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Abstract: In this study, mono-digestion of the organic fraction of municipal solid waste (OFMSW) and co-digestion of OFMSW with beech sawdust, simulating green waste, were used to investigate the maximum operational total solid (TS) content in semi-continuous high-solids anaerobic digestion (HS-AD). To alleviate substrate overloading in HS-AD, the effluent mass was relatively reduced compared to the influent mass, extending the mass retention time. To this aim, the reactor mass was daily evaluated, permitting to assess the reactor content removal by biogas production. During mono-digestion of OFMSW, the NH3 inhibition and the rapid TS removal prevented to maintain HS-AD conditions (i.e. TS ≥ 10 %), without exacerbating the risk of reactor acidification. In contrast, the inclusion of sawdust in OFMSW permitted to operate HS-AD up to 30 % TS, before acidification occurred. Therefore, including a lignocellulosic substrate in OFMSW can prevent acidification and stabilize HS-AD at very high TS contents (i.e. 20-30 %).

Semi-continuous Mono-digestion of OFMSW and Co-digestion of OFMSW with Beech Sawdust: Assessment of the Maximum Operational Total Solid Content

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2nd May 2018

Prof. R. Dewil,
Co-Editor-in-Chief
Journal of Environmental Management

Cover letter - manuscript submission

Dear Prof. R. Dewil,

Please find attached the manuscript with the title *"Semi-continuous Mono-digestion of OFMSW and Co-digestion of OFMSW with Beech Sawdust: Assessment of the Maximum Operational Total Solid Content"* by Vicente Pastor Poquet, Stefano Papirio, Eric Trably, Jukka Rintala, Renaud Escudié and Giovanni Esposito.

High-solids anaerobic digestion (HS-AD) is a well-established treatment technology for the organic fraction of municipal solid waste (OFMSW), being operated at a total solid (TS) content ≥ 10 %. Thus, HS-AD enhances the overall economy of the process, while contributes to the abatement of the uncontrolled greenhouse gases emissions associated to the landfilling of OFMSW.

The semi-continuous HS-AD reactor setup is widely used for OFMSW treatment. However, the design and operation of HS-AD reactors strongly depend on the OFMSW composition and/or the inclusion of lignocellulosic substrates to OFMSW, which might be associated to regional/seasonal variances and/or the local strategies for waste management (i.e. recycling). Moreover, since the early HS-AD studies for OFMSW treatment (i.e. back to the 80's), the increasing awareness about sustainable development and global warming have triggered the implementation of new policies and/or waste management strategies, influencing the OFMSW composition. For example, a progressive reduction in the inert content (i.e. plastics, metals) of OFMSW has been observed in Europe during the last 30 years associated to the European Waste Framework Directive.

Therefore, understanding the effect of the OFMSW composition to determine the TS content in semi-continuous HS-AD reactors is crucial to take advantage of the benefits of this biotechnology. In this scheme, the laboratory-scale experiments permitted to highlight invaluable aspects of semi-continuous HS-AD as, for example, the need to reduce the effluent in comparison to the influent to maintain the reactor content constant, extending the biomass retention time and avoiding the reactor failure.

The present study relates the OFMSW composition with the goal of maintaining a high operating TS content in laboratory-scale semi-continuous HS-AD reactors. The results suggest that the maximum TS to be used in HS-AD depends on the OFMSW biodegradability and presence of inhibitory compounds (i.e. NH_3), being the addition of lignocellulosic substrates an adequate strategy to overcome acidification and stabilize HS-AD of OFMSW at very high TS contents (i.e. 20-30 %).

The authors consider that the present study address the *Journal of Environmental Management* requirements, regarding the implementation of environmental technologies for organic waste treatment and resource recovery. Particularly, the study links together the OFMSW composition, the process economy and operation, and the NH_3 inhibition, being this information particularly important for scientists and engineers, as well as for the development of mathematical models for HS-AD of OFMSW.

All the authors mutually agreed to submit this manuscript to *Journal of Environmental Management*. We confirm that the manuscript is an original work, that it has not been previously submitted to the *Journal of Environmental Management*, and that it is not under consideration for publication anywhere else.

We kindly thank you for your time and consideration. In case you need to contact me, please feel free to use the below address, phone or e-mail address.

Sincerely yours,

Vicente Pastor Poquet

On behalf of all the co-authors

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RESPONSE TO THE EDITOR'S AND REVIEWERS' COMMENTS

Title: Semi-continuous Mono-digestion of OFMSW and Co-digestion of OFMSW with Beech Sawdust: Assessment of the Maximum Operational Total Solid Content
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Date: 7th September 2018

Dear reviewers and manuscript editor,

We would like to express our sincere appreciation for all the time and effort that you devoted to the examination of this manuscript. All the comments have been carefully considered and amended accordingly. Please, find below the due answers and proposed modifications for each of your previous comments. We believe that after these modifications, the manuscript has enhanced its quality and readability considerably. Please, do not hesitate to contact me/us any time you consider it necessary.

Sincerely Yours,

Vicente Pastor Poquet

On behalf of all the co-authors

Editor Recommendation: "Due to space limitations in the printed journal, we are requesting that all authors reduce the length of their papers by at least 10% if possible. If your paper includes large tables or datasets, it is preferred that these be published as supplementary material in Science Direct rather than in print".

Following with the recommendation for reducing the length of the manuscript, several References from the original manuscript have been removed, while Table 1 has been moved to Supplementary Information (as Table A1). Importantly, removing some references did not affect the overall content of the manuscript. Those references were also modified to address the first comment of Reviewer #2. Please see these comments below. Meanwhile, the corrections proposed by the reviewers required extending slightly the manuscript. Thus, all these corrections were kept as short as possible, while some minor modification thorough the text permitted also to reduce slightly the length of the manuscript. The authors consider that reducing further the manuscript might result in a loose of important information. In either case, we could address any further suggestion in this regard, whether the editor and/or reviewers consider it necessary.

Lines 211-212 previously stating: "A summary of the weekly operational variables is presented in Table 1"

were substituted by

"A summary of the weekly operational variables is presented as Supplementary Information".

Meanwhile, Table 2 and Table 3 were renamed as Table 1 and Table 2 throughout the manuscript.

With all the above, the original version of this manuscript contained 7777 words (including 45 references) + 3 tables + 5 figures.

In this version, after addressing all the due corrections, the manuscript contains 7991 words (but only 36 references) + 2 tables + 5 figures.

Reviewer #1: This is an interesting study. Like many in this area, it is quite empirical and observational and does not address underlying mechanism. I do not think this is a reason for rejecting the work since the results are, nevertheless, interesting.
I suggest that the authors incorporate some statistical treatment of the data - for example:

a. **Include some details in the Methods about how the data were treated statistically**

To address this comment, a new subsection within the methodology was added in Lines 254-258:

“2.5 Statistical Analyses

The Dixon’s test for BMP outliers was applied as recommended by Holliger et al. (2016). The unpaired t-test of Microsoft Excel 2016 (Microsoft, USA) was applied to determine the statistical significance of experimental data, using the two-tail p-value at 95 % confidence.”.

b. **In Tables 2 and 3 how were the errors estimated? Why do only some values have errors estimated?**

In this study, results in Tables 2 and 3 were averaged and the standard deviation calculated with Excel®. On the other hand, the BMP values were calculated as recommended by Holliger et al. (2016).

In this manuscript version, the BMP values in Table 2 have been updated, since BMP outliers have been removed as also recommended by Holliger et al. (2016).

BMP values have been also modified in Lines 269-270 and Line 434.

Meanwhile, in Table 3, since only TS and VS of the co-digestion inoculum were obtained in duplicate, the standard deviation of these two analyses has been removed to homogenize the results format.

c. **Where appropriate, use statistical tests to compare values**

Please, see comment (a) above. In this manuscript version, the unpaired t-test has been carried for all the previously-used comparisons between semi-continuous reactors.

Particularly, p-values have been added in Lines 302-303, 305-306, 322-325, 358-359, 360-361, 366-367, 374-375, 440-442 and 504-505.

Reviewer #2: The aim of the current manuscript by Pastor-Poquet et al was to investigate the high solids-AD performance using the OFMSW and the addition of a green lignocellulosic waste (sawdust) as a strategy to achieve a more stable and efficient biogas production. The work presents results including TS, nitrogen, % CH4 and VFA to support the performance of the AD processes in two groups of reactor, including mono and co-digestion. The results provided are interest to the wider readership of the journal, since its interesting approach. Nevertheless, it is my opinion that addressing a few points will improve the overall quality of the manuscript. They are as follows:

General recommendations:

- There are many references from 1990, 1995 and 200 for example. Actually, references from the past 15 years represents 40% of the overall references used. I recommend updating them, since many interesting contributions were published after this time.

The summary of references within the original manuscript version was:

No. References = 45
Year ≤ 2000 → 15 Ref. (33 %);
Year ≤ 2005 → 24 Ref. (53 %);
Year ≤ 2008 → 30 Ref. (66 %);

In this version, the references summary is as follows:

No. References = 36
Year ≤ 2000 → 5 Ref. (14 %);
Year ≤ 2005 → 12 Ref. (33 %);
Year ≤ 2008 → 18 Ref. (50 %);

As it can be observed, several references from year ≤ 2000 have been removed and/or updated as recommended. Importantly, all the added references condense the same information of the previously-used references. On the other hand, some references were also added to support some of the information needed to address all the rest of comments throughout this revision document.

- There are many interesting data regarding reactor performance, so I strongly believe that a table including the main results (averages from a week with the same OLR for example) would helping the readers understanding the specific discussions.

All coauthors agree that including more experimental data could enhance the quality of the manuscript. However, it would extend also the length of the manuscript. Therefore, to address this particular comment, 4 more tables were added as Supplementary Information. In Tables A2 and A3 the weekly-averaged results of 7-d OLR and 7-d MRT are presented for mono-digestion and co-digestion reactors, respectively. On the other hand, Tables A4 and A5 include the weekly averaged dynamic variables (i.e. TS, VS, pH, TAN, NH_3, total VFA and CH_4 content) for mono-digestion and co-digestion reactors, respectively. These tables could be further modified and/or included within the due manuscript, whether the manuscript editor and/or reviewers consider it necessary.

Noteworthy, in Lines 285-286, the following sentence was also added: “The weekly-averaged results were also included as Supplementary Information”.

Since Tables A2-A5 contain all the experimental results until Week No. 16, Table A1 (previously Table 1) was extended accordingly to ease comparison of results.

- Please add a comma (,) in the thousand units in the text and figures.

We have modified the VFA units from mg/kg to g/kg throughout the text.

In Lines 277 to 279, “2300 and 3000 mg/kg” was substituted by “2.30 and 3.00 g/kg”.

In Line 281, “20 mg/kg” was substituted by “0.02 g/kg”.

In Line 473, “3000 to 9000 mg/kg” was substituted by “3.00 to 9.00 g/kg”.

In Line 475, “700 mg/kg” was substituted by “0.70 g/kg”.

In Line 476, “150 mg/kg” was substituted by “0.15 g/kg”.

In Lines 476-477, “5000, 4000 and 1100 mg/kg” was substituted by “5.00, 4.00 and 1.10 g/kg”.

In Line 478, “3000 to 5000 mg/kg” was substituted by “3.00, to 5.00 g/kg”.

In Line 482, “4000 mg/kg” was substituted by “4.00 g/kg”.

In Line 483, “1200 (day 7) to 5800 mg/kg” was substituted by “1.20 (day 7) to 5.80 g/kg”.

In Line 490, “2700 to 5800 mg/kg” was substituted by “2.70 to 5.80 g/kg”.

In Line 494, “8300 mg/kg” was substituted by “8.30 g/kg”.

In Lines 494-495, “360 mg/kg” was substituted by 0.36 g/kg”.

In Line 497, “8200 mg/kg” was substituted by “8.20 g/kg”.

In Line 498, “350 mg/kg” was substituted by “0.35 g/kg”.

In Line 499, “7200 mg/kg” was substituted by “7.20 g/kg”.

In Line 501, “1000 to 3700 mg/kg” was substituted by “1.00 to 3.70 g/kg”.

In Lines 501-502, “500, 140 and 5 mg/kg” were substituted by “0.50, 0.14 and 0.00 g/kg”

In Lines 502-503, “3000-3500, 2900-3200 and 2500-2600 mg/kg” was substituted by “3.00-3.50, 2.90-3.20 and 2.50-2.60 g/kg”.

Noteworthy, in Figures 3 and 5, the VFA units have been modified from mg/kg to g/kg, and the figures slightly enlarged.

Specific recommendations:
Key-words: rep;ave OFMSW since it already appears on the title.

In this version, Keyword “OFMSW” has been substituted by “Acidification”.

P6 L3: The substrate were able to homogenization by itself? Was there any difficulties by doing so or extra water was added? It is important to register it.

To address this comment, Line 114 previously stating

“fully homogenized and stored in 5L buckets”

was substituted by

“fully homogenized manually and stored in 5L buckets”.

On the other hand, the following sentence was also included in Lines 115-116: “During mincing and homogenization, no extra water was added to the raw substrate”.

P6 L16: Is it possible to provide the OFMSW and inoculum collection point (city, names...)?

To address this comment, Lines 109-111 previously stating

“OFMSW consisted of a mixture of household waste, restaurant waste, spent coffee, and garden waste”

were substituted by

“OFMSW consisted of a mixture of household waste collected in Cassino (Italy), restaurant waste, spent coffee, and garden waste collected at the university facilities”

Similarly, Lines 123-124 previously stating

“sludge collected from a mesophilic (35ºC) digester treating buffalo manure and mozzarella whey”

were substituted by

“sludge collected from a mesophilic (35ºC) digester treating buffalo manure and mozzarella whey (Capaccio, Italy)”.

P6 L33: Is there any explanation for this 1 month period without feeding the 'source reactor? It seems that the 7 and 15 days from feeding restart was used for degassing period (which is more than necessary), so it is recommended to explain this 'extra' 30 days.

To address this comment, Lines 128-131 previously stating

"Prior to start the mono-digestion experiments, the source reactor was kept unfed for 1 month and, subsequently, the feeding with diluted OFMSW was resumed to recover the methanogenesis activity"

were substituted by

"Prior to start the mono-digestion experiments, the source reactor was kept unfed for 1 month and, to consume/reduce the organic content, while continuing with the inoculum adaptation to the new substrate. Subsequently, the feeding with diluted OFMSW was resumed to recover methanogenesis"

P7 L35: It is better to change 'or' to 'and' since the reactors were all operated at the same time.

Dear reviewer, we apologize but we consider that the word 'or' condenses better the fact that mono-digestion and co-digestion reactors were not operated at the same time. Thus, in Lines 138-140, it was previously mentioned that "Once the mono-digestion experiments ended, the source reactor was kept unfed for 1 month to serve as inoculum for the co-digestion experiments".

P7 L40: I recommend: "The semi-continuous reactors and reactor influents/effluents were weighted on a ±0.01 precision scale."

Lines 156-159 previously stating

"The semi-continuous reactors were weighted on a ± 0.01 kg precision scale, before/after the discharge/loading operations, while the reactor influents/effluents were weighted on a ± 0.01 g precision scale"

were substituted by

“The semi-continuous reactors (i.e. kg) and the reactor influents/effluents (i.e. g) were weighed on a ± 0.01 precision scale”.

P7 L45: add OLR unit

Lines 159-163 previously stating

“The OLR was evaluated as the daily VS substrate addition divided by the reactor mass content, while the mass retention time (MRT) was evaluated as the quotient between the reactor mass and reactor effluent mass”

were substituted by

“The OLR was evaluated as the daily substrate addition in terms of volatile solids (VS) divided by the reactor mass content (i.e. g VS/kg·d), while the MRT was evaluated as the quotient between the reactor mass and the daily effluent mass (i.e. days)”.

P7 L50: add MRT unit

Please, see previous comment.

P8 L33-45: This paragraph is quite confusing. I recommend clarifying the ideas here or adding an extra explanation closer to the results.

To address this comment, Lines 186-190 previously stating

“The methanogenic activity was roughly associated to the relative increase of the pH and inorganic carbon alkalinity (ALK_P), the reduction of the reactor mass content and the biogas production compared to previous operational values”

were substituted by

"In each reactor, the methanogenic activity was roughly associated to the relative increase of the pH and inorganic carbon alkalinity (ALK_P), the reduction of the reactor mass content and the biogas production compared to previous operational values, as also mentioned in section 3.2".

Meanwhile, Lines 292-293, previously stating

"As pH recovered in reactor A (i.e. pH = 7.6, day 29), feeding was also resumed"

were substituted by

"As pH recovered in reactor A (i.e. from 6.4 on day 21, to 7.6 on day 29) due to methanogenesis activity, feeding was resumed.".

Similarly, in Lines 293-295, the following sentence was also added: "During the same period, ALK_P in reactor A increased alongside pH from 1.1 to 2.7 g $CaCO_3$/kg (data not shown), as an indicator of ongoing methanogenesis".

Finally, in Lines 320-322, the following sentence was also added: "As an example, the occurrence of methanogenesis led to a 60 g removal of the reactor mass content in both mono-digestion reactors from day 37 to 41 (data not shown).".

Table 1: Why the mass added in the reactors A and B for mono and A, B and C for co-digestion are different throughout the same week if there were to be operated at the same conditions?

In this study, parallel reactors were conceived to be operated under slightly different conditions, particularly regarding the use of a relatively higher OLR, to observe potential differences in the reactor performance. For example, in Lines 302-304, it was mentioned that "The OLR in (mono-digestion) reactor B was averagely about 1.5 g VS/kg·d higher than that used in reactor A during the whole experiment, explaining the relatively faster acidification observed in reactor B".

To address this comment, Lines 183-186 previously stating

“Mono-/co-digestion reactors were fed in parallel though different OLR/MRT were used, as shown in section 3.2”

were substituted by

“To evaluate the differences in the reactor performance, mono-digestion reactors were fed in parallel using different OLR/MRT in each reactor, as shown in section 3.2. Subsequently, co-digestion reactors were also operated in parallel at three different OLR/MRT”.

P10 L8: Why extra water was used for the BMP test of OFMSW, since the mass of substrate could be reduced to keep the same ISR? Why NaHCO3 was added? Please provide the guidelines used as a reference here.

In Lines 234-240, the following paragraph was added: “BMP tests were performed according to Angelidaki and Sanders (2004) and Holliger et al. (2016). In the BMP test for OFMSW, the distilled water and $NaHCO_3$ addition served to minimize the chances of inhibition (i.e. by NH_3) and acidification, respectively. In contrast, NH_3 build-up and acidification were not expected in the BMP test of sawdust, due to the low nitrogen content and the reduced biodegradability of sawdust, as thoroughly discussed in next section, permitting also to use a lower ISR”.

P10 L18: Why ISR of OFMSW and sawdust were different? The biological activity of the inoculum was evaluated using a reference sample?

Please, see previous comment.

In Lines 242-243, the following sentence was added: “Inoculum activity assays using a reference substrate were not performed”.

Table 2: How the alkalinity of the solid substrate (sawdust) was determined? Provide a simple procedure here since it is not very usual.

In this study, solid and semi-solid samples were previously diluted as recommended by the EPA (2015) guidelines. Thus, to address this comment, <u>Lines 215-222</u> previously stating

“The pH, TS, volatile solids (VS), total Kjeldahl (TKN) and ammonia (TAN) nitrogen, and the total hydrogen sulfide were determined by the standard methods (APHA, 1999). The partial (ALK_P) and intermediate (ALK_I) alkalinity were determined as proposed by Lahav et al. (2002)”

were substituted by

“The pH, ALK_P and ALK_I were determined from the supernatant of solid and semi-solid samples, as proposed by Lahav et al. (2002), after diluting the sample with distilled water, homogenization and centrifugation at 6000 rpm for 15 min (EPA, 2015). The TS and VS content, total Kjeldahl (TKN) and ammonia (TAN) nitrogen, and the total H_2S were determined by the standard methods (APHA, 1999)”.

P11 L13: Minor modifications were related to the analytical methods or the results obtained? Please clarify this topic.

The small differences in the physical-chemical analysis for OFMSW (i.e. TS, TKN and BMP) were associated to the waste heterogeneity. Thus, TS and TKN were normally conducted in duplicate, while 6 replicates were used for the BMP tests. To address this comment, <u>Lines 271-274</u> previously stating

“Minor modifications observed in the OFMSW characterization were attributed to the substrate heterogeneity”

were substituted by

“Despite the thorough mincing and homogenization, minor modifications were observed in the OFMSW characterization (i.e. TS, TKN or BMP), mainly attributed to the substrate heterogeneity”.

P11 L42-57 and P12 L1-13: How the authors explain the effect of no operation for 2 days a week? The lack of feeding during this period can create special conditions regarding 'reactor recovering' in terms of VFA consumption (see Fig 3a and Fig 3b) and this trend is not being showed in the figures. How the average 5days to 7 days can really avoid this? Moreover, if pH was under 6.5 and CH4 content under 40% why data are showed in the figures after day 78, since there was no feeding? It seems these extra data do not say much.

The absence of feeding in AD normally triggers an increase in pH, TAN, %CH_4 and biogas production, while TS and acetate decrease. Thus, the influence of various days with no operation can be ‘easily’ spotted within the data trends, particularly in the rapid pH increases.

Meanwhile, 7-d-averaged OLR and MRT were thought to be more convenient operative parameters, since the cascade of bio-physical-chemical processes occurring in the digester at different time-scales normally results in a delay response in biogas production and/or VFA buildup. Moreover, a 7-d-average ‘smooths’ the digester operational parameters (i.e. OLR and MRT), easing the comparison among reactors operated under slightly different conditions. In short, a 7-d-average condenses both the operation of that particular operation day, but also the 6 days immediately preceding. Using moving-average is a common practice in environmental modelling of wastewater treatment plants and/or weather events.

On the other hand, even if after day 78 (approx.) there was no feeding, operation days from 78 to 100 were used to assess potential recovery strategies for these semi-continuous reactors, as condensed in <u>Lines 209-211</u> (“Semi-continuous reactors were fed until acidification occurred. From this point, feeding was stopped and reactor dilution and/or inorganic salt addition (i.e. $NaHCO_3$ and $FeCl_2$) were tested as recovering strategies”). The recovering strategies are also explained in Section 3.5.

To address this particular comment, in Lines 164-169, the following paragraph was added:

“Moving-average operational variables are well suited indicators of the immediately preceding operations (i.e. feeding, dilution, reactor content removal) to discern about the risk of VFA buildup in semi-continuous digesters. Moreover, expressing the operational conditions as a moving-average eases the comparison of digesters, when feeding days are not the same or an important mass removal occurs”.

Meanwhile, in Lines 511-518, the following paragraph was also added:

“Feeding the reactors a maximum of 5 days per week influenced the reactor dynamics, since pH increased and VFA – mainly acetic acid – decreased during the periods with no feed. The pH and VFA modifications [Figure 3 and 5] were associated to the TS removal, as mentioned before, and also affected the biogas production/composition, and the TAN buildup [Figures 2 and 4]. As an example, in co-digestion reactor A, pH increased from 7.0 to 7.9 from day 37 to 41, while acetic acid decreased from 4.30 to 2.40 g/kg, triggering a biogas production of 0.6 L/kg Reactor Content and a methane content increase from 59 to 70 % [Figure 4 and 5]”.

Figure 4a: Why there are two lines for both reactors A, B and C? The second group of lines are related only to OFMSW in the mixture? Express that in the subtitle.

Figure Caption 4a previously indicating

“Organic loading rate”

was substituted by

“Organic loading rate – parentheses indicate the sole addition of OFMSW”.

P14 L16-18: Same here. Why the results after day 76 on reactors A, B and C are important since there is no feeding after this time.

Operation days from 78 to 100 were used to assess potential recovery strategies for these semi-continuous reactors, as mentioned in the second comment above.

P18 L21-45: Too general discussion of the results. I recommend eliminating this topic and discuss only data trend.

In the revised version of this manuscript, Lines 460-470 have been removed as recommended.

P20 L23: Change 'Reactor Content' to 'reactor content'

Capitalized “Reactor Content” was substituted by “reactor content” in Lines 192-193, 522, 524-525, 543 and 546-547.

Topic about recovering strategies: Why NaHCO3 and FeCl2 were used specifically? Had the authors consider using a trace elements solution instead of single compound? Please add in the figures the specific times when extra buffering solution was added. You can use an arrow for example.

The following sentence was added in Lines 566-568: “Adding $NaHCO_3$ is normally used to counteract acidification when digesters show a reduced ALK_P (Chen et al., 2008; Holliger et al., 2016)”.

The following sentence was also added in Lines 575-578: “Both Fe^{2+} and/or Fe^{3+} can be used to precipitate sulfide in AD, but Fe^{2+} was preferred in this study to avoid the inclusion of a strong electron acceptor (i.e. Fe^{3+}) that could react with organic compounds in the anaerobic digester (i.e. $Fe^{3+} + 1/2\ H_2 \rightarrow Fe^{2+} + H+$, $\Delta G^{o\prime} << 0$) (Fermoso et al., 2015; Rittman & McCarty, 2001).

The following paragraph was also added in Lines 599-601 “Another strategy to prevent reactor acidification and/or enhance the digester performance is trace element (i.e. Se, Ni, Co, Mo, W) addition (Fermoso et al., 2015; Mancini et al., 2018). However, this was out of the scope of this manuscript”.

In Figure 2 and 3, arrows representing the $NaHCO_3$, $FeCl_2$ or inoculum additions were added.

In Figure Captions 3 and 4 the following sentence was also added: “Black arrows represent the addition of $NaHCO_3$ in reactor A, while dotted arrows represent the $FeCl_2$ or inoculum addition in reactor B”.

Conclusions: This topic must be improved. The comparison between reactors performance regarding the influence of sawdust addition could really be evaluated in terms of 'specific biogas production' (L biogas kg VS-1 added), since operation conditions were very different in terms of strategy.

To address this comment, Lines 659-662 previously stating “Overloading was related to the substrate biodegradability and inhibitory content, since the maximum TAN content was 4.8 and 3.6 g N/kg, while the biogas production was 166-192 and 59-71 L/kg VS Fed, for mono-digestion of OFMSW and co-digestion of OFMSW and sawdust, respectively” were removed.

Instead, Lines 649-654 previously stating

“In this study, reducing the effluent compared to the influent mass (i.e. 1-38 %) permitted to extend the biomass retention time in semi-continuous mono-digestion of OFMSW. However, the sole implementation of influent/effluent uncoupling was not sufficient to avoid reactor overload/acidification when reaching HS-AD conditions (i.e. TS ≥ 10 %)”

were substituted by

“In this study, reducing the effluent compared to the influent mass (i.e. 18 %) permitted to extend the MRT in semi-continuous mono-digestion of OFMSW, and obtain a specific biogas production of 229 L/kg VS added, due to the high biodegradability of OFMSW. However, the sole implementation of influent/effluent uncoupling was not sufficient to avoid reactor overload and acidification when reaching HS-AD conditions (i.e. TS ≥ 10 %)”.

Moreover, in Lines 654-659 the following sentence was added: “The average OLR was 4.5 g VS/kg·d, whereas a maximum 11.5 % TS was reached. In contrast, the addition of beech sawdust to OFMSW allowed to operate co-digestion reactors with an average OLR of 8.3 g VS/kg·d, and reach a maximum 29.0 % TS. Co-digestion lowered by 22 % the TAN content, though an average 186 L/kg VS added of biogas was obtained”.

Finally, in Lines 665-667 the following sentence was added: “Nonetheless, a compromise must be found between increasing the TS content and reducing the specific biogas production by co-digestion, since both aspects strongly determine the HS-AD economy for OFMSW treatment”.

Minor changes, not suggested by the reviewers, were also added throughout the manuscript to enhance its readability and/or reduce the overall length:

In Line 23, “biomass” was substituted by “mass”.

In Lines 25-26, previously stating “the NH_3 inhibition and the rapid solid removal observed prevented to maintain HS-AD conditions” was substituted by “the NH_3 inhibition and the rapid TS removal prevented to maintain HS-AD conditions”.

In Line 27, “Meanwhile” was substituted by “In contrast”.

In Lines 54-55, “potential” was substituted by “risk”.

In Lines 62-63, “among other advantages” was removed.

In Lines 68-71, previously stating “Furthermore, overloading is in many cases related to the presence of methanogenic inhibitors. For example, particular attention must be paid upon the inhibitory effects of NH_3 in HS-AD, due to the high protein content of OFMSW” were substituted by “Furthermore, overloading is in many cases related to the presence of methanogenic inhibitors, such as NH_3, due to the high protein content of OFMSW”.

In Lines 73-75, previously stating “HS-AD is a mature technology, while most of the recently-constructed industrial plants for OFMSW treatment have targeted the semi-continuous HS-AD process” were substituted by “HS-AD of OFMSW is a mature technology, with most of the recently-constructed industrial plants targeting the semi-continuous HS-AD process”.

In Lines 81, “extensively” was removed.

In Lines 88, “encountered” was removed.

In Line 98, “two or three parallel” was substituted by “semi-continuous”.

In Lines 102-103, “biomass retention time” was substituted by “mass retention time (MRT)”.

In Line 104, “these” was substituted by “the”.

In Line 109, “main” was removed.

In Line 122, “working” was substituted by “operated”.

In Line 134, “showed” was substituted by “shown”.

In Line 137, “and/or” was substituted by “and”.

In Line 140-141, previously stating “3.4 kg portions of reactor content were filtered through a 1 mm mesh and used to inoculate co-digestion reactors” were substituted by “3.4 kg of reactor content were filtered through a 1 mm mesh and used to inoculate each co-digestion reactor”.

In Line 176, “and/or” was substituted by “or”, and “supplemented” was substituted by “fed”.

In Line 194, “and/or” was substituted by “or”.

In Lines 207-208, “the MRT was relatively extended in the semi-continuous reactors” was substituted by “the MRT was relatively extended”.

In Line 222, “the free ammonia nitrogen (NH_3)” was substituted by “the NH_3”.

In Lines 231 and 234 “– the” was substituted by “, with an”, while “was” was substituted by “of”.

In Line 247, “with the” was added.

In Line 248, “the” was added.

In Line 250, “Helium” was substituted by “helium”.

In Line 288, “at” was substituted by “on”.

In Lines 290-291, “After two days with no operation (day 20), feeding was resumed in reactor B on day 20, but not in reactor A due to the low pH (i.e. 6.4) observed” was substituted by “After two days with no feed, feeding was resumed in reactor B on day 20, but not in reactor A due to the low pH (i.e. 6.4)”.

In Lines 297 and 355, “Last” was substituted by “The last”.

Lines 315-320, previously stating: “The hydraulic retention time (HRT) and MRT are equivalent concepts defining the average time a fluid and a mass particle, respectively, remain within a continuous reactor. However, the MRT was considered as a more suited indicator in HS-AD, since both the specific weight of the influent/effluent and the reactor mass content might vary, in contrast to ‘wet’ AD were substituted by “In this study, the MRT was considered as a more suited indicator in HS-AD than the hydraulic retention time (HRT), since both the specific weight of the influent/effluent and the reactor mass content varied, in contrast to ‘wet’ AD”

In Line 329, “also” was removed.

In Line 332, “was aimed to promote” was substituted by “promoted”.

In Line 338, “potentially results” was substituted by “resulted”.

In Line 341, “in this study” was removed.

In Line 368, “OLR used” was substituted by “VS fed”.

In Lines 391-393, “The highest TS and VS/TS observed in semi-continuous HS-AD of OFMSW being related to acidification indicate a reduced VS degradation” was substituted by “The highest TS and VS/TS observed in semi-continuous HS-AD of OFMSW were associated with acidification and indicates a reduced VS degradation”.

In Line 403, “might have affected” was substituted by “affects”.

In Line 412, “Further implying” was substituted by “This is a further indication”.

In Line 416, “of” was added.

In Line 429, “Thus, co-digestion” was substituted by “Co-digestion”.

In Line 435, “Moreover” was substituted by “Indeed”.

Paragraph in Lines 447-456 was removed.

In Line 493, “an acetic peak of” was substituted by “a peak of acetic acid”.

In Line 498, “again” was added.

In Line 521, “showed” was substituted by “of OFMSW resulted in”.

In Lines 523-524, “as mentioned in previous subsection” was added.

In Line 525, “of biogas” was added.

In Line 528, “of” was added.

In Lines 531-534, “The CH_4 content reduction is also an indicator of AD imbalance, though it might fail” was substituted by “The reduction of CH_4 content in the biogas is also an indicator of AD imbalance, though it might be inappropriate”.

In Line 536, “cases” was substituted by “reactors”.

In Line 542, “Co-digestion reactor A showed” was substituted by “Co-digestion in reactor A led to”.

In Lines 545-546, “the biogas production” was substituted by “that”.

In Lines 563-564, “(i.e. reactor dilution)” was removed.

In Line 572, “hydrogen sulfide” was substituted by “H_2S”.

In Lines 584-586, “Aiming to recover methanogenesis, water was progressively added to dilute the effect of potential methanogenic inhibitor(s) in all co-digestion reactors.” was substituted by “Aiming to recover methanogenesis in all co-digestion reactors, water was progressively added to dilute the effect of potential methanogenic inhibitor(s)”.

In Line 591, “of” was added, and “were” was substituted by “was”.

In Line 594, “In this study” was substituted by “In conclusion”.

In Lines 596-597, “Therefore” was substituted by “In these conditions”.

In Line 604, “Overloading in this study” was substituted by “In this study, overloading”

In Line 605, “Thus, the” was substituted by “The”.

In Line 616, “The initial TAN in co-digestion” was substituted by “In co-digestion, the initial TAN”.

Finally, “associated to” was substituted by “associated with” in Lines 187, 193, 279, 385, 395-396, 401, 436, 493, 500, 538, 581, 604, 605 and 629.

Semi-continuous Mono-digestion of OFMSW and Co-digestion of OFMSW with Beech Sawdust: Assessment of the Maximum Operational Total Solid Content

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ABSTRACT

In this study, mono-digestion of the organic fraction of municipal solid waste (OFMSW) and co-digestion of OFMSW with beech sawdust, simulating green waste, were used to investigate the maximum operational total solid (TS) content in semi-continuous high-solids anaerobic digestion (HS-AD). To alleviate substrate overloading in HS-AD, the effluent mass was relatively reduced compared to the influent mass, extending the ~~bio~~mass retention time. To this aim, the reactor mass was daily evaluated, permitting to assess the reactor content removal by biogas production. During mono-digestion of OFMSW, the NH_3 inhibition and the rapid TS~~solid~~ removal ~~observed~~ prevented to maintain HS-AD conditions (i.e. TS ≥ 10 %), without exacerbating the risk of reactor acidification. ~~Meanwhile~~In contrast, the inclusion of sawdust in OFMSW permitted to operate HS-AD up to 30 % TS, before acidification occurred. Therefore, including a lignocellulosic substrate in OFMSW can prevent acidification and stabilize HS-AD at very high TS contents (i.e. 20-30 %).

Keywords: High-Solids Anaerobic Digestion; ~~OFMSW;~~ Influent/Effluent Uncoupling; Substrate Overloading; Acidification; Ammonia Inhibition.

1 INTRODUCTION

Anaerobic digestion (AD) of the organic fraction of municipal solid waste (OFMSW), including food waste (FW) and green waste (GW), is a particularly suited treatment biotechnology for energy and by-product recovery (Clarke, 2018; Mata-Álvarez, 2003). ~~(Clarke, 2018; Christensen, 2011; Mata-Álvarez, 2003).~~ In AD, an organic waste is degraded to biogas, mainly composed by CH_4 and CO_2, and a partially stabilized organic digestate, by consortia of different microorganisms working in absence of oxidative species (i.e. O_2 and NO_3^-) (Astals et al., 2015; Gerardi, 2003). ~~(Gerardi, 2003; Switzenbaum et al., 1990).~~

The sequential steps in AD include hydrolysis, acidogenesis, acetogenesis and methanogenesis, during which different inhibitory substances can be formed leading to inhibitory effects for the anaerobic microorganisms and/or even a complete AD failure. Depending on the concentration, free ammonia (NH_3), hydrogen sulfide (H_2S) and free ions (i.e. Na^+) are some of the inhibitory substances in AD, affecting predominantly the methanogenic stage, either acetoclastic and/or hydrogenotrophic, and potentially resulting in the buildup of volatile fatty acids (VFA) and H_2 in the system (Astals et al., 2015; Chen et al., 2008).~~(Chen et al., 2008; Gerardi, 2003; Liu & Boone, 1991; Park & Novak, 2013).~~ Meanwhile, the acetoclastic activity results into inorganic carbon (i.e. HCO_3^-) release in AD, as an important source of pH buffering, minimizing the ~~potential~~ risk of reactor acidification (i.e. $\mathrm{pH} \leq 6.0$) by VFA accumulation (Gerardi, 2003).

The interrelationship between the organic waste characteristics, operational conditions and reactor design determines the AD potential (Karthikeyan & Visvanathan, 2013;

Mata-Álvarez, 2003). AD can be differentiated depending on the operational total solid (TS) content into ’wet’ (i.e. TS < 10 %) and high-solids AD (HS-AD, i.e. TS ≥ 10 %) (Benbelkacem et al., 2015). HS-AD allows the use of a smaller reactor, reducing the need for water addition and minimizing the digestate production~~, among other advantages~~ (Karthikeyan & Visvanathan, 2013; Pastor-Poquet et al., 2018). However, HS-AD drawbacks include the pervasive chances of reactor acidification due to substrate overload (Benbelkacem et al., 2015).~~(Benbelkacem et al., 2015; Kayhanian, 1995).~~ Overloading is the consequence of the slow-growing methanogens being unable to cope with the rapid VFA and/or H_2 buildup resulting from acidogenesis/acetogenesis in HS-AD (Pavan et al., 2000). Furthermore, overloading is in many cases related to the presence of methanogenic inhibitors (Drosg, 2013)~~. For example, particular attention must be paid upon the inhibitory effects of~~ , such as NH_3 ~~in HS-AD,~~ due to the high protein content of OFMSW (Kayhanian, 1999).

HS-AD of OFMSW is a mature technology, with~~hile~~ most of the recently-constructed industrial plants ~~for OFMSW treatment have targeted~~ targeting the semi-continuous HS-AD process (Mattheeuws, 2016).~~(De Baere & Mattheeuws, 2013; Mattheeuws, 2016).~~ The focus of semi-continuous HS-AD lies on the maximization of the organic loading rate (OLR) that optimizes the methane yield and ensures an adequate organic removal at high TS contents (Benbelkacem et al., 2015; Hartmann & Ahring, 2006). ~~(Benbelkacem et al., 2015; Hartmann & Ahring, 2006; Owens & Chynoweth, 1993; Rivard et al., 1990).~~ In this line, depending on the organic waste used in HS-AD, the operational TS content is substantially lower than the feed TS, as the organic substrate is ~~extensively~~

converted to biogas by methanogenesis (Pastor-Poquet et al., 2018). ~~(Kayhanian & Hardy, 1994; Mata-Álvarez, 2003)~~

Therefore, HS-AD lies on a balance between maximizing the OLR and TS content, while minimizing the chances of reactor failure. Particularly, in order to startup HS-AD, the OLR needs to be increased relatively slowly, permitting the methanogens to grow and adapt to the new conditions~~ encountered~~. The transient (non-steady) OLR modification in HS-AD aims to find an optimum stationary (steady-state) operation to be used, avoiding acidification and maximizing the economy of the process (Angelidaki et al., 2006; Bolzonella et al., 2003). However, the risk of inhibition and failure is undesirably high under HS-AD startup, potentially requiring the implementation of recovering strategies (i.e. reactor content dilution) to minimize the influence of inhibitory substances, or even restarting the process when a significant methanogenic imbalance occurs (Fricke et al., 2007; Kayhanian, 1999).

This study evaluated the highest tolerable TS content in semi-continuous HS-AD of OFMSW, by gradually increasing the OLR in ~~two or three parallel~~semi-continuous reactors operated at 55°C, until process failure occurred by acidification. Two feeding strategies were used: mono-digestion of OFMSW and co-digestion of OFMSW and beech sawdust – as a model lignocellulosic substrate, simulating the inclusion of GW in OFMSW. Aiming to minimize the risk of substrate overload, the ~~bio~~mass retention time (MRT) was relatively extended by reducing the effluent compared to the influent mass, according to the daily mass content removal by biogas production observed in the~~se~~ semi-continuous reactors.

2 MATERIALS AND METHODS

2.1 Substrates and Inoculum

The ~~main~~ substrates used in this study were OFMSW and beech sawdust. OFMSW consisted of a mixture of household waste collected in Cassino (Italy), restaurant waste, spent coffee, and garden waste collected at the university facilities, with an approximated wet weight proportion of 45, 35, 15 and 5 % (w/w), respectively. OFMSW was minced twice to a particle size ≤ 5-10 mm by an industrial mincer [REBER 9500NC, Italy], fully homogenized manually and stored in 5 L buckets at -20ºC. During mincing and homogenization, no extra water was added to the raw substrate. A single 5 L bucket of OFMSW was thawed at room temperature overnight, as required to feed the semi-continuous reactors. Goldspan® beech sawdust with 1.0-2.8 mm particle size was used as co-substrate, to simulate biodegradable green/lignocellulosic waste.

The inoculum for semi-continuous experiments was obtained from a pre-adapted ‘wet’ AD (i.e. TS ≤ 5 %) source reactor ~~working~~ operated at 55ºC. The pre-adaptation of 20 L sludge, collected from a mesophilic (35ºC) digester treating buffalo manure and mozzarella whey (Capaccio, Italy), consisted of a 4-month progressive feeding of tap-water-diluted OFMSW at 55ºC, in order to adapt the inoculum to the new substrate and temperature.

Prior to start the mono-digestion experiments, the source reactor was kept unfed for 1 month ~~and,~~ to consume/reduce the organic content, while continuing with the inoculum adaptation to the new substrate. S~~s~~ubsequently, the feeding with diluted OFMSW was resumed to recover ~~the~~ methanogenesis~~ activity~~. After 7 and 15 days from the feeding restart, 4 kg of sludge were taken from the source reactor, filtered through a 1 mm mesh and used to inoculate the mono-digestion reactors “A” and “B”, respectively. Therefore, the inoculum was slightly different in reactors A and B, as ~~showed~~ shown in section 3.1.

During the mono-digestion experiments, the source reactor was periodically fed with diluted OFMSW and~~/or~~ the mono-digestion reactors effluents, to maintain the reactor volume and methanogenic activity. Once the mono-digestion experiments ended, the source reactor was kept unfed for 1 month to serve as inoculum for the co-digestion experiments. Thus, 3.4 kg ~~portions~~ of reactor content were filtered through a 1 mm mesh and used to inoculate each co-digestion reactor~~s~~ “A”, “B” and “C”.

2.2 Experimental Setup

The laboratory-scale semi-continuous reactors consisted of 5 L polyethylene terephthalate (PET) bottles with a modified head allowing the (semi-)solid waste input, reactor content withdrawal and biogas measurement [Figure 1]. The reactor port was a polyvinyl chloride (PVC) flexible hosepipe with two valves, easing the reactor loading/unloading while avoiding air intrusion. The biogas output, containing a sampling septum, was connected to 5 L Tedlar® bags [Sigma-Aldrich, USA]. All reactors were maintained at 55ºC within a temperature-controlled TCF 400 oven [ARGOLAB, Italy].

2.3 Operation Strategy

Two semi-continuous reactors for mono-digestion of OFMSW or three reactors for co-digestion of OFMSW and sawdust were operated simultaneously in a drag-and-fill mode. The semi-continuous reactors (i.e. kg) and the reactor influents/effluents (i.e. g) were weighed on a ± 0.01 precision scale. ~~The semi-continuous reactors were weighted on a ± 0.01 kg precision scale, before/after the discharge/loading operations, while the reactor influents/effluents were weighted on a ± 0.01 g precision scale.~~ The OLR was evaluated as the daily ~~VS~~ substrate addition in terms of volatile solids (VS) divided by the reactor mass content (i.e. g VS/kg·d), while the ~~mass retention time (~~MRT~~)~~ was evaluated as the quotient between the reactor mass and ~~reactor~~ the daily effluent mass (i.e. days). Since the reactors were fed a maximum of 5 days per week, 7-days moving average OLR and MRT were estimated. Moving-average operational variables are well suited indicators of the immediately preceding operations (i.e. feeding, dilution, reactor content removal) to discern about the risk of VFA buildup in semi-continuous digesters. Moreover, expressing the operational conditions as a moving-average eases the comparison of digesters, when feeding days are not the same or an important mass removal occurs.

During each drag-and-fill operation, the reactor content was 1) homogenized before opening the system, 2) sampled and 3) analyzed mainly for pH and alkalinity – since pH had to be maintained over 6.5, as an important methanogenic inhibition might take place below this threshold (De Vrieze et al., 2012; Gerardi, 2003). ~~(Gerardi, 2003; Switzenbaum et al., 1990)~~ Depending on the pH and alkalinity, 4) the proper amount of substrate was used ~~and/~~or diluted as needed, 5) prior to be ~~supplemented~~ fed to the reactors. Finally, 6) the reactor content was homogenized once again, while the Tedlar® bags were checked for biogas production and subsequently emptied.

To increase the reactor TS content from ‘wet’ AD (i.e. TS < 5 %) to HS-AD (i.e. TS ≥ 10 %), the OLR was controlled by increasing/decreasing the daily amount of substrate and/or tap water addition based on the methanogenic activity, and aiming to minimize the substrate overload. To evaluate the differences in the reactor performance, m~~M~~ono-~~/co-~~digestion reactors were fed in parallel ~~though~~ using different OLR/MRT ~~were used~~in each reactor, as shown in section 3.2. Subsequently, co-digestion reactors were also operated in parallel at three different OLR/MRT. In each reactor, t~~T~~he methanogenic activity was roughly associated ~~to~~ with the relative increase of the pH and inorganic carbon alkalinity (ALK$_P$), the reduction of the reactor mass content and the biogas production compared to previous operational values, as also mentioned in section 3.2. For example, a relative pH and ALK$_P$ increase of approximately 0.5 pH units and 0.3 g CaCO$_3$/kg, respectively, alongside a reactor mass removal of about 30-50 g/d and a specific biogas production higher than 250 mL/kg ~~Reactor~~ reactor ~~Content~~content·d were associated ~~to~~ with ongoing methanogenesis, indicating that the OLR could be maintained ~~and/~~or relatively increased. Similarly, the relative increase of intermediate alkalinity (ALK$_I$) (i.e. 0.5 g Acetic Acid/kg) was used as a preliminary indicator of the potential VFA buildup and risk of substrate overload (Lahav et al., 2002).

All these parameters were further complemented with the user’s evaluation of the previous operation, in order to decide for the daily feed/dilution to be used. Thus, all reactors were started with a low OLR (i.e. 2 g VS/kg·d) that was gradually increased to increase the TS content. As reactor performance deteriorated with increasing OLR, the reactor feeding was reduced/stopped to prevent acidification (i.e. pH ≤ 6.0).

The reactor mass was maintained constant by reducing the effluent compared to the influent mass, according to the observed reactor mass content removed by biogas production from the previous operation. With this strategy, the MRT was relatively extended~~ in the semi-continuous reactors~~, aiming to promote the methanogenic adaptation in case of overloading. Semi-continuous reactors were fed until acidification occurred. From this point, feeding was stopped and reactor dilution and/or inorganic salt addition (i.e. $NaHCO_3$ and $FeCl_2$) were tested as recovering strategies. A summary of the weekly operational variables is presented -as Supplementary Information~~in Table 1~~.

2.4 Bio-Physical-Chemical Analyses

~~The pH, TS, volatile solids (VS), total Kjeldahl (TKN) and ammonia (TAN) nitrogen, and the total hydrogen sulfide were determined by the standard methods (APHA, 1999).~~ The pH, ~~The partial (~~ALK_P~~)~~ and ~~intermediate (~~ALK_I~~)~~ ~~alkalinity~~ were determined from the supernatant of solid and semi-solid samples ~~as proposed by~~ (Lahav et al., 2002), after diluting the sample with distilled water, homogenization and centrifugation at 6000 rpm for 15 min (EPA, 2015). The TS and VS content, total Kjeldahl (TKN) and ammonia (TAN) nitrogen, and the total H_2S were determined by the standard methods (APHA, 1999). The ~~free ammonia nitrogen (~~NH_3~~)~~ was approximated as a function of TAN and pH~~, as shown by~~ (Astals et al., 2015). ~~(Angelidaki & Ahring, 1993).~~ The VFA (acetic, propionic, butyric and valeric acids) were measured with an LC-20AD HPLC

[Shimadzu, Japan], mounting a Rezex ROA-Organic Acids 8+ column coupled to a 210 nm UV detector, and using 0.0065 M H_2SO_4 at 0.6 mL/min as mobile phase. The biogas composition (CH_4, CO_2 and H_2) was analyzed with a 3400 GC-TCD [Varian, USA], using argon as carrier gas.

The biomethane potential (BMP) test for OFMSW used 3.0 g of substrate, 50.0 g of source inoculum, 40.0 g of distilled water and 0.10 g of $NaHCO_3$ in 280 mL bottles (6 replicates)~~— the~~, with an inoculum-to-substrate ratio (ISR) ~~was~~ of 2.0 g VS/g VS. The BMP test for sawdust used 1.0 g substrate and 50.0 g of inoculum in 160 mL bottles (3 replicates)~~—~~, with an ~~the~~ ISR ~~was~~ of 1.0 g VS/g VS. BMP tests were performed according to Angelidaki and Sanders (2004) and Holliger et al. (2016). In the BMP test for OFMSW, the distilled water and $NaHCO_3$ addition served to minimize the chances of inhibition (i.e. by NH_3) and acidification, respectively. In contrast, NH_3 build-up and acidification were not expected in the BMP test of sawdust, due to the low nitrogen content and the reduced biodegradability of sawdust, as thoroughly discussed in next section, permitting also to use a lower ISR. Both BMP tests lasted longer than 100 days. Blank assays included the inoculum and further distilled water compensating for the absence of substrate, using three replicates in each BMP. Inoculum activity assays using a reference substrate were not performed.

The BMP was the normalized methane production (P = 1 bar, T = 0ºC), excluding the methane production of the inoculum, per unit of substrate VS added. The gas production was evaluated with a two-vessel displacement system, with the first vessel containing 4 N NaOH to capture CO_2 and the second vessel containing water to be ‘displaced’. The bottles were sealed with butyl rubber stoppers and aluminum crimps and flushed with ~~Helium~~helium, before adding 0.2 mL of 10 g/L Na_2S piercing the septum to ensure an adequate redox potential (Angelidaki & Sanders, 2004). All bottles were incubated at 55ºC and agitated only while measuring the gas production.

2.5 Statistical Analyses

The Dixon's test for BMP outliers was applied as recommended by Holliger et al. (2016). The unpaired t-test of Microsoft Excel 2016 (Microsoft, USA) was applied to determine the statistical significance of experimental data, using the two-tail p-value at 95 % confidence.

3 RESULTS AND DISCUSSION

3.1 Bio-Physical-Chemical Characterization of Substrates and Inoculum

OFMSW showed a TS of 26 %, a VS/TS ratio of 0.93 and a TKN of 24.8 g N/kg TS, in agreement with real source-sorted OFMSW (Angelidaki et al., 2006; Bolzonella et al., 2006; Jokela & Rintala, 2003). ~~(Cecchi et al., 2002; Jokela & Rintala, 2003).~~ The high VS/TS ratio of OFMSW (i.e. > 0.9) indicated minimal presence of inert materials (Pavan et al., 2000). Sawdust showed a TS of 94 % and a VS/TS ratio of 0.99, similar to those obtained by Brown and Li (2013) for 40ºC-dried yard waste, suggesting that beech sawdust could simulate GW. The BMP of OFMSW and sawdust was 497~~457~~ and ~~148~~ 161 NmL CH_4/g VS, respectively. Table ~~2~~ 1 shows the bio-physical-chemical characterization of OFMSW and sawdust. Despite the thorough mincing and homogenization, ~~Minor~~ minor modifications were observed in the OFMSW characterization (i.e. TS, TKN or BMP),~~were~~ mainly attributed to the substrate heterogeneity.

The inoculum in mono-digestion reactors A and B showed a common TS and TKN of 2.8 % and 161 g N/kg TS, respectively. An initial acetic acid concentration of 2.30~~0~~ and 3.30~~0~~ ~~m~~g/kg was observed in reactors A and B, respectively, being this difference associated ~~to~~ with the later inoculation of reactor B than reactor A. The inoculum used in co-digestion reactors showed a TS of 2.5 %, a TKN of 139 g N/kg TS and an acetic acid concentration of 0.02~~0~~ ~~m~~g/kg. The inoculum compositions are shown in Table ~~3~~2.

3.2 Semi-continuous Operation – Increasing the TS Content

3.2.1 Mono-digestion of OFMSW

Mono-digestion results are summarized in Figures 2 and 3. The weekly-averaged results were also included as Supplementary Information. The 7-days average OLR in reactors A and B was varied from an initial 2.4 (day 6) and 6.0 (day 13) g VS/kg·d to 4.9 and 5.5 g VS/kg·d, respectively, ~~at~~ on day 17 [Figure 2a]. Thus, a common OLR (i.e. around 5 g VS/kg·d) was achieved, aiming to compensate for the 1-week-lagged inoculation in reactor B. After two days with no ~~operation~~ feed~~(day 20)~~, feeding was resumed in reactor B on day 20, but not in reactor A due to the low pH (i.e. 6.4) ~~observed~~ [Figure 3a]. As pH recovered in reactor A (i.e. from 6.4 on day 21, to ~~pH =~~7.6~~,~~ on day 29) due to methanogenesis activity, feeding was ~~also~~ resumed. During the same period, ALK$_P$ in reactor A increased alongside pH from 1.1 to 2.7 g CaCO$_3$/kg (data not shown), as an indicator of ongoing methanogenesis. By day 45, a maximum OLR of 6.8 and 8.5 g VS/kg·d was reached in reactors A and B, respectively. After day 48, the OLR required progressive reduction to minimize the risk of acidification. The l~~L~~ast feeding in reactors A and B was implemented on days 78 and 73, respectively, as both reactors showed pH $\leq$ 6.5 and CH$_4$ content $\leq$ 40 % [Figure 2f]. From this point, mono-digestion reactors were left unfed aiming to promote the recovery of methanogenesis.

The OLR in reactor B was averagely about 1.5 g VS/kg·d higher than that used in reactor A during the whole experiment ($p < 0.001$), explaining the relatively faster acidification observed in reactor B. Thus, prior to the occurrence of acidification, reactor B was fed with a~~ daily ~~n average ~~20 ~~35 g VS/d, significantly higher ~~compared to~~than the ~~15 ~~26 g VS/d used for reactor A ($p = 0.03$). The initial MRT was 55 (day 6) and 29 days (day 13) for reactors A and B, respectively, and was gradually increased to maintain the methanogenic performance at higher OLR [Figure 2b]. Noteworthy, the MRT and OLR in these semi-continuous reactors did not show an inverse pattern, since the dilution as well as the influent and effluent mass flows used were different to account for the organic removal.

Uncoupling the influent and effluent mass flows in the semi-continuous reactors, based on the HS-AD reactor content removal by methanogenesis, permitted to increase the MRT and OLR simultaneously. ~~The hydraulic retention time (HRT) and MRT are equivalent concepts defining the average time a fluid and a mass particle, respectively, remain within a continuous reactor. However, ~~In this study, the MRT was considered as a more suited indicator in HS-AD than the hydraulic retention time (HRT), since both the specific weight of the influent/effluent and the reactor mass content ~~might vary~~varied, in contrast to ‘wet’ AD (Pastor-Poquet et al., 2018). ~~(Rivard et al., 1990).~~

As an example, the occurrence of methanogenesis led to a 60 g removal of the reactor mass content in both mono-digestion reactors from day 37 to 41 (data not shown). ~~Thus,~~ Prior to the occurrence of reactor acidification, the weekly effluent mass was ~~1-38 %~~significantly higher than the influent (i.e. 18 %; p = 0.03) to maintain the mono-digestion reactors mass content constant. ~~[Table 1], with the exception of weeks 5 and 12 in reactor A, when a reduced influent was used to prevent acidification but the reactor was sampled for physical-chemical analyses.~~

The MRT-uncoupling concept was proposed by Richards et al. (1991) and was ~~also~~ used by Kayhanian and Rich (1995) to operate a pilot-scale semi-continuous HS-AD reactor fed with OFMSW. In this study, uncoupling the influent and effluent in HS-AD ~~was aimed to~~ promoted the methanogenic adaptation to overloading conditions and/or the buildup of inhibitors (i.e. NH_3) during the OFMSW degradation. Noteworthy, the MRT must be longer than the doubling time of methanogens (i.e. 20-30 days) to avoid their 'washout' from continuous HS-AD reactors, while the methanogenic doubling time might lengthen considerably in presence of inhibitory substances (i.e. NH_3) (Drosg, 2013; Gerardi, 2003; Rittman & McCarty, 2001). Therefore, extending the MRT ~~potentially results~~ resulted in a more stable HS-AD operation (Hartmann & Ahring, 2006; Rajagopal et al., 2013), ~~(Climenhaga & Banks, 2008; Hartmann & Ahring, 2006; Rajagopal et al., 2013),~~ though the sole implementation of influent-effluent uncoupling ~~in this study~~ was not sufficient to avoid HS-AD overloading and acidification during mono-digestion of OFMSW.

3.2.2 Co-digestion of OFMSW and Sawdust

Co-digestion results are summarized in Figures 4 and 5. The 7-days average OLR was increased from 4.5-4.9 g VS/kg·d (day 6) up to 10.9, 12.1 and 12.6 g VS/kg·d (day 23) in reactors A, B and C, respectively. To avoid acidification, feeding was stopped in reactors A and B from day 26, while the OLR was only reduced to 5.0 g VS/kg·d in reactor C [Figure 4a]. As pH recovered (i.e. ≥ 7.0) [Figure 5], feeding was resumed in reactors A and B. A maximum OLR of 14.8 g VS/kg·d was reached in reactor C (day 47) using a sawdust/OFMSW ratio of 2.1 g VS/g VS, prior to the occurrence of reactor acidification (day 56). The maximum OLR in reactor B was 15.1 g VS/kg·d (day 55) using a sawdust/OFMSW ratio of 1.6 g VS/g VS, while an OLR of 16.0 g VS/kg·d was reached in reactor A during the same period, using a sawdust/OFMSW ratio of 1.3 g VS/g VS. The l~~L~~ast feeding in reactors A and B was performed on day 76, as a slight but continued drop in pH [Figure 5] and CH_4 [Figure 4f] was observed in both reactors.

The ~~maximum~~average- OLR used for co-digestion was two times higher than that for mono-digestion (i.e. 8.3~~13.0~~ *vs.* ~~6.5~~4.5 g VS/kg·d, respectively; $p < 0.001$), due to the lower biodegradability of sawdust, though the ~~maximum~~ OLR only due to OFMSW was similar in both cases (i.e. ~~7.5~~4.1 *vs.* 4.5 g VS/kg·d, respectively; $p = 0.07$). Thus, a ~~common~~ maximum OLR of 7.5-8.0 g VS/kg·d related to the sole supplementation of OFMSW was ~~reached~~ used in the three co-digestion reactors on day 21, while the OLR solely due to OFMSW was subsequently maintained below 6.0 g VS/kg·d, as sawdust was increased in the feeding mixture [Figure 4a]. In terms of average VS fed, reactor C was operated under relatively more stressing feeding conditions than reactors A and B (i.e. ~~35~~53~~,~~ ~~11 and~~ *vs.* ~~14~~ 44 g VS/d, respectively; $p = 0.15$), being again the fastest occurrence of reactor acidification related to the highest ~~OLR used~~VS fed.

The initial MRT was higher than 168 days (day 6) and was decreased to 30 days (day 17), similarly in the three reactors [Figure 4c]. From this point, the MRT reached an average of 85 days (day 35) and was subsequently reduced to an average of 37 days (day 53) in all reactors, before being progressively increased to minimize the substrate overload. The MRT was ~~relatively~~ significantly lower in co-digestion than mono-digestion (i.e. 69 *vs.* 92 days, respectively; $p < 0.001$), as lower MRT were predominantly linked to the higher OLR used in co-digestion.

3.3 Influence of the Substrate Composition on the TS Increase

The OLR/MRT control in the mono-digestion reactors fed with OFMSW permitted to increase the TS content, balancing the VFA accumulation with the rapid organic degradation observed [Figures 2 and 3]. Reactors A and B were started at TS = 2.8 % and reached a maximum of 10.7 (day 79) and 11.7 % (day 69), respectively [Figure 2c], being these TS slightly higher than the lower HS-AD threshold (i.e. TS ≥ 10 %). The highest TS in the semi-continuous reactors did not coincide with the maximum OLR, but were predominantly associated ~~to~~ with low pH (i.e. ≤ 6.5), when methanogenesis was potentially inhibited. In this line, a gradual increase of the VS/TS ratio (data not shown) was observed in both reactors from 0.69 (day 0) to 0.82 (day 40), reaching a maximum value of 0.87, prior acidification occurred on days 79 and 76 in reactors A and B, respectively.

The highest TS and VS/TS observed in semi-continuous HS-AD of OFMSW ~~being related to acidification~~were associated with acidification and indicate~~s~~ a reduced VS degradation alongside inhibitory conditions. Particularly, the lowest HS-AD threshold (i.e. TS = 10 %) using OFMSW was reached only under extreme overloading. A more stable HS-AD fed with an easily biodegradable OFMSW (i.e. FW) is also associated ~~to~~ with a TS increase alongside overloading/inhibitory conditions. For example, Tampio et al. (2014) reported a TS increase from 7 to 8 % during 400 days of semi-continuous AD fed with FW, though TS rapidly reached 11 % during the next 50 days of operation, when reactor inhibition was likely occurring. In the same line, Bolzonella et al. (2003) reported a TS increase from 5 to 15 % during the initial 60 days of continuous AD pilot-scale startup fed with OFMSW, being the maximum TS associated ~~to~~ with the highest total VFA observed (i.e. 2.8 g Acetic Acid/L). All these results were likely related to methanogenesis inhibition, since the VFA accumulation ~~might have affected~~affects the hydrolysis/acidogenesis rates, hampering the organic removal in HS-AD (Vavilin et al., 2008).

The maximum TS obtained in this study for semi-continuous HS-AD of OFMSW should be considered as indicative (only) of those obtainable in steady-state digesters, since the transient/acidification conditions potentially reduced the VS removal. Thus, the operational TS content of stable digesters fed with the easily biodegradable content of OFMSW (i.e. FW) might be lower than those observed along non-steady-state conditions. ~~Further implying~~This is a further indication that a steady-state semi-continuous reactor using an easily biodegradable OFMSW as a substrate might not be operated within the HS-AD threshold (i.e. TS $\geq$ 10 %).

Co-digestion permitted to increase TS from 2.5 % (day 0) up to a maximum of 33.2 (day 79), 26.7 (day 76) and 27.0 % (day 57) in reactors A, B and C, respectively [Figure 4c]. Hence, the maximum TS reached in co-digestion before the reactors acidified (i.e. 29.0 ± 2.8 %) was considerably higher than the lower HS-AD threshold (i.e. TS ≥ 10 %) and the maximum TS of mono-digestion (i.e. 11.5 ± 0.5 %), due to the addition of sawdust to OFMSW. The highest TS was related again to acidified (i.e. pH < 6.5) or acidifying (i.e. downward trend on pH/CH_4 content) conditions, as observed for mono-digestion. Thus, the VS/TS ratio in co-digestion (data not shown) increased from 0.65 (day 0) to 0.90 (day 40), reaching a maximum of 0.95 before reactors acidified (day 76), due to both the higher VS/TS ratio of sawdust and the reduced VS removal during inhibitory conditions.

These results showed that the particular characteristics of OFMSW determined the maximum operating TS content in semi-continuous HS-AD. ~~Thus, ~~C~~c~~o-digestion of OFMSW and sawdust resulted in ~~a ~~approximately three times higher TS than mono-digestion (i.e. ~~33 ~~29.0 and 11.5 %, respectively). The inclusion of sawdust in OFMSW favored the rapid TS and OLR increase compared to mono-digestion due to the higher TS and the lower biodegradability of sawdust, as demonstrated by the substantially lower BMP of sawdust than that of OFMSW (i.e. ~~148 ~~161 and ~~457 ~~497 NmL CH_4/g VS, respectively) [Table ~~2~~1]. ~~Moreover~~Indeed, lignocellulosic materials (i.e. GW) are normally associated ~~to ~~with a reduced biodegradation rate, compared to more easily degradable substrates (i.e. FW), due to the high lignin content hampering hydrolysis (Brown & Li, 2013; Mancini et al., 2018a; Vavilin et al., 2008), ~~(Brown et al., 2012; Chavez-Vazquez & Bagley, 2002; Mancini et al., 2018a), ~~being also beneficial to limit the VFA buildup in HS-AD. On the other hand, TAN was ~~approximately 30~~ 22 % lower during co-digestion than mono-digestion (i.e. 2.9 *vs.* 3.7 g N/kg, respectively; $p < 0.001$) [Figures 2 and 4], due to the lower TKN of sawdust [Table ~~2~~1]. Noteworthy, the TAN accumulation was likely promoting methanogenic inhibition in this study, as further discussed in section 3.5. Therefore, using sawdust – as GW – was also adequate to adjust the carbon-to-nitrogen (C/N) ratio in HS-AD of OFMSW.

~~Introducing a lignocellulosic substrate in OFMSW is a well-suited strategy to operate semi-continuous reactors in HS-AD conditions (i.e. 20-30 % TS), since the maximum operational OLR and TS in semi-continuous HS-AD of OFMSW is determined by the substrate characteristics (i.e. TS, VS/TS ratio and biodegradability) and/or the buildup of inhibitors (i.e. NH_3) (Bolzonella et al., 2006; Kayhanian & Hardy, 1994; Mata-Alvarez et al., 2014; Pavan et al., 2000; Rivard et al., 1990). Meanwhile, the TS content is further determined by the organic removal, since a TS removal ranging from 30 to 80 % has been reported in semi-continuous HS-AD of OFMSW, depending on the substrate characteristics, but also reactor design and operation (Bolzonella et al., 2006; Mata-Álvarez, 2003; Pavan et al., 2000)~~

3.4 Main Indicators of Substrate Overload

3.4.1 Evolution of pH and VFA

~~The start-up of biogas plants requires a gradual and slow OLR increase to adapt progressively the methanogens, avoiding VFA accumulation in the digester . (Angelidaki et al., 2006; Drosg, 2013; Marchaim & Krause, 1993). However, all the semi-continuous reactors in this study had to be operated under overloading conditions,~~

~~to increase TS from ‘wet’ AD to HS-AD. Thus, acidification was expected due to the high OLR used, but also because acidification is likely to occur in HS-AD due to the high organic content in the reactor (Kayhanian, 1995). Particularly, pH fluctuated as a result of the OLR modification and the activity of microorganisms, showing a downward trend associated to the VFA accumulation, until a sharp acetic acid buildup occurred. This rapid acetic acid buildup indicated a significant inhibition of acetoclastic methanogenesis, subsequently resulting in reactor acidification (i.e. pH ≤ 6.0).~~

pH in mono-digestion reactor A decreased from 8.1 to 6.4 due to the rapid acetic acid buildup (i.e. from 3.00~~0~~ to 9.00~~0~~ ~~m~~g/kg) observed during the initial 20 days of operation [Figure 3a]. As feeding was stopped from day 20 to 29, pH reached 7.6, while acetic acid decreased below 0.70~~0~~ ~~m~~g/kg right afterwards (day 34). Propionic, butyric and valeric acids gradually increased from < 0.15~~0~~ ~~m~~g/kg (day 0) to 5.00~~0~~, 4.00~~0~~ and 1.10~~0~~ ~~m~~g/kg (day 79), respectively. From day 79, pH dropped from 7.1 to 6.1, linked to a sudden acetic acid increase from 3.00~~0~~ to 5.00~~0~~ ~~m~~g/kg, and the subsequent CH_4 content drop from 56 to 37 % [Figure 2f].

Mono-digestion reactor B was relatively more stressed than reactor A, as indicated by the wider acetic acid fluctuations (i.e. ± 4.00~~0~~ ~~m~~g/kg) and the rapid accumulation of propionic acid from 1.20~~0~~ (day 7) to 5.80~~0~~ ~~m~~g/kg (day 73) [Figure 3b]. The VFA fluctuation is in line with the fact that methanogens grow relatively slower than the hydrolytic/acidogenic microorganisms in AD (De Vrieze et al., 2012; Gerardi, 2003). ~~(Gerardi, 2003; Marchaim & Krause, 1993).~~ Thus, the higher OLR used in reactor B led to a more pronounced methanogenic/acidogenic imbalance, exacerbating the VFA accumulation. The VFA buildup led to a pH decrease from 8.4 to 6.2 in reactor B during the whole experimental period, while a significant acetoclastic inhibition occurred from day 70 to 73, when acetic acid abruptly increased from 2.70~~0~~ to 5.80~~0~~ ~~m~~g/kg.

pH in co-digestion reactor A gradually decreased from 8.7 to 6.4 along the experimental period, showing a minimum of 6.1 associated ~~to~~ with a~~n acetic~~ peak of acetic acid of 8.30~~0~~ ~~m~~g/kg (day 26) [Figure 5a]. Acetic acid was considerably consumed (i.e. < 0.36~~0~~ ~~m~~g/kg) by day 47 due to ongoing methanogenesis, and progressively increased thereafter by overloading. Similarly, pH in reactor B showed a minimum of 6.3 when acetic acid peaked at 8.20~~0~~ ~~m~~g/kg (day 26) [Figure 5b], while the acetic acid was extensively consumed (i.e. < 0.35~~0~~ ~~m~~g/kg) by day 41 prior to increase again steadily. In reactor C, acetic acid had a similar evolution with a maximum of 7.20~~0~~ ~~m~~g/kg (day 26) [Figure 5c], while pH dropped to 6.0 on day 57, associated ~~to~~ with a sharp acetic acid build-up from 1.00~~0~~ to 3.70~~0~~ ~~m~~g/kg. Propionic, butyric and valeric acids increased from 0.50~~0~~, 0.14~~0~~ and 0.00~~5~~ ~~m~~g/kg (day 0) to a maximum range of 3.00~~0~~-3.50~~0~~, 2.90~~0~~-3.20~~0~~ and 2.50~~0~~-2.60~~0~~ ~~m~~g/kg, respectively, obtained right after acidification occurred on day 79 in reactors A and B, and on day 56 in reactor C. The pH was relatively lower (i.e. 2 %; p = 0.13) and total VFA was relatively higher (i.e. 5 %; p = 0.25) ~~profiles showed wider fluctuations~~ during mono-digestion than co-digestion, likely due to the faster degradation rates but also the higher release of inhibitory compounds related to OFMSW than sawdust~~inhibitory content of OFMSW compared to sawdust~~, as discussed ~~before~~in section 3.3.

Feeding the reactors a maximum of 5 days per week influenced the reactor dynamics, since pH increased and VFA – mainly acetic acid – decreased during the periods with no feed. The pH and VFA modifications [Figures 3 and 5] were associated with the TS removal, as mentioned before, and also affected the biogas production/composition, and the TAN buildup [Figures 2 and 4]. As an example, in co-digestion reactor A, pH increased from 7.0 to 7.9 from day 37 to 41, while acetic acid decreased from 4.30 to 2.40 g/kg, triggering a biogas production of 0.6 L/kg reactor content and a methane content increase from 59 to 70 % [Figure 4 and 5].

3.4.2 Biogas Production and Composition

Mono-digestion ~~showed~~ of OFMSW resulted in a cumulative biogas production of 65 and 66 L/kg ~~Reactor~~ reactor ~~Content~~ content in reactor A and B, respectively [Figure 2e]. Biogas production was mainly correlated to the acetic acid consumption [Figure 3a], as mentioned in the previous subsection. For example, 21 L/kg ~~Reactor~~ reactor ~~Content~~ content of biogas were measured during the initial 20 days of reactor A, before acetic acid accumulated and biogas production slowed down.

Biogas composition measurements started on day 60 showing an average of 63 % CH_4 in both mono-digestion reactors [Figure 2f], which subsequently fluctuated showing a downward trend alongside the VFA accumulation. The CH_4 content dropped below 40 % in both reactors right after biogas production definitely ceased on days 78-79. The reduction of CH_4 content ~~reduction~~ in the biogas is also an indicator of AD imbalance, though it might ~~fail~~ be inappropriate to assess rapid changes in the reactor performance (Drosg, 2013). The highest H_2 concentration (data not shown) was 1.8 and 1.1 % on day

59 in reactors A and B, respectively, while H_2 remained below 0.8 % in both ~~cases~~reactors~~,~~ during the rest of the experiment. The presence of H_2 indicated that the hydrogenotrophic methanogens were unable to cope with the rapid H_2 production from acidogenesis, since H_2 higher than 1-2 % in the gas phase is normally associated ~~to~~ with AD overloading (Drosg, 2013; Molina et al., 2009). ~~(Drosg, 2013; Marchaim & Krause, 1993; Switzenbaum et al., 1990).~~

Co-digestion in reactor A ~~showed~~ led to a cumulative biogas production of 48 L/kg ~~Reactor~~ reactor ~~Content~~content, while 49 and 27 L/kg ~~Reactor~~ reactor ~~Content~~ content were observed in reactors B and C, respectively [Figure 4e]. In spite of the higher OLR used in co-digestion, the biogas production was considerably lower than ~~the biogas production~~that obtained with mono-digestion (i.e. 65 L/kg ~~Reactor~~ reactor ~~Content~~content). Thus, the specific biogas production was ~~166-192~~229 ± 20 L/kg VS added~~Fed~~ in mono-digestion and ~~59-71~~86 ± 18 L/kg VS added ~~Fed~~ in co-digestion (i.e. 62 % lower), due to the reduced biodegradability of sawdust.

The CH_4 content [Figure 4f] reached a peak of 75 % during the first two weeks of operation in the three co-digestion reactors, but it decreased subsequently as VFA accumulated [Figure 5]. A minimum 43 % CH_4 was detected in reactor A associated with the last biogas production observed (day 82), while a sharp drop from 60 to 29 % CH_4 was observed in reactor C right after day 60. H_2 was detected at 0.3 % in the three co-digestion reactors on day 23 (data not shown). Thereafter, H_2 was not detected in reactor A, while reactor B showed a single H_2 peak of 1.5 % on day 70, right after the reactor was accidentally opened to the atmosphere. In reactor C, H_2 peaks of 1.7, 1.2

and 1.6 % were observed on days 41, 47 and 58, respectively, supporting the occurrence of a more extensive overload in this reactor.

3.5 Testing Recovering Strategies

Once acidification occurred, feeding was stopped and some recovering strategies ~~(i.e. reactor dilution)~~ were tested to resume methanogenesis. In mono-digestion reactor A, a 3 M $NaHCO_3$ buffer solution was added on days 83 and 84 to raise the pH (i.e. from 6.2 to 6.8)~~, and return the pH~~ within a suitable range for methanogens (i.e. 6.5-7.0). Adding $NaHCO_3$ is normally used to counteract acidification when digesters show a reduced ALK_P (Chen et al., 2008; Holliger et al., 2016). However, methanogenesis did not recover after more than 20 days.

On day 76, $FeCl_2$ was supplemented to mono-digestion reactor B in a higher amount than the stoichiometric, to precipitate the total ~~hydrogen sulfide~~H_2S in the system (i.e. 30 mg H_2S/kg, data not shown). However, $FeCl_2$ overdosing resulted in a pH drop from 6.3 to 5.7 (days 76-77).~~,~~ ~~and~~ Thus, 2 M $NaHCO_3$ solution was rapidly added to recover the pH to 6.6 (day 77). Both Fe^{2+} and/or Fe^{3+} can be used to precipitate sulfide in AD, but Fe^{2+} was preferred in this study to avoid the inclusion of a strong electron acceptor (i.e. Fe^{3+}) that could react with organic compounds in the anaerobic digester (i.e. $Fe^{3+} + 1/2\ H_2 \rightarrow Fe^{2+} + H^+$, $\Delta G^{o\prime} << 0$) (Fermoso et al., 2015; Rittman & McCarty, 2001). After 2 weeks of methanogenic inhibition (day 90), 200 g of ‘wet’ AD inoculum from the source reactor were added to reactor B, allowing a gradual methanogenic recovery, associated ~~to~~ with an increase of pH from 6.9 to 7.3 [Figure 3b] and CH_4 content from 20 to 52 % [Figure 2f], until the end of the reactor operation.

Aiming to recover methanogenesis in all co-digestion reactors, water was progressively added to dilute the effect of potential methanogenic inhibitor(s) ~~in all co-digestion reactors~~. The progressive addition of low amounts of water in co-digestion reactors permitted to maintain HS-AD conditions (i.e. TS ≥ 10 %), thanks to the elevated TS content reached before reactors acidified (i.e. TS ≥ 30 %). Dilution was performed in reactors A and B from day 79 and in reactor C from day 62. In reactor A and B an average of 180 and 170 mL of water was used, respectively, on days 79, 82, 84, 91 and 98, while an average of 160 mL of water ~~were~~ was added to reactor C on days 62, 63, 68 and 91.

In ~~this study~~conclusion, neither water, nor buffer addition permitted to recover acidified/acidifying HS-AD reactors, probably because of the important imbalance between methanogens and acid-producers in the system (Gerardi, 2003). ~~Therefore~~In these conditions, inoculum addition might be the only way to recover an acidified HS-AD reactor, though emptying and re-inoculating the reactor might be necessary (Fricke et al., 2007). Another strategy to prevent reactor acidification and/or enhance the digester performance is trace element (i.e. Se, Ni, Co, Mo, W) addition (Fermoso et al., 2015; Mancini et al., 2018b). However, this was out of the scope of this manuscript.

3.6 Ammonia Buildup

In this study, o~~O~~verloading ~~in this study~~ was associated ~~to~~ with the high OLR used, but also ~~to~~ with the NH_3 buildup, in the semi-continuous reactors. T~~hus, t~~he high OLR and the degradation of the protein content of OFMSW increased the TAN content in both mono-digestion reactors [Figure 2d]. TAN ranged from 3.4 g N/kg (day 0) to a maximum of 4.8 and 4.9 g N/kg in reactors A and B (day 104), respectively, with both reactors showing a minimum TAN of 3.0 g N/kg around day 20. The initial NH_3 was 1.1 and 1.7 g N/kg in reactors A and B, respectively. Subsequently, NH_3 fluctuated with an overall decreasing trend along the pH modification in both reactors, showing peaks higher than 1.0 g N/kg mainly when pH was relatively high (i.e. ≥ 8.0) [Figure 3]. In reactor A, NH_3 reached peaks of 1.4 (day 7) and 1.5 g N/kg (day 34), while NH_3 higher than 1.5 g N/kg was repeatedly observed in reactor B (i.e. days 20, 27, 34 and 41).

In co-digestion, ~~The~~ the initial TAN ~~in co-digestion~~ was 3.0 g N/kg and slightly increased to a maximum of 3.3, 3.6 and 3.3 g N/kg (day 61) in reactors A, B and C, respectively [Figure 4d]. TAN subsequently decreased due to the reduced OFMSW feeding and the progressive dilution used for HS-AD recovering, until a minimum of 1.9, 2.3 and 2.8 g N/kg was reached in reactors A, B and C, respectively (day 112). The initial NH_3 was 2.0 g N/kg and progressively decreased in the three reactors alongside pH. NH_3 peaked at 1.5 g N/kg (day 12) and 1.2-1.7 g N/kg (day 19), rapidly decreasing to ≤ 0.1 g N/kg (day 23), similarly in all reactors. From this point, NH_3 was maintained below 1.0 g N/kg in the three reactors. Thus, NH_3 was considerably reduced during co-digestion alongside the reduction of OFMSW in the feed, since peaks higher than 1.0 g N/kg were not observed from day 20 onwards, in contrast to mono-digestion reactors.

NH_3 inhibition was likely one of the main triggers of overloading in this study, since the high NH_3 levels observed (i.e. ≥ 1.0 g N/kg) are normally associated ~~to~~ with methanogenic inhibition and VFA accumulation in AD (Drosg, 2013; Rajagopal et al., 2013). ~~(Drosg, 2013; Jokela & Rintala, 2003; Poggi-Varaldo et al., 1997)~~ Thus, despite each AD system might show particular NH_3 inhibition thresholds depending on the anaerobic consortia (Fricke et al., 2007; Westerholm et al., 2016), a gradual methanogenic adaptation to high levels of TAN (i.e. ≥ 4.0 g N/kg) might be crucial to increase OLR in semi-continuous HS-AD of OFMSW (Hartmann & Ahring, 2006; Rajagopal et al., 2013).

In this study, a tradeoff was needed between the ‘undesired’ TAN buildup and the rapid TS removal observed, to reach HS-AD conditions (i.e. TS ≥ 10 %) with mono-digestion of OFMSW. For example, the different TS and TAN dynamics can be appreciated in mono-digestion reactor A from day 30, when TS fluctuated while TAN steadily increased [Figure 2]. Potential ammonia contingency strategies in AD, as increasing the substrate dilution, reducing the OLR, and/or increasing the MRT (Kayhanian, 1999; Rajagopal et al., 2013), would have lengthened considerably the experimental time, or even prevented to achieve HS-AD conditions (i.e. TS ≥ 10 %) with mono-digestion of OFMSW.

4 CONCLUSIONS

In this study, reducing the effluent compared to the influent mass (i.e. ~~1 38~~18 %) permitted to extend the ~~biomass retention time~~MRT in semi-continuous mono-digestion of OFMSW, and obtain a specific biogas production of 229 L/kg VS added, due to the high biodegradability of OFMSW. However, the sole implementation of influent/effluent uncoupling was not sufficient to avoid reactor overload and acidification~~/acidification~~ when reaching HS-AD conditions (i.e. TS ≥ 10 %). The average OLR was 4.5 g VS/kg·d, whereas a maximum 11.5 % TS was reached. In contrast, the addition of beech sawdust to OFMSW allowed to operate co-digestion reactors with an average OLR of 8.3 g VS/kg·d, and reach a maximum 29.0 % TS. Co-digestion lowered by 22 % the TAN content, though an average 186 L/kg VS added of biogas was obtained. ~~Overloading was related to the substrate biodegradability and inhibitory content, since the maximum TAN content was 4.8 and 3.6 g N/kg, while the biogas production was 166-192 and 59-71 L/kg VS Fed, for mono-digestion of OFMSW and co-digestion of OFMSW and sawdust, respectively.~~ Therefore, the addition of sawdust, as an example of lignocellulosic substrate, to OFMSW (i.e. 1-2 g VS-Sawdust/g VS-OFMSW) is an adequate strategy to stabilize HS-AD at very high TS contents (i.e. 20-30 %). Nonetheless, a compromise must be found between increasing the TS content and reducing the specific biogas production by co-digestion, since both aspects strongly determine the HS-AD economy for OFMSW treatment.~~.~~

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TABLE AND FIGURE CAPTIONS

~~**Table 1**: Summary of weekly operating parameters used in the semi-continuous reactors until last feeding was implemented (i.e. week 12)~~

Table ~~2~~1: Bio-physical-chemical characterization of substrates.

Table ~~3~~2: Physical-chemical characterization of inoculums.

Figure 1: Experimental setup. 1) Reactor body; 2) reactor head; 3) feeding port; 4) gas output; 5) gas measuring port; and 6) opening valves.

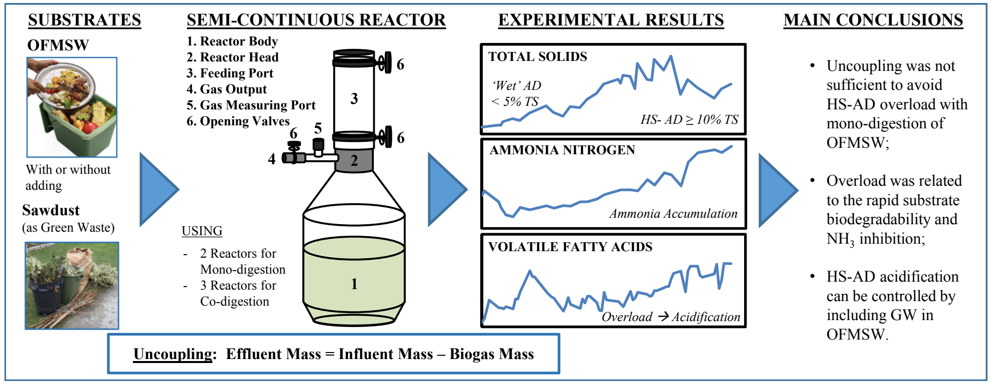
Figure 2: Mono-digestion of OFMSW: a) Organic loading rate; b) mass retention time; c) total solids; d) total and free ammonia nitrogen (NH_3); e) cumulative biogas production; and f) methane content. Black arrows represent the $NaHCO_3$ addition in reactor A, while dotted arrows represent the $FeCl_2$ or inoculum addition in reactor B.

Figure 3: Mono-digestion of OFMSW: Volatile fatty acids and pH in a) reactor A; and b) reactor B. Black arrows represent the $NaHCO_3$ addition in reactor A, while dotted arrows represent the $FeCl_2$ or inoculum addition in reactor B.

Figure 4: Co-digestion of OFMSW and sawdust: a) Organic loading rate – parentheses indicate the sole addition of OFMSW; b) mass retention time; c) total solids; d) total and free ammonia nitrogen (NH_3); e) cumulative biogas production; and f) methane content.

Figure 5: Co-digestion of OFMSW and sawdust: Volatile fatty acids and pH for a) reactor A; b) reactor B; and c) reactor C.

Graphical Abstract



1. Reactor Body
2. Reactor Head
3. Feeding Port
4. Gas Output
5. Gas Measuring Port
6. Opening Valves

USING

- 2 Reactors for Mono-digestion
- 3 Reactors for Co-digestion

Uncoupling: Effluent Mass = Influent Mass – Biogas Mass

- Uncoupling was not sufficient to avoid HS-AD overload with mono-digestion of OFMSW;
- Overload was related to the rapid substrate biodegradability and NH_3 inhibition;
- HS-AD acidification can be controlled by including GW in OFMSW.

Highlights

- A reduced effluent compared to the influent extended the MRT in HS-AD of OFMSW.
- Uncoupling was not sufficient to avoid overload/acidification in HS-AD of OFMSW.
- Substrate overload in HS-AD of OFMSW was exacerbated by NH_3 inhibition.
- HS-AD overload can be controlled by adding green waste to OFMSW.

Semi-continuous Mono-digestion of OFMSW and Co-digestion of OFMSW with Beech Sawdust: Assessment of the Maximum Operational Total Solid Content

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ABSTRACT

In this study, mono-digestion of the organic fraction of municipal solid waste (OFMSW) and co-digestion of OFMSW with beech sawdust, simulating green waste, were used to investigate the maximum operational total solid (TS) content in semi-continuous high-solids anaerobic digestion (HS-AD). To alleviate substrate overloading in HS-AD, the effluent mass was relatively reduced compared to the influent mass, extending the mass retention time. To this aim, the reactor mass was daily evaluated, permitting to assess the reactor content removal by biogas production. During mono-digestion of OFMSW, the NH_3 inhibition and the rapid TS removal prevented to maintain HS-AD conditions (i.e. TS ≥ 10 %), without exacerbating the risk of reactor acidification. In contrast, the inclusion of sawdust in OFMSW permitted to operate HS-AD up to 30 % TS, before acidification occurred. Therefore, including a lignocellulosic substrate in OFMSW can prevent acidification and stabilize HS-AD at very high TS contents (i.e. 20-30 %).

Keywords: High-Solids Anaerobic Digestion; Influent/Effluent Uncoupling; Substrate Overloading; Acidification; Ammonia Inhibition.

1 INTRODUCTION

Anaerobic digestion (AD) of the organic fraction of municipal solid waste (OFMSW), including food waste (FW) and green waste (GW), is a particularly suited treatment biotechnology for energy and by-product recovery (Clarke, 2018; Mata-Álvarez, 2003). In AD, an organic waste is degraded to biogas, mainly composed by CH_4 and CO_2, and a partially stabilized organic digestate, by consortia of different microorganisms working in absence of oxidative species (i.e. O_2 and NO_3^-) (Astals et al., 2015; Gerardi, 2003).

The sequential steps in AD include hydrolysis, acidogenesis, acetogenesis and methanogenesis, during which different inhibitory substances can be formed leading to inhibitory effects for the anaerobic microorganisms and/or even a complete AD failure. Depending on the concentration, free ammonia (NH_3), hydrogen sulfide (H_2S) and free ions (i.e. Na^+) are some of the inhibitory substances in AD, affecting predominantly the methanogenic stage, either acetoclastic and/or hydrogenotrophic, and potentially resulting in the buildup of volatile fatty acids (VFA) and H_2 in the system (Astals et al., 2015; Chen et al., 2008). Meanwhile, the acetoclastic activity results into inorganic carbon (i.e. HCO_3^-) release in AD, as an important source of pH buffering, minimizing the risk of reactor acidification (i.e. $pH \leq 6.0$) by VFA accumulation (Gerardi, 2003).

The interrelationship between the organic waste characteristics, operational conditions and reactor design determines the AD potential (Karthikeyan & Visvanathan, 2013; Mata-Álvarez, 2003). AD can be differentiated depending on the operational total solid (TS) content into ’wet’ (i.e. TS < 10 %) and high-solids AD (HS-AD, i.e. TS ≥ 10 %)

(Benbelkacem et al., 2015). HS-AD allows the use of a smaller reactor, reducing the need for water addition and minimizing the digestate production (Karthikeyan & Visvanathan, 2013; Pastor-Poquet et al., 2018). However, HS-AD drawbacks include the pervasive chances of reactor acidification due to substrate overload (Benbelkacem et al., 2015). Overloading is the consequence of the slow-growing methanogens being unable to cope with the rapid VFA and/or H_2 buildup resulting from acidogenesis/acetogenesis in HS-AD (Pavan et al., 2000). Furthermore, overloading is in many cases related to the presence of methanogenic inhibitors (Drosg, 2013), such as NH_3, due to the high protein content of OFMSW (Kayhanian, 1999).

HS-AD of OFMSW is a mature technology, with most of the recently-constructed industrial plants targeting the semi-continuous HS-AD process (Mattheeuws, 2016). The focus of semi-continuous HS-AD lies on the maximization of the organic loading rate (OLR) that optimizes the methane yield and ensures an adequate organic removal at high TS contents (Benbelkacem et al., 2015; Hartmann & Ahring, 2006). In this line, depending on the organic waste used in HS-AD, the operational TS content is substantially lower than the feed TS, as the organic substrate is converted to biogas by methanogenesis (Pastor-Poquet et al., 2018).

Therefore, HS-AD lies on a balance between maximizing the OLR and TS content, while minimizing the chances of reactor failure. Particularly, in order to startup HS-AD, the OLR needs to be increased relatively slowly, permitting the methanogens to grow and adapt to the new conditions. The transient (non-steady) OLR modification in HS-AD aims to find an optimum stationary (steady-state) operation to be used, avoiding acidification and maximizing the economy of the process (Angelidaki et al., 2006; Bolzonella et al., 2003). However, the risk of inhibition and failure is undesirably high under HS-AD startup, potentially requiring the implementation of recovering strategies (i.e. reactor content dilution) to minimize the influence of inhibitory substances, or even restarting the process when a significant methanogenic imbalance occurs (Fricke et al., 2007; Kayhanian, 1999).

This study evaluated the highest tolerable TS content in semi-continuous HS-AD of OFMSW, by gradually increasing the OLR in semi-continuous reactors operated at 55ºC, until process failure occurred by acidification. Two feeding strategies were used: mono-digestion of OFMSW and co-digestion of OFMSW and beech sawdust – as a model lignocellulosic substrate, simulating the inclusion of GW in OFMSW. Aiming to minimize the risk of substrate overload, the mass retention time (MRT) was relatively extended by reducing the effluent compared to the influent mass, according to the daily mass content removal by biogas production observed in the semi-continuous reactors.

2 MATERIALS AND METHODS

2.1 Substrates and Inoculum

The substrates used in this study were OFMSW and beech sawdust. OFMSW consisted of a mixture of household waste collected in Cassino (Italy), restaurant waste, spent coffee, and garden waste collected at the university facilities, with an approximated wet weight proportion of 45, 35, 15 and 5 % (w/w), respectively. OFMSW was minced twice to a particle size ≤ 5-10 mm by an industrial mincer [REBER 9500NC, Italy], fully homogenized manually and stored in 5 L buckets at -20ºC. During mincing and homogenization, no extra water was added to the raw substrate. A single 5 L bucket of OFMSW was thawed at room temperature overnight, as required to feed the semi-continuous reactors. Goldspan® beech sawdust with 1.0-2.8 mm particle size was used as co-substrate, to simulate biodegradable green/lignocellulosic waste.

The inoculum for semi-continuous experiments was obtained from a pre-adapted 'wet' AD (i.e. TS ≤ 5 %) source reactor operated at 55ºC. The pre-adaptation of 20 L sludge, collected from a mesophilic (35ºC) digester treating buffalo manure and mozzarella whey (Capaccio, Italy), consisted of a 4-month progressive feeding of tap-water-diluted OFMSW at 55ºC, in order to adapt the inoculum to the new substrate and temperature.

Prior to start the mono-digestion experiments, the source reactor was kept unfed for 1 month to consume/reduce the organic content, while continuing with the inoculum adaptation to the new substrate. Subsequently, the feeding with diluted OFMSW was resumed to recover methanogenesis. After 7 and 15 days from the feeding restart, 4 kg of sludge were taken from the source reactor, filtered through a 1 mm mesh and used to inoculate the mono-digestion reactors "A" and "B", respectively. Therefore, the inoculum was slightly different in reactors A and B, as shown in section 3.1.

During the mono-digestion experiments, the source reactor was periodically fed with diluted OFMSW and the mono-digestion reactors effluents, to maintain the reactor volume and methanogenic activity. Once the mono-digestion experiments ended, the source reactor was kept unfed for 1 month to serve as inoculum for the co-digestion experiments. Thus, 3.4 kg of reactor content were filtered through a 1 mm mesh and used to inoculate each co-digestion reactor “A”, “B” and “C”.

2.2 Experimental Setup

The laboratory-scale semi-continuous reactors consisted of 5 L polyethylene terephthalate (PET) bottles with a modified head allowing the (semi-)solid waste input, reactor content withdrawal and biogas measurement [Figure 1]. The reactor port was a polyvinyl chloride (PVC) flexible hosepipe with two valves, easing the reactor loading/unloading while avoiding air intrusion. The biogas output, containing a sampling septum, was connected to 5 L Tedlar® bags [Sigma-Aldrich, USA]. All reactors were maintained at 55ºC within a temperature-controlled TCF 400 oven [ARGOLAB, Italy].

2.3 Operation Strategy

Two semi-continuous reactors for mono-digestion of OFMSW or three reactors for co-digestion of OFMSW and sawdust were operated simultaneously in a drag-and-fill mode. The semi-continuous reactors (i.e. kg) and the reactor influents/effluents (i.e. g) were weighed on a ± 0.01 precision scale. The OLR was evaluated as the daily substrate addition in terms of volatile solids (VS) divided by the reactor mass content (i.e. g VS/kg·d), while the MRT was evaluated as the quotient between the reactor mass and the daily effluent mass (i.e. days). Since the reactors were fed a maximum of 5 days per week, 7-days moving average OLR and MRT were estimated. Moving-average operational variables are well suited indicators of the immediately preceding operations (i.e. feeding, dilution, reactor content removal) to discern about the risk of VFA buildup in semi-continuous digesters. Moreover, expressing the operational conditions as a moving-average eases the comparison of digesters, when feeding days are not the same or an important mass removal occurs.

During each drag-and-fill operation, the reactor content was 1) homogenized before opening the system, 2) sampled and 3) analyzed mainly for pH and alkalinity – since pH had to be maintained over 6.5, as an important methanogenic inhibition might take place below this threshold (De Vrieze et al., 2012; Gerardi, 2003). Depending on the pH and alkalinity, 4) the proper amount of substrate was used or diluted as needed, 5) prior to be fed to the reactors. Finally, 6) the reactor content was homogenized once again, while the Tedlar® bags were checked for biogas production and subsequently emptied.

To increase the reactor TS content from ‘wet’ AD (i.e. TS < 5 %) to HS-AD (i.e. TS ≥ 10 %), the OLR was controlled by increasing/decreasing the daily amount of substrate and/or tap water addition based on the methanogenic activity, and aiming to minimize the substrate overload. To evaluate the differences in the reactor performance, mono-digestion reactors were fed in parallel using different OLR/MRT in each reactor, as shown in section 3.2. Subsequently, co-digestion reactors were also operated in parallel at three different OLR/MRT. In each reactor, the methanogenic activity was roughly associated with the relative increase of the pH and inorganic carbon alkalinity (ALK_P), the reduction of the reactor mass content and the biogas production compared to previous operational values, as also mentioned in section 3.2. For example, a relative pH and ALK_P increase of approximately 0.5 pH units and 0.3 g $CaCO_3$/kg, respectively, alongside a reactor mass removal of about 30-50 g/d and a specific biogas production higher than 250 mL/kg reactor content·d were associated with ongoing methanogenesis, indicating that the OLR could be maintained or relatively increased. Similarly, the relative increase of intermediate alkalinity (ALK_I) (i.e. 0.5 g Acetic Acid/kg) was used as a preliminary indicator of the potential VFA buildup and risk of substrate overload (Lahav et al., 2002).

All these parameters were further complemented with the user's evaluation of the previous operation, in order to decide for the daily feed/dilution to be used. Thus, all reactors were started with a low OLR (i.e. 2 g VS/kg·d) that was gradually increased to increase the TS content. As reactor performance deteriorated with increasing OLR, the reactor feeding was reduced/stopped to prevent acidification (i.e. $pH \leq 6.0$).

The reactor mass was maintained constant by reducing the effluent compared to the influent mass, according to the observed reactor mass content removed by biogas production from the previous operation. With this strategy, the MRT was relatively extended, aiming to promote the methanogenic adaptation in case of overloading. Semi-continuous reactors were fed until acidification occurred. From this point, feeding was stopped and reactor dilution and/or inorganic salt addition (i.e. $NaHCO_3$ and $FeCl_2$) were tested as recovering strategies. A summary of the weekly operational variables is presented as Supplementary Information.

2.4 Bio-Physical-Chemical Analyses

The pH, ALK_P and ALK_I were determined from the supernatant of solid and semi-solid samples (Lahav et al., 2002), after diluting the sample with distilled water, homogenization and centrifugation at 6000 rpm for 15 min (EPA, 2015). The TS and VS content, total Kjeldahl (TKN) and ammonia (TAN) nitrogen, and the total H_2S were determined by the standard methods (APHA, 1999). The NH_3 was approximated as a function of TAN and pH (Astals et al., 2015). The VFA (acetic, propionic, butyric and valeric acids) were measured with an LC-20AD HPLC [Shimadzu, Japan], mounting a Rezex ROA-Organic Acids 8+ column coupled to a 210 nm UV detector, and using 0.0065 M H_2SO_4 at 0.6 mL/min as mobile phase. The biogas composition (CH_4, CO_2 and H_2) was analyzed with a 3400 GC-TCD [Varian, USA], using argon as carrier gas.

The biomethane potential (BMP) test for OFMSW used 3.0 g of substrate, 50.0 g of source inoculum, 40.0 g of distilled water and 0.10 g of $NaHCO_3$ in 280 mL bottles (6 replicates), with an inoculum-to-substrate ratio (ISR) of 2.0 g VS/g VS. The BMP test for sawdust used 1.0 g substrate and 50.0 g of inoculum in 160 mL bottles (3 replicates), with an ISR of 1.0 g VS/g VS. BMP tests were performed according to Angelidaki and Sanders (2004) and Holliger et al. (2016). In the BMP test for OFMSW, the distilled water and $NaHCO_3$ addition served to minimize the chances of inhibition (i.e. by NH_3) and acidification, respectively. In contrast, NH_3 build-up and acidification were not expected in the BMP test of sawdust, due to the low nitrogen content and the reduced biodegradability of sawdust, as thoroughly discussed in next section, permitting also to use a lower ISR. Both BMP tests lasted longer than 100 days. Blank assays included the inoculum and further distilled water compensating for the absence of substrate, using three replicates in each BMP. Inoculum activity assays using a reference substrate were not performed.

The BMP was the normalized methane production (P = 1 bar, T = 0ºC), excluding the methane production of the inoculum, per unit of substrate VS added. The gas production was evaluated with a two-vessel displacement system, with the first vessel containing 4 N NaOH to capture CO_2 and the second vessel containing water to be ‘displaced’. The bottles were sealed with butyl rubber stoppers and aluminum crimps and flushed with helium, before adding 0.2 mL of 10 g/L Na_2S piercing the septum to ensure an adequate redox potential (Angelidaki & Sanders, 2004). All bottles were incubated at 55ºC and agitated only while measuring the gas production.

2.5 Statistical Analyses

The Dixon’s test for BMP outliers was applied as recommended by Holliger et al. (2016). The unpaired t-test of Microsoft Excel 2016 (Microsoft, USA) was applied to determine the statistical significance of experimental data, using the two-tail p-value at 95 % confidence.

3 RESULTS AND DISCUSSION

3.1 Bio-Physical-Chemical Characterization of Substrates and Inoculum

OFMSW showed a TS of 26 %, a VS/TS ratio of 0.93 and a TKN of 24.8 g N/kg TS, in agreement with real source-sorted OFMSW (Angelidaki et al., 2006; Bolzonella et al., 2006; Jokela & Rintala, 2003). The high VS/TS ratio of OFMSW (i.e. > 0.9) indicated minimal presence of inert materials (Pavan et al., 2000). Sawdust showed a TS of 94 % and a VS/TS ratio of 0.99, similar to those obtained by Brown and Li (2013) for 40ºC-dried yard waste, suggesting that beech sawdust could simulate GW. The BMP of

OFMSW and sawdust was 497 and 161 NmL CH_4/g VS, respectively. Table 1 shows the bio-physical-chemical characterization of OFMSW and sawdust. Despite the thorough mincing and homogenization, minor modifications were observed in the OFMSW characterization (i.e. TS, TKN or BMP), mainly attributed to the substrate heterogeneity.

The inoculum in mono-digestion reactors A and B showed a common TS and TKN of 2.8 % and 161 g N/kg TS, respectively. An initial acetic acid concentration of 2.30 and 3.30 g/kg was observed in reactors A and B, respectively, being this difference associated with the later inoculation of reactor B than reactor A. The inoculum used in co-digestion reactors showed a TS of 2.5 %, a TKN of 139 g N/kg TS and an acetic acid concentration of 0.02 g/kg. The inoculum compositions are shown in Table 2.

3.2 Semi-continuous Operation – Increasing the TS Content

3.2.1 Mono-digestion of OFMSW

Mono-digestion results are summarized in Figures 2 and 3. The weekly-averaged results were also included as Supplementary Information. The 7-days average OLR in reactors A and B was varied from an initial 2.4 (day 6) and 6.0 (day 13) g VS/kg·d to 4.9 and 5.5 g VS/kg·d, respectively, on day 17 [Figure 2a]. Thus, a common OLR (i.e. around 5 g VS/kg·d) was achieved, aiming to compensate for the 1-week-lagged inoculation in reactor B. After two days with no feed, feeding was resumed in reactor B on day 20, but not in reactor A due to the low pH (i.e. 6.4) [Figure 3a]. As pH recovered in reactor A (i.e. from 6.4 on day 21, to 7.6 on day 29) due to methanogenesis activity, feeding was resumed. During the same period, ALK_P in reactor A increased alongside pH from 1.1

to 2.7 g $CaCO_3$/kg (data not shown), as an indicator of ongoing methanogenesis. By day 45, a maximum OLR of 6.8 and 8.5 g VS/kg·d was reached in reactors A and B, respectively. After day 48, the OLR required progressive reduction to minimize the risk of acidification. The last feeding in reactors A and B was implemented on days 78 and 73, respectively, as both reactors showed pH ≤ 6.5 and CH_4 content ≤ 40 % [Figure 2f]. From this point, mono-digestion reactors were left unfed aiming to promote the recovery of methanogenesis.

The OLR in reactor B was averagely about 1.5 g VS/kg·d higher than that used in reactor A during the whole experiment ($p < 0.001$), explaining the relatively faster acidification observed in reactor B. Thus, prior to the occurrence of acidification, reactor B was fed with an average 35 g VS/d, significantly higher than the 26 g VS/d used for reactor A ($p = 0.03$). The initial MRT was 55 (day 6) and 29 days (day 13) for reactors A and B, respectively, and was gradually increased to maintain the methanogenic performance at higher OLR [Figure 2b]. Noteworthy, the MRT and OLR in these semi-continuous reactors did not show an inverse pattern, since the dilution as well as the influent and effluent mass flows used were different to account for the organic removal.

Uncoupling the influent and effluent mass flows in the semi-continuous reactors, based on the HS-AD reactor content removal by methanogenesis, permitted to increase the MRT and OLR simultaneously. In this study, the MRT was considered as a more suited indicator in HS-AD than the hydraulic retention time (HRT), since both the specific weight of the influent/effluent and the reactor mass content varied, in contrast to ‘wet’

AD (Pastor-Poquet et al., 2018). As an example, the occurrence of methanogenesis led to a 60 g removal of the reactor mass content in both mono-digestion reactors from day 37 to 41 (data not shown). Prior to the occurrence of reactor acidification, the weekly effluent mass was significantly higher than the influent (i.e. 18 %; $p = 0.03$) to maintain the mono-digestion reactors mass content constant.

The MRT-uncoupling concept was proposed by Richards et al. (1991) and was used by Kayhanian and Rich (1995) to operate a pilot-scale semi-continuous HS-AD reactor fed with OFMSW. In this study, uncoupling the influent and effluent in HS-AD promoted the methanogenic adaptation to overloading conditions and/or the buildup of inhibitors (i.e. NH_3) during the OFMSW degradation. Noteworthy, the MRT must be longer than the doubling time of methanogens (i.e. 20-30 days) to avoid their ‘washout’ from continuous HS-AD reactors, while the methanogenic doubling time might lengthen considerably in presence of inhibitory substances (i.e. NH_3) (Drosg, 2013; Gerardi, 2003; Rittman & McCarty, 2001). Therefore, extending the MRT resulted in a more stable HS-AD operation (Hartmann & Ahring, 2006; Rajagopal et al., 2013), though the sole implementation of influent-effluent uncoupling was not sufficient to avoid HS-AD overloading and acidification during mono-digestion of OFMSW.

3.2.2 Co-digestion of OFMSW and Sawdust

Co-digestion results are summarized in Figures 4 and 5. The 7-days average OLR was increased from 4.5-4.9 g VS/kg·d (day 6) up to 10.9, 12.1 and 12.6 g VS/kg·d (day 23) in reactors A, B and C, respectively. To avoid acidification, feeding was stopped in reactors A and B from day 26, while the OLR was only reduced to 5.0 g VS/kg·d in reactor C [Figure 4a]. As pH recovered (i.e. ≥ 7.0) [Figure 5], feeding was resumed in reactors A and B. A maximum OLR of 14.8 g VS/kg·d was reached in reactor C (day 47) using a sawdust/OFMSW ratio of 2.1 g VS/g VS, prior to the occurrence of reactor acidification (day 56). The maximum OLR in reactor B was 15.1 g VS/kg·d (day 55) using a sawdust/OFMSW ratio of 1.6 g VS/g VS, while an OLR of 16.0 g VS/kg·d was reached in reactor A during the same period, using a sawdust/OFMSW ratio of 1.3 g VS/g VS. The last feeding in reactors A and B was performed on day 76, as a slight but continued drop in pH [Figure 5] and CH_4 [Figure 4f] was observed in both reactors.

The average OLR used for co-digestion was two times higher than that for mono-digestion (i.e. 8.3 *vs.* 4.5 g VS/kg·d, respectively; $p < 0.001$), due to the lower biodegradability of sawdust, though the OLR only due to OFMSW was similar in both cases (i.e. 4.1 *vs.* 4.5 g VS/kg·d, respectively; $p = 0.07$). Thus, a maximum OLR of 7.5-8.0 g VS/kg·d related to the sole supplementation of OFMSW was used in the three co-digestion reactors on day 21, while the OLR solely due to OFMSW was subsequently maintained below 6.0 g VS/kg·d, as sawdust was increased in the feeding mixture [Figure 4a]. In terms of average VS fed, reactor C was operated under relatively more stressing feeding conditions than reactors A and B (i.e. 53 *vs.* 44 g VS/d, respectively; $p = 0.15$), being again the fastest occurrence of reactor acidification related to the highest VS fed.

The initial MRT was higher than 168 days (day 6) and was decreased to 30 days (day 17), similarly in the three reactors [Figure 4c]. From this point, the MRT reached an average of 85 days (day 35) and was subsequently reduced to an average of 37 days (day 53) in all reactors, before being progressively increased to minimize the substrate overload. The MRT was significantly lower in co-digestion than mono-digestion (i.e. 69 *vs.* 92 days, respectively; $p < 0.001$), as lower MRT were predominantly linked to the higher OLR used in co-digestion.

3.3 Influence of the Substrate Composition on the TS Increase

The OLR/MRT control in the mono-digestion reactors fed with OFMSW permitted to increase the TS content, balancing the VFA accumulation with the rapid organic degradation observed [Figures 2 and 3]. Reactors A and B were started at TS = 2.8 % and reached a maximum of 10.7 (day 79) and 11.7 % (day 69), respectively [Figure 2c], being these TS slightly higher than the lower HS-AD threshold (i.e. TS ≥ 10 %). The highest TS in the semi-continuous reactors did not coincide with the maximum OLR, but were predominantly associated with low pH (i.e. ≤ 6.5), when methanogenesis was potentially inhibited. In this line, a gradual increase of the VS/TS ratio (data not shown) was observed in both reactors from 0.69 (day 0) to 0.82 (day 40), reaching a maximum value of 0.87, prior acidification occurred on days 79 and 76 in reactors A and B, respectively.

The highest TS and VS/TS observed in semi-continuous HS-AD of OFMSW were associated with acidification and indicate a reduced VS degradation alongside inhibitory conditions. Particularly, the lowest HS-AD threshold (i.e. TS = 10 %) using OFMSW was reached only under extreme overloading. A more stable HS-AD fed with an easily biodegradable OFMSW (i.e. FW) is also associated with a TS increase alongside overloading/inhibitory conditions. For example, Tampio et al. (2014) reported a TS increase from 7 to 8 % during 400 days of semi-continuous AD fed with FW, though TS rapidly reached 11 % during the next 50 days of operation, when reactor inhibition was likely occurring. In the same line, Bolzonella et al. (2003) reported a TS increase from 5 to 15 % during the initial 60 days of continuous AD pilot-scale startup fed with OFMSW, being the maximum TS associated with the highest total VFA observed (i.e. 2.8 g Acetic Acid/L). All these results were likely related to methanogenesis inhibition, since the VFA accumulation affects the hydrolysis/acidogenesis rates, hampering the organic removal in HS-AD (Vavilin et al., 2008).

The maximum TS obtained in this study for semi-continuous HS-AD of OFMSW should be considered as indicative (only) of those obtainable in steady-state digesters, since the transient/acidification conditions potentially reduced the VS removal. Thus, the operational TS content of stable digesters fed with the easily biodegradable content of OFMSW (i.e. FW) might be lower than those observed along non-steady-state conditions. This is a further indication that a steady-state semi-continuous reactor using an easily biodegradable OFMSW as a substrate might not be operated within the HS-AD threshold (i.e. TS ≥ 10 %).

Co-digestion permitted to increase TS from 2.5 % (day 0) up to a maximum of 33.2 (day 79), 26.7 (day 76) and 27.0 % (day 57) in reactors A, B and C, respectively [Figure 4c]. Hence, the maximum TS reached in co-digestion before the reactors acidified (i.e. 29.0 ± 2.8 %) was considerably higher than the lower HS-AD threshold (i.e. TS ≥ 10 %) and the maximum TS of mono-digestion (i.e. 11.5 ± 0.5 %), due to the addition of sawdust to OFMSW. The highest TS was related again to acidified (i.e. pH < 6.5) or acidifying (i.e. downward trend on pH/CH_4 content) conditions, as observed for mono-digestion. Thus, the VS/TS ratio in co-digestion (data not shown) increased from 0.65 (day 0) to 0.90 (day 40), reaching a maximum of 0.95 before reactors acidified (day 76), due to both the higher VS/TS ratio of sawdust and the reduced VS removal during inhibitory conditions.

These results showed that the particular characteristics of OFMSW determined the maximum operating TS content in semi-continuous HS-AD. Co-digestion of OFMSW and sawdust resulted in approximately three times higher TS than mono-digestion (i.e. 29.0 and 11.5 %, respectively). The inclusion of sawdust in OFMSW favored the rapid TS and OLR increase compared to mono-digestion due to the higher TS and the lower biodegradability of sawdust, as demonstrated by the substantially lower BMP of sawdust than that of OFMSW (i.e. 161 and 497 NmL CH_4/g VS, respectively) [Table 1]. Indeed, lignocellulosic materials (i.e. GW) are normally associated with a reduced biodegradation rate, compared to more easily degradable substrates (i.e. FW), due to the high lignin content hampering hydrolysis (Brown & Li, 2013; Mancini et al., 2018a; Vavilin et al., 2008), being also beneficial to limit the VFA buildup in HS-AD. On the other hand, TAN was 22 % lower during co-digestion than mono-digestion (i.e. 2.9 *vs.* 3.7 g N/kg, respectively; $p < 0.001$) [Figures 2 and 4], due to the lower TKN of sawdust [Table 1]. Noteworthy, the TAN accumulation was likely promoting methanogenic inhibition in this study, as further discussed in section 3.5. Therefore, using sawdust – as GW – was also adequate to adjust the carbon-to-nitrogen (C/N) ratio in HS-AD of OFMSW.

3.4 Main Indicators of Substrate Overload

3.4.1 Evolution of pH and VFA

pH in mono-digestion reactor A decreased from 8.1 to 6.4 due to the rapid acetic acid buildup (i.e. from 3.00 to 9.00 g/kg) observed during the initial 20 days of operation [Figure 3a]. As feeding was stopped from day 20 to 29, pH reached 7.6, while acetic acid decreased below 0.70 g/kg right afterwards (day 34). Propionic, butyric and valeric acids gradually increased from < 0.15 g/kg (day 0) to 5.00, 4.00 and 1.10 g/kg (day 79), respectively. From day 79, pH dropped from 7.1 to 6.1, linked to a sudden acetic acid increase from 3.00 to 5.00 g/kg, and the subsequent CH_4 content drop from 56 to 37 % [Figure 2f].

Mono-digestion reactor B was relatively more stressed than reactor A, as indicated by the wider acetic acid fluctuations (i.e. ± 4.00 g/kg) and the rapid accumulation of propionic acid from 1.20 (day 7) to 5.80 g/kg (day 73) [Figure 3b]. The VFA fluctuation is in line with the fact that methanogens grow relatively slower than the hydrolytic/acidogenic microorganisms in AD (De Vrieze et al., 2012; Gerardi, 2003). Thus, the higher OLR used in reactor B led to a more pronounced methanogenic/acidogenic imbalance, exacerbating the VFA accumulation. The VFA buildup led to a pH decrease from 8.4 to 6.2 in reactor B during the whole experimental period, while a significant acetoclastic inhibition occurred from day 70 to 73, when acetic acid abruptly increased from 2.70 to 5.80 g/kg.

pH in co-digestion reactor A gradually decreased from 8.7 to 6.4 along the experimental period, showing a minimum of 6.1 associated with a peak of acetic acid of 8.30 g/kg (day 26) [Figure 5a]. Acetic acid was considerably consumed (i.e. < 0.36 g/kg) by day 47 due to ongoing methanogenesis, and progressively increased thereafter by overloading. Similarly, pH in reactor B showed a minimum of 6.3 when acetic acid peaked at 8.20 g/kg (day 26) [Figure 5b], while the acetic acid was extensively consumed (i.e. < 0.35 g/kg) by day 41 prior to increase again steadily. In reactor C, acetic acid had a similar evolution with a maximum of 7.20 g/kg (day 26) [Figure 5c], while pH dropped to 6.0 on day 57, associated with a sharp acetic acid build-up from 1.00 to 3.70 g/kg. Propionic, butyric and valeric acids increased from 0.50, 0.14 and 0.00 g/kg (day 0) to a maximum range of 3.00-3.50, 2.90-3.20 and 2.50-2.60 g/kg, respectively, obtained right after acidification occurred on day 79 in reactors A and B, and on day 56 in reactor C. The pH was relatively lower (i.e. 2 %; $p = 0.13$) and total VFA was relatively higher (i.e. 5 %; $p = 0.25$) during mono-digestion than co-digestion, likely due to the faster degradation rates but also the higher release of inhibitory compounds related to OFMSW than sawdust, as discussed in section 3.3.

Feeding the reactors a maximum of 5 days per week influenced the reactor dynamics, since pH increased and VFA – mainly acetic acid – decreased during the periods with no feed. The pH and VFA modifications [Figures 3 and 5] were associated with the TS removal, as mentioned before, and also affected the biogas production/composition, and the TAN buildup [Figures 2 and 4]. As an example, in co-digestion reactor A, pH increased from 7.0 to 7.9 from day 37 to 41, while acetic acid decreased from 4.30 to

2.40 g/kg, triggering a biogas production of 0.6 L/kg reactor content and a methane content increase from 59 to 70 % [Figure 4 and 5].

3.4.2 Biogas Production and Composition

Mono-digestion of OFMSW resulted in a cumulative biogas production of 65 and 66 L/kg reactor content in reactor A and B, respectively [Figure 2e]. Biogas production was mainly correlated to the acetic acid consumption [Figure 3a], as mentioned in the previous subsection. For example, 21 L/kg reactor content of biogas were measured during the initial 20 days of reactor A, before acetic acid accumulated and biogas production slowed down.

Biogas composition measurements started on day 60 showing an average of 63 % CH_4 in both mono-digestion reactors [Figure 2f], which subsequently fluctuated showing a downward trend alongside the VFA accumulation. The CH_4 content dropped below 40 % in both reactors right after biogas production definitely ceased on days 78-79. The reduction of CH_4 content in the biogas is also an indicator of AD imbalance, though it might be inappropriate to assess rapid changes in the reactor performance (Drosg, 2013). The highest H_2 concentration (data not shown) was 1.8 and 1.1 % on day 59 in reactors A and B, respectively, while H_2 remained below 0.8 % in both reactors during the rest of the experiment. The presence of H_2 indicated that the hydrogenotrophic methanogens were unable to cope with the rapid H_2 production from acidogenesis, since H_2 higher than 1-2 % in the gas phase is normally associated with AD overloading (Drosg, 2013; Molina et al., 2009).

Co-digestion in reactor A led to a cumulative biogas production of 48 L/kg reactor content, while 49 and 27 L/kg reactor content were observed in reactors B and C, respectively [Figure 4e]. In spite of the higher OLR used in co-digestion, the biogas production was considerably lower than that obtained with mono-digestion (i.e. 65 L/kg reactor content). Thus, the specific biogas production was 229 ± 20 L/kg VS added in mono-digestion and 86 ± 18 L/kg VS added in co-digestion (i.e. 62 % lower), due to the reduced biodegradability of sawdust.

The CH_4 content [Figure 4f] reached a peak of 75 % during the first two weeks of operation in the three co-digestion reactors, but it decreased subsequently as VFA accumulated [Figure 5]. A minimum 43 % CH_4 was detected in reactor A associated with the last biogas production observed (day 82), while a sharp drop from 60 to 29 % CH_4 was observed in reactor C right after day 60. H_2 was detected at 0.3 % in the three co-digestion reactors on day 23 (data not shown). Thereafter, H_2 was not detected in reactor A, while reactor B showed a single H_2 peak of 1.5 % on day 70, right after the reactor was accidentally opened to the atmosphere. In reactor C, H_2 peaks of 1.7, 1.2 and 1.6 % were observed on days 41, 47 and 58, respectively, supporting the occurrence of a more extensive overload in this reactor.

3.5 Testing Recovering Strategies

Once acidification occurred, feeding was stopped and some recovering strategies were tested to resume methanogenesis. In mono-digestion reactor A, a 3 M $NaHCO_3$ buffer solution was added on days 83 and 84 to raise the pH (i.e. from 6.2 to 6.8) within a suitable range for methanogens (i.e. 6.5-7.0). Adding $NaHCO_3$ is normally used to counteract acidification when digesters show a reduced ALK_P (Chen et al., 2008; Holliger et al., 2016). However, methanogenesis did not recover after more than 20 days.

On day 76, $FeCl_2$ was supplemented to mono-digestion reactor B in a higher amount than the stoichiometric, to precipitate the total H_2S in the system (i.e. 30 mg H_2S/kg, data not shown). However, $FeCl_2$ overdosing resulted in a pH drop from 6.3 to 5.7 (days 76-77). Thus, 2 M $NaHCO_3$ solution was rapidly added to recover the pH to 6.6 (day 77). Both Fe^{2+} and/or Fe^{3+} can be used to precipitate sulfide in AD, but Fe^{2+} was preferred in this study to avoid the inclusion of a strong electron acceptor (i.e. Fe^{3+}) that could react with organic compounds in the anaerobic digester (i.e. $Fe^{3+} + 1/2\ H_2 \rightarrow Fe^{2+} + H^+$, $\Delta G^{o\prime} << 0$) (Fermoso et al., 2015; Rittman & McCarty, 2001). After 2 weeks of methanogenic inhibition (day 90), 200 g of ‘wet’ AD inoculum from the source reactor were added to reactor B, allowing a gradual methanogenic recovery, associated with an increase of pH from 6.9 to 7.3 [Figure 3b] and CH_4 content from 20 to 52 % [Figure 2f], until the end of the reactor operation.

Aiming to recover methanogenesis in all co-digestion reactors, water was progressively added to dilute the effect of potential methanogenic inhibitor(s). The progressive addition of low amounts of water in co-digestion reactors permitted to maintain HS-AD conditions (i.e. TS ≥ 10 %), thanks to the elevated TS content reached before reactors acidified (i.e. TS ≥ 30 %). Dilution was performed in reactors A and B from day 79 and in reactor C from day 62. In reactor A and B an average of 180 and 170 mL of water was used, respectively, on days 79, 82, 84, 91 and 98, while an average of 160 mL of water was added to reactor C on days 62, 63, 68 and 91.

In conclusion, neither water, nor buffer addition permitted to recover acidified/acidifying HS-AD reactors, probably because of the important imbalance between methanogens and acid-producers in the system (Gerardi, 2003). In these conditions, inoculum addition might be the only way to recover an acidified HS-AD reactor, though emptying and re-inoculating the reactor might be necessary (Fricke et al., 2007). Another strategy to prevent reactor acidification and/or enhance the digester performance is trace element (i.e. Se, Ni, Co, Mo, W) addition (Fermoso et al., 2015; Mancini et al., 2018b). However, this was out of the scope of this manuscript.

3.6 Ammonia Buildup

In this study, overloading was associated with the high OLR used, but also with the NH_3 buildup, in the semi-continuous reactors. The high OLR and the degradation of the protein content of OFMSW increased the TAN content in both mono-digestion reactors [Figure 2d]. TAN ranged from 3.4 g N/kg (day 0) to a maximum of 4.8 and 4.9 g N/kg in reactors A and B (day 104), respectively, with both reactors showing a minimum TAN of 3.0 g N/kg around day 20. The initial NH_3 was 1.1 and 1.7 g N/kg in reactors A and B, respectively. Subsequently, NH_3 fluctuated with an overall decreasing trend along the pH modification in both reactors, showing peaks higher than 1.0 g N/kg mainly when pH was relatively high (i.e. ≥ 8.0) [Figure 3]. In reactor A, NH_3 reached peaks of 1.4 (day 7) and 1.5 g N/kg (day 34), while NH_3 higher than 1.5 g N/kg was repeatedly observed in reactor B (i.e. days 20, 27, 34 and 41).

In co-digestion, the initial TAN was 3.0 g N/kg and slightly increased to a maximum of 3.3, 3.6 and 3.3 g N/kg (day 61) in reactors A, B and C, respectively [Figure 4d]. TAN subsequently decreased due to the reduced OFMSW feeding and the progressive dilution used for HS-AD recovering, until a minimum of 1.9, 2.3 and 2.8 g N/kg was reached in reactors A, B and C, respectively (day 112). The initial NH_3 was 2.0 g N/kg and progressively decreased in the three reactors alongside pH. NH_3 peaked at 1.5 g N/kg (day 12) and 1.2-1.7 g N/kg (day 19), rapidly decreasing to ≤ 0.1 g N/kg (day 23), similarly in all reactors. From this point, NH_3 was maintained below 1.0 g N/kg in the three reactors. Thus, NH_3 was considerably reduced during co-digestion alongside the reduction of OFMSW in the feed, since peaks higher than 1.0 g N/kg were not observed from day 20 onwards, in contrast to mono-digestion reactors.

NH_3 inhibition was likely one of the main triggers of overloading in this study, since the high NH_3 levels observed (i.e. ≥ 1.0 g N/kg) are normally associated with methanogenic inhibition and VFA accumulation in AD (Drosg, 2013; Rajagopal et al., 2013). Thus, despite each AD system might show particular NH_3 inhibition thresholds depending on the anaerobic consortia (Fricke et al., 2007; Westerholm et al., 2016), a gradual methanogenic adaptation to high levels of TAN (i.e. ≥ 4.0 g N/kg) might be crucial to increase OLR in semi-continuous HS-AD of OFMSW (Hartmann & Ahring, 2006; Rajagopal et al., 2013).

In this study, a tradeoff was needed between the ‘undesired’ TAN buildup and the rapid TS removal observed, to reach HS-AD conditions (i.e. TS ≥ 10 %) with mono-digestion of OFMSW. For example, the different TS and TAN dynamics can be appreciated in mono-digestion reactor A from day 30, when TS fluctuated while TAN steadily increased [Figure 2]. Potential ammonia contingency strategies in AD, as increasing the substrate dilution, reducing the OLR, and/or increasing the MRT (Kayhanian, 1999; Rajagopal et al., 2013), would have lengthened considerably the experimental time, or even prevented to achieve HS-AD conditions (i.e. TS ≥ 10 %) with mono-digestion of OFMSW.

4 CONCLUSIONS

In this study, reducing the effluent compared to the influent mass (i.e. 18 %) permitted to extend the MRT in semi-continuous mono-digestion of OFMSW, and obtain a specific biogas production of 229 L/kg VS added, due to the high biodegradability of OFMSW. However, the sole implementation of influent/effluent uncoupling was not sufficient to avoid reactor overload and acidification when reaching HS-AD conditions (i.e. TS ≥ 10 %). The average OLR was 4.5 g VS/kg·d, whereas a maximum 11.5 % TS was reached. In contrast, the addition of beech sawdust to OFMSW allowed to operate co-digestion reactors with an average OLR of 8.3 g VS/kg·d, and reach a maximum 29.0 % TS. Co-digestion lowered by 22 % the TAN content, though an average 186 L/kg VS added of biogas was obtained. Therefore, the addition of sawdust, as an example of lignocellulosic substrate, to OFMSW (i.e. 1-2 g VS-Sawdust/g VS-OFMSW) is an adequate strategy to stabilize HS-AD at very high TS contents (i.e. 20-30 %). Nonetheless, a compromise must be found between increasing the TS content and reducing the specific biogas production by co-digestion, since both aspects strongly determine the HS-AD economy for OFMSW treatment.

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TABLE AND FIGURE CAPTIONS

Table 1: Bio-physical-chemical characterization of substrates.

Table 2: Physical-chemical characterization of inoculums.

Figure 1: Experimental setup. 1) Reactor body; 2) reactor head; 3) feeding port; 4) gas output; 5) gas measuring port; and 6) opening valves.

Figure 2: Mono-digestion of OFMSW: a) Organic loading rate; b) mass retention time; c) total solids; d) total and free ammonia nitrogen (NH_3); e) cumulative biogas production; and f) methane content. Black arrows represent the $NaHCO_3$ addition in reactor A, while dotted arrows represent the $FeCl_2$ or inoculum addition in reactor B.

Figure 3: Mono-digestion of OFMSW: Volatile fatty acids and pH in a) reactor A; and b) reactor B. Black arrows represent the $NaHCO_3$ addition in reactor A, while dotted arrows represent the $FeCl_2$ or inoculum addition in reactor B.

Figure 4: Co-digestion of OFMSW and sawdust: a) Organic loading rate – parentheses indicate the sole addition of OFMSW; b) mass retention time; c) total solids; d) total and free ammonia nitrogen (NH_3); e) cumulative biogas production; and f) methane content.

Figure 5: Co-digestion of OFMSW and sawdust: Volatile fatty acids and pH for a) reactor A; b) reactor B; and c) reactor C.

Figure 1

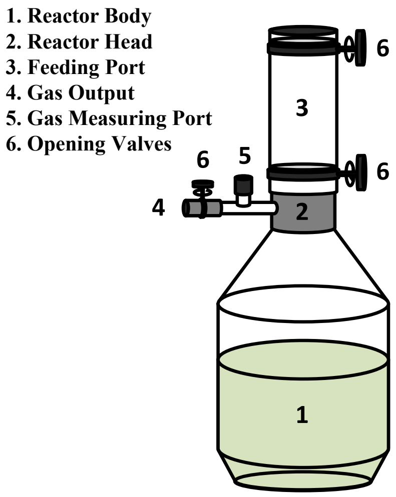

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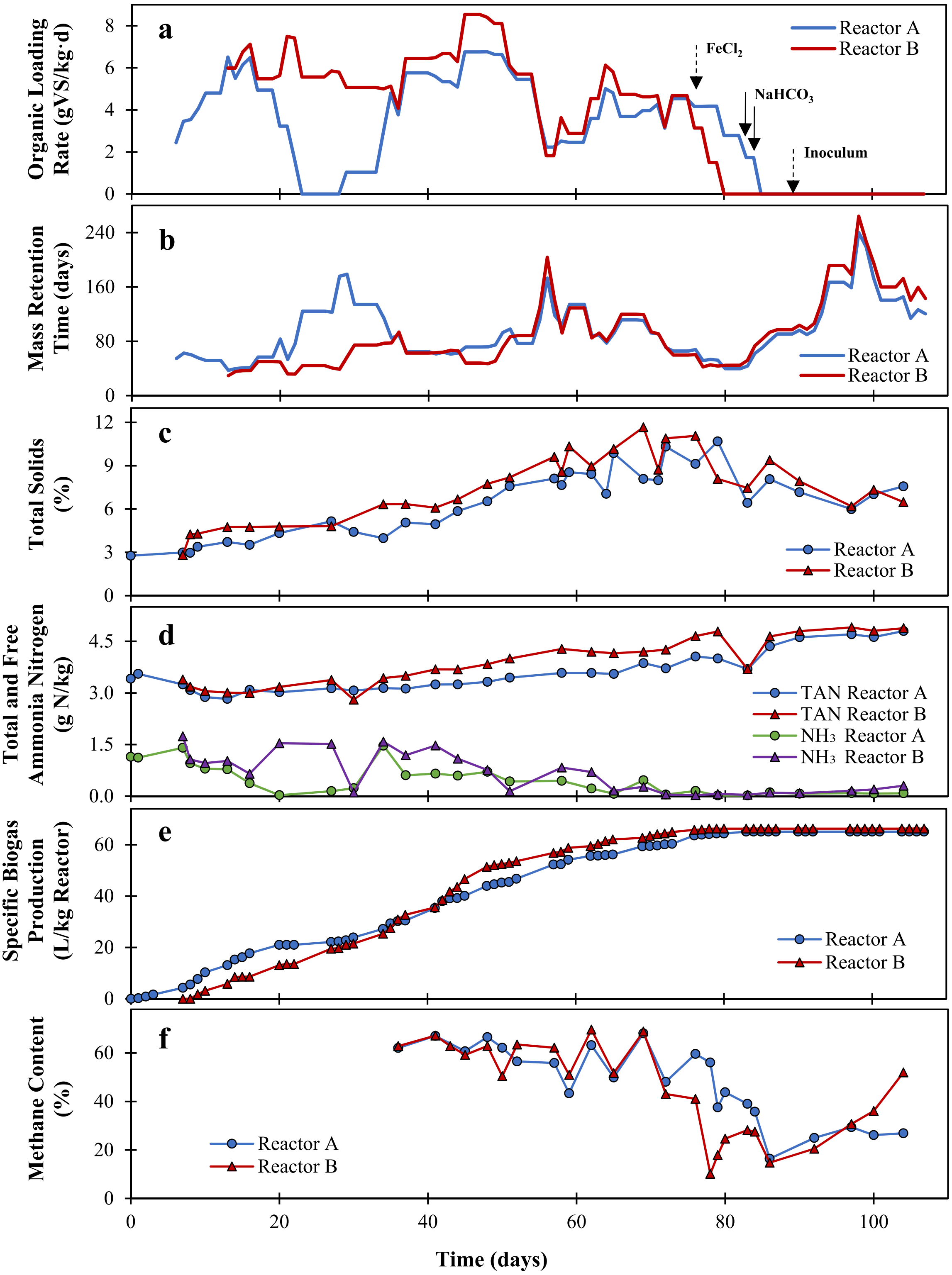
Figure 2



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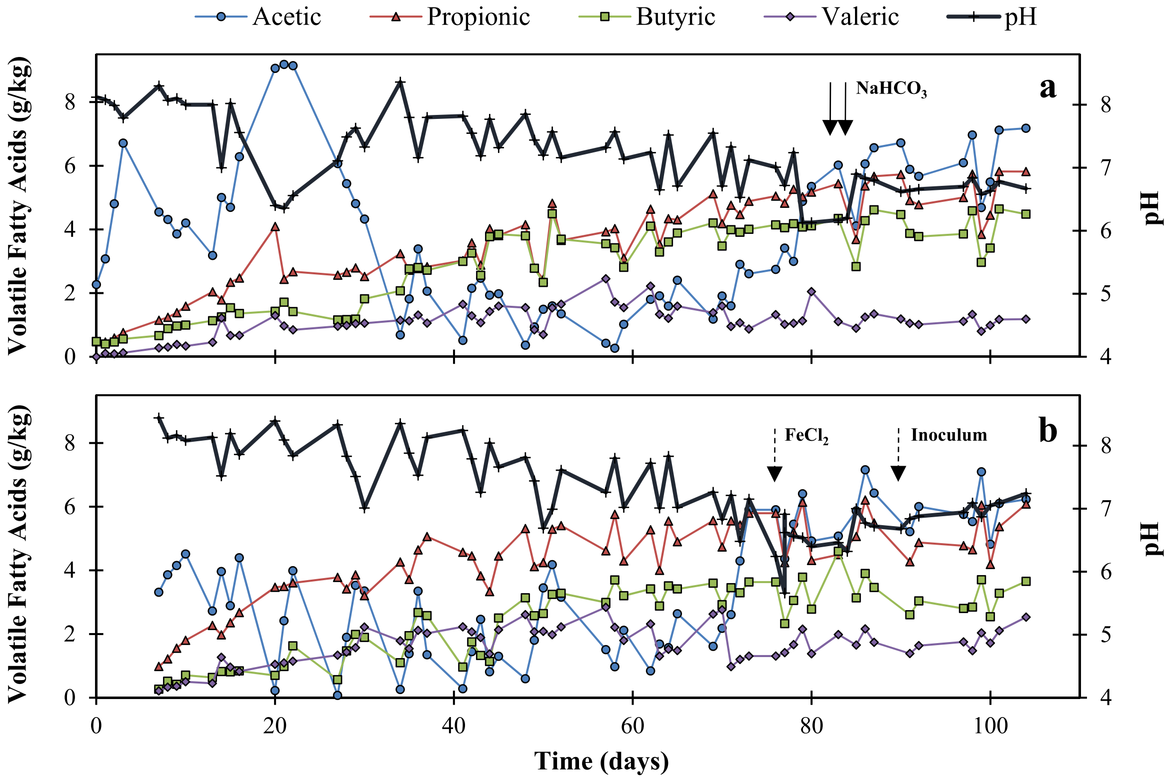

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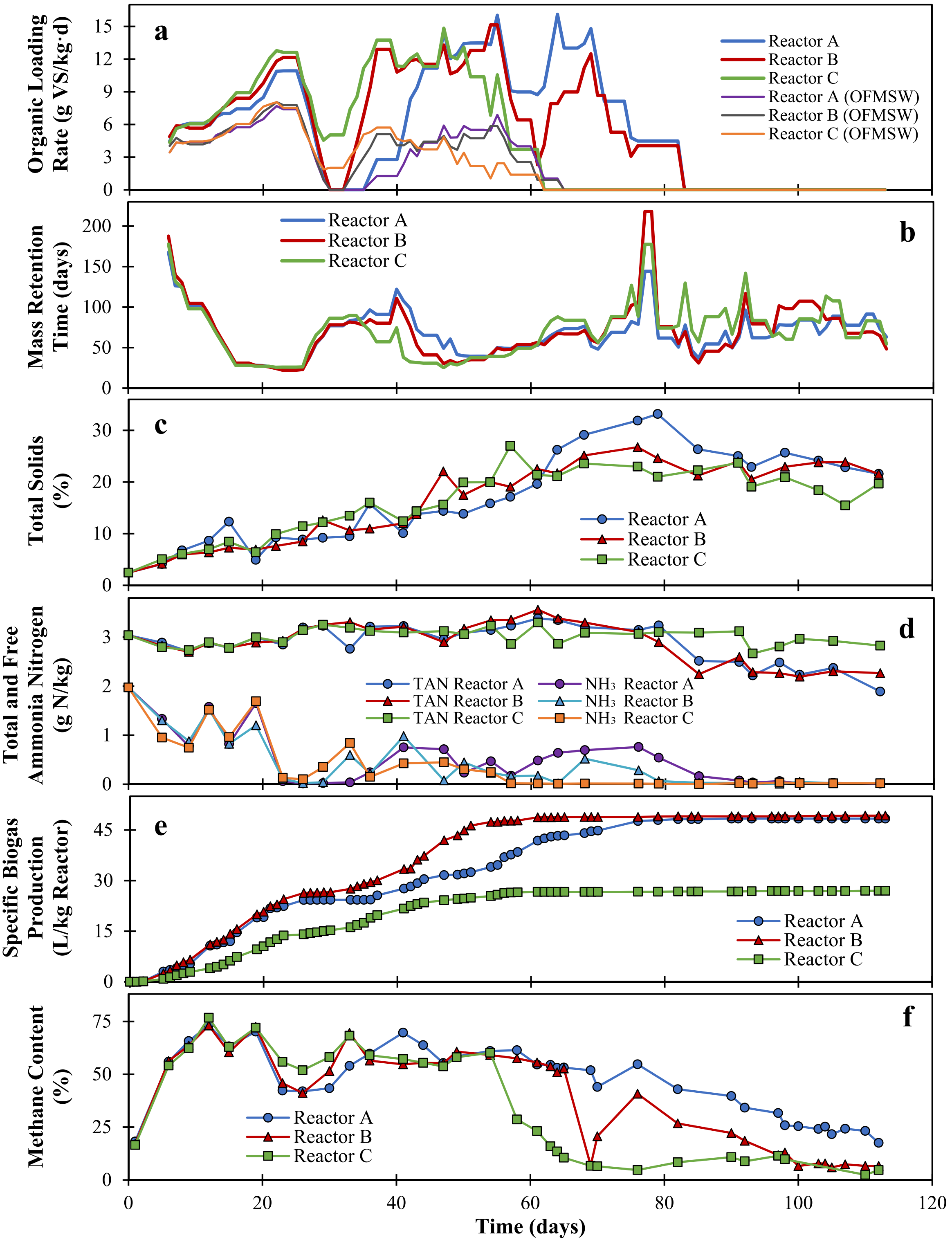

Figure 4: Co-digestion of OFMSW and sawdust: a) Organic loading rate – parentheses indicate the sole addition of OFMSW; b) mass retention time; c) total solids; d) total and free ammonia nitrogen (NH_3); e) cumulative biogas production; and f) methane content.

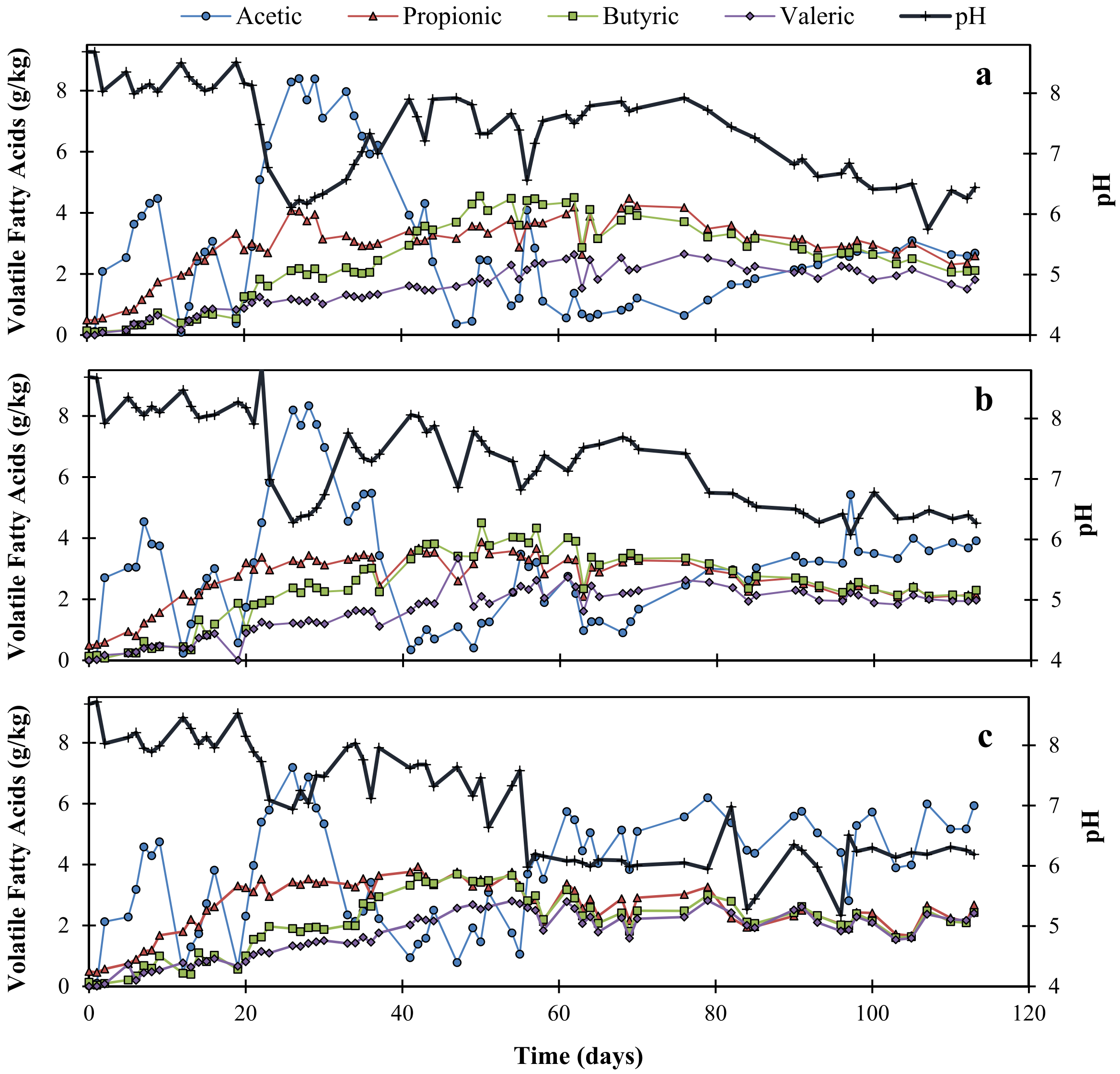

Figure 5: Co-digestion of OFMSW and sawdust: Volatile fatty acids and pH for a) reactor A; b) reactor B; and c) reactor C.

Table 1: Bio-physical-chemical characterization of substrates.

	OFMSW	**Sawdust**
TS (%)	26.52 ± 1.35	93.69 ± 0.42
VS (%)	24.62 ± 1.27	92.64 ± 0.70
VS/TS	0.93 ± 0.02	0.99 ± 0.01
TKN (g N/kg TS)	24.78 ± 1.50	0.98 ± 0.17
TAN (g N/kg TS)	4.92 ± 0.06	0.12 ± 0.01
pH	4.40 ± 0.14	5.65 ± 0.06
ALK_I (g Acetic/kg)	1.17 ± 0.82	1.50 ± 0.26
BMP (NmL CH_4/g VS)	497 ± 58	161 ± 12

Table 2: Physical-chemical characterization of inoculums.

	Mono-digestion		Co-digestion
	Reactor A	**Reactor B**	**Reactors A, B & C**
TS (%)	2.8	2.8	2.5
VS (%)	1.9	2	1.6
VS/TS	0.69	0.70	0.64
TKN (g N/kg TS)	161	161	139
TAN (g N/kg TS)	122	121	122
pH	8.12	8.44	8.69
ALK$_P$ (g CaCO$_3$/kg)	9.6	9.6	9.3
ALK$_I$ (g Acetic/kg)	5.3	6.3	3.2
Acetic (mg/kg)	2260	3310	20
Propionic (mg/kg)	470	980	490
Butyric (mg/kg)	480	260	140
Valeric (mg/kg)	0	210	0

Supplementary Information