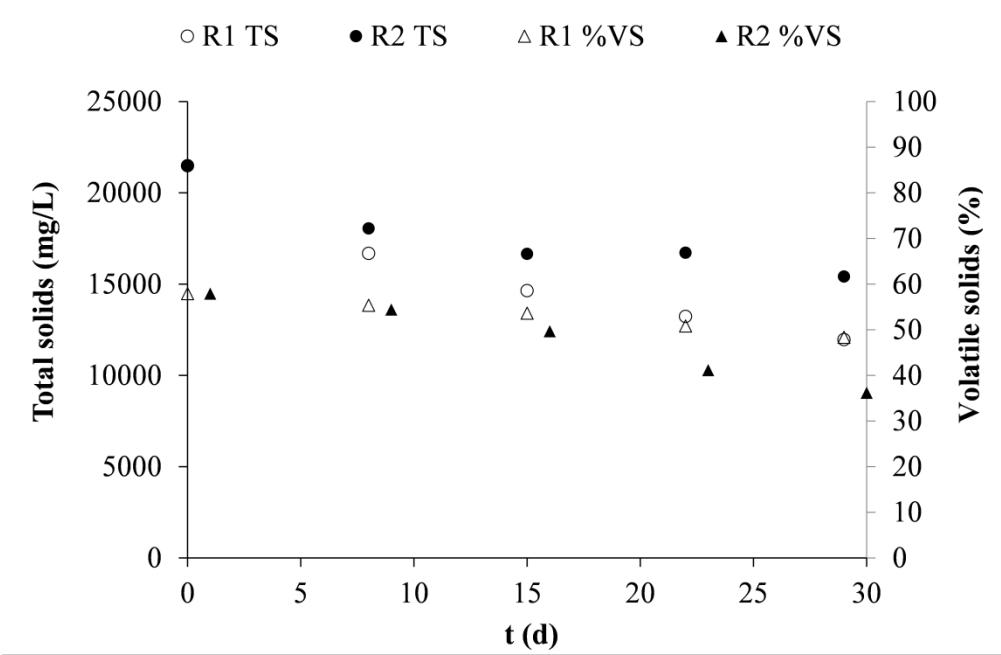
258x169mm (500 x 500 DPI)



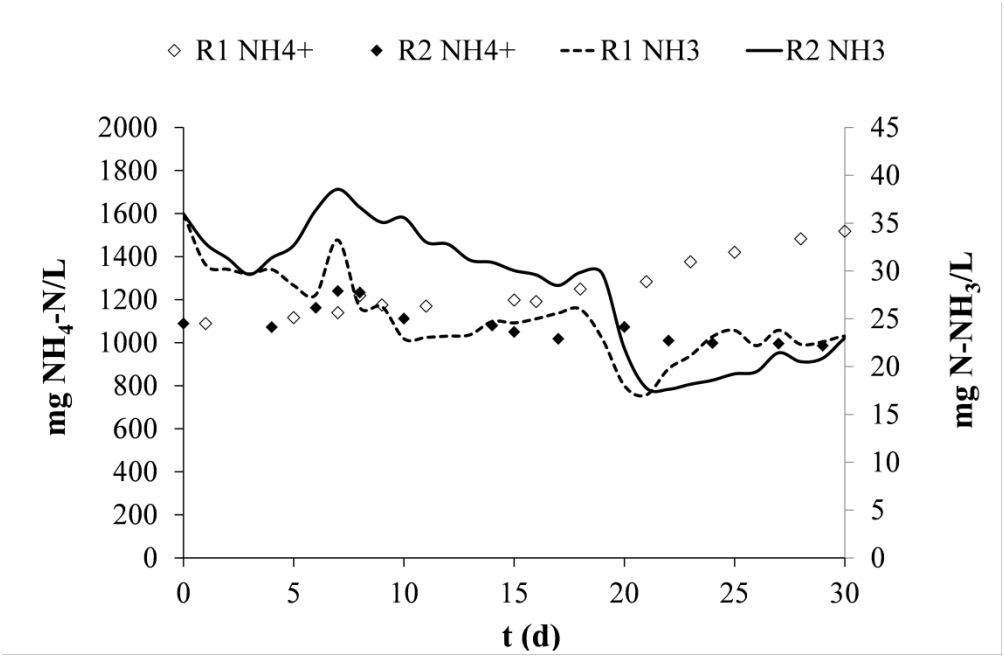
258x168mm (500 x 500 DPI)



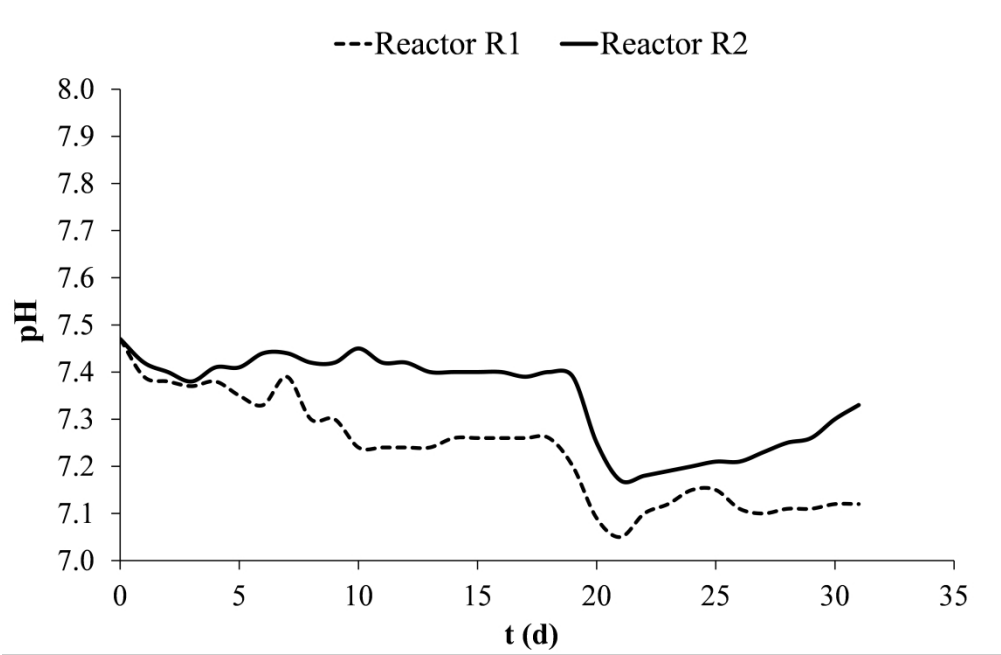
258x169mm (500 x 500 DPI)



258x168mm (500 x 500 DPI)



258x168mm (500 x 500 DPI)



258x168mm (500 x 500 DPI)

Influence of free ammonia extraction in methane production from human urine

Ana Ruiz-Martínez*[,a], Silvia Greses[a,1], Emérita Jiménez[a,2], Joaquín Serralta[b], Javier Claros[b,3], José Ferrer[b] and Aurora Seco[a]

[a]CALAGUA Unidad Mixta UV-UPV, Department of Chemical Engineering, School of Engineering, Universitat de València. Av. Universitat, 46100 Burjassot, Spain.

(email: ana.ruiz-martinez@uv.es, silvia.greses@uv.es, emerita.jimenez@uv.es, aurora.seco@uv.es)

[b]CALAGUA Unidad Mixta UV-UPV, Research institute of Water and Environmental Engineering – IIAMA, Universitat Politècnica de València. Camino de Vera, 46022 Valencia, Spain (e-mail: jserralt@hma.upv.es, jferrer@hma.upv.es).

*Corresponding author. Tel. +34 963 877 000 ext. 76176; Fax +34 963 877 618, e-mail address: ana.ruiz-martinez@uv.es

[1]Present address: Fundación Imdea energía, Avda. Ramón de la Sagra, 3. Parque tecnológico de Móstoles, 28935 Madrid, Spain. silvia.greses@imdea.org

[2]Present address: FCC Aqualia SA. Plaza del Carmen, 6, bajo. 46120 Alboraya, Valencia, Spain. ejimenezdo@fcc.es

[3]Present address: Ciclagua SA. Calle Cronista Carreres 11, 1E. 46003 Valencia, Spain. jaclaros@ciclagua.com

Abstract

Human urine has a high chemical oxygen demand (COD) content which makes anaerobic treatments potentially appropriate for the management of yellow waters, allowing for energy recovery. However, its high N content makes this treatment challenging. The present work studied the viability of performing an anaerobic digestion process for COD valorization on a real (not synthetic) urine stream at laboratory scale. To deal with nitrogen inhibition, two different ammonia extraction systems were proposed and tested. With them, a proper evolution of acidogenesis and methanogenesis was observed. Nitrogen was recovered in the form of ammonium sulphate, which could be used for agriculture, in two different ways: ammonia extraction from the urine stream before feeding the reactor and *in situ* extraction in the reactor. The first method, which proved to be a better strategy consisted in a desorption process (NaOH addition, air bubbling and acid (H_2SO_4) absorption column, HCl for final pH adjustment) whereas the *in situ* extraction in the reactor consisted of an acid (H_2SO_4) absorption column installed in the biogas recycling line of both reactors. Stable methane production over 220 mL/g COD was achieved and methane content in the biogas was stable around 71 %.

For Peer Review Only

Keywords

anaerobic digestion, free ammonia inhibition, nitrogen recovery, urine, yellow water

Acknowledgements

This research project has been supported by the Spanish Ministry of Economy, Industry and Competitiveness (MINECO, project CTM2017- 86751-C2), whose support is gratefully acknowledged.

For Peer Review Only

1. Introduction

Global demand of water, energy, fertilizers and other materials is constantly increasing due to the rapid growth of world population since the twentieth century. This can derive in severe environmental problems such as depletion of resources, increase of pollution and accumulation of residues. At the same time, and as a reaction to this, the last decades have seen an increasing interest on resource recovery from different kind of wastes. The trending concept of Circular Economy, which has gained very much attention in the last years [1], reflects the objective of accomplishing sustainable development by replacing the "end-of-life" concept with reducing, reusing, recycling and recovering materials. The application of the Circular Economy principles in the field of urban wastewater treatment encourages to recover not only water but also energy and nutrients from the wastewater.

Urban wastewaters can be classified into yellow water (urine), brown water (feces), black water (toilet wastewater, i.e. yellow water and brown water) and grey water (domestic wastewater that does not come from toilets). Source separation constitutes a promising solution for facing current environmental problems derived from wastewater generation, since treating concentrated and unmixed solutions is more resource efficient than treating highly diluted combined solutions [2]. For example, grey water constitutes 70 % of the generated volume of wastewater and has a high reuse potential due to its low pollution level. On the other hand, black waters show a high organic matter content, thus making anaerobic treatment a very advisable option for them. De Graaf et al. [3] achieved 78 % COD removal treating black waters in an anaerobic UASB reactor, while recovering the wastewater energy content in the form of biogas.

Yellow waters contain around 70 % of the total urban wastewater nitrogen content and 40 % of the phosphorus [4], which makes them very suitable for nutrient recovery processes following the principles of circular economy. The main application for recovering nutrients in yellow waters is the production of fertilizers, mainly via struvite precipitation [5]. Struvite crystallization is a fast and reliable process that allows phosphorus recovery and has been studied by several authors. See [6-8]. At the same time, however, human urine has a high COD content of about 7-11 g COD/L, which also needs to be removed during its treatment and makes an anaerobic treatment appropriate for it. COD removal improves the performance of further struvite

For Peer Review Only

crystallization process. However, an issue needs to be taken into consideration: the presence of high nitrogen concentration could cause the inhibition of the anaerobic process because of high free ammonia concentrations. See [9-10].

Anaerobic processes have been widely used in the field of wastewater treatment. They generate biogas that can be used for electricity generation, therefore reducing the carbon footprint of the process. Methane content of the biogas depends on different parameters (temperature, pH, TSS concentration and acclimation, waste and reactor characteristics, etc.). Apart from black waters, also sewage sludge, industrial wastewaters with high organic loads and some farm residues have proved to be good substrates for anaerobic digestion [8, 11, 12]. Different authors have reported a biogas production increase after the addition of human urine to the anaerobic digestion process of other wastes (Liu et al., 2022, Haque, 2006). Eduok et al. (2018) also proved urine to be a promising wetting and buffering agent to enhance biogas production. However, to our knowledge, and in spite of its high occurrence and availability, no studies on anaerobic digestion of yellow water exist to this date. On the other hand, the influence of urine characteristics on process stability and performance is still not well known. For instance, studies on free ammonia inhibition on methanogenic archaea show very different values, proving that this phenomenon depends on different factors such as the *inoculum* used or the acclimation period. See [8, 9].

The present work studied the viability of performing an anaerobic digestion process for COD valorization and N recovery on a non-synthetic yellow water stream at laboratory scale. In order to deal with nitrogen inhibition, two different ammonia extraction strategies were proposed and tested: *in situ* (i.e., from the reactor) and in feed (from the urine, before it was fed to the reactor). The influence of different operational parameters such as sludge retention time or nitrogen extraction system, and environmental factors such as pH or ammonium content was identified and quantified.

2. Material and methods

2.1 Setup descriptions and experimental procedure

Yellow wastewater

For Peer Review Only

Source separated yellow wastewater was obtained from collecting campaigns performed every two weeks. Urine was analyzed and stored at 4 ºC after subjecting it to dilution 1:4, which is a typical value for separated toilets.

Average values for COD, pH and nutrient content of the mixed urine collected during this work are shown in **Error! Reference source not found.**. As expected, TN and COD values were high. Nitrogen is present in recently collected urine in the form of organic compounds and therefore the difference between parameters TN and NH_4 is considerable. Analysis demonstrated that storage did not change the urine Total Nitrogen nor COD content (*data not shown*), although it increased ammonium concentration as well as pH, due to the hydrolysis process that took place.

Study of the effect of ammonium concentration on methanogenic activity

Four batch experiments were carried out in duplicate with the automatic biomethanation potential analyzer AMPTS ® II (BPC Instruments, Sweden) in order to determine the effect of pH and nitrogen content on methane production. The multi-channel analyzer consisted of 15 parallel reactors (500 mL glass bottles with mechanical agitation at 200 rpm) and the same number of gas flow meters attached to a data acquisition system. Temperature in all reactors was kept at 35 ± 0.1 ºC and the experiment was extended for 21 days. This allowed for the analysis of the Biomethanation Potential (BMP) of fresh and hydrolyzed human urine. The *inoculum* used was sludge from the conventional anaerobic digester (AD-S) of the "Conca del Carraixet" WWTP (Valencia, Spain), which treats primary and secondary sludge produced during wastewater treatment. This biomass was acclimated to NH_4-N concentrations around 1000 mg NH_4-N/L.

For batch 1 and batch 2 urine hydrolysis process was promoted during a 12-day storage period prior to the test, in order to increase the initial ammonium concentration in the substrate. For these experiments pH values of 6.7 ± 0.01 and 7.5 ± 0.01 were established, respectively. Batch 3 and batch 4 were prepared with urine which had been stored for only 2 days and therefore presented a lower ammonium content. Again, pH values of 6.7 ± 0.01 and 7.5 ± 0.01 were set. A blank bottle was set up for each type of batch (1+3 and 2+4) to determine methane production due to the consumption of the organic matter present in the *inoculum*. Initial and final pH and ammonium concentration in the bottles were analyzed.

Continuous reactors

For Peer Review Only

Continuous experiments were performed in two lab-scale methacrylate cylindrical reactors which were operated simultaneously (Figure 1). The experimental period for both reactors lasted 200 days and was divided in three phases: Phase I without ammonia extraction, Phase II and III with two different ammonia extraction processes (see **Error! Reference source not found.**).

Each reactor had a total volume of 14 L (20 cm diameter, 50 cm height) of which 4 L was headspace. Headspace gas was recirculated to the bottom of the reactor and injected using fine bubble diffusers for agitation. The tanks were hermetically sealed.

The reactors were equipped with sensors for continuous monitoring of pH, temperature and oxidation reduction potential (ORP). The sensors were connected to a PC through a multiparametric analyzer (Orion Versastart, Thermo Scientific). Pressure was also measured (Sintrans P, Siemens) and transmitted to the PC with a Picolog Datalogger 1216 (Pico Technology). All the obtained information was recorded by a custom data logging script written in Visual Basic (Microsoft). Reactor temperature was maintained at 35 ºC with a water jacket connected to a temperature-controlled water bath (LAUDA Alpha RA 8).

Each reactor had two lateral hand valves for purging and feeding the system, and two valves on the top for biogas discharge and measurement of biogas composition. Biogas production was measured with a gas flow meter (µFlow, Bioprocess Control. Lund, Sweden). Reactors were fed once a day.

To avoid inhibitions during the anaerobic digestion process, ammonia was extracted from the system using two strategies: *in situ* ammonia extraction and extraction in the feed stream (see **Error! Reference source not found.**). The *in situ* ammonia extraction system (during digestion process) consisted of an acid (H_2SO_4) absorption column installed in the biogas recycling line of both reactors, which enabled ammonia recovery as $(NH_4)_2SO_4$. A condenser was installed before the absorption column in order to protect it from the humidity present in the biogas. Ammonia extraction from the feed stream (urine after each collecting campaign) was carried out with a desorption process that comprised the following steps: i) NaOH was added to the urine to enhance the extraction process by rising the pH; ii) air was bubbled through urine; iii) the obtained ammonia-rich air was bubbled through an acid (H_2SO_4) absorption column where > 90 % nitrogen was recovered as $(NH_4)_2SO_4$. Therefore, in this case, the urine nitrogen

For Peer Review Only

content was lower than 200 mg N/L before it entered the reactors. HCl was used for final pH adjustment.

Two different *inoculum* were used:

- PigMan-S: Sludge from a pilot scale anaerobic digester treating pig manure (UPV, Valencia, Spain), acclimated to high NH_4-N concentrations (around 3000 mg NH_4-N/L).
- AD-S: Sludge from the conventional anaerobic digester of the "Conca del Carraixet" WWTP (Valencia, Spain), which is fed with primary and secondary sludge produced during wastewater treatment. This biomass was acclimated to NH_4-N concentrations around 1000 mg NH_4-N/L.

Designated phases and operational conditions are summarized in **Error! Reference source not found.**.

2.2 Analytical methods

Reactor samples were regularly analyzed to monitor the biological process. Generated biogas and the urine fed to the reactor were also analyzed. **Error! Reference source not found.** shows the analyzed parameters and the equipment and methods used.

Once ammonium concentration was measured along with pH and temperature, the free ammonia concentration in the reactors was calculated using the equilibrium equation (Eq. 1) proposed by [15], in which TAN is the total ammonium nitrogen concentration and temperature (T) was expressed in Kelvin.

$$NH_3 + H^+ \rightarrow NH_4^+ \qquad \text{eq 1}$$

$$[NH_3] = \frac{TAN}{1 + \frac{10^{-pH}}{10^{-(0.09018 + 2729.92/T)}}}$$

The methane fraction in biogas was measured three times a week using a Gas Chromatograph fitted with a Flame Ionization Detector (GC-FID, Agilent Technologies 6890N). For this purpose, a volume of 0.5 mL of biogas was sampled from the headspace of the reactor through a septum by gas-tight syringe, and then injected into a 15 m × 0.53 mm × 1 µm TRACE TR-FFAP column (Thermo Fisher), which was

URL: http:/mc.manuscriptcentral.com/tent

For Peer Review Only

maintained at 40 °C. Helium was used as carrier gas with a flow rate of 5 mL/min and the calibration standard was pure methane (> 99.9995 %, Air Products Inc.). All the analyses carried out for every sampling point were performed in triplicate in order to calculate the average and the standard deviation shown in tables and graphs (section 3).

3. Results and Discussion

3.1 Effect of ammonium concentration on methanogenic activity

During the storage of urine, urea is hydrolyzed and therefore its ammonium concentration increases. To study the effect of this process on methanogenic activity, different batch experiments were performed. The Biomethanation Potential (BMP) of human urine with different initial ammonium levels was evaluated by analyzing 2-day stored urine and 12-day stored urine. These initial ammonium concentrations were combined with different pH values, resulting in the different ammonium levels reported in Table 4, which also shows final pH and ammonium levels in the bottles, together with methane production. Figure 2 shows the biogas production of all batch experiments (total CH_4 production) and their blanks (*inoculum* CH_4 production).

Batch 1, consisting of processing hydrolyzed urine at a pH of 6.7 ± 0.01, was the only one to show as much biogas production as expected, considering the theoretical value of 0.35 m^3 CH_4/kg COD. Initial NH_3 concentration was relatively low and final NH_3 concentration was the lowest from all batch experiments (20.7 mg N/L), which proved to be lower than the methanogenesis inhibiting concentration.

Although batch 2 and batch 3 had different initial pH and substrate hydrolysis level, both presented a similar final NH_3 concentration (the second lowest of all batch experiments). This is probably the reason why methane production was also similar (34.9 and 32.1 mL CH_4). The slope of the represented curves in Figure 2, for the first hours of the batch experiments, dropped between 54 % and 78 %. This means that methanogenesis in these tests was partially inhibited: in these cases, only 50 % of the expected biogas production was achieved after 21 days. It could therefore be concluded that, whereas 20 mg N/L did not have any effect on the methanogenic activity, at 45-50 mg N/L, for the studied biomass (not adapted) and under the given conditions, biogas production was already hampered.

For Peer Review Only

In batch 4 biogas production was significantly lower than the obtained in the blank experiment, indicating the inhibition of the process due to the high level of ammonia. Initial slope for batch 4 was 95 % smaller than for batch 1. This confirmed the need for ammonia extraction in the system to achieve a proper anaerobic digestion process. Ammonia was therefore extracted from the system in the continuous experiments.

pH evolution during the 21 days of the batch experiments was different depending on the digested substrate. In batch 3, where the substrate was 2-days stored urine and pH 6.7 ± 0.01, pH rose considerably, whereas in batch 1 and 2, with hydrolyzed urine, the difference between initial and final pH was smaller. The reason for this is that hydrolysis process increases pH and therefore free ammonia concentration, which must be taken into account in order to avoid inhibition.

3.2 Continuous experiments

Start-up of the reactors

Prior to this study, R1 and R2 were inoculated with the sludge of an Anaerobic Membrane Bioreactor treating urban wastewater, not adapted to high Nitrogen levels. In these previous phase methanogenesis was inhibited (*data not shown*) and thus 50 % of their total volume was substituted for a different *inoculum*: sludge from an anaerobic digester treating pig manure (PigMan-S). As commented before, the biomass of this sludge was adapted to high Nitrogen concentrations (3000 mg NH_4-N/L). The sludge presented a content of 30 ± 0.5 g TSS/L and 33 ± 0.6 g COD/L.

Phase I – days 1 to 69

During the first 69 days of the experiment ammonia extraction was not performed. The evolution of R1 and R2, which improved with respect to the start-up phase, can be observed in Figures 3Figure to 6.

VFAs had been accumulating in the reactor during the start-up and reached a value of 1500 mg HAc/L at the beginning of this experiment (Figure 3). The VFA concentration decreased during the first 40 days of the experiment and stayed stable around 1000 mg HAc/L, indicating that organic matter was not completely degraded and only the acidification step was taking place (averaged values were 982 ± 66 mg HAc/L for R1 and 949 ± 76 mg HAc/L for R2). Figure 3 also shows how total COD progressively descended during the experiment, whereas soluble COD stayed at values around 2 g

For Peer Review Only

COD/L (averaged values were 1976 ± 131 mg HAc/L for R1 and 1904 ± 152 mg COD/L for R2). This might be a consequence of the inhibition of the fermentation process, due to the high VFAs content.

Figure 4 shows how biogas production peaked at the beginning of the experiment, due to *inoculum* degradation, and stabilized around day 20 at 7.97 ± 1.96 mL/d for R1 and 9.98 ± 2.97 mL/d for R2. Methane % increased during the first 35 days and stabilized at 38.59 ± 1.62 % for R1 and 46.04 ± 3.18 % for R2 (average measures for the period 35-60 d). It was assumed that this difference was due to the different SRT with which the reactors operated: a SRT of 40 d was more beneficial for methane production than 30 d. Produced methane corresponded only to 1 % and 3 % of the total influent COD in R1 and R2 respectively, calculated after the stabilization period (days 35-60). Such a small percentage of methanogenesis achieved is consistent with the observed accumulation of VFAs in the reactor, indicating that the digestion process stopped after the acidification step. Indeed, on average, VFAs concentration in R1 and R2 accounted for 43 % and 50 % of the total incoming COD, respectively. On the other hand, sulphate reducing bacteria accounted for the elimination of 17 % and 16 % of the total influent COD, in R1 and R2 respectively. These results indicate that the organic compounds present in human urine could be anaerobically degraded, although the high N content inhibited the methanogenic archaea.

During Phase I total ammonium nitrogen accumulated in the reactors (Figure 5). Maximum ammonium concentration was 2000 mg N/L in R1 and 2200 mg N/L in R2. Free Ammonia concentrations reached values around 350 mg N/L, which were responsible for methanogenesis inhibition, as the previous batch experiments demonstrated. In order to address the nitrogen accumulation problem in the reactors, an ammonia extraction system was setup and operated during Phase II and III.

Phase II - days 80 to 169

As previously explained, the ammonia extraction system was installed on the biogas line of both reactors on day 69. After some adjustments, the system was continuously working from day 80 of the experiment. Biogas was bubbled through a sulfuric acid dilution and ammonia was retained in it due to its high water solubility. Due to the low pH, free ammonia transformed into ammonium and the system stayed far from the

For Peer Review Only

saturation point. Thus, considerable amounts of ammonium were removed from the biogas.

As it can be seen in Figures 5 and 6, ammonium and free ammonia concentrations progressively decreased, as well as pH, which shifted from 8.3 to 7.4 while free ammonia was being retrieved from the reactor. Biogas production raised to an average of 22 mL/d (R1) and 18 mL/d (R2) for the period 90-120 d (Figure 4). Nevertheless, the methanogenic process remained inhibited, possibly due to the previous high ammonia levels reached in the reactors. Thus, produced methane corresponded to 3.2 % and 3.9 % of the total available COD in R1 and R2 respectively, calculated for period 90-120 d.

It can be concluded from Phase II of the experiments, where PigMan-S was used as *inoculum*, that although ammonia content decreased due to the extraction system, the system remained inhibited and organic matter degradation was still incomplete. VFA still accounted for 48 % and 43 % of total incoming COD to the reactor. It was also observed that a SRT of 40 days rendered slightly better results than a SRT of 30 days.

Phase III

In the last phase of the experiments the reactors were re-seeded with AD-S, sludge from a conventional WWTP anaerobic digester adapted to relatively low ammonium concentration (1 g NH_4-N/L) and with no VFAs accumulation. The ammonia extraction system was kept in operation in R1, to avoid nitrogen inhibition. As explained in section 2, ammonia was extracted directly from the collected urine stream before introducing it in R2. Thus, nitrogen concentration in the substrate was around 200 mg NH_4-N/L. A SRT of 40 days was chosen based on the preliminary results of previous phases. The reactors run for 30 days and a proper evolution of acidogenesis and methanogenesis was observed, since VFAs concentration stayed below 100 mg HAc/L. After the high production peak of 2100 mL/d observed at inoculation (Figure 7), a stable biogas production of 222 mL/d (R1) and 223 mL/d (R2) was achieved (averaged for days 8 to 30), with a methane content of around 60.9 3.92 ± % (R1) and 71.4 ± 2.01 % (R2). A COD balance showed that methane production corresponded to 43 % and 65 % of the influent COD in the reactors R1 and R2, respectively. The difference amongst them can be explained from the ammonia removal system used: *in situ* ammonia removal caused a pH decrease in R1 that was detrimental to the anaerobic digestion whereas extraction

For Peer Review Only

in feed proved to be a better strategy. Total suspended solids also decreased along the experiment, due to hydrolysis processes (Figure 8).

The ammonia extraction system in R1 resulted insufficient for the existing nitrogen load, since ammonium concentration increased along the experiment, due to the high nitrogen content in the urine and the hydrolysis of the *inoculum* (Figure 9). The progressive pH drop provoked by ammonia extraction from the biogas (from 7.4 to 7.0, Figure 10) caused the low efficiency of the extraction system, since free ammonia concentration in the reactor and therefore in the biogas descended too. Slower ammonia extraction velocity allowed the system to remain at equilibrium. However, free ammonia concentration remained under 30 mg NH_3-N/L which, according to previous results, was not detrimental to acetotroph methanogens. This was confirmed by the percentage of influent organic matter converted into methane (43 %)

On the other hand, ammonium concentration in R2 followed a decreasing trend due to the lower Nitrogen content of the feed. Ammonia concentration, however, was similar to that in R1 (Figure 10), given that pH was slightly higher (Figure 11). It can be concluded that it is more advisable to perform ammonia extraction on the feed than on the reactor, and the proposed system in R2 is a viable way of doing so, since process inhibition is better controlled. The percentage of biomethanation was significantly higher in R2 (65 %) than in R1(43 %).

One aspect that has to be taken into account, however, is that a high amount of salts are generated during urine hydrolysis and salinity can negatively affect the biomass in the reactor by osmotic stress [16]. The AD-S presented a salinity between 8-12 mS/cm whereas urine contributed with 13-15 mS/cm, therefore progressively increasing conductivity in the reactor. Moreover, the urine extraction process made use of NaOH and HCl for pH control, which increased even more the salinity of the feed.

Another aspect affecting the methanogenic process is the high sulphate concentration in urine, since sulphate-reducing bacteria (SRB) compete with methanogens for the organic matter present in the urine. COD consumed by SRB during the experimental period oscillated between 10 % and 20 % of the total reactor load, a small percentage that can be explained by the high COD to SO4-S ratio in the feed, which reached values greater than 12.

4. Conclusions

For Peer Review Only

Anaerobic digestion of human urine is not feasible without a free ammonia extraction system. However, an adequate organic matter degradation and methane production can be obtained during the anaerobic digestion process of human urine with the application of two ammonia removal strategies: in the urine prior to be fed into the reactors and *in situ* (from the reactors sludge). Both mechanisms allowed for a proper process performance although better results were obtained with the first one.

Therefore, this study showed that bubbling the biogas through sulfuric acid is a valid alternative for yellow wastewater treatment. Nitrogen can be recovered for agriculture in the form of ammonium sulphate and ammonia inhibition in the reactor is reduced. However, an off-line ammonia extraction system (i.e., in the feed stream) is preferred due to the higher nitrogen recovery rates and higher biomethanation grade that was achieved (65 % vs 43 %).

On the other hand, results showed that after a long period of time under inhibiting conditions, biomass was not able to completely recover and thus its performance, despite the lower ammonium concentrations achieved, was still poor. This suggests that preventing ammonia inhibition in the reactor can be a more advisable strategy than varying the conditions in the reactor once the inhibition has already taken place.

5. Statements and Declarations

5.1 Funding

This research work was possible thanks to the project IISIS IPT-20111023, an INNPRONTA 2011 project granted to FCC Aqualia, partially funded by the Centre for Industrial Technological Development (CDTI) and supported by the Spanish Ministry of Economy and Competitiveness.

5.2 Competing Interests

Authors declare they have no financial or non-financial interests directly or indirectly related to the work submitted for publication.

5.3 Author Contributions

J. Serralta, J. Ferrer and A. Seco contributed to the study conception and design. Material preparation, data collection and analysis were performed by S. Greses, E. Jiménez, and J. Claros. A. Ruiz-Martinez performed data analysis and wrote the first

For Peer Review Only

draft of the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

5.4 Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

[1] Kirchherr J, Reikert D, Hekkert M (2017) *Conceptualizing the circular economy: An analysis of 114 definitions*. Resour Conserv Recy 127, 221-232. https://doi.org/10.1016/j.resconrec.2017.09.005

[2] Larsen TA, Alder AC, Eggen RIL, Maurer M, Lienert J (2009) *Source separation: Will we see a paradigm shift in wastewater handling?* Environ Sci Technol 43 (16), 6121-6125. https://doi.org/10.1021/es803001r

[3] de Graaff MS, Temmink H, Zeeman G, Buisman CJN (2010) *Anaerobic Treatment of Concentrated Black Water in a UASB Reactor at a Short HRT*. Water 2, 101-119. https://doi.org/10.3390/w2010101

[4] Kujawa-Roeleveld K, Zeeman G (2006) *Anaerobic treatment in decentralised and source-separation-based sanitation concepts*. Rev Environ Sci 5, 115–139. https://doi.org/10.1007/s11157-005-5789-9

[5] Etter B, Tilley E, Khadka R, Udert KM (2011) *Low-cost struvite production using source-separated urine in Nepal*. Water Res 45, 852-862. https://doi.org/10.1016/j.watres.2010.10.007

[6] Aguado D, Barat R, Bouzas A, Seco A, Ferrer J (2019) *P-recovery in a pilot-scale struvite crystallisation reactor for source separated urine systems using seawater and magnesium chloride as magnesium sources*. Sci Total Environ 672, 88-96. https://doi.org/10.1016/j.scitotenv.2019.03.485Wei et al. 2018

[7] Ronteltap M, Maurer M, Gujer W (2007) *Struvite precipitation thermodynamics in source-separated urine*. Water Res 41 (5), 977-984. https://doi.org/10.1016/j.watres.2006.11.046

[8] Garcia ML, Angenent LT (2009) *Interaction between temperature and ammonia in mesophilic digesters for animal waste treatment*. Water Res 43, 2373–2382. https://doi.org/10.1016/j.watres.2009.02.036

[9] Liu ZG, Zhou XF, Zhang YL, Zhu HG (2012) *Enhanced anaerobic treatment of CSTR-digested effluent from chicken manure: The effect of ammonia inhibition*. J Waste Manag 32, 137–143. https://doi.org/10.1016/j.wasman.2011.09.015

[10] Cardona L, Levrard C, Guenne A, Chapleur O, Mazéas L (2019) *Co-digestion of wastewater sludge: Choosing the optimal blend.* J Waste Manag, 87, 772-781. https://doi.org/10.1016/j.wasman.2019.03.016

[11] Rahman MA, Møller HB, Saha CK, Alam MM, Wahid R, Feng L (2017) *Optimal ratio for anaerobic co-digestion of poultry droppings and lignocellulosic-rich substrates for enhanced biogas production*. Energy Sustain Dev 39, 59-66. https://doi.org/10.1016/j.esd.2017.04.004

For Peer Review Only

[12] Akbay HEG, Dizge N, Kumbur H (2021) *Enhancing biogas production of anaerobic co-digestion of industrial waste and municipal sewage sludge with mechanical, chemical, thermal, and hybrid pretreatment*. Bioresour Technol 340, 125688. https://doi.org/10.1016/j.biortech.2021.125688

[13] Moosbrugger RE, Wentzel MC, Ekama GA, Marais GvR (1992) Simple Titration Procedures To Determine H2CO3* Alkalinity And Short-chain Fatty Acids In Aqueous Solutions Containing Known Concentrations Of Ammonium, Phosphate And Sulphide Weak Acid/Bases. WRC Report No. TT 57/92, UCT Research Report W 74.

[14] APHA (2005) American Public Health Association/American Water Works Association/Water Environmental Federation . Standard methods for the Examination of Water and Wastewater, 21st edition, Washington DC, USA.

[15] Zhang L, Lee YW, Jahng D (2012) Ammonia stripping for enhanced biomethanization of piggery wastewater. J Hazard Mater 199-200 36–42. https://doi.org/10.1016/j.jhazmat.2011.10.049

[16] Chen Y, Cheng JJ, Creamer KS (2008) Inhibition of anaerobic digestion process: A review. Bioresour Technol 99, 4044–4064. https://doi.org/10.1016/j.biortech.2007.01.057

For Peer Review Only

Figure 1: Setup for the continuous reactors, with (a) and without (b) *in situ* ammonia extraction

Figure 2: Accumulated biogas production during the batch tests

Figure 3: COD and VFAs concentration in R1 and R2 during phases I and II. Grey area represents the establishment of the ammonia extraction system

Figure 4: Biogas production and methane content in R1 and R2 during phases I and II. Grey area represents the establishment of the ammonia extraction system

Figure 5: Ammonium and free ammonia concentration in R1 and R2 during phases I and II. Grey area represents the establishment of the ammonia extraction system

Figure 6: pH in R1 and R2 during phases I and II. Grey area represents the establishment of the ammonia extraction system

Figure 7: Biogas production and methane content in R1 and R2 during phase III

Figure 8: Total and % volatile suspended solids in R1 and R2 during phase III

Figure 9: Ammonium and ammonia concentration in R1 and R2 during phase III

Figure 10: pH in R1 and R2 during phase III

Table 1: Fresh urine characterization (before dilution)

Table 2: Operational conditions of the continuous experiments

Table 3: Used equipment and methods for determination of the analyzed parameters

Table 4: Operational conditions of the batch experiments conducted in this study and measured values for pH, ammonium and methane production

For Peer Review Only